FUNGICIDES
An Advanced Treatise

VOLUME I
Agricultural and Industrial Applications
Environmental Interactions
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FUNGICIDES
An Advanced Treatise

Edited by
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BOYCE THOMPSON INSTITUTE
FOR PLANT RESEARCH, INC.
YONKERS, NEW YORK

VOLUME I
Agricultural and Industrial Applications
Environmental Interactions

1967
ACADEMIC PRESS New York and London
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Preface

The aim of this treatise is to present an integrated and complete discussion of all aspects of agricultural and industrial fungicides except for the readily available and continually changing handbook-type of information on what to spray or apply for the control of a particular fungus. Each subject is comprehensively treated and the discussion synthesized so as to be informative of the present state of knowledge and thought provoking when areas of divergent opinion are encountered or when gaps in our knowledge are pointed out. Although each is complete within itself, the chapters were written as far as possible with an understanding of their relationships to each other.

Twenty-five years ago, at the beginning of the organic fungicide era, it would have been possible for a well-informed worker in the fungicide field to intelligently review and discuss the then current status of knowledge. During the ensuing years the scientific discoveries made through the combined research efforts of plant pathologists and other biologists, chemists, biochemists, physicists, and other specialists have proliferated in many directions our information on fungicides. It was, therefore, immediately obvious when the present treatise was conceived that the comprehensive work desired could be achieved only through the use of multi-authors. Since research knows no boundaries the contributions were selected internationally to obtain a group of individuals capable of giving the broadest possible coverage in depth of the various subject areas.

Volume I is concerned principally with the application, use, and environmental interactions of fungicides. Chapters on the history, usage and how usage is influenced by epidemiology, measurement of fungitoxicity, procedures followed in the commercial development of a fungicide, and the significance of formulation on effectiveness serve to introduce a series of chapters on the status of fungicide research in various areas of use. The last four chapters are concerned with the various interactions between fungicides and the environment which may cause them to be more or less effective. Volume II is devoted to the chemistry and physiology of fungicides. These aspects are discussed for inorganic, synthetic organic, and antibiotic fungicides and naturally occurring antifungal compounds.

It is hoped that the treatise will be useful to the advanced student and professional worker in the fungicide area. It should serve as a source of information and to stimulate the generation of new ideas and approaches to researchers concerned with the synthesis and development of new and better fungicides or their mode of action. Since a basic under
standing of principles is necessary for the most advantageous use of fungicides the information presented here should also be useful to research and extension workers interested in developing better fungus control practices.

The aid and advice of Drs. G. L. McNew, S. E. A. McCallan, L. P. Miller, and R. G. Owens of Boyce Thompson Institute in the general organization of the treatise are gratefully acknowledged. Without the pleasant working environment of Boyce Thompson Institute the editorship would have been most difficult. The greatest share of work in the preparation of the treatise has been borne by the contributors. Their thoroughness and patience in performing their tasks are greatly appreciated. I am also grateful to my wife, Kathryn, for her patience, encouragement, and assistance during the editorship of these volumes.

Yonkers, New York
August, 1967

D. C. TORGESON
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FUNGICIDES
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CHAPTER 1

History of Fungicides

S. E. A. McCallan

I. Introduction

The history of fungicides falls logically into three distinct eras based on the discovery and development of a new kind of fungicide. These are the...
First or Sulfur Era from ancient times to 1882, the Second or Copper Era from 1882 to 1934, and the Third or Organic Fungicide Era beginning in 1934. Naturally the interest and use of the older fungicides carried over into the following eras but their dominance waned with the respective discoveries of Bordeaux mixture in 1882 and of the dithiocarbamate fungicides in 1934. Interestingly, though perhaps not surprisingly, a close parallel exists in the history of insecticides. Thus, Hough and Mason (1931) begin the Second Period of insecticides with the discovery of Paris green in 1868 to control the Colorado potato beetle, and the Third Period with the discovery of DDT in 1939.

The movements of peoples and susceptible crops into new areas precipitated crises with a new pest and a new disease which resulted respectively in the discoveries of 1868 and 1882. The continuing and increasing pressure for better fungicides and insecticides, together with interest in the potential of organic compounds led to the closely timed discoveries of 1934 and 1939. Following on the heels of these events, a new era was also begun with herbicides upon the discovery of the growth-regulating properties of 2,4-D by Zimmerman and Hitchcock (1942). Finally, it is to be noted that just prior to these three significant events, Fleming (1929) had discovered penicillin and so ushered in a new era in medicine. The phenomenon of four era-initiating discoveries in disease and pest control thus appeared within a period of 13 years.

II. THE FIRST OR SULFUR ERA (TO 1882)

A. Ancient Knowledge

The first use of a fungicide is unknown. Although the Old Testament contains many references to blights, blasts, mildews, rusts, and smuts, there seems to have been no effort to control them and these tribulations were accepted as an expression of the wrath of the Deity (Whetzel, 1918).

In the Homeric poems which were written about the eighth century B.C. and described legends and customs of several centuries earlier, sulfur was used as a purifying agent. These are the first known references to a pesticide. Two specific accounts are described. In "The Iliad" XVI, Achilles is preparing for a libation to Zeus and withdraws a sacred cup from a storage chest containing also "tunics, wind-warding cloaks, and fleecy warm blankets." But first "he cleansed it with sulphur and rinsed it well in pure running water" (Homer, Rees' translation, 1953). This cleansing was perhaps necessary because the cup had become contaminated from mildewed leather and fabrics stored in the chest. This reasoning also
1. HISTORY OF FUNGICIDES

seems strengthened because other references to libation in Homer do not mention cleansing with sulfur. Second, in “The Odyssey” XXII, Odysseus, after slaying the suitors, calls for “sulphur for purging and fire,” and he “purified hall, house and courtyard” (Homer, Rees’ translation, 1960). In the prose translations of Rees (Homer, Rees’ translations, 1946, 1950) the modern words fumigate and disinfectant are used in the above two passages.

The next record we have is that of Dioscorides, a Greek physician, who during the first century of the Christian era, advocated the dermatological use of sulfur ointments (Texas Gulf Sulphur Co., 1934). At about the same time Pliny the Elder in his “Natural History,” a collection of Greek folklore, recommended the steeping of seed grain in wine or in a mixture of bruised cypress leaves in order to control “mildew” which presumably was smut (Mason, 1928).

From the time of the Greeks and Romans to well into the eighteenth century there are many references to the use of weird materials and concoctions to control insects and what we now know to be fungus diseases. Common ingredients were the dung and urine of farm animals and occasionally lime or ashes. These fantastic remedies and gropings in the dark are of course to be expected since fungi were not recognized as separate entities nor were fungi clearly established as a cause of plant diseases until Prévost’s classical studies on smut in 1807. However, no advantage was taken of this fact until the time of the Tulane’s brothers and De Bary, 40 or more years later (Keitt, 1959).

While six fungicidal chemicals were known prior to the time of De Bary and Pasteur, Horsfall (1956) states that only two were used solely as fungicides, namely, copper sulfate and zinc chloride. The other four, sulfur, arsenic, mercury, and the glycerides (specifically amurca or drops of olive oil), he suggests were probably first known as insecticides. However, as shown above, sulfur was a general disinfectant and possibly even first used as a fungicide. Shepard (1961) records the first use of mercuric chloride as an insecticide in 1822, but its wood-preserving, i.e., fungicidal, properties were known by 1705 (Boulton, 1930).

The diseases of most concern from ancient times to well into the seventeenth century seem to have been the smuts and rusts of grain. In any case, most of the early discoveries of fungicidal materials were concerned with the control of smut or bunt of wheat. These early records on bunt control have been collected by Woolman and Humphrey (1924). The first recorded experiments to control a recognizable disease, bunt, were not made until 1637. In that year Remant in England tried soaking wheat in various steeps to control bunt. Apparently none was effec-
tive and the nature of the steeps was not disclosed. The well-known English agriculturist, Jethro Tull, reported in 1733 that the use of salt brine as a steep for seed grain originated about 1650. At that time a shipload of wheat was beached near Bristol and the cargo was salvaged, but being unfit for milling was used for seed. The resulting crop was clean and unlike the neighboring stands of smutty wheat. However, over the years the brine treatment was generally ineffective. Tull, unaware of the true nature of infection, came to the astute conclusion that the shipload of wheat from another area probably did not have the smut in the first place. Aucante in 1756 reported that the use of lime and arsenic, and corrosive sublimate as treatments to control wheat smut was generally ineffective, while Bryant in 1784 confirmed the general ineffectiveness of the above treatments (Woolman and Humphrey, 1924). Such candor and objectiveness at this early date by these four is refreshing. Tillet's classic study on the cause of bunt (1755) is of especial interest because of his brilliant plot designs which included seed treatments with saltpeter and lime. Such sophistication in field plots probably was not to be repeated until the founding of Rothamsted a century later.

B. Copper Sulfate

The first use of copper sulfate as a treating medium for bunt, and thus as a fungicide, was probably by Schulthes in 1761. His work, however, was not generally known and Prévous credits Tessier with the discovery in 1788, while Kuhn in turn credits Prévous with the discovery of copper sulfate as a seed steep (Woolman and Humphrey, 1924). Prévous critically studied the effect of copper sulfate at different concentrations, temperatures, and duration of treatments on the viability of the spores of the bunt fungus. This is considered by Horsfall (1956) to be the first example of laboratory testing. A translation of Prévous's classic studies by Keitt is available in the Phytopathological Classics (Prévous, 1807). Julius Kuhn, the "Father of Plant Pathology" (Whetzel, 1918) in 1866 was apparently the first to give a specific practical recommendation for the control of bunt, namely an immersion of the grain for 12 to 14 hours in a 0.5% solution of copper sulfate (Woolman and Humphrey, 1924).

C. Rediscovery of Sulfur

Although sulfur had been known as a pesticide in the time of the ancient Greeks, no mention of it as a fungicide appeared again until the
beginning of the nineteenth century. The treatise of Forsyth (1802) appears to be the earliest record known so far (Lodeman, 1896; McCallan, 1956). This first preparation of lime sulfur was intended to control mildew on fruit trees. William Forsyth, gardener to His Majesty, reported:

"Take tobacco one pound, sulphur two pounds, unslaked lime one peck, and about a pound of elderbuds; pour on the above ingredients ten gallons of boiling water; cover it close, and let stand till cold; then add as much cold water as will fill a hogshead. It should stand two or three days to settle, then take off the scum, and it is fit to use."

The cover and pertinent page of the second edition of 1803 are reproduced by Horsfall (1956).

Twelve years later Weightoll (1814) also reported on a mixture of sulfur and lime water for mildew control. Another seven years later Robertson (1821), who has been generally credited with the rediscovery of sulfur, read a paper before the Royal Horticultural Society recommending the use of sulfur and soap for peach mildew.

Kenrick in 1833 in the United States probably prepared the first self-boiled lime sulfur in which the heat was furnished by the slaking lime (Lodeman, 1896). Among other early reports on the use of sulfur were those of Knight in 1834 (Horsfall, 1956) and Kyle in 1846 (G. H. Cunningham, 1934). The former advocated its use for peach leaf curl and the latter for powdery mildew of grapes.

In the middle of the nineteenth century the powdery mildew of grapes was introduced, probably from America, into England and M. J. Berkeley, the mycologist, advised Edward Tucker, a gardener of Margate, to try sulfur as a remedy (Berkeley, 1846; see reprint preface). The application made in 1847 was a success. Tucker's name became noted when M. J. Berkeley named the new fungus Oidium Tuckeri. The disease spread across the English Channel and ravaged the vines of France. About 1850 Duchatel found that sulfur dust could be applied to grape leaves moistened with dew and thereby fungicide application by dusting was discovered (Large, 1940). The most notable of these early lime sulfur preparations was probably that of Grison, head gardener of the vegetable houses at Versailles. This mixture became known as Eau Orison or Grison's Liquid. Various other early sulfur preparations are described in some detail by Lodeman (1896).

Thus by the middle of the nineteenth century sulfur, the first fungicide in elemental form or as a crude lime sulfur preparation, was more or less commonly used on fruit trees, grapes, and hops to control powdery mildews and on peaches for leaf curl, while copper sulfate was used as a steep for the control of wheat bunt.
III. The Second or Copper Era (1882–1934)

A. Bordeaux Mixture

About 1870 nursery stock of grape resistant to the Phylloxera root louse was introduced into France from the United States. Unfortunately the stock was infected with Plasmopara viticola, the fungus which causes downy mildew. This disease spread with alarming rapidity. Sulfur was ineffective and copper sulfate would burn the leaves. Millardet, a prominent French botanist who had introduced the Phylloxera-resistant stock, was now in the awkward position of having to find a remedy for the downy mildew. For a long time a poisonous-looking mixture of lime and copper sulfate had been dumped on the vines along the roadways in the Gironde to discourage petty thieving. Millardet observed in 1882 that the vines covered with the mixture remained green and healthy while the others were defoliated. The significance of this observation was recognized by Millardet and he immediately began to experiment with mixtures of copper sulfate and lime, aided by the chemist Gayon. Three years later a satisfactory mixture was announced (Millardet and Gayon, 1885; Millardet, 1885a,b) and Boullie Bordelaise or bordeaux mixture, as it became called in 1886 (G. F. Johnson, 1935), was formally introduced. Further improvements followed and a standard ratio of 3 parts copper sulfate and 1 part calcium oxide to 100 parts of water was commonly adopted in France (Millardet and Gayon, 1887a,b). The use of bordeaux mixture rapidly spread and the wine industry of France was saved. Millardet died in 1902 and a grateful people raised a monument to him in Bordeaux—a unique honor among plant pathologists (see Fig. 1).

Millardet has been criticized for promoting a mixture already more or less known (Horsfall, 1956) and engaging in an unseemly scramble for credit (Lange, 1940). While Proust, a professor of chemistry, studying copper reactions in the laboratory, had prepared some basic cupric sulfates as early as 1800, there is no evidence that he had any concept of its use as a fungicide (Proust, 1800), or that the term "Proust's mixture" (Horsfall, 1956) is appropriate. On the other hand, Boucherie's wood preservative treatment of grape posts with copper sulfate had been introduced in 1839 and was in common use in French vineyards. As a result it was a frequent observation that there was less mildew adjacent to the treated posts (G. F. Johnson, 1935). Nevertheless, before the bordeaux episode Millardet was already one of the most noted and respected botanists of France. He seems to have been the right man in the right place at the right time. Without his talents, both scientific and
Fig. 1. Monument to Millardet in Bordeaux, France. Translation of inscription on front: "P. M. Alexis Millardet, Professor of the Faculty of Sciences of the University of Bordeaux, Correspondent of the Institute of France. 1838-1902. Grateful viticulture," on side: "In memory of the work on the maladies of the vine, Bordeaux mixture, the American vines, hybridization." (Photograph courtesy of Mr. Pierre Jacques.)
promotional, a solution to the mildew problem may well have lagged for years. The dramatic story of Millardet and bordeaux mixture may be seen in more detail in Lodeman (1896), in the translations of Millardet's papers by Schneiderhan (Millardet, 1885a,b; Millardet and Gayon, 1885), in G. F. Johnson (1935), in Large (1940), and in an anniversary publication "Le Cinquantenaire de la Bouillie Bordelaise" (1935).

From the time of Millardet until at least as late as 1958 (Burchfield et al., 1957; Magdoff et al., 1958) there were tremendous efforts to improve the formulation of bordeaux mixture and to understand the nature of its fungicidal action. The activity was to be expected, for bordeaux mixture possessed the title of universal fungicide. This universality was first curtailed by the reintroduction of lime sulfur in 1906, later by the fixed copper fungicides, and the final blow came with the development of the dithiocarbamate fungicides. So at the present time, in the United States at least, bordeaux has become more of a specialty fungicide. However, in many countries bordeaux mixture, which dominated an era, is still a fairly important fungicide.

Following Millardet's triumphs rival preparations soon appeared in France (Lodeman, 1886). One of the better known was Eau Celeste in which ammonia was substituted for lime and which was introduced by Audoynaud in 1886. This was too caustic on foliage. Burgundy mixture or soda bordeaux developed by Masson in 1887 with sodium carbonate substituted for lime was more satisfactory. A combination of these two prepared by Patrigeon and known as Modified Eau Celeste had a limited success. A further modification, developed in Delaware by Chester in 1891, was based on copper carbonate dissolved in ammonium carbonate solution.

Workers in the United States were not slow to take advantage of the newly discovered bordeaux mixture. In 1886 Colman, the Commissioner of Agriculture (United States Department of Agriculture) distributed 3000 pamphlets recommending its use for downy mildew and black rot of grapes (Lamson-Scribner, 1887). Lamson-Scribner and Galloway in the Section of Vegetable Pathology experimented with many formulations of copper and lime and U.S.D.A. Circular No.5 on "Fungicides" appeared in 1888 (Lamson-Scribner). By the summer of 1889 bordeaux mixture was being tried, mainly on grapes and potatoes, in at least four Agricultural Experiment Stations—Connecticut, New Jersey, Ohio, and Vermont. However, according to Large (1940) its adoption in Great Britain was much more conservative. A most practical discovery of this period was the successful mixing of a fungicide (bordeaux) with an insecticide (an arsenical) by Weed of Ohio (Weed, 1889).
A problem immediately created by the development of bordeaux mixture and its use on such a large scale, was how to apply the sludgy mixture. Labor in France was cheap and Millardet had it whisked on with a crude brush of heath. Soon, however, there was interest in developing a mechanical sprayer with a nozzle. The most successful was that of Vermorel. And so the discovery of bordeaux mixture led to a new industry, the manufacture of spraying machines. Some early models are described and illustrated by Large (1940).

A question often asked is why, with the knowledge that copper sulfate kills spores, an attempt was not made to control the late blight of potatoes which was causing the Irish famine. The blight appeared in the summer of 1845 in epidemic proportions; within a few months a Royal Commission was at work, and by 1846 three technical books on the blight appeared (Ordish, 1965). Unfortunately, and despite such rapid communication, the books were incorrect. In general, no one would accept the fact that such devastation could be caused by a minute fungus. As early as 1844 Teschemacher in Boston had “satisfied himself that a fungus is the cause of the disease” (Anonymous, 1846). Montagne in 1845 had identified and named the fungus and Berkeley in England had confirmed the cause a year later. Also in 1845 Morren, studying the disease in Belgium, had identified a “Botrydix” as the probable cause and concluded, “the itch is not the less to be feared because the minuscule which produces it is a microscopic being” (Anonymous, 1846). Furthermore, Morren had recommended a mixture of copper sulfate, lime, and table salt to prevent tuber rot. Unfortunately he applied the mixture to the soil instead of on the foliage and thereby missed an even greater fame than that which fell to Millardet.

Among the many who later studied bordeaux mixture two teams in England must be mentioned, Barker and Gimingham at Long Ashton and the Duke of Bedford and Pickering at Woburn. From 1907 to 1914 a total of ten papers by these workers appeared. Much of this effort was an attempt to explain the chemistry of bordeaux mixture and how such an insoluble product could become solubilized and fungicidally effective. The idea of a solubilizing product first advanced by Swingle (1896) came in for much discussion. One result was the introduction of the low-lime Woburn bordeaux. These studies and those in related papers which appeared prior to 1930 are summarized by McCallan (1930b).

Attempts to bring out a bordeaux-type dust have not been particularly successful. The first apparently was Podechard’s mixture developed at the same time but independently of Millardet’s work. Lime was slaked with copper sulfate in the presence of ashes, the mixture dried, pulver-
ized, and applied as a thick cloud of dust (G. F. Johnson, 1935). The most successful dust was the copper-lime mixture which was reported from Nova Scotia by Sanders and Kelsall (1918). In the presence of moisture on the leaf this physical mixture reacted to give an inferior bordeaux.

B. Fixed-Copper Fungicides

Despite its fungicidal effectiveness bordeaux mixture was too damaging to the foliage of many plants, and interest was generated for a less toxic form of copper. A partial answer was the so-called fixed-copper fungicides. In general, the goal of less phytotoxicity was attained but at the price of less fungitoxicity. Both of these characteristics were due in part to the appreciably lower tenacity to foliage. These preparations were developed mostly by industry in the 1920’s and 1930’s. They fall into four groups: (1) the basic sulfates, (2) the basic chlorides, (3) the oxides (introduced by Horsfall, 1932), and (4) miscellaneous groups of copper silicates, phosphates, and zeolites.

C. Revival of Lime Sulfur and Sulfur

Lime sulfur used as a sheep dip in Australia was introduced into California and applied successfully in combating San José scale on peaches. As early as 1880 or 1881 the peach growers began to use it as a dormant spray to control peach leaf curl (Pierce, 1900). Scott (1908) devised a so-called self-boiled lime sulfur mixture in which the sulfur was added to slaking lime. Lime sulfur of course had long been used as a dormant insecticide. As McNew (1959) has pointed out, Lowe and Parrott (1902) discovered that a much diluted lime sulfur could be used with safety in summer applications to control apple scab. However, this observation apparently was not generally recognized until the work of Cordley (1908) in Oregon 6 years later. These discoveries were immediately followed up and within 5 years lime sulfur had largely replaced bordeaux mixture on apples, peaches, and some other crops. Following this lead Blodgett, Reddiek, and Stewart at Cornell University during the period 1911 to 1917 introduced dusting with finely divided sulfur (Whetzel, 1918). Shortly after, Farley (1923) in New Jersey brought out the dry-mix sulfur lime. This was a sulfur preparation made wettable by the addition of hydrated lime and calcium caseinate. From here on industry developed a number of wettable sulfur fungicides which became the major form in which inorganic sulfur is applied as a fungicide.
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D. Seed Treatments

Formaldehyde in a 40% solution known as formalin was developed as a seed treatment for wheat smut by Bolley (1897). This method became standard for seed grains for many years; however, this entailed wetting and then drying the seed—a messy procedure. Darnell-Smith (1915) in Australia recommended a dry treatment with copper carbonate dust and thus rediscovered Tubeuf's first application made in 1902 (Martin, 1961). This simple and effective method was a huge success and by 1925 it was estimated that in the United States one-tenth of all the seed wheat was treated with copper carbonate dust to control bunt.

Organic Mercurials

Of even greater significance was the introduction of the first organic mercury seed treatment by Riehm (1913) in Germany. This application was a direct outcome of Ehrlich's successful control of syphilis with the organic arsical arsphenomine. The first seed disinfestant called Uspulun was a chlorophenol mercury compound. The organic mercury treatments were destined to become standard for practically all kinds of seeds until their dominant position was reduced by the introduction of the dithiocarbamate and other organic compounds. The development of the organic mercurials was entirely industrial, first by the I. G. Farbenindustrie A.-G. and Fr. Bayer in Germany and later by E. I. du Pont de Nemours in the United States, Imperial Chemical Industries in England, and other companies. A number of different chemicals have been made available and are generally satisfactory for the specified uses. Unfortunately, very little has been published on the general chemical and fungicidal background of many of these materials so the correlation between structure and activity remains somewhat obscure.

E. Other Significant Events

1. The American Phytopathological Society

The founding of this society in 1908 and of its journal Phytopathology in 1911 did much to encourage an exchange of ideas and results on fungicides along with all other areas of plant pathological interest.

2. Crop Protection Institute

This institute, established in 1920 at the University of New Hampshire, fostered the improvement and discovery of new fungicides and insecticides by bringing together industry and the agricultural experiment stations.
3. The Close of an Era

As the second era in fungicides was about to come to a close in the early 1930's bordeaux mixture was still the most versatile fungicide although the fixed coppers were becoming more widely used. Lime sulfur, sulfur dust, and the wettable sulfur were generally used on fruit crops and for powdery mildews and the inorganic mercurial disinfectants were secure, especially in the seed treatment of grains. These materials were to endure for a time as important fungicides, but their loss in popularity was about to begin.

IV. The Third or Organic Fungicide Era (1934-)

Two factors set this third era apart from the previous one. First and foremost was the dramatic development of highly effective and, in some instances, highly specific organic fungicides. Second, there was the increasing awareness and resultant emphasis on better methods and more precision for the evaluation of fungicides in the laboratory and in the field.

A. The First Dithiocarbamates

In 1931 Tisdale and Williams, assignors to E. I. du Pont de Nemours & Co., applied for a patent on "disinfectants" to control and prevent the growth of fungi and microbes. These chemicals were derivatives of dithiocarbamic acid

\[ \text{H}_2\text{N}\text{C(S-H)}\text{S-S-H} \]

such as tetramethyldithiuram disulfide and various metal salts, which had previously been known as rubber accelerators. United States Patent 1,972,961 was granted in 1934. This was not an auspicious start for a new era. The company did not have the courage of its convictions and let the compounds lie dormant, presumably for economic reasons. Furthermore, there was never published from this source an adequate scientific report. Interestingly, that same year Martin (1934) reported from Wye, "Preliminary work was also made on the fungicidal properties of certain organic sulphur compounds produced commercially as rubber accelerators and of the effects of these derivatives on the fungicidal action..."
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of sulphur. Independent studies appeared later in England (Montgomery et al. 1936; Marsh, 1938) and Northern Ireland (Muskett and Col­houn, 1940).

Following Tisdale and Williams' patent of 1934 there were no further reports for 7 years from the United States until those of Harrington (1941), Anderson (1942), Kincaid (1942), Dunegan (1943), Goldsworthy et al. (1943), and Hamilton and Palmer (1943). This delay had been enhanced by the economic depression. The awakening can be attributed first to the advent of World War II which placed a high priority on copper and mercury and the demand for adequate substitutes, and second, to the unexpected success and competition of a new type of organic fungicide from a new quarter. This was the quinone, chloranil, the story of which will be told later.

1. Thiram

The first derivative of a dithiocarbamate to achieve prominence was tetramethylthiuram disulfide, subsequently to be called thiram, a white crystalline material consisting of two dimethylthiocarbamates joined at the sulfur bond. Actually this compound was first found to have insect-repellant properties as the result of a du Pont-sponsored fellowship at the Delaware Agricultural Experiment Station. Here Guy (1937) reported that tetramethylthiuram disulfide was an effective repellant for adult Japanese beetles. The related chemical, tetramethylthiuram sulfide, had been reported to be unsatisfactory for the control of apple scab in small-scale field tests by Montgomery et al. (1936). Likewise, Marsh (1938) found that the monosulfide and disulfide, first tested on glass slides and then on leaves in the laboratory, gave no promise of being useful in the field. After another two years, however, Muskett and Colhoun (1940) reported that tetramethylthiuram disulfide furnished by Imperial Chemical Industries gave very promising results as a seed dressing to prevent seedling blight in flax. From that time on, tetramethylthiuram disulfide, known as Thad's, TMD, and finally and officially as thiram in 1949, (see Section IV, M), established itself as one of the most successful and universal of seed-treatment chemicals. The first report from the United States, that of Harrington (1941), showed thiram to be very effective for the control of turf diseases. Although various experimenters had found that thiram was a protectant spray chemical of high value, it was not until a decade or so later that it was formulated and made available for use as a protectant spray. This delay may have been due in part to its dermatitic effect on a comparatively few sensitive individuals.
2. Ferbam

The first reports of the metal salts of dithiocarbamic acid as successful field fungicides were those of Anderson (1942) and Kincaid (1942) who independently found that the ferric dimethyldithiocarbamate successfully controlled downy mildew of tobacco in seed beds. Hamilton and Palmeter (1943) issued a brief report of work begun in 1940 under the prophetic title, “Fermate—A Promising Fungicide.” Fermate, in 1944 to be called by the common name ferbam, was an unattractive black powder consisting of three dimethyldithiocarbamate groups attached to the ferric iron. In spite of its unattractiveness, however, it gave good control of orchard diseases and was found to be almost specific for cedar apple rust and the anthracnose diseases. In comparison with the older recommendations of 12–16 lb of sulfur per 100 gal of spray (Mason, 1928), the use of ferbam at 1½ lb/100 gal seemed strikingly effective. Ferbam also found wide acceptance as a spray for ornamentals. Also its potential for injury was so much less than that of the copper sprays or even sulfur.

3. Ziram

The companion fungicide to black ferbam is the white zinc salt, zinc dimethyldithiocarbamate, known as ziram, which received less attention than ferbam and was not mentioned in the early reports. It was found to be more successful on vegetable crops, however. The promising control of early blight and anthracnose of tomatoes and early blight of potatoes was reported independently and at the same time and place by Heuberger and Wolfenbarger (1944) and by Wilson (1944). For a time ziram threatened to replace bordeaux mixture and the fixed-copper formulations but it was succeeded by an even more effective dithiocarbamate fungicide.

B. Quinones

1. Chloranil

While the first dithiocarbamate fungicides were getting off to a wavering start under the auspices of the du Pont Co. and I.C.I. of England, in 1938 the United States Rubber Co. sponsored a fellowship with the Crop Protection Institute at the New York (Geneva) Agricultural Experiment Station under the initial direction of J. G. Horsfall with E. G. Sharvelle as fellow. In what is almost surely a record, a compound with the low number of 120 (but actually the twentieth compound submitted)
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came to the fore and became the first tonnage organic fungicide. Number 120 was chloranil, tetrachloro-1,4-benzoquinone, a compound with a long history as an oxidant or rubber accelerator. The preliminary testing had included pea seed treatments and in this area chloranil found its forte. The first report, an abstract, was published by H. S. Cunningham and Sharvelle (1940) on the treatment of lima bean seed. A detailed report by Sharvelle et al. (1942) describing a seed treatment for peas appeared later under the trade name Spergon. Beginning in 1941 McNew (1941, 1942) published a series of reports on chloranil as a seed treatment. In 1944 U.S. Patent 2,349,771 was granted to the chemist who had first suggested chloranil as a possible fungicide, W. P. ter Horst.

Actually the antibacterial action of the quinones was known as early as 1906 according to Browne (1945) who used this property to prevent the decomposition of sugarcane juice. Later there was a report on its bactericidal action by Thalheimer and Palmer (1911), while U.S. Patent 1,752,293 was granted to Brodersen and Ext (1930) covering quinone compounds as fungicides based on the toxicity to spores of Ustilago avenae and Ustilago levis. No practical development came from these discoveries, however. In the opinion of Horsfall (1956) the marked success of chloranil accelerated the faltering industrial program on the di­thiocarbamate fungicides and "opened a new era in plant pathology. It made organic fungicides possible."

Chloranil did not show promise as a foliage fungicide although an analog did when tested for the control of cherry leaf spot by Hamilton in 1938 at Geneva. These results encouraged Hamilton to attempt to develop an organic foliage fungicide and accordingly he arranged for extensive field tests with ferrie dimethylthiocarbamate beginning in 1940. This led to the report of 1943 mentioned above, “Fermate—A Promising New Fungicide.”

2. Dichlone

Following in the footsteps of chloranil, ter Horst and Felix (1943) introduced 2,3-dichloro-1,4-naphthoquinone as a fungicide. Their results indicated that this compound was four to eight times as effective as chloranil and included in the report was its recommended use for the mildew-proofing of textiles. The material under the trade name Phygon, and subsequently designated dichlone in 1953, proved to be an effective seed treatment. Early promise as a foliage fungicide was somewhat curtailed by its tendency to phytotoxicity even though used at dosages as low as ½ lb/100 gal. Also there was a dermatitic effect resembling sunburn in sensitive persons.
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C. Later Dithiocarbamates—the Ethylenebisdithiocarbamates

1. Nabam

The first dithiocarbamates had been prepared from a monoamine and carbon disulfide. In 1940 W. F. Hester of the Rohm & Haas Co. prepared a dithiocarbamate from a diamine. A cooperative fellowship had been established at the Connecticut Agricultural Experimental Station in conjunction with the Crop Protection Institute. This new compound, disodium ethylenebisdithiocarbamate was submitted for laboratory evaluation, and showing promise, received its first field test in 1941 (Horsfall, 1956). Early in 1943 U.S. Patent 2,317,765 was awarded to Hester. Later in the year the first scientific report appeared by Dimond et al. (1943) under the intriguing title, “A Water Soluble Protectant Fungicide with Tenacity”—usually two incompatible characteristics. As was shown much later (McCallan, 1957) disodium ethylenebisdithiocarbamate had an even more unusual property; it was nonfungicidal. Only when this compound is exposed to the air and decomposes does it exhibit fungitoxicity. Because of the marked instability of this compound first known as Djthane and later assigned the common name of nabam, despite most promising results would never have become a practical fungicide in the opinion of Ludwig and Thorn (1960). Fortunately one of the original workers Heuberger, together with Mans (1943), discovered the stabilizing effect of adding zinc sulfate to the spray tank. From then on its progress was rapid and it became widely used for many vegetable diseases, in particular for potato diseases, for which it almost completely replaced the old standard, bordeaux mixture, in the United States.

2. Zineb

The reaction in the spray tank of zinc sulfate with sodium ethylenebisdithiocarbamate results in the formation of zinc ethylenebisdithiocarbamate, a stable and superior fungicide. The next step naturally followed, the factory production of zinc ethylenebisdithiocarbamate. These fungicides were introduced under the trade names Dithane Z-78 and Parzate, and the common name zineb was assigned in 1949.

3. Maneb

The latest and perhaps most effective of the dithiocarbamate fungicides is manganese ethylenebisdithiocarbamate. It appears to have been field tested first on potatoes in 1946, and is commonly used on vegetable
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crops in general. The common name is maneb, assigned in 1954. Maneb is protected by a du Pont patent (Flomec, 1950).

The dithiocarbamate fungicides are without question the most important and most versatile group of organic fungicides yet discovered. An immense amount of literature has been built up on these compounds, first to report on their effectiveness, later to explain their action. For almost two decades there has been a great research effort attempting to explain the nature of their fungicidal action. This effort possibly exceeds that devoted to elucidating the nature of the action of all other fungicides combined. Not only have these studies been well covered in general reviews, but also in the specific reviews of McCallan (1946), van der Kerk (1959), and Ludwig and Thorn (1960). Finally there has appeared, a book on "The Dithiocarbamates and Related Compounds" by Thorn and Ludwig (1962).

D. Imidazolines

Another cooperative project initiated by the Crop Protection Institute was established in 1939 at the Boyce Thompson Institute, Yonkers, New York, under the sponsorship of the Carbide and Carbon Chemicals Corporation. As a result of these studies a new group of fungicides was reported by Wellman and McCallan (1946). These were the imidazolines or glyoxalidines which are heterocyclic nitrogen compounds. The most interesting compound, No. 341, was 2-heptadecyl-2-imidazoline acetate which in 1953 was given the common name glyodin. This compound was found to be effective in the control of apple scab and cherry leaf spot. An aspect of this cooperative effort was that although this discovery was protected by U.S. Patent 2,540,170 issued in 1951 to Law and Wellman, this was not the sole or even the first source of information. The above report of 1946 gave full data on the effect of the length of the side chain on fungistatic action and phytotoxicity. At the same time details of field tests on several of the more promising compounds were reported by Thurston et al. (1946).

Glyodin is prepared from stearic acid and ethylenediamine. Difficulty with phytotoxicity was encountered in some early preparations. Subsequently this was shown to be due to the use of impure stearic acid which resulted in the formation of toxic heptadecenyl imidazoline (Chandler and Thurston, 1949; Thurston, 1950). This phytotoxicity was also accelerated under conditions of high temperature and high humidity (McCallan, 1950b). The use of purified stearic acid eliminated this problem.
E. Trichloromethylthiodicarboximides

1. Captan

Another new class of organic fungicides, the trichloromethylthiodicarboximides were announced by Kittleson (1952). As events have subsequently shown, this is the most important group of fungicides since the advent of the dithiocarbamates. This discovery was the result of a cooperative effort by the Standard Oil Development Co. and Rutgers University, New Jersey, where the biological evaluations were performed by R. H. Daines and L. E. Hagmann. Kittleson was searching for new pesticides. It seems that a bottle of N'-cyclohexene-1,2-dicarboximide had been standing on the bench top and he thought that replacing the hydrogen on the imide nitrogen with some other group might make an interesting new structure which would be biologically active. Later seeing a bottle of trichloromethyl sulfuryl chloride in another laboratory he reasoned this might be a suitable candidate for the reaction. This reaction was performed satisfactorily to give the \( \text{NSCl}_3 \) group in the compound \( N\text{-trichloromethylmercapto-4-cycloheximide-1,2-dicarbox­} 

imide \) (Kittleson, 1965). Thus compound SR-406 ("S" for Standard and "R" for Rutgers) was submitted for testing at Rutgers where it performed excellently as a fungicide. U.S. Patent 2,553,770 was issued to Kittleson in 1951 and the common name captan was assigned the following year, Captan was developed commercially by the California Spray Chemical Company. An extensive program of field testing demonstrated excellent control of a large number of fungus diseases of fruits, vegetables, and ornamentals in foliage, seed, and soil applications.

2. Folpet

A closely related compound is folpet, recently introduced under the trade name Phaltan. Folpet, the common name assigned in 1951, has given good control of a wide variety of diseases.

F. Guanidines

The most recent addition to the list of important foliage fungicides is \( \text{n-dodecylguanidine acetate} \) which was developed by the American Cyanamid Co. and released under the trade name Cyprex. It is protected by U.S. Patent 2,867,562 (Lamb, 1959). In 1959 it was given the common name dodine. In the results of general field testing which first appeared in 1957 dodine has shown unusual effectiveness in controlling apple scab.
There have been no publications giving any background on the relation of its chemical structure to fungicidal activity.

G. Miscellaneous Fungicides

Among the various new fungicides that have not attained great usage are several of special interest.

1. Copper Oxinate

Copper 8-quinolinate, apparently first discovered as a fungicide by Meyer (1932) at the close of the second era, is of interest for two reasons. First, by practically all kinds of laboratory tests it has been found to be a most promising fungicide. Furthermore, it has performed well under various field conditions in the control of disease and for mildew-proofing of fabrics, paints, etc. (Horsfall, 1956; Martin, 1961). For economic reasons it has not had wide use, however, at least in agriculture. Second, the parent compound oxine, and copper oxinate have stimulated much research on the chelation theory of fungicidal action originally proposed by Zentmyer (1944).

2. Dimethocaprylphenyl Crotonate

This compound, known as an acaricide, was first reported as a protectant for apple scab and late blight of potatoes (Rich and Horsfall, 1949). However, it was subsequently found to be a specific for the control of powdery mildews. The substitution of various organic fungicides for sulfur had created a problem with the increase in powdery mildew. This brought compound CR 1639, or Karathane, a product of Rohm & Haas Co., to the fore. There is presently no common name. The specificity for powdery mildews appears to have been first emphasized by Yarwood (1951).

3. Chromate Complexes

Amid the vast interest and development of organic fungicides a class of unusual inorganic chromate complexes came to light and had this happened in the bordeaux mixture era they might well have been of major significance. These are non-Daltonian complexes of chromate with cadmium, copper, mercury, zinc, etc. Their development was a result of a cooperative effort by the Union Carbide and Carbon Corp., the Boyce Thompson Institute and Pennsylvania State College. Certain copper-zinc-chromate complexes were effective potato fungicides (Harry et al., 1948) while other compounds showed promise as seed treatments.
H. Soil Fungicides

The history of soil fungicides, as well as that of three other areas of application—wood preservatives, antibiotics, and systemic fungicides—has developed under somewhat special circumstances. Accordingly, these areas will be discussed separately from the main chronological order.

The development of soil fungicides is closely interwoven with that of soil disinfestation in general since any chemicals would have more or less of an effect on the whole biological population of the soil—-insects, nematodes, fungi, bacteria, and even weed seeds. Even to this day, most popular soil fumigants are noted for their nematocidal properties rather than their fungicidal ones. The first fumigant was carbon disulfide which was injected into the soil to control the grape Phylloxera in France and Germany (Newhall, 1955). It will be recalled that attempts to control this pest also led indirectly to the discovery of Bordeaux mixture. According to Newhall (1955), carbon disulfide was the most widely used of all fumigant chemicals until 1945. Carbon disulfide, however, is not a very effective fungicide.

The first successful and strictly fungicidal application is believed to be sulfur which Thaxter (1891) applied to the soil at seeding time in an attempt to control onion smut.

The second soil fungicide which attained more general use was formaldehyde which Selby (1900) introduced into the planting row to control onion smut and in 1906 applied as a drench to tobacco seed beds. Thus all present methods of applying fungicides directly to the soil were known by 1906 (Kreutzer, 1960, 1964).

At the end of World War I there were available large stocks of a tear gas, chloropicrin. This chemical was known to have general biocidal properties but it remained for M. O. Johnson and Godfrey (1932) to introduce it into practice as a general soil fumigant. Later Godfrey (1936) demonstrated its high fungicidal value. While chloropicrin remains the most toxic of soil fungicides, its high cost and disagreeable properties led to a search for other chemicals. The first of these was the so-called DD mixture (a mixture of 1,3-dichloropropane and 1,2-dichloropropane) developed by the Shell Chemical Corp. Carter (1943) discovered that this mixture was most effective in treating the “sick soils” of the pineapple fields in Hawaii. The discovery of DD mixture is credited with stimulating an increased interest in soil fumigation (New-
This mixture, however, is primarily a nematocide with only restricted fungicidal value.

The insecticidal properties of ethylene dibromide were long known (Neifert et al., 1925) when its nematocidal properties were discovered by Christie (1945). It was then introduced as a general soil fumigant by the Dow Chemical Co. and rapidly rose in favor. According to Lear (1951), it is the most powerful of all nematocides but has even more restricted fungicidal use than DD. The third new soil fumigant to attain general use was methyl bromide which was first uncovered by Le Goupil (1922) and possessed a long history as a space fumigant for insects. Its nematocidal properties were discovered by Taylor and McBeth (1940) and its fungicidal value was demonstrated by Newhall and Lear (1948). As with DD mixture and ethylene dibromide, its nematocidal and insecticidal properties far surpass its fungicidal effectiveness.

All of the above materials are highly volatile and eradicant in action. The protectant soil fungicides include a number of compounds discussed earlier such as copper sulfate, the organic mercury preparations, the di-thiocarbamates, especially thiram and captan. To this group may be added pentachloronitrobenzene or PCNB which was developed in Germany by I. G. Farbenindustrie shortly before World War II (Martin, 1961).

Interest in developing new volatile soil fungicides continues at a high level; for example, Schmitt (1949) some time ago reported on tests of 600 compounds. Many of the newer chemicals are discussed by Kendrick and Zentmyer (1957) and Kreutzer (1960). Among these materials are sodium N-methylthiocarbamates dehydrate (Vapam) and 3,5-dimethyl-1,3,5,2H-tetrahydrothiadiazine-2-thione (Mylone) which together with 3-p-chlorophenyl-5-methylrhodamine are of interest in that they are believed to function by virtue of their breakdown product—methylisothiocyanate (van der Kerk, 1956, Torgeson et al., 1957). Kreutzer (1964) in his recent review concludes that while there is need for more efficient and specific soil fungicides, perhaps more important is the need for more information on the complex which is treated—the soil and its microflora.

1. Wood Preservatives

Interest in wood preservatives dates back to the Greeks and Romans who referred to such ingredients as “astringent portions of the oil expressed from olives,” and also oils from cedar, larch, juniper, etc., as may be noted in the writings of Cato Censorius and Pliny the Elder (Boulton,
The first specific chemical to be used was mercuric chloride by Romberg in France in 1705. This application of mercuric chloride pre-dates its use for plant disease control. In 1767 de Boissieu also recommended mercuric chloride as well as copper sulfate, while Bordenave likewise suggested copper sulfate. The first report from America was probably that of Crook who was granted a patent in 1716 by the Province of South Carolina for the protection of ship's planking against ship worms and decay by a preservative, "one part of which is oyle or spirit of tar" (Hunt and Garratt, 1953). Zinc chloride, later to become one of the most important preservatives, was recommended as early as 1815 by Wade.

Interest and use of wood preservatives began to expand in the 1830s, first with Ryan's British patent of 1832 on a mercuric chloride treatment, the so-called Kyanizing process. This was followed by patents on copper sulfate issued to Margary in 1837 and to Boucherie in 1839 and on zinc chloride in 1838 to Burnett. Later Burnett in 1847 developed a pressure treatment. In 1836 Moll obtained a French patent on oils of coal tar which he called "krescat" or "creosate," but the method was impractical. However, this was followed by Bethell's British patent of 1838 for injecting what we now know as creosote into wood under considerable pressure. The method was immensely successful and is the basis of most modern day treatments (Boulton, 1930; Hunt and Garratt, 1953). With the development of the railroads at about this same time wood preservation expanded rapidly, especially for the treatment of cross ties. According to S. B. Boulton, "from the year 1768 up to the present time (1884) the records of the British patent office contain lists of almost every conceivable antiseptic suitable or unsuitable for the preservation of wood." However, only creosote and the above salts of mercury, copper, and zinc were of any importance. These early details may be seen in the report of Sir Henry Boulton the first president of the British Wood Preserving Association (Boulton, 1930).

The American Wood-Preservers' Association was founded in 1906 and has done much to promote the standardization of methods of testing, and of treatment, as well as the exchange of information in their annual Proceedings.

Creosote in various forms remains by far the most important of all treatments and the history of its compositions is given by Rhodes (1951). Since the early developments many important preservatives have come to the fore. Among the oil-borne preservatives, pentachlorophenol and copper naphthenate can be mentioned. Important proprietary preservatives are: Woolman Salts or Tanalith, a mixture of fluoride, arsenate,
chromates, and dinitrophenol; the somewhat similar Osmose Salts; chromated zinc chloride; Celcurc, an acid copper chromate; and Borden Salts, a chromated zinc arsenate. Details of the history of these and methods of application may be found in Hunt and Garrett (1953).

J. Antibiotics

Modern interest in antibiotics began with the discovery of penicillin by Fleming (1929) who noted that the growth of *Staphylococcus aureus* in a culture contaminated with a *Penicillium* was gradually disappearing. The material secreted by the fungus and antagonistic to the bacteria he named penicillin. Following this lead Raistrick and his associates (Clutterbuck et al., 1932) undertook not very successfully to produce more of the penicillin and then turned to more promising pursuits. Not until 10 years after Fleming's discovery were real clinical tests made and a start on the volume production of penicillin begun by Florey and his associates (Chain et al., 1940). Thus began a new era in medical therapy.

The tremendous interest was shown by the widespread collection of all manner of microorganisms, particularly from the soil, and their testing for antibiotic properties, and also by the development and production of antibiotics by industry. The second landmark was the discovery of streptomycin by Schatz et al. (1944). This discovery and its immense success, among other things, made possible the founding of the Institute of Microbiology at Rutgers University (Waksman, 1949).

The origin of the word antibiotic is from the term antibiosis first used by Vuillemin in 1889 (Ward, 1899) and adopted a decade later by the plant pathologist H. Marshall Ward. Antibiosis, meaning "against life" is the converse of symbiosis. The word antibiotic was hardly to be found in the literature until 1941 when Waksman and Woodruff (1941) named a new species of *Actinomyces, A. antibioticus*; thereafter Waksman (1949) and his associates began using the word antibiotic in their publications. The choice of this word, defined as a chemical substance produced by a living microorganism (later any plant) and toxic to other microorganisms, was not a particularly happy one since all fungicides and bactericides are against life. However, the term antibiotic is undoubtedly here to stay along with the unfortunate combination of numbers and initials, 2,4-D.

Actually the use of microorganisms and their crude products is well recognized in the folklore of medicine (Florey, 1949). According to Waksman (1941), in 1879 De Bary was the first to emphasize the antagonistic
relations among microorganisms, an opinion with which Smith (1905) agreed. The first attempt to control a plant disease with an antibiotic substance was that of Potter (1908). He found that the concentrated liquid on which *Pseudomonas destructans*, the cause of turnip rot, had grown would kill the organism itself when it was invading the turnip tissue. Likewise, he found that the products of *Penicillium italicum* would eliminate the fungus from infected orange rind. Potter stated, "many other parasites may be checked if not effectively prevented from further progress by the adoption of this principle." Sanford (1926) concluded that when potato scab is controlled by green manuring on some soils, "the antibiotic qualities of certain predominant soil microorganisms" may influence the development of *Actinomyces scabies*.

A phenomena long known, even to beginning graduate students handling cultures, was the inhibiting effect of one microorganism upon another in contaminated cultures. However, usually nothing was done about it. Porter (1924) has presented an illustrated and detailed report of the effect of mixed cultures of organisms upon the growth of one another. Prior to Fleming's discovery of penicillin, and in increasing numbers since, there have been reports of the antagonism of plant pathogens by other microorganisms, and in some instances laboratory control. Reviews by Waksman (1941), Florey (1949), and Sanford (1959) may be consulted.

Of special interest to plant pathologists is the series of papers beginning in 1932 by Weindling and associates (Weindling, 1932, et seq.) in which *Trichoderma lignorum* (later identified as *Gliocladium fimbriatum*) was shown to be parasitic on *Rhizoctonia solani* and other fungi. This culminated in the identification of the antibiotic gliotoxin (Weindling and Emerson, 1936; J. R. Johnson et al., 1943).

While antibiotics have had a tremendous impact on the control of human and animal diseases their use in the control of plant diseases is still limited. No less than 13 different antibiotics are listed by McGrath (1964) as having been used in the control of plant diseases. However, there are at present only two of practical importance—streptomycin, primarily a bacteriostatic compound and cyclohexamide, an antifungal antibiotic. Interestingly both streptomycin and cyclohexamide are produced by the same actinomycete, *Streptomyces griseus*. This was first observed by Whiffen et al. (1946) of the Upjohn Co. in Michigan. A year later its empirical formula was determined by Leach et al. (1947) who named it Acti-dione; subsequently it received the common name cycloheximide. According to Goodman (1962), its application in plant pathology was first made by Felber and Hammer (1948) who used it to eradicate...
the powdery mildew, *Erysiphe polygoni*, from beans. Cycloheximide has shown its greatest effectiveness in eradicating the *Coccomyces hiemalis* leaf spot of cherry at very low dosages as first demonstrated by Petersen and Cation (1950). Perhaps of greater significance is the observation of Westcott that the innate toxicity of cycloheximide to cells of *Saccharomyces pastorianus* is as low as 0.38 μg per gram (ED₅₀), thus demonstrating a breakthrough to a new order of toxicity for fungicides (McCallan and Miller, 1963).

**K. Systemic Fungicides**

The idea of controlling diseased plants, particularly trees, by the injection of chemicals is not new and efforts at injection were even earlier. In the fifteenth century Leonardo da Vinci, for example, described a method of injecting arsenic into apple trees (Roach, 1939). The objective, however, was not to control diseases or even insects, but apparently one's enemies. Excluding the attempted control of deficiency diseases, the first effort to control fungus diseases was probably that of Bolley (1905) on plum trees infected by the plum pockets disease. Unfortunately no details are available. Attempts were made to control the chestnut blight by injection (Rumbold, 1915) but nothing seems to have come of this. Nor were the efforts of Brooks and Bailey (1919) to control the silver leaf of plums consistently successful. Miller (1926) conducted extensive experiments toward controlling insects, pests, and diseases but there was little interest.

A revival of interest in systemic fungicides is to be attributed to Howard (1941) who successfully treated the bleeding canker of hard maple caused by *Phytophthora cactorum* with the dye diaminoazobenzene dehydrochloride. This dye acted as an antidote to the toxin produced by the fungus. This success, together with Howard's new concept and the emergency of the Dutch elm disease, soon led to many experiments with possible systemic fungicides. Notable among this work was that of Dimond, Horsfall, and their associates. While there is yet no outstanding commercial control of plant diseases by systemic fungicides, interest continues and the practical solution is probably only a matter of time. Details of the history may be seen in the reviews by Stoddard and Dimond (1949), Dimond (1959), and Howard and Horsfall (1959).

**L. The Quantitative Approach**

The growing interest in developing new fungicides led to a demand for better methods of evaluating potential compounds, which in turn was de-
ependent on a more quantitative approach. Better procedures for measuring fungitoxicity would also contribute to a better understanding of the nature of fungicidal action.

In 1938 a committee on The Standardization of Fungicidal Tests was appointed by The American Phytopathological Society, the original members being J. G. Horsfall, K. J. Kadlo, W. H. Martin, J. W. Roberts, C. E. Yarwood, H. C. Young, and S. E. A. McCallan, Chairman. During its 11 years of existence the committee prepared a number of recommended and tentative methods including those for evaluation of fungicides for cereal smuts, vegetable seeds, and fruit trees. The most notable and widely used procedure, however, was the slide germination method (American Phytopathological Society, 1943, 1947). The elements of this procedure were first suggested by Reddick and Wallace (1910) and later the method was enlarged and improved by McCallan (1930a). The formation of this committee stimulated more extensive and analytical studies of the method by McCallan and associates, and Horsfall and associates (see McCallan, 1947). Basic among these were the principles of precision, statistical evaluation, and the dosage-response curve (McCallan and Wilcoxon, 1932; Wilcoxon and McCallan, 1939). These adaptations to fungicide evaluation followed the concepts of “Student” (1908) and Fisher (1925) on statistical methods and Trevan (1927), Gaddum (1933), and Bliss (1935) on the dosage-response curve. Thus, comparisons of fungitoxicity at the level of the ED$_{30}$ or ED$_{90}$ became standard. These developments of quantitative assay in the laboratory also influenced the field evaluation of fungicides.

With increasing data it became evident that in vitro evaluations alone were not sufficient and these were supplemented or even replaced by greenhouse tests. Notable among these were the general tomato foliage disease method (McCallan and Wellman, 1943) and the specialized apple scab test of Hamilton (1959).

M. Other Significant Developments

1. Common Names for Fungicides

The increasing emergence of new organic fungicides had for some time posed a problem in nomenclature. The correct chemical names were far too cumbersome for ordinary use while code or trade names were too restrictive. The American Phytopathological Society, recognizing the problem, in 1947 designated a subcommittee on Fungicide Nomenclature, with Dwight Powell (1948) as chairman. To meet this general problem of pesticide nomenclature an Interdepartmental Committee on Pest Control,
representing several departments of the United States Government, had just been organized under the initiative and chairmanship of S. A. Rower. The subcommittee in cooperation with the Interdepartmental Committee announced in 1949 its first five names: ferbam, ziram, nabam, zineb, and thiram. These Old Testament-sounding names are almost household words. Later five additional names were announced: chloranil, captan, dichlone, glyodin, and maneb (Zentmyer, 1955). In 1954 this function was taken over by the American Standards Association Sectional Committee K62 on Common Names for Pest Control Chemicals. This committee, composed of representatives from interested professional societies and industrial groups, has drawn up a set of detailed procedures and specifications. The committee also works in cooperation with a similar committee of the International Standardization Organization.

2. Summaries of Information on Fungicides

The assembling of information on fungicides, as in any field, is always desirable and as the volume of literature grows it becomes imperative. The first collection of data on fungicides together with other pesticides was Lodeman's (1896) "The Spraying of Plants," a volume in which one can find much early history. This was followed by "Insecticides, Fungicides, and Weed Killers" by Bourcart in 1911, with several English editions (Bourcart, 1925). Later, general texts were those of Martin (1928), Mason (1928, revised by Hough and Mason, 1951), and G. H. Cunningham (1933) and still later, various others. Martin's "Scientific Principles of Plant Protection" now in its fifth edition remains a standard for detailed information on chemistry and mode of action. A text limited to chemistry only is that of Frear (1942). The first text wholly on fungicides was the readable and provocative "Fungicides and their action" by Horsfall (1945), which was followed by "Principles of Fungicidal Action" (1956). The latest fungicide text, stressing the applied phase, is that of Sharvelle (1961).

A listing of commercial pesticides "The Pesticide Handbook" was begun by Frear in 1949, revised annually, and is now in its 18th edition. It has since merged with Entoma (Frear, 1966). This handbook is supplemented by the "Pesticide Index" (Frear, 1961). The "Guide to the Chemicals Used in Crop Protection" with background details was first brought out by Martin and Miles in 1952 and is now in its 4th edition (Martin, 1961).

The question of how to keep up on the merits of the new fungicides was resolved in 1945 by a mimeographed annual report issued by the Potomac Division of The American Phytopathological Society. Later the reports
were sponsored by the whole Society and issued in the Plant Disease Re­porter, still later in "Agricultural Chemicals," and currently as an independent publication of the Society ("Fungicide-Nematocide Tests. Results of 1966"). These reports on field performance by objective experimenters provide up-to-date information on the newer fungicides and nematocides.

In the area of review journals Advances in Pest Control Research appeared in 1957 and World Review of Pest Control in 1961. By 1963 the science of plant pathology, including the study of fungicides, had attained a degree of sophistication to warrant the launching of the Annual Review of Phytopathology (1963).

3. The Residue Problem

The publication of the controversial best seller "Silent Spring" by Rachel Carson (1962) focused popular attention on the residue problem with a vengeance. While residues are of paramount importance with insecticides they cannot be ignored with fungicides. Since the tonnage fungicides sulfur and copper are exempt from tolerances, most organic fungicides have a relatively high tolerance and only the mercury fungicides have zero tolerance. The outcome of this emphasis has undoubtedly slowed down and increased the cost of bringing out a new pesticide mainly because of long-range feeding tests. But the general welfare of populations, both human and animal, has been increased by a tightening up of residue tolerances and a special emphasis on the correct use of pesticides.

V. Conclusion

In concluding this history of fungicides it is fitting to mention the growth of centers of activity. The early lead of France is outstanding with the discovery of bordeaux mixture, various other copper and sulfur preparations, pressure spraying, dusting, the first soil fumigant, and several initial wood preservatives. This initiative in the practical discoveries of new fungicides is now mainly in the United States.

The growth of centers of research especially for the study of the nature of fungicidal action is to be noted. Of especial interest are those which have maintained sustained programs. The senior center is that at Long Ashton, England, where the first reports, on bordeaux mixture, appeared in 1911 and from which there continues to flow a steady stream of basic papers. Work at the South Eastern Agricultural College, Wye, England was begun in 1914 on fungicidal action and at East Malling about 1943. In the United States Department of Agriculture there was a period of ac-
tivity in the early days of bordeaux mixture and this interest was revived again in the 1930's and 1940's. The oldest sustained program in the United States is that which began at the Boyce Thompson Institute in 1929. This was followed a decade later by research at the Connecticut Agricultural Experiment Station which has been a continuing source of stimulating ideas. Two new groups especially noted for their work on the dithiocarbamate fungicides are those at Utrecht, The Netherlands, and the Canadian Department of Agriculture at London, Ontario. Papers began to appear from these sources in the early 1950's. The latest center is at the University of Maryland.

The contributions from industry which began in Germany and extended to England and especially to the United States have been mentioned. The industrial companies have provided new types of compounds covering the whole field of foliage fungicides, seed and soil treatments, antibiotics, and wood preservatives.

These various groups together with individuals from many other laboratories, whose work is described in the following chapters, are slowly but surely finding out what makes a good fungicide work. Thus are we aided in our search for better fungicides to carry on the fight with the fungi first reported by Homer.

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1. HISTORY OF FUNGICIDES


1. HISTORY OF FUNGICIDES


I. HISTORY OF FUNGICIDES


Porter, C. L. (1924). Concerning the characters of certain fungi as exhibited by their growth in the presence of other fungi. *Am. J. Botany* 11, 186-188.


1. HISTORY OF FUNGICIDES


Whitman, J. D. (1944). The zinc salt of dimethyl disulfocarbamie acid (metalaxyl and azelite) as a fungicide on vegetables. *Phytopathology* 24, 1014.


CHAPTER 2

World Fungicide Usage

GEORGE ORDISH AND J. F. MITCHELL

I. Introduction

Quantities of Fungicides Used

II. Major Diseases on Major Crops

Individual Crops

III. Noncrop Uses of Fungicides

IV. Trends in Fungicide Usage

A. Economics of Fungicide Use

B. Changes in Fungicide Use

C. Systemic Fungicides

References

1. Introduction

The problem of fungicide usage facing the world in the latter part of the twentieth century can be stated as follows: How is food to be produced for 4 billion when at least half the world's crops cannot be grown without fungicides? It may be difficult to justify a statement so simple, and yet one with extensive implications, with precise detail. There may be reservations about the assessment of the total numbers of crops affected. Calculation of disease loss remains at best a mere estimate, and the margin of error that attends all valuation of "untaken harvest" fluctuates with crop, season, and environment. Of the increasing demands imposed on plant protection by the world's increasing millions, however, there is no doubt.

To meet this demand, thousands of chemicals are annually screened for fungitoxic properties. The cost of producing a single successful fungicide is said to approach 3 million dollars (see Chapter 5) even before the
first large-scale use by the farmer materializes. McGrath (1964) has recently stated that there are 280 chemicals marketed for use in plant disease control. These are available in approximately 500 formulations, and are very largely an indication of the extensive and intensive research carried out over the last 20 years.

It is not a mere proliferation of compounds that has occurred over the years in fungicide usage. Among the ancients, Homer refers to the “all curing sulphur,” and this substance remains an important source of fungicide. The copper ion also dominates, even though we are often inclined to think we have come a long way from Millardet’s bordeaux mixture which received prominence as a preventative treatment in the vineyards of the Gironde in the 1880’s. Mercury compounds have been long established as seed dusts. Manganese, tin, zinc, and nickel, together with the dithiocarbamates and a variety of other organic compounds and protective oils, provide the remainder of the range of source materials now in use as fungicides.

**Quantities of Fungicide Used**

The assessment of world fungicide usage is complicated by a number of factors. The first of these is related to the fact that satisfactory statistics are available for very few countries; and second, many of the published statistics embrace other pest control chemicals such as insecticides, herbicides, fumigants, and in some cases, unspecified disinfectants. Such imprecise records contribute unnecessarily to the inefficiency in any country’s planning and assessing of their agricultural potential. The Food and Agricultural Organization of the United Nations gives this problem a great deal of attention, but the general categorization of quantities of fungicides “sold to or used in agriculture” leaves much to be desired. Many governments, it appears, do not record the percentage of active ingredient in the compounds reported.

A pattern of usage in a particular country, however, i.e., its domestic consumption of fungicide, may be evaluated by assessing the balance between production, import, and export data. By this method, Barnes (1964) has illustrated how consumption of inorganic pesticides has declined in the United States since 1950, and leveled off after 1955 (Fig. 1).

The statistical data on fungicide consumption shows many gaps. An analysis of that available is given in Table 1.

It is not at present possible to assess the entire pattern of world trade, due mainly to the system adopted in many countries where import and
export figures include other pesticides. Some idea of the direction of the trade, however, can be obtained from the analysis of the United States and British exports for 1962 (Figs. 2 and 3).

The total value of United States exports was 15 million dollars, a figure that includes neither the half-million dollars' worth of copper sulfate separately classified and probably used industrially, nor oil and fumigants. The figure for Britain in the same year is 2.94 million dollars. By far the greatest proportion of United States trade is with Latin America, and that of Britain with the British Commonwealth.

Europe is essentially self-sufficient in fungicide production in terms of quantities used. This is particularly true of the Netherlands, West Germany, and Switzerland. A different picture, however, emerges with an importing country such as Ceylon. Here the cost of 1250 tons of fungicide imports in 1963 amounted to $720,000, a figure that represents 0.002% of the value of total imports, but which is adequately compensated for by the significance of fungicide usage in the production of 10% of the world's natural rubber and 20% of the world's tea.

---

**Fig. 1.** Domestic consumption of inorganic pesticides in the United States. (E. H. Barnes, 1964.)
<table>
<thead>
<tr>
<th>Country</th>
<th>Copper compounds</th>
<th>Sulfur</th>
<th>Dithiocarbamates</th>
<th>Mercury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>expressed as copper sulfate 25% Cu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Austria</td>
<td>1,568</td>
<td>867</td>
<td>363</td>
<td>185</td>
</tr>
<tr>
<td>Czechoslovakia</td>
<td>244</td>
<td>54</td>
<td>—</td>
<td>417</td>
</tr>
<tr>
<td>Finland</td>
<td>40</td>
<td>9.6</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>France</td>
<td>10,415</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Germany (West)</td>
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<td>1,256</td>
<td>—</td>
<td>1,609</td>
</tr>
<tr>
<td>Greece</td>
<td>6,996</td>
<td>12,524</td>
<td>1,659</td>
<td>1.3</td>
</tr>
<tr>
<td>Hungary</td>
<td>11,797</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Iceland</td>
<td>0.2</td>
<td>2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Italy</td>
<td>63,796</td>
<td>83,948</td>
<td>16,444</td>
<td>17</td>
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<td>261</td>
<td>660</td>
<td>10.5</td>
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<td>24,904</td>
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<td>439</td>
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<tr>
<td>Portugal</td>
<td>84</td>
<td>1,579</td>
<td>—</td>
<td>625</td>
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<td>Sweden</td>
<td>1,540</td>
<td>1,129</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Switzerland</td>
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<td></td>
<td>—</td>
</tr>
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<td>U.S.S.R.</td>
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<td></td>
<td>6,639</td>
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<td>—</td>
</tr>
<tr>
<td>Canada</td>
<td>1,709</td>
<td>15,648</td>
<td>481</td>
<td>8</td>
</tr>
<tr>
<td>United States</td>
<td>36,130</td>
<td>78,289</td>
<td>3,726</td>
<td>777</td>
</tr>
<tr>
<td>Jamaica</td>
<td>816</td>
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<td>—</td>
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</tr>
<tr>
<td>Argentina</td>
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<td>224</td>
<td>2</td>
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<tr>
<td>Chile</td>
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<td>1,228</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ecuador</td>
<td>143</td>
<td>17</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Paraguay</td>
<td>3</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Peru</td>
<td>20</td>
<td>3,014</td>
<td>—</td>
<td>5</td>
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<tr>
<td>Uruguay</td>
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<td>—</td>
<td></td>
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<td>23,242</td>
<td>—</td>
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<td>Cyprus</td>
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<td>14</td>
<td>—</td>
</tr>
<tr>
<td>Ethiopia</td>
<td>32</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Libya</td>
<td>14</td>
<td>—</td>
<td>9</td>
<td>—</td>
</tr>
<tr>
<td>Uganda</td>
<td>—</td>
<td>2,715</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>United Arab Republic</td>
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<td>1,149</td>
<td>—</td>
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</tr>
<tr>
<td>Israel</td>
<td>215</td>
<td>132</td>
<td>162</td>
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</tr>
<tr>
<td>India</td>
<td>10,654</td>
<td>572</td>
<td>—</td>
<td>408</td>
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<tr>
<td>Indonesia</td>
<td>—</td>
<td>200</td>
<td>3</td>
<td>—</td>
</tr>
<tr>
<td>Jordan</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Japan</td>
<td>7,350</td>
<td>1,138</td>
<td>1,006</td>
<td>50,929</td>
</tr>
<tr>
<td>Korea</td>
<td>174</td>
<td>336</td>
<td>—</td>
<td>4,025</td>
</tr>
<tr>
<td>Lebanon</td>
<td>34</td>
<td>305</td>
<td>12</td>
<td>—</td>
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2. WORLD FUNGICIDE USAGE

<table>
<thead>
<tr>
<th>Country</th>
<th>Copper compounds</th>
<th>Sulfur</th>
<th>Dithiocarboxamides</th>
<th>Mercury</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>expressed as</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>copper sulfate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>25% Cu</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pakistan</td>
<td>20</td>
<td>76</td>
<td>134</td>
<td>29°</td>
</tr>
<tr>
<td>Malaysia</td>
<td>85</td>
<td>—</td>
<td>—</td>
<td>8°</td>
</tr>
<tr>
<td>New Zealand</td>
<td>2,200</td>
<td>—</td>
<td>—</td>
<td>15°</td>
</tr>
</tbody>
</table>

* Source: "F.A.O. Production Yearbook" (1963), and The Commercial Attaché French Embassy, London.

The following interpretation has been put on the figures collected from the F.O.A. yearbook:

* 1959.
* 1960.
* Some 11% products converted to 25%.
* Includes copper dusts with insecticides.
* Includes 191 tons copper oxychloride.
* Taken as 1433 tons copper sulfate plus 411 tons other Cu compounds with 50% Cu content.
* Adjusted to 25% Cu as 3 tons at 75% and 234 tons at 75%.
* Adjusted from:
  * Copper sulfate 5000 tons
  * Other copper compounds 1900 tons
  * Copper dust 2% 1976 tons 490 tons (approx.)
* 7350 tons

Note that there is no entry for Australia where there is a considerable usage of copper compounds.

1. Seed dressing.
2. 100%.
3. 11% to 2%.
4. 2.4%.
5. Total production.
6. Fungicides.
7. Technical.
8. 1965.
9. Dressing 1.157 tons and dust 0.4 to 0.24% 49,772 tons.
10. 2.5%.
11. 7.7%.
Fig. 2. United States fungicide exports, 1962.

Fig. 3. Great Britain fungicide exports, 1962.
II. MAJOR DISEASES ON MAJOR CROPS

Less than a dozen of the 200 fungi recognized to cause severe plant disease losses, and of the 100,000 fungi classified, create demand for the bulk of the world's fungicide tonnage. The world's supply of fungicides is largely based on the three elements, copper, sulfur, and mercury, and more recently the dithiocarbamates.

The demand for fungicides for the control of these fungi has not declined over the years. Such changes as have occurred have been largely in the choice of fungicide. The use of resistant varieties remains a hopeful alternative; rust-resistant wheats are commonplace; the leaf-spot-resistant banana (Alta Fort) exists, but does not have other desired characteristics. In the meantime, growers rely on greater efficiency in fungicide use for disease control.

A survey of the use of fungicides against the more significant diseases follows (Table II). It should be pointed out that no reference is made to important bacterial or viral infections, which tend to come to mind in association with pest problems. Reference is made where necessary to the magnitude of loss caused by the fungus, as this provides a measure of the task to be overcome by fungicidal treatment.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Crop</th>
<th>Crop Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>Grape</td>
<td>Downy mildew</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>Late blight</td>
</tr>
<tr>
<td></td>
<td>Cocoa</td>
<td>Black pod</td>
</tr>
<tr>
<td></td>
<td>Tea</td>
<td>Blister blight</td>
</tr>
<tr>
<td></td>
<td>Cereals</td>
<td>Seed-borne diseases</td>
</tr>
<tr>
<td>Sulfur</td>
<td>Grape</td>
<td>Powdery mildew</td>
</tr>
<tr>
<td>Mercury</td>
<td>Cereals</td>
<td>Seed-borne diseases</td>
</tr>
<tr>
<td></td>
<td>Cotton</td>
<td>Seed- and soil-borne diseases</td>
</tr>
<tr>
<td></td>
<td>Rice</td>
<td>Paddy blast</td>
</tr>
<tr>
<td>Dithiocarbamates</td>
<td>Grape</td>
<td>Downy mildew</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>Late blight</td>
</tr>
<tr>
<td>Mineral oil</td>
<td>Banana</td>
<td>Leaf spot (sigatoka)</td>
</tr>
</tbody>
</table>
Individual Crops

1. Apples and Pears

   - Apple scab  *Ventura inaequalis* (Cooke) Wint.
   - Pear scab  *Ventura pirina* Adenr.

Commercial apples cannot now be grown without the use of a fungicide, and most pear orchards have to be treated as well. Timing of the protective sprays is of great importance in scab control, and advice on this point is a regular feature of most extension services. For instance, Mills (1944) laid down the foundation for such a service in New York State as far back as 1943 and similar services exist in most large apple- and pear-producing areas.

Scab can be controlled with the simple basic fungicides such as bordeaux mixture, lime sulfur, and sulfur, but the "finish" of the commercial apple is of the utmost importance today, so that fungicides such as dodine acetate, glyodin, captan, organomercurials, and thiram, which are kinder to the skin of the fruit are increasing in popularity.

2. Bananas

   - Leaf spot or Sigatoka disease  *Mycosphaerella musicola* Leach.
   - Panama disease  *Fusarium oxysporum* Schlecht f. *cubense* (E. F. Smith) Synd and Hans.

Bananas are an important cash crop in several regions throughout the tropics. This crop may represent 80% by value of certain areas of production, e.g., some islands in the Caribbean (Biggs et al., 1963). Unlike many other tropical crops, such as coffee, cocoa, and sugar cane, the banana has always been grown with reasonable expectancy of a profitable market. (Problems of overproduction are quite recent.) Wherever bananas are cultivated there is the attendant problem of one or both of these diseases, and their epidemiology governs the history of banana production.

Gros Michel, and clones of the Cavendish group—Giant Cavendish, Dwarf Cavendish, and Robusta—are the main cultivars used. Gros Michel is susceptible to Panama disease, and all are susceptible to leaf spot.

The fact that clones of the Cavendish group are resistant to Panama disease has meant extensive reliance on them in Latin America and the Caribbean. Panama disease wiped out plantations throughout the banana growing world in the 1930's, and in 1937 after the epidemic of leaf spot, many plantations were abandoned. Wardlaw (1961) considers the control of *M. musicola* to be probably one of the greatest achievements in plant pathology.
Prior to 1953 Dunegan and Doolittle (1953) estimated that 45 million lb of copper sulfate were used in tropical America. The discovery by Guyot and Cuillé of the use of oil sprays, or oil with copper or zinc fungicides, has considerably altered the techniques in leaf spot control (Guyot, 1953). It is doubtful if the world's growers and the world's eaters of bananas really appreciate the immense debt they owe to these two men. The present world area and production of bananas (20 million tons) could never have been reached at present prices if the spraying against leaf spot had to be done with the difficult Bordeaux mixture. For instance in 1941 Wardlaw reported that 60 sq mi of the Republic of Honduras were under permanent pipeline spray control for the regular application of Bordeaux mixture (Wardlaw, 1961). This has now been replaced by aerial application, usually 1 gal per acre of mineral oil.

The usage of spray oil in Latin America and the Caribbean is shown in Table III. Methods of application range from the use of portable mist-blowing machines to extensively organized aerial application from helicopters and fixed-wing aircraft. It is estimated that the consumption of mineral oil fungicide in the other banana-producing areas, such as the Camerouns and Oceania, is another 2 million gal. At an approximate price of $0.35 per gallon, the annual expenditure on oil would be 5½ million dollars. The crop is hardly ever grown without fungicidal treatment, and this cost represents 10% of the price paid to farmers.

### Table III

<table>
<thead>
<tr>
<th>Country</th>
<th>Quantity (gal)</th>
<th>U.S. gallons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil</td>
<td>300,000</td>
<td>300,000</td>
</tr>
<tr>
<td>Columbia</td>
<td>1,000,000</td>
<td>1,000,000</td>
</tr>
<tr>
<td>Costa Rica</td>
<td>1,000,000</td>
<td>1,000,000</td>
</tr>
<tr>
<td>Dominican Republic</td>
<td>130,000</td>
<td>130,000</td>
</tr>
<tr>
<td>Ecuador</td>
<td>6,000,000</td>
<td>6,000,000</td>
</tr>
<tr>
<td>Guatemala</td>
<td>100,000</td>
<td>100,000</td>
</tr>
<tr>
<td>Rep. of Honduras</td>
<td>900,000</td>
<td>900,000</td>
</tr>
<tr>
<td>Nicaragua</td>
<td>35,000</td>
<td>35,000</td>
</tr>
<tr>
<td>Paraguay</td>
<td>200,000</td>
<td>200,000</td>
</tr>
<tr>
<td>Panama</td>
<td>1,700,000</td>
<td>1,700,000</td>
</tr>
<tr>
<td>Windward Islands</td>
<td>1,014,000</td>
<td>1,014,000</td>
</tr>
<tr>
<td>French Antilles</td>
<td>1,000,000</td>
<td>1,000,000</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12,929,000</strong></td>
<td></td>
</tr>
</tbody>
</table>
In passing it is interesting to note that "sigatoka" is not known in the Canary Islands, the limiting factor there being water. Should cheap desalination of sea water become a possibility the Canaries would be well placed economically for increasing their production. On the other hand, an outbreak of a new disease has been reported from Fiji (Leach, 1964). Known as black leaf streak Mycosphaerella fijiensis, it is said to be very virulent. Fiji is not an important banana area, but it is a sobering thought to reflect that sigatoka was first reported from Fiji and that the word "sigatoka" is Fijian.

3. Cereals

The world's basic foods are cereals, and these are attacked by a series of diseases the spores of which are frequently carried on or in the seed. Seed treatment consequently is a powerful aid in securing a disease-free cereal crop. It can take three forms:

1. Cleaning the seed to remove broken, discolored or "light" seeds. This removes much potentially diseased material and can also remove the seeds of parasitic plants, such as dodder (of clover) and witchweed of corn.

2. Chemical treatment of the seed to kill spores on the seed, or to protect the seed in the soil from pathogenic fungi. These are the common bunts, smuts, and foot rots. The chemicals used fall into a number of groups, i.e., copper, sulfur, organomercurials (by far the most used group) and miscellaneous organic chemicals, such as thiram (tetramethylthiuram disulfide).

3. There are certain smuts such as loose smut of wheat (Ustilago tritici), and loose smut of barley (Ustilago nuda) which cannot be controlled by seed dressings because the mycelium of the fungus is within the seed itself. These can be destroyed by heat treatment usually accomplished by immersing the seed in hot water for a certain period.

Most of the cereal-growing areas of the world are sown with chemically treated seed. The quantities of mercury reported in the F. A. O. yearbook are given in Table I, Section I. The table is incomplete and again indicates the unsatisfactory nature of the available statistics. There is no entry for Australia, which uses a large quantity of seed dressings, or for the United Kingdom, where all cereal crops are dressed. We are convinced that Canada uses far more than 8 tons of "fungicide," and in fact estimate that she uses about 8 tons of metallic mercury per year as seed dressing for grain crops on 17.7 million hectares.

Seed dressing has no effect on the rust diseases of cereals. Up to the present the breeding of resistant varieties of grains faster than the rust
could breed new races of fungi capable of attacking the hitherto resistant variety have been relied upon. This is a constant battle as the rust can breed at a faster rate than can wheat. Attention is now being given to the use of fungicides to control rust. Dusting with sulfur has proved successful, as have nickel salts, such as the chloride and sulfate, and mixtures of maneb and nickel sulfate. In some experiments by the U.S. Department of Agriculture in Oregon one application of dichlorotetrafluoroacetone hydrate at 4.5 kg per hectare in 190 liters of water (4 lb per acre in 20 gal water) at the stem-elongation stage gave effective control of stripe rust \((Puccinia striiformis)\) (George, 1964). Oil is being tried as a carrier and a mixture of nickel sulfate hexahydrate plus maneb has shown promise. Unfortunately oil alone did not prove effective in the field (Rowell, 1964). This dual approach to the rust control problem, that is, resistant varieties and fungicide use, promises well for the future of cereal production.

4. Cocoa

Black pod \(Phytophthora palmivora\) (Butl.) Butl. Hickman (1958) points out that \(Phytophthora palmivora\) parasitizes 51 genera in 29 families of flowering plants including cocoa, rubber, various palms, cotton, citrus, papaw, pineapple, pepper, breadfruit, mango, guava, elinchona, and a number of orchids. Research among such a vast usage of crops, as expected with their varying economics, has been quite uneven. It was suggested by Orellana (1959) that isolates of \(P.\ palmivora\) from cocoa and rubber warranted elevation to varietal names, but the work of Turner (1961) indicates that there is no such justification.

The disease has a worldwide distribution (Turner, 1961), and existing cocoa in many parts of the world is highly susceptible to black pod. Infection is severe in Nigeria with losses between 40% and 50% occurring in many areas (Hislop, 1963). National losses in that country have been estimated between 15,000 and 30,000 tons of dry cocoa annually (Anonymous, 1954; Johns and Gibberd, 1951). Some relevance of this figure to the world situation is obtained when one recognizes that Nigeria produces approximately 17% of the world's supply. Similar losses, approximately 47%, have been reported in the Western Hemisphere (Orellana, 1954).

\(Phytophthora\) control has received appropriate attention in Nigeria, and in its Western province over 180,000 farmers had accordingly been trained in the proper application of fungicide by the end of 1962. (Hislop, 1963, 1964). Fungicidal treatment against \(P.\ palmivora\) in cocoa reaches back to 1907 when von Faber obtained 50% increases in yield using
The bordeaux mixture in the Cameroons. Fowler (1948) put yield increases due to this treatment at 200% to 300% in Costa Rica. A number of fungicides have been tested (Hislop and Park, 1962; Hislop, 1963), but copper formulations, mainly bordeaux mixture, regularly sprayed throughout the tropical wet season has proved the most consistently and economically effective. It has been successfully used in Brazil, Ecuador, Central America, the Caribbean, Fernando Po, the Cameroons, Nigeria, and the Philippines (Orellana, 1954). Carbide bordeaux was introduced in Kenya (McDonald, 1937) and into Nigeria by Thorold (1953).

5. Coffee

Leaf rust *Hemileia vastatrix* Berk. and Br.

Berry disease *Colletotrichum coffeae-num* Noack

Coffee leaf rust is one of the classics of plant pathology. Berry disease is a more recent hazard.

After rust was introduced in Ceylon in 1867, it reduced yields from 450 to 200 lb per acre in less than 10 years, with an estimated annual loss of 5.5 million dollars (Large, 1940). Consequently coffee was abandoned, and Ceylon became the tea-producing country it is today. Haarer (1962), however, emphasizes that the disease did not wipe out coffee in Ceylon; it ravaged plantations where knowledge of disease control measures was limited. Wellman (1954) indicated that the disease is still severe in the Eastern Hemisphere, but through efficient quarantine it has not been introduced into the New World.

Both diseases are severe problems in East Africa. Nutman and Roberts (1964) estimate that in Kenya alone preventable losses exceed 7000 tons annually, which at current prices are equivalent to 5.6 million dollars. Spraying with copper formulations is standard practice. About 20,000 acres are affected by berry disease and 45,000 acres affected by rust; control measures on this scale cost 1.3 million dollars (Nutman and Roberts, 1962). It was estimated by Wellman (1954) that one-third of the 3 million acres of coffee cultivation where rust was endemic required treatment. At a cost of treatment varying between 5 to 9 dollars per acre, Vallega and Chiarappa (1963) consider that 10 to 27 million dollars would need to be expended.

6. Cotton

Various soil- and seed-borne diseases

Le Clerc (1964), interpreting data from the Statistical Service of U.S.D.A., estimated that the average annual loss from potential production due to diseases in cotton in the United States is about 300 million...
dollars. When we recognize that United States production of cotton annually approximates 29% of the world's production, and that distribution of cotton diseases closely follows the worldwide pattern of cotton cultivation, then we have a relevant picture of the enormity of loss in cotton harvests due to disease.

The main use of fungicides in cotton is in seed treatment. For this, a number of mercury-based dusts are marketed. More limited amounts of organic fungicides are used in soil and seed treatments. There are two categories of virulent organisms against which protection is taken. The first includes diseases transmitted by seeds but also present in soil, such as Glomerella gossypii, Fusarium oxysporum, Verticillium albo-atrum, and Fusarium moniliforme. The second comprises fungi which are essentially soil-borne and practically never transmitted by seeds, such as Rhizoctonia solani, Macrophomina phaseoli, Sclerotium rolfsii, and several Pythium spp. (Cognee, 1963).

There is very little of the world's cotton which is not grown from treated seed. Estimating that 17 gm of seed dressing per hectare are used on cotton. With 80% of the world area treated, one gets a figure of

\[
\text{33.7 million hectares} \times 0.08 \times 17 \text{ gm} = \frac{\text{33.7 million hectares} \times 0.08 \times 17 \text{ gm}}{100} = \text{3.40 tons of seed dressing as world usage}
\]

About a quarter of this is based on copper and three quarters are organo-mercurial preparations. It will be seen that a small tonnage of seed dressing is performing a great service to cotton growers.

7. Potato Blight  
*Phytophthora infestans* (Mont.) de Bary.

The potato blight is one of the world's most devastating diseases. The history of the epidemic of the 1840's, as well as the far-reaching economic and social effects, is well known. It was Millardet's discovery of bordeaux mixture in 1884 that made all the difference to potato blight control.

Blight can attack potatoes wherever they are grown throughout the world, and the attack can be overcome by preventative treatment with fungicides. However such treatments are not only costly but may cause damage to the crop even by the mere passage of the machine giving the treatment. Consequently the aim of the modern potato grower is to treat the fields only when it is necessary. The "insurance" treatment is no longer considered to be desirable. It is quite another matter with seed disinfection of grain; here an "insurance" treatment is simple and cheap.
The amount of damage done by blight is a function of the amount of leaf destroyed by the disease; hence the object of spraying is to keep the leaf growing and healthy as long as possible. The fungus and its host have been extensively studied and it is now known under just what conditions the disease will spread. All major potato areas now run a forecasting service and advise potato growers when to spray. Large sums of money and much effort are saved in this way as unnecessary spraying is avoided. Twenty years ago we estimate that some 20% of the potato acreage was sprayed. Today the area under scientific pest control treatment is much greater and only some 15% of the area is treated because much unnecessary spraying is avoided. The standard copper sprays give a control of blight, but much less harm is done to the leaves by dithiocarbamate products.

A new technique in potato blight control is the use of infrared aerial photographs. This permits the detection of an incipient blight attack at once, even if it be in the center of a remote field. Spraying can be started at once to prevent such infection from becoming a contamination center.

The present system of blight control is an admirable example of how a disease should be controlled, for intensive study by many disciplines has led to a rational system. No doubt it will be improved still further; one day we shall have the blight-immune potato.

8. Tobacco

Blue mold. *Peronospora tabacina* Adam

Wolf (1957), discussing blue mold in the United States, reported that the outbreak of no other tobacco disease had affected prices on the Stock Exchange. In Australia, the tobacco industry has recognized the severity of the disease for many years. Hughes (1963) states that in Queensland, in particular, there are 50% leaf losses, and crop lodging losses up to 80% due to stem infections. Throughout Europe, in the period between 1958 and 1961, severe outbreaks of *P. tabacina* have limited production (Peyrot, 1962); damage to the 1960 crop was estimated at 50 million dollars (Todd, 1961). The total area cultivated in Europe including the U.S.S.R. has risen from an estimated 570,000 to 612,000 hectares. Meanwhile, in France the cultivated area of tobacco has been reduced by 12%, in Germany 18%, and in the U.S.S.R. approximately 8.5% (FAO, 1963a).

Treatment with fungicides in seed beds and the field are therefore standard practice throughout infected tobacco growing countries (Rayner and Hopkins, 1962). Copper-based fungicides have not proved efficient, but the dithiocarbamates—ferbam, maneub, and zineb—give adequate,
2. World Fungicide Usage

though expensive, control. In the field, problems of phytotoxicity arise with maneb; consequently this fungicide is recommended for later spraying, zineb being the more suitable earlier treatment. At an approximate cost of spraying at 200 dollars per hectare (Todd, 1961; Peyrot, 1962), and estimating the relevance of treatment being necessary for 80% of Europe's crop, the magnitude of annual cost is approximately 120 million dollars.

Zambia, Malawi, Rhodesia, and South Africa now produce 4% of the world supply; this area reports no depredations from *P. tabacina*.

9. Grapes

Powdery mildew *Uncinula necator* (Schu.) Burr.
Downy mildew *Plasmopara viticola* Berl. and de Toni

The European grape *Vitis vinifera* suffered three major attacks in the 19th century from fungi and an insect living on the American Vitaceae, all introduced accidentally from America. They were the powdery mildew, the *Phylloxera* (an aphid) and the downy mildew. These are three striking examples of the success of a life form when introduced to a new environment. Of course the same thing was happening with European forms introduced to America such as the fungus causing Dutch elm disease and the European red mite. In passing one should note the delicate international pejorative nuances of the common names of these pests, more noticeable with insects than plant pathogens. Our favorite is the Argentine ant, known the world over by this name, except in Argentina, where it is called the Brazilian ant.

The powdery mildew of grape was first reported about 1846 by M. Grison, a royal head-gardener at the palace of Versailles, France. The disease spread rapidly: a few years later the grapes became Republican but were still as badly attacked and Grison looked for a remedy. He invented lime-sulfur, which became known as "Eau Grison" and was a cure, but it was not a practical proposition in those days to spray extensive vineyards. By the time the grapes were Imperial (1852) and just as badly threatened by the fungus, it had been found that dusting with powdered sulfur was the remedy and it largely remains so to this day.

The *Phylloxera* attack on the European grape was overcome by grafting this grape onto certain American roots. This meant that many American plants were brought to France, and although Cornu issued a warning against bringing the downy mildew disease with them this was not heeded, so eager were the grape growers to get stocks which would save the vineyards.

Downy mildew, the third disaster to strike the European vineyards,
was well established by 1880. The cure was found accidentally by Millardet in 1884, with the invention of bordeaux mixture. It and Burgundy mixture, while still the standard treatments, are being replaced by other chemicals. There are also modern improvements in the method of application. Aircraft, helicopter and fixed wing, and machines straddling the rows of vines are now being tried. As with potatoes, an advisory service is maintained in all large grape areas to warn growers when spraying is necessary, and consequently much unnecessary spraying is avoided.

Four years of tests with helicopters conducted by Audibert (1964) during which much economic data was accumulated, showed that helicopter spraying of vineyards could be done for from 18 to 30 francs per hectare ($1.46 to $2.43 per acre). This was almost the same price as hand treatment. It was said not to be as effective but, the author points out, conditions in the hand- and aerial-treated areas were not strictly comparable. It is interesting to note that ferry time, the item that so adds to the cost of aerial application, was reduced from 8.7% of flying time in 1960 to 2.7 in 1963.

The advantages of aerial, particularly helicopter, application are so great that we foresee an increasing use of this method of applying fungicides to vineyards. The main advantages are speed of operation and saving of labor. Aircraft enable an area to be done quickly after a spell of bad weather and good spraymen for hand working are increasingly difficult to find. Audibert finds that one helicopter can serve some 3700 acres of vineyard and that about 700 machines would be needed to cover all the French vineyards. With increasing use and experience the cost of aerial treatment will drop.

It should be noted that experiments are being made in Italy (Turin University, 1962) on oil sprays for downy mildew control. If oil controls downy mildew as well as it does banana leaf spot, the control of the former disease would be much simplified.

Before 1939 copper sprays, mostly bordeaux and Burgundy mixture, were used on 99% of French vineyards. Today only 20% of the area is treated with copper alone and the rest is divided between sprays of zineb, zineb and copper, maneb, and maneb and copper. Other European vineyard areas follow a similar pattern. The Californian and Middle East vineyards have the advantage of requiring no spraying against downy mildew.

III. NONCROP USES OF FUNGICIDES

The greatest noncrop use of fungicides is the treatment of timber, which is dealt with in Chapter 11. In addition, large quantities of fungi-
sides are used to prevent deterioration of fabric, paper, leather, etc. These uses are discussed in Chapter 10.

IV. TRENDS IN FUNGICIDE USAGE

A. Economics of Fungicide Use

Diseases of crops cause a loss; remedial measures prevent this loss, whether these measures be cultural, biological, or chemical, and in the case of crop diseases by far the commonest remedial measure is the use of fungicides. It is consequently quite possible to establish a relationship between the cost of the remedial measure and the value of the crop saved by the measure. This is called the cost/potential benefit ratio. If the cost of measures against virus and blight in potatoes is $20.00 per acre and a potential loss of 20% of a crop worth $300.00 is saved, that is $60.00, then the ratio of cost to potential benefit is 20 : 60 or 1 : 3. The operation is well worthwhile for the individual concerned. This is the commonplace commercial calculation every farmer makes when faced with a disease control problem. Diseases and pests cause losses; remedies overcome them and usually pay handsome dividends, but not always—there is often much uncertainty. The remedy may not always be successful; in certain years the spraying may not be needed at all and the treatment is a waste of money.

The economic effect of pest control on the individual was discussed above. Let us now consider the effect of the use of fungicides on an industry and the real economic nature of the loss. The United States potato acreage and yields for 1948-1952 and 1962 are compared in Table IV. Over this period the yield has increased by 5.6 tons per hectare or about 35%. This is due to the use of more fertilizer and better disease control and has been induced very largely by acreage restriction programs. For

<table>
<thead>
<tr>
<th>Year</th>
<th>Area (1000 hectares)</th>
<th>Production (1000 metric tons)</th>
<th>Yield (metric tons per hectare)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1948-1952</td>
<td>662</td>
<td>10.676</td>
<td>16.1</td>
</tr>
<tr>
<td>1962</td>
<td>557</td>
<td>12.097</td>
<td>21.7</td>
</tr>
</tbody>
</table>

*From "F.A.O. Production Yearbook" (1963b).
example, suppose that half of the improvement (2.8 tons per hectare) is due to better pest control; with no disease the 1948-1952 crop could have been 18.9 tons per hectare or one can then say that, in the United States, diseases consumed $2.8 \times 662,000 = 1.8$ million tons of potatoes. At $30.00$ per ton this comes to a lot of money, in fact, $54$ million. This is a deceptive figure, however. Consumers wanted only about $10\frac{1}{2}$ million tons of potatoes; had there been no disease the pressure of nearly $2$ million extra tons of potatoes would have depressed prices very considerably and the growers would have been worse off, not better off, by $54$ million. In other words, a short crop may at times pay growers as a group better than an abundant crop, a fact well known to agricultural economists.

The real economic loss, in the above examples, is the land and the effort put into potato production that is going to be consumed by disease. Had there been no disease the yield could have been $18.9$ tons per hectare instead of $16.1$ and the $10\frac{1}{2}$ million tons the market wanted could have been grown on $555,000$ hectares instead of $662,000$ hectares. The difference, $107,000$ hectares of good potato land, is the real economic loss, the "untaken harvest" that the diseases consumed.

The cost of controlling a disease by the use of fungicides is proportionate to the area to be treated. Some crops produce a big cash return per acre and some but little; one has but to think of the different cash yield per acre between fruit and wheat. Though the percentage loss may be the same in both high and low value per acre crops, it is the former crops that tend to receive fungicide sprays. Hence it is particularly fortunate that so many cereal diseases can be controlled by dressing the seed, a very cheap operation.

The control of diseases is improving all the time; new fungicides are developed, better means of applying them and better timing are the order of the day. This will lead to increased yields per unit area and to problems of surplus crops in the "have" nations and to a real improvement in the standard of living in the developing areas.

A remarkable change in fungicide usage over the last 10 years is from bordeaux mixture to oil for leaf spot control in bananas, and the use of this old standard is declining in many other crops as well, for the new organics are much less harmful to foliage. The modern pest control expert seeks to work with nature, that is to say he quite ruthlessly exploits natural conditions to the advantage of man. Integrated pest control is part of this exploitation so that in the struggle between man and "the rival world"* the former may continue victorious.

II. Changes in Fungicide Use

On the basis of their application to plants, fungicides are classified in three groups: protectant, eradicant, and systemic. Attention is drawn to the fact that most plants are usually able to protect themselves, and the purpose of fungicidal treatment, often ignored, is to protect plants rather than to kill fungi. The emphasis, however, is somewhat different in mammalian pathology, where fungal infection is rare, and therefore the emphasis must be on therapy. The individual person, or animal, is not as expendable as a tree.

A look at Table I, indicates the still dominant position of inorganic copper, mercury compounds, along with sulfur, in terms of quantities in general use. The dithiocarbamates developed by Tisdall and Williams (1931), and introduced as fungicides by Dimond et al. (1943) have provided the basis of important change in usage throughout the world. An interesting pattern of change is to be noted in France, in reference to the production of grapes (Table V). The table shows a steady decline in copper sulfate usage. The area treated does not explain this decline. It also reflects the increasing usage of dithiocarbamates.

In applied pathology, as in other disciplines, one can measure efficiency in terms of the assessment of effect, and in quantity of material used. The value of the new fungicides is that a smaller quantity of active ingredient of dithiocarbamate may produce a fungitoxic effect equivalent to a larger volume of sulfur or copper sulfate. This trend toward the development of more active fungicides (gram for gram) coincides with the pat- 

<table>
<thead>
<tr>
<th>Year</th>
<th>Area cultivated (1000 hectares)</th>
<th>Copper sulfate used (1000 metric tons)</th>
<th>Yield (1000 metric tons grapes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1958</td>
<td>1402</td>
<td>37.4</td>
<td>7348</td>
</tr>
<tr>
<td>1959-1960</td>
<td>1464</td>
<td>28.2</td>
<td>9228</td>
</tr>
<tr>
<td>1960-1961</td>
<td>1462</td>
<td>24.92</td>
<td>9677</td>
</tr>
<tr>
<td>1961-1962</td>
<td>1418</td>
<td>18.33</td>
<td>7491</td>
</tr>
<tr>
<td>1962-1963</td>
<td>1414</td>
<td>9.6</td>
<td>11504</td>
</tr>
</tbody>
</table>

*From "F.A.O. Production Yearbook" (1963), and by courtesy of the Agricultural Attache, French Embassy, London.*
term of research into selective control with specific chemotherapeutants. It is, of course, the basis of the interest of chemical manufacturers in the pesticide market. A fungicide with three times the activity of copper sulfate can be sold for three times the price of the latter, in fact for more than three times if it has other advantages, such as doing less damage to foliage. Against the downy mildew of grape certain dithiocarbamates have three times the activity of copper sulfate, and are less damaging to foliage. The price relationship for bulk lots is about 70 cents per pound.

### TABLE VI

<table>
<thead>
<tr>
<th>Present-day fungicides</th>
<th>Group</th>
<th>Individual types</th>
<th>Major applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foliage</td>
<td>Dithiocarbamates</td>
<td>Dimethyl dithiocarbamates</td>
<td>Very wide, except powdery mildews</td>
</tr>
<tr>
<td></td>
<td>Ethylendimethio-</td>
<td>Ethylendimethio-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>carbamates</td>
<td>carbamates</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Captan</td>
<td></td>
<td>Fruit trees</td>
</tr>
<tr>
<td></td>
<td>Folpet</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Triphenyltin acetate</td>
<td></td>
<td>Potato blight</td>
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<td></td>
<td>Triphenyltin oxide</td>
<td></td>
<td>Sugar beets and celery</td>
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<td></td>
<td>Dodecylguanidine acetate</td>
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<td>Sugar on fruit trees</td>
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<td>Dinocap</td>
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<td>Powdery mildew</td>
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<td>Dichloriene</td>
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<td>Powdery mildew</td>
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<td></td>
<td>Dichlorine</td>
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<td>Fruits and vegetables</td>
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<td>Soil and seed disinfectants</td>
<td>Organomercurials</td>
<td>Many ethyl- and phenyl-mercury derivatives</td>
<td>Seed disinfection</td>
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<td>Chlorinated quinones</td>
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<td>Rhizoctonia control</td>
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<td>Soil fungicide</td>
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<td>Chloropicrin</td>
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<td>Dithiocarbamates</td>
<td>Methyl isothiocyanate</td>
<td>Soil fumigant</td>
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<td>Isothiocyanates</td>
<td>Methyl isothiocyanate</td>
<td>Soil fumigant</td>
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* By courtesy of Dr. G.J.M. van der Kerk and the editor of World Review of Pest Control.
C. Systemic Fungicides

In the past the emphasis in fungicidal treatment has been on external application. This has somewhat altered since success in providing the plant with internal protection against disease was obtained with 8-hydroxyquinoline against Dutch elm disease. Research in systemic compounds proceeds along three lines:

1. Changing the plant’s metabolism
2. Reorienting the physiological relationship between the host and pathogen
3. Introducing deactivating tissue-destroying enzymes.

Among the compounds tested are: S-carboxymethyl dimethyldithiocarbamate in cucumbers (Van der Kerk, 1963); phenylalanine, alanine (Koe et al., 1957) and o-coumaric acid (Kirkham and Hunter, 1964) against apple scab; procaine and 6-azanacil against powdery mildews; and rufianic acid against Fusarium wilt in tomatoes (Grossman, 1962).

This field of research has been dominated by work at the Connecticut Agricultural Experiment Station and Wageningen in The Netherlands, but the quantity of compounds definitively marketed as systemics is not yet of worldwide significance.

While satisfactory progress is being made in understanding the internal physiology of the plant, some significance is to be recognized in the studies of plant waxes, the cuticle in particular. The work of Martin (1964) at Long Ashton Research Station in Britain provides the avenue for exploring the interrelationships between externally applied fungicides and the plant surface. This, conceivably, will have a significant bearing on the efficiency and economics of much fungicidal usage on an extensive range of plants in the future.

Also of importance in future control of fungus diseases is the observation of Grainger (1965) that to suffer from a disease, three conditions must be favorable: (1) the parasite must be present, (2) conditions suitable for the parasite to attack must reign, and (3) the host plant must be receptive. Grainger points out that there are long periods when the host is not receptive because it cannot supply the large quantities of carbohydrate needed for growth of the fungal mycelium. Grainger further postulates that this availability can be tested by estimating the $C_p/R$. 

for maneb and 20 cents for copper sulfate, a ratio of 1:3.5, about the figure postulated above. Van der Kerk (1963) has summarized the present-day application of the more recently developed fungicides (see Table VI).
ratio, where $C_p$ is the weight of total carbohydrate in the plant and $R$, the residual (carbohydrate-free) dry weight of the shoot. Using the economic study of crop losses in combination with technical biological studies, Grainger foresees costless control of certain diseases in the future. Already Epicure, an early potato, if planted early in West Scotland, can produce a commercial crop in eelworm-infested land.

References


Chapter 3

Epidemiology of Fungicidal Action

J. E. Van Der Plank

1. Introduction

Fungicide research follows two directions. In one direction we study differences among the various fungicides and the special properties and features of particular fungicides: the chemical structure, the physical and chemical properties, and the fungicidal activity of the dithiocarba-
mates, for example. It is in this direction the great discoveries of old and new fungicides have been made. It is also in this direction that the farmer thinks when he chooses a fungicide for use in his apple orchard of some particular variety at some particular stage of seasonal development. In this direction, on the special properties and features of particular fungicides, most chapters of any comprehensive treatise on fungicides must necessarily be written. This is not, however, the direction of this chapter.

In the other direction one studies properties shared by all fungicides. Primary among them is the central property all fungicides share: they kill fungi. It is around this central property that this chapter is written. We shall be concerned not with the properties of individual fungicides, but with the effects of fungicidal action in general.

The definition of fungicides as fungus-killing substances is somewhat oversimplified. To allow for fungistasis and other processes, we must change the definition somewhat, and consider fungicides as inoculum-reducing substances. Fungicides stop the fungus from forming viable spores, or they stop spores from germinating, or they stop germ tubes or other mycelium from infecting the host plants; in all these ways they reduce inoculum. Even this wider definition of fungicides as inoculum-reducing substances does not cover all the ways in which fungicides act. It covers most of them, however, and it is the definition we shall use.

Our theme, then, is the effect of reduced inoculum on the development and course of epidemics. To determine the effect, three questions must be answered.

First, what is the relation between the number of spores and the number of lesions or systemic infections they produce? We refer here to the number of spores that escape the fungicide, i.e., the number that survive the attack. If, for example, the fungicide inactivates nine-tenths of the spores, how does reducing the proportion of active survivors to one-tenth affect the number of lesions or systemic infections produced? Is the number reduced to one-tenth of what it would have been without the fungicide? If not, by how much is it reduced?

Second, what is the effect of reducing the number of spores on the infection rate? The rate brings the element of time into the discussion; the essential element of how disease builds up as the season advances. If a fungicide inactivates nine-tenths of the spores, how does this affect the rate? Sometimes it reduces the rate by much less than nine-tenths; at other times it stops disease developing altogether, i.e., it reduces the rate to zero.
Third, how do the environment and the susceptibility of the host plants affect the relations mentioned above? Fungicidal activity is not just an interaction between fungicide and fungus. It involves the whole fungicide square: host plants, fungus, fungicides, and environment. Factors such as the susceptibility of the host plants are intimately involved in the result of a spray program; how they are involved is discussed briefly in the last part of this chapter.

II. RELATION BETWEEN THE AMOUNT OF INOCULUM AND THE AMOUNT OF DISEASE IT PRODUCES

A. The General Problem

Inoculum produces disease. Fungicides reduce inoculum. The relation between the amount of inoculum and the amount of disease it produces is basic to any study on the effect of fungicides on disease. Therefore, no apology is made for discussing the matter more critically and in greater detail than has been done before, even though it is relevant not just to the study of fungicidal action but also to many other areas of plant pathology as well. Everything discussed later in this chapter is based on the simple relations between inoculum and disease discussed here.

Data show that spores, as inoculum, act independently of one another if they are in concentrations likely to be found in natural epidemics. The condition with regard to concentrations is important. Much of the confusion in the literature about the relation between the number of spores and the amount of disease they produce arises from the use of abnormally high concentrations which have little meaning epidemiologically.

Independent action of spores means that there is a simple relation between spore numbers and disease, except in some systemic diseases, or when lesions overlap or compete with each other. Evidence for independent action is also necessarily evidence against the concept of a numerical threshold of infection, a concept which, if it were true, would bear considerably on the theory of fungicidal action.

B. Infection by Two Rust Fungi

Petersen (1959) allowed uredospores of Puccinia graminis tritici to fall on wheat plants, which he then kept for 24 hours in an incubation chamber and a further 4 days in a greenhouse. He then stained the
leaves and counted the infection points. Figure 1 shows the relation between the number of spores and the number of infection points. The first nine points, up to 2810 spores and 330 infection points/cm² have been treated as a group. The regression coefficient of the number of infection points on the number of spores is 0.108, and the regression line passes practically through the origin. The last six points, up to 5640 spores and 1520 infection points/cm² have also been treated as a group. The regression coefficient is 0.490, which is significantly higher than the previous figure. The regression line cuts the x-axis well to the right of the origin.

A straight line passing through the origin means that the number of infection points is proportional to the number of spores; in other words that the ratio of infections to spores is independent of the number of spores. This can happen only if the spores do not interact with one another; it is what happened in Petersen’s experiment when the concentration of spores was low.

In contrast with this, a line cutting the x-axis far to the right of the origin means that the ratio of infections to spores increases with...

![Graph showing the relation between the number of infection points and the number of spores per cm².](image)
3. EPIDEMIOLOGY OF FUNGICIDAL ACTION

the concentration of spores. In the example, there were about 620 infection points with 3600 spores/cm², a ratio of 17:100, and 1470 infection points with 5400 spores/cm², a ratio of 27:100. At these high concentrations the spores helped one another to infect; they acted synergistically. There is other evidence for this. Petersen (1959) found that high concentrations of spores stimulated germination. With 850 spores/cm², 28% of the spores germinated and produced appressoria; with 3800 spores/cm², 52% germinated and formed appressoria.

At high concentrations, spores interacted synergistically. This is irrelevant to our problem, because the high concentrations needed to bring about this synergistic interaction are outside the range of concentrations likely to be found in natural epidemics. The first nine points of Fig. 1, up to 2810 spores/cm², are more in line with natural epidemics. These 2810 spores/cm² produced 18 pustules/cm², which is equivalent to 100% infection on the modified Cobb scale so widely used to assess wheat rust. They settled on the leaves in a single, simultaneous salvo, whereas in natural epidemics spores settle over days and weeks, so that at any one time the concentration of spores is relatively low. Since they did not interact synergistically in Petersen's experiment even when they settled simultaneously in numbers large enough to cause 100% infection, we can infer that stem rust spores would not interact synergistically while disease increases gradually in natural epidemics. The data of Rowell and Olien (1957) also show that wheat stem rust spores act independently of one another up to the highest incidence of disease studied by them—about 20 pustules per leaf.

Now consider bean rust. Davison and Vaughan (1964) added a suspension of uredospores of *Uromyces phaseoli* to bean leaves, held the plants in a moist chamber for 16 to 18 hours and then in a greenhouse for two weeks, after which they counted the pustules. Figure 2 relates the number of pustules per square centimeter of leaf surface to the number of spores per square centimeter.

The first two points in the graph are in line with the origin. There was therefore no evidence of interaction at this stage. The third point, with about 27 pustules/cm², reflects unnaturally high concentrations of disease; the pustules were crowded and below average size, and unlike the pustules that develop in natural epidemics. With still greater numbers of spores the number of pustules decreased sharply, considerably fewer pustules being formed by 3400 than by 1100 spores/cm².

The first two points of Fig. 2, showing that spores act independently
of one another, are the only two points to concern this chapter directly, because they are the only two points that represent spore concentrations likely to be found in natural epidemics. Schein's (1964) results, with bean rust spores suspended in dilute agar solutions, also show interaction to be absent.

The evidence for two opposite sorts of interaction, for synergistic interaction in the last six points of Fig. 1 and antagonistic interaction in the last two points of Fig. 2, tallies with findings of another kind. Uredospores of _P. graminis_ produce a substance that promotes germination (Allen, 1957). This could explain the synergistic effect recorded in Fig. 1 at high spore concentrations. They also produce a substance that inhibits germination (Allen, 1955). If uredospores of _U. phaseoli_ behave similarly, this could explain the antagonistic effect recorded in Fig. 2 at high spore concentrations.

Although experimental results obtained with abnormally high spore concentrations can generally be ignored in considering the progress of natural epidemics, they nevertheless bear on fungicide research. Various research workers have tested the efficiency of fungicides against disease by spraying plants or detached leaves with fungicides and then inoculating them with spores. If the spores are applied at normal concentrations, results so obtained indicate fungicidal potency in a general way. If the spores are applied at abnormally high concentrations, however, the results may be misleading, since the potency of the fungicide may be overrated if the spores interact synergistically and underrated if they interact antagonistically. Indeed, if spores interact only while

![Graph](image-url)

**Fig. 2.** The relation between the number of pustules and the number of _Uromyces phaseoli_ per square centimeter. (Data of Davison and Vaughan, 1964.)
they are alive, one could surmise from Fig. 2 that a potent fungicide could increase the infection of bean plants inoculated with high concentrations of rust spores.

C. Multiple Infections

In Fig. 1 the number of infection points was plotted and in Fig. 2 the number of lesions per square centimeter. More commonly, information about the relation between inoculum and disease is given in terms of the percentage of diseased plants. This section concerns the transformation of this information into the required form.

Suppose, considering systemic disease first, tobacco plants are inoculated with mosaic virus in two separate places on opposite sides of the plant. Later it will be difficult to tell that infection began in two places; it could just as well have begun at one place, or at three or more. However many acts of infection there were to begin with, the result will be the same: a systemically infected plant. The tobacco plant is multiply infected, but each act of infection is independent, the infections occurring on opposite sides of the plant. To avoid misunderstanding, it should be stressed that the term "multiple infection" is used to cover independent acts of infection. (Sometimes the term has been used to mean that several spores acted in concert, synergistically, helping one another to infect. This meaning is outside the present definition.)

What happened to the tobacco plant can happen to any plant susceptible to a systemic disease. An oat seedling, for example, can be infected more than once, independently, by smut. This was neatly proved by Person and Cherewick (1964) for *Ustilago kolleri* and *Ustilago avenae*. With genetic markers they showed that as many as four separate infections can participate to develop disease in a single oat plant.

To allow for multiple infections in a population of homogeneous plants, the proportion \( y \) of infected plants is changed to \(-\log_e(1 - y)\), which is the average number of infections per plant. For example, if \( y = 0.2 \) then \(-\log_e(1 - y) = 0.223\). That is, on an average 20% of the plants will be infected when there are 223 infections randomly scattered among every 1000 plants.

The principle is this: with infections distributed randomly among homogeneous plants, the probability of any one plant being healthy is \( e^{-m} \), where \( m \) is the mean number of infections per plant. But when the
number of plants is large the probability of a plant being healthy is also the proportion \(1 - y\) of healthy plants.

\[
1 - y = e^{-m} \\
- \log(1 - y) = m 
\]

A table of \(-\log(1 - y)\), that is, \(\log[1/(1 - y)]\), is given by Van der Plank (1963, Appendix Table 3). For a comment on signs, see Section II.E.

Consider now Heald's (1921) data on the amount of bunt in wheat plants grown from grain artificially contaminated with spores of Tilletia spp. The data are for the very susceptible variety Jenkins Club. Figure 3 shows \(-\log(1 - y)\) plotted against the number of spores per grain. Only data for up to 40,000 spores per grain are included; from Heald's survey it is known that higher loads are abnormal on grain used for seed, and, as Figs. 1 and 2 have shown, abnormally high loads of inoculum can confuse the issue. The line in Fig. 3 is fitted by the usual statistical procedure.

Figure 3 can be read in the same way as Figs. 1 and 2. If spores act independently of one another, one expects a straight line fitted to the points to pass through the origin. This is practically what is shown.

---

**Fig. 3.** The relation between the proportion \(y\) of smutted plants and the number of spores of *Tilletia* spp. per grain. In order to allow for multiple infections \(-\log(1 - y)\) is plotted instead of \(y\). (Data of Heald, 1921.)
in Fig. 3. When \(-\log_e(1 - y) = 1\), there is an average of one infection per plant, and in Fig. 3 this corresponds to a load of 15,000 spores per grain. That is, an average of one spore in 15,000 managed to infect. Similarly, when \(-\log_e(1 - y) = 2\) there is an average of two infections per plant, and in Fig. 3 this corresponds to a load of 31,000 spores per grain, which tallies well enough with the previous estimate that one spore in 15,000 managed to infect.

As it is with systemic diseases, so it is when infection produces local lesions. If one is simply told the proportion of infected plants, without being told how many lesions there actually are on the infected plants, one must transform the data as before. Figure 4 uses Glynne's (1925) data for wart disease of potatoes caused by *Synchytrium endobioticum*. The proportion \(y\) of infected plants is transformed into \(-\log_e(1 - y)\) and plotted against the number of sporangia per gram of soil. This allows for some infected plants having one wart, some two warts, some three, and so on. A straight line fitted to the points passes practically through the origin, indicating that the myxamoebae did not interact during the infection process; they neither helped nor hindered one another. Quantitatively, \(-\log_e(1 - y) = 1\) when there were 1000 sporangia per gram of soil. That is, it required a concentration of 1000 sporo-

---

**Fig. 4.** The relation between the proportion \(y\) of infected plants and the number of sporangia of *Synchytrium endobioticum* per gram of soil. In order to allow for multiple infections \(-\log_e(1 - y)\) is plotted instead of \(y\). (Data of Glynne, 1925.)
ranging per gram of soil to cause an average of one wart per plant. The proportion of myxamoebae that developed and managed to infect was therefore very small; but this has nothing to do with the evidence for independent action.

For this sort of evidence about independent action there is a condition: \(-\log(1 - y)\) allows accurately for multiple infections only if conditions for infection are uniform. That is, straight lines in graphs such as Figs. 3 or 4 can be expected only if all plants are equally susceptible or vulnerable to infection, if the soil is uniformly moist, and so on. All departures from uniformity, whatever their source, will cause the line to curve to the right. A similar curvature can be expected when spores interact antagonistically; and from the curvature one cannot always easily distinguish between lack of uniformity and antagonism between spores. Figure 6, to be discussed later, is an example.

D. Evidence Against a Numerical Threshold of Infection

Gaumann (1946), Sadasivan and Subramanian (1960), Nutman and Roberts (1963), and others have claimed that a minimum number of individual propagules is necessary, even in favorable conditions, to establish infection. This is the numerical threshold of infection. Thus, citing Gaumann, 200 resting sporangia of *S. endobioticum* per gram of soil are necessary to cause wart disease in potatoes; an infection spot caused by a single myxamoeba does not lead to disease, because the morphogenic stimulus is too weak. Similarly, with bunt in wheat, 100 spores per grain are needed to infect even a very susceptible variety such as Jenkins Club, and as many as 500 to 5000 to infect a more resistant variety such as Marquis.

All this bears directly on the theory of fungicide usage. Thus wart disease was found in potatoes in some mining villages in Pennsylvania, but not elsewhere in the United States. To prevent the disease from spreading, infected soils were treated with fungicides to eradicate the pathogen. On the theory of a numerical threshold of infection, it would have been necessary only to reduce the number of sporangia below 200 per gram of soil, and the disease would have been eradicated. So, with bunt, if chemical treatment reduced the number of surviving spores below 100 per grain, all infection from contaminated seed would disappear, even from a very susceptible variety. Unfortunately it is not as simple as that.

A numerical threshold of infection implies that there is a synergistic
interaction between propagules, but no evidence for it is known. Briefly, the evidence cited by Gaumann and by Nutman and Roberts will be reproduced and the conclusions they drew from it disputed.

Haydell's (1921) results with bunt of wheat and Glynne's (1925) with wart disease of potatoes, two diseases quoted by Gaumann, are shown in Figs. 3 and 4. There is no evidence in them of synergistic interaction. Haydell's (1928) results with fusarium wilt of tomato, also quoted by Gaumann, are analyzed in Fig. 5. A straight line fitted to the points by normal statistical procedure passes practically through the origin, and there is no evidence for synergistic interaction. Finally, Gaumann cited Dickson's (1923) results with seedling blight of maize caused by Gibberella saubinetii. These results are analyzed in Fig. 6. The relation between \(-\log(1 - y)\) and the number of spores is not linear. At first, the curve rises steeply; relatively few spores, 13,200/cubic cm produced a relatively high incidence of disease. Higher concentrations of spores, however, produced diminishing increments of disease. This is precisely the opposite of what the theory of a numerical threshold of infection demands. On this theory there should have been no disease or little disease, with low concentrations of spores, and an intensified response later. The line curves to the right, which is incompatible with the theory.

Nutman and Roberts (1963) inoculated coffee leaves with spores of the rust fungus Hemileia vastatrix. They used varying numbers of spores in drops of water, and counted the percentage of successful transfers. Their results are plotted in Fig. 7. The data are not very precise; 10 to 20 spores per drop is interpreted as 15 spores per drop. The percentage of successful transfers are too low to need transformation to allow for multiple infections. The straight line fitted to the points by standard statistical procedure cuts the y-axis above the origin, and there is no hint of evidence for a numerical threshold of infection. Figure 7 only indicates that the proportion of spores that lead to infection is very small, irrespective of the number of spores per drop. There is nothing to suggest that any one spore has a better chance of producing infection.

One can explain the curvature of the line in Fig. 6 in two ways. Either the spores interact antagonistically at high concentrations or, alternatively, conditions in the experiment were variable; all seedlings were not equally vulnerable to infection, for example. In the first alternative, of antagonism at high spore concentrations, one expects disease to increase to a maximum and then decrease as spore concentration increases. (The behavior of bean rust, shown in Fig. 2, is an example.) In the second alternative, of variable conditions, one expects a continuous, but diminishing, increment of disease without any decrease at high spore concentrations. The evidence of Fig. 6 thus favors the second alternative.
chance to infect when it is with 14 others in a drop than when it is alone.

![Graph](image)

**FIG. 5.** The relation between the proportion $y$ of tomato plants that become infected with fusarium wilt and the number of spores of *Fusarium oxysporum* f. *lycopersici* per cubic centimeter of inoculum. In order to allow for multiple infections, $-\log(1 - y)$ is plotted instead of $y$. (Data of Haymaker, 1928.)

![Graph](image)

**FIG. 6.** The relation between the proportion $y$ of severely diseased maize plants and the number of conidia of *Gibberella subminuta* per cubic centimeter of inoculum. To allow for multiple infections $-\log(1 - y)$ is plotted instead of $y$. (Data of Dickson, 1923.)
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There is indeed a threshold condition for disease to develop; and it is of capital importance in the theory of fungicidal action, because it determines how much fungicide is needed to stop further development of disease. However, it has nothing to do with interaction between propagules; it stems from the removal of infectious tissue, as when lesions dry up and stop forming spores. This condition is discussed in Section IIIJ.

E. Tests for Independent Action of Propagules; The Misuse of Logarithms and Probits

To summarize, the number of infection points, as in Fig. 1, or the number of pustules, as in Fig. 2, or \(-\log_e(1 - y)\), as in Figs. 3 to 6, is plotted against the number of spores used as inoculum. If the spores act independently of one another, a straight line fitted to the points passes through the origin; in other words, the number of infection points or pustules or \(-\log_e(1 - y)\) is directly proportional to the number of spores. The conditions are that all plants should be homogeneously susceptible or vulnerable to infection and that the number of plants or lesions should not be small.

Druett (1952) and Peto (1953) have used a similar test for independent action. They showed that when homogeneous test animals are exposed to infection by microorganisms there should be a linear rela-
tion between the logarithm of the proportion of survivors, i.e., animals not infected, and the number of microorganisms, if the microorganisms infect independently of one another. The proportion of survivors is $1 - y$. Druett and Peto plot $+ \log(1 - y)$ with ordinates negative. I plot $- \log(1 - y)$ with ordinates positive. Otherwise the tests are identical. I prefer $- \log(1 - y)$ because it is more easily interpreted and because it brings Figs. 3 to 6 into line with Figs. 1 and 2. A simple change from plus to minus changes $+ \log(1 - y)$, the logarithm of the proportion of survivors, to $- \log(1 - y)$, the average number (not the logarithm of the number) of multiple infections per plant or animal. The change takes logarithms out of our mental picture of the relationship. For the nonlogarithmic meaning of $- \log(1 - y)$ see Eq. (1).

In either case, irrespective of the sign of the ordinates, the abscissas are the numbers, not the logarithms of the numbers, of spores. I know of no reason, practical or theoretical, for using logarithms on the $x$-axis, when one relates the number of spores (or other propagules) to the amount of disease they cause directly.

I state this flatly (that I know of no reason for using logarithms on the $x$-axis for the sort of direct relation we are discussing), despite the fact that the test for independent action currently prescribed in the literature of plant pathology uses logarithms. The probit of the proportion of infected plants—which we shall call probit disease—on the $y$-axis is plotted against the logarithm of the number of spores—which we shall call log dose—on the $x$-axis. If the dose is expressed by multiples of $ED_{50}$, then the slope of the curve at $ED_{50}$ will be approximately 2 if spores do not interact. (The logarithms here are to the base 10).

The reason for using probits and logarithms here is obscure. Probits are a useful way of straightening one form of sigmoid curve. The curve relating the proportion of infected plants—disease—to the number of spores—the dose—is not sigmoid, however, so why probits were ever chosen is unknown. Whatever the reason for it, the choice of probits necessitates further changes: dose has to be changed to log dose to try to straighten out the complication probits introduce. Yet even this double transformation—from disease to probit disease and dose to log dose—still leaves the relation nonlinear and complex. It must not be thought that the simple relation between disease and dose used by Druett and Peto and in this chapter is crude and inexact and needs to be replaced by a more refined probit-disease/log-dose relation. Historically, probit disease and log dose were used earlier, and it was
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Druett and Peto's purpose to show that they could be replaced by the simpler forms.*

In the understanding of fungicidal action two separate relations are involved. One is the relation between spores killed or inactivated and the dose of fungicide. The other is the relation between disease and the dose of surviving spores or other propagules. This chapter deals with the second relation, not the first. Chapter 4 deals with the first.

III. THE PROGRESS OF AN EPIDEMIC AND THE EFFECT OF FUNGICIDES ON IT

A. The General Problem

The problem discussed in Section II was relatively uncomplicated. To put it in terms of a foliage disease, spores fall on leaves, some of them germinate there and establish infections. The relation between the number of spores and the number of infections they establish was discussed. The reference here is to infections established directly, initiated where the spores settled. Now the matter must be considered further. With diseases such as the cereal rusts or potato blight, spores settle, initiate lesions which in the course of time produce another generation of spores, which scatter to initiate another generation of lesions, and so on through many generations of spores and lesions. If the foliage is kept covered with a fungicide, the fungicide acts not just against one set of spores—the problem of Section II—but continuously during the epidemic. To determine the effect of this continuous action is the basic task of Section III.

Figure 8 summarizes data (Anonymous, 1954) for the progress of blight in a very susceptible potato variety, Bintje. The data for unsprayed fields are for 29 fields in the sand area of the Netherlands. The data for the sprayed fields are the combined data for all fields receiving 3, 4, or 5 sprays during the season. The fungicide was mostly copper oxychloride. The fields were inspected and blight recorded at weekly intervals.

* In the course of his work Peto proved the relation given in the previous paragraph; that, with independent action, the slope of the probit-disease/log-dose curve at ED₅₀ should be 2. This was incidental to showing the value of the simple log survival/dose relation. It testifies to a widespread love of embroidery that the incidental has come into the literature of plant pathology but the simple relation has generally been forgotten.
The top half of Fig. 8 records the proportion \( x \) of foliage that was infected at the various times. In the unsprayed fields disease mounted fast in the second half of July and in the first half of August practically destroyed the foliage. In the sprayed fields the amount of disease was negligible during most of July and was still fairly low in early
September when the fields ripened. The problem is to interpret these curves in terms of fungicidal action.

Before the problem is considered further the change from \( y \) to \( x \) must be explained. In Section II the proportion \( y \) of infected plants was discussed. In Section III the proportion \( x \) of susceptible tissue, foliage in our present example, that is infected is discussed. To illustrate the difference, assume that it takes 1000 blight lesions to destroy a potato plant completely, i.e., to cause 100% infection; this is a realistic figure for large plants. If 50% of the plants were entirely healthy, with no lesions at all, and 50% had one or more lesions, one could write \( y = 0.5 \). If there were an average of 500 lesions per plant, one would write \( x = 0.5 \). Only with systemic disease does \( x = y \); for local lesion diseases \( x \) and \( y \) necessarily differ.

**B. The Apparent Infection Rate \( r \)**

An infection rate \( r \) is defined by Eq. 2,

\[
\frac{dx}{dt} = r x (1 - x) \tag{2}
\]

where \( t \) is the time at which the proportion \( x \) of infected tissue is assessed. The rate \( dx/dt \) is related both to \( x \) and to \( 1 - x \), the proportion of tissue still healthy and available for infection; and \( r \) is the regression coefficient of \( \log \left[ \frac{x}{1 - x} \right] \) on time.

In the bottom half of Fig. 8 \( \log \left[ \frac{x}{1 - x} \right] \) is plotted instead of \( x \) against time, and straight regression lines fitted to the points. The regression coefficients with time measured in days are 0.42 and 0.082 for the unsprayed and sprayed fields, respectively. Hence \( r = 0.42 \) and 0.082 per day. This gives a clearer picture of the problem. Protective copper fungicides reduced \( r \) from 0.42 to 0.082 per day. How can this protection be analyzed quantitatively?

**C. The Basic Infection Rate \( R \)**

Equation (2) relates \( r \) to the proportion of infected tissue, but "infected tissue" must be defined further. For the purpose of the present analysis, infected tissue is of three sorts: (1) newly infected tissue that has not yet had time to become infectious, (2) infectious tissue, and (3) tissue that was infectious previously but has since ceased to be so. Newly infected tissue takes a period \( p \) to become infectious (e.g., with potato blight to form sporangia which disperse to propagate the
fungus anew); this is called the latent period. Thereafter it remains infectious only for a period $i$, after which it is "removed" from the epidemic.

Suppose $x(t)$ is the proportion of susceptible tissue infected at time $t$. Then $x(t - p)$ was the proportion infected at time $t - p$, and at time $t$ is the proportion that has passed through the latent period $p$ and is either infectious or removed. Similarly $x(t - i - p)$ was the proportion infected at time $t - i - p$, and at time $t$ is the proportion that has been removed. Hence $x(t - p) - x(t - i - p)$ is the proportion infectious at time $t$.

A new infection rate $R$, based specifically on infectious tissue and not just infected tissue, is defined by

$$\frac{dx(t)}{dt} = R[x(t - p) - x(t - i - p)][1 - x(t)]$$

$R$ is determined by two factors: (1) the infectiousness of the infectious tissue (e.g., the number of spores produced per unit area of sporulating leaf tissue), and (2) the susceptibility and vulnerability of the healthy tissue to infection (e.g. the proportion of spores which after falling on healthy leaves germinate and start lesions there). Both factors are sensitive to the environment, to the susceptibility of the host and the virulence of the pathogen, and—what concerns us primarily in this Section—to the presence of fungicides.

In regard to the first factor, the infectiousness of infectious tissue, Hodgson (1963) inoculated three potato varieties with Phytophthora infestans and then sprayed some of them with maneb a day later. When the leaves began to sporulate he washed them and counted the number of sporangia per cubic centimeter of washings from similar sized leaves. His results are given in Table I. Maneb reduced spora-

<table>
<thead>
<tr>
<th>Variety</th>
<th>Susceptibility to blight</th>
<th>Number of sporangia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Maneb</td>
</tr>
<tr>
<td>Green Mountain</td>
<td>High</td>
<td>33,000</td>
</tr>
<tr>
<td>Sebago</td>
<td>Medium</td>
<td>14,000</td>
</tr>
<tr>
<td>Libertas</td>
<td>Low</td>
<td>2,000</td>
</tr>
<tr>
<td>Average</td>
<td>16,300</td>
<td>1,800</td>
</tr>
</tbody>
</table>

*Data of Hodgson (1963). The numbers of sporangia are per cubic centimeter of washings from similar-sized leaflets.*
3. EPIDEMIOLOGY OF FUNGICIDAL ACTION

...tion from 16,300 to 1800; it reduced it to one-ninth. If one assumes a similar action in the field, maneb by its effect on sporulation (and quite apart from any other effect it might have on blight) would reduce $R$ to one-ninth. (The differences between the three varieties will be discussed in Section IV.)

Similarly, Albert and Lewis (1962) sprayed captan and dodine on to apple leaves with established scab lesions caused by Venturia inaequalis. The fungicides reduced the number of conidia produced in the lesions. Further, not only was the number reduced, but the few conidia that formed were abnormal and germinated poorly when removed from the lesions and tested in a fungicide-free medium. Powell (1960), Albert and Heuberger (1961), and Heuberger and Jones (1962) also added fungicides—captan, dodine, dyrene, folpet, phalan, and phenyl-mercuric acetate—to apple leaves with established scab lesions on them, and found that the ability of the conidia in these lesions to germinate was impaired. Here again, $R$ would be reduced in direct proportion to the combined reduction of numbers and percentage germination of conidia; that is, $R$ would be proportional to the number of viable spores produced per lesion.

In regard to the second factor, the susceptibility and vulnerability of healthy tissue to infection, Björling and Selgren (1957) sprayed healthy potato leaves with bordeaux mixture and, after the fungicide deposit was dry, inoculated them with spores of $P$ infestans, and later counted the lesions. There were 12,732 lesions in the unsprayed leaves but only 1193 on the sprayed leaves. The bordeaux mixture reduced infection to roughly one-tenth. It reduced the vulnerability of the healthy leaves to infection—it protected them—and the same amount of protection in the field would reduce $R$ to roughly one-tenth. Protection of this sort gives its name to the largest group of fungicides: the protectants. Plants are covered with fungicide and thus protected against infection. This process is so well known that it needs no further examples.

D. The Logarithmic Phase of an Epidemic: Its Relevance to Studies on Fungicidal Action

Fungicides reduce $R$ in direct proportion to their effect in reducing the number of viable spores produced in the lesions and in reducing the proportion of spores that manage to germinate and start new lesions. Essentially, then, the concept of $R$ is simple, and the effect of fungi-
J. E. VAN DER PLANK

cides on $R$ easily grasped. Usually, however, $R$ is not a parameter one estimates directly.*

Normally, the experimental data from disease progress curves, as in Fig. 8, give estimates only of $r$. To assess fungicidal activity one must therefore relate $r$ to $R$.

From Eqs. (2) and (3), remembering that $x$ in Eq. (2) is $x(t)$, one obtains

$$r = \frac{R[x(t - p) - x(t - i - p)]}{x(t)}$$

This equation states that the ratio of $r$ to $R$ is the ratio of the proportions of infectious to infected tissue. It holds for all phases of epidemics, but is not generally suitable for our purpose. To adapt it, one must turn to the logarithmic phase of an epidemic. The logarithmic phase is when $x$ is small enough for $1 - x$ to be near enough to unity for practical purposes. During this phase Eq. (2) reduces to

$$\frac{dx}{dt} = \frac{T}{x}$$

where the subscript $l$ shows that $r_l$ is the estimate of $r$ appropriate to this phase.

During the logarithmic phase there are $e^{-r_l}$ units of tissue which are either infectious or removed and $e^{-(i + p)r_l}$ units which are removed, for every unit of infected tissue. Hence, for every unit of infected tissue there are $e^{-r_l} - e^{-(i + p)r_l}$ units of infectious tissue, so (because the ratio $r : R$ is the ratio infectious : infected tissue)

$$r_l = R(e^{-r_l} - e^{-(i + p)r_l})$$

This equation has been used elsewhere (Van der Plank, 1963, 1965) in the form

$$r_l = \frac{R(e^{r_l} - 1)}{e^{(i + p)r_l}}$$

Equation (4) will be used to relate $r$ and $R$. At first it would seem that to restrict ourselves to an equation developed for the logarithmic phase would severely hamper research into the effect of fungicides. This would be so except for the fortunate circumstance that in an epidemic proceeding at constant values of $R$, $i$, and $p$, the value of $r$ stays substantially constant at its logarithmic value $r_l$ until $x$ reaches 0.15 (or even 0.35, if too fine a degree of accuracy is not needed). This

*When the data are obtained after artificial inoculation they are sometimes suitable for estimating $R$ directly. An example is given by Van der Plank (1963, Section 5.4).
has been discussed elsewhere (Van der Plank, 1961, 1963, 1965). In other words, one may estimate \( r \) at any time during the epidemic until \( x \) reaches 0.15 (or more), and use the estimate as an estimate of \( r \) for the purpose of using Eq. (4). Normally, in fields and orchards treated with fungicides \( x \) does not exceed 0.15, so Eq. (4) provides a relationship that can be used widely enough for most purposes of fungicide research.

**E. Difficulties Arising from Variation and from the Growth of Lesions**

Equations (2) and (3) are simply definitions; they define \( r \) and \( R \), respectively. Equation (4) connects Eqs. (2) and (3). There is no need to prove it. The difficulty is to apply it, and especially to have accurate estimates of \( p \) and \( i \) to use with it.

First consider some simple uncomplicated data. Cammack (1961) studied the development of pustules and spores on maize plants inoculated with *Puccinia polysora*. Under favorable conditions pustules develop and start to form spores 9 days after inoculation. They continue to form spores at a constant rate—a rate constant for the variety and for the environmental conditions—for a further 18 to 20 days. This is easily interpreted: \( p = 9 \) days and \( i = 18 \) to 20 days.

Usually matters are not so simple, however. Lesions start to form spores after a variable period, and continue to produce them at a variable rate for a variable period. Thus Yarwood's (1961) results with bean rust differ from those of Cammack with tropical rust of maize in that spore production in pustules rose gradually, not abruptly, to a peak. Complications in another form occur when lesions increase in size and the zone of spore production moves outward. Lesions of potato blight expanding over the foliage are examples.

There are no hard and fast equations for dealing with these complications. One must adapt the method of analysis to the data as one finds them. A method is selected here which seems apt for the data that are available on potato blight.

**F. The Latent Period**

Consider some of Lapwood's (1961b) data on potato blight. Lapwood cut disks out of potato leaves, sprayed them with *P. infestans*, kept them in conditions favorable for blight, and then noted when they first started to sporulate. Some started after 4 days, some after 5 days,
some after 6 or more days. Those of us who considered the latent period of blight fell into the error of assuming that these figures indicate the latent period. That is wrong. Quite apart from the fact that the disks were inoculated with many spores whereas a lesion probably usually starts from a single spore, a difference not pursued here, a disk inoculated by covering it with spores is unlike a lesion expanding as mycelium spreads laterally. A lesion must be considered as an whole which can be traced back to the spore from which it started; and one must measure the latent period of all parts of the lesion from a single zero time: the time the lesion started from the spore.

Consider a lesion. If $a_1, a_2, \ldots, a_n$ are the proportions of tissue with latent periods $p_1, p_2, \ldots, p_n$, the latent period $p$ of the lesion as a whole is given by

$$ a_1 + a_2 + \ldots + a_n = 1 $$

$$ e^{-p_1 t} = a_1 e^{-p_1 t} + a_2 e^{-p_2 t} + \ldots + a_n e^{-p_n t} $$

This is the value of $p$ to be used in Eq. (4) (Van der Plank, 1965).

The terms in the equation are not of equal weight, and if a lesion goes on growing for many days, as when a potato blight lesion starts to grow down the petiole, the last terms become increasingly unimportant numerically.

Primarily, $p$ is affected by the susceptibility of the host to infection and by the temperature and other environmental conditions. With expanding lesions such as those of potato blight it is also affected by the size and shape of the leaflets, the positions on the leaflets where lesions mostly start, and other incidental factors.

**G. An Indirect Beneficial Effect of Fungicides on the Latent Period**

It follows from Eq. (5) that what reduces $r_1$ must also increase $p$. Fungicides reduce $r_1$, they therefore increase $p$. This is quite apart from any direct effect a fungicide might have in delaying sporulation.

The reason, stated without mathematics, is this: a high value of $r_1$ gives extra weight to those parts of a lesion which start sporulating first. A low value spreads the weight more evenly, and therefore increases $p$.

Fungitidal activity is thus put into high gear. Fungicides are doubly beneficial. They reduce $R$ directly (in ways discussed in Section IIIC). They increase $p$ indirectly (because by reducing $R$ they reduce $r_1$).
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Failure to use fungicides is doubly harmful. It leaves R large and p small. The rule is that p is most sensitive to changes of \( r_t \) when \( r_t \) is great. The beneficial effect is therefore greatest when fungicides are used against explosive epidemics.

H. The Infectious Period

Consider some other findings of Lapwood (1961a,b,c) about potato blight. A typical lesion has three zones: an outer zone into which mycelium has penetrated but in which no sporulation yet occurs, a sporulation zone, and an inner zone which has stopped sporulating. As the lesion grows older the sporulation zone moves outward, leaving an ever-widening sterile zone within. In very susceptible varieties in weather favorable to disease, the outer boundary of the sporulation zone on one day practically coincides with the outer boundary of the sterile zone (which is the inner boundary of the sporulation zone) on the next day. That is, any particular small element of tissue forms spores for one day; for any small element of tissue the infectious period \( i = 1 \) day.

What is \( i \) for the lesion as a whole? To give the simplest possible answer, \( i \) for all the lesion's elements will be assumed to be constant (e.g., at 1 day, as in the previous paragraph).

The proportion of infected tissue that has passed the latent period and is therefore either infectious or removed, is

\[
a_1e^{-r_1} + a_2e^{-r_2} + \ldots + a_ne^{-r_n}
\]

when \( a_1, a_2, \ldots, a_n \) and \( p_1, p_2, \ldots, p_n \) have the meaning given for Eq. (5). The proportion of infected tissue that has been removed is

\[
a_1e^{-(p_1r_1)} + a_2e^{-(p_2r_2)} + \ldots + a_ne^{-(p_nr_n)}
\]

The proportion of infected tissue that is infectious is therefore

\[
a_1e^{-r_1}(1 - e^{-r_1}) + a_2e^{-r_2}(1 - e^{-r_2}) + \ldots + a_ne^{-r_n}(1 - e^{-r_n})
\]

\[
= (1 - e^{-r_1})(a_1e^{-r_1}) + a_2e^{-r_2} + \ldots + a_ne^{-r_n}
\]

\[
= (1 - e^{-r_1})e^{-r_1}
\]
by Eq. (5). The amount of infectious tissue per unit of infected tissue is also \( r_i/R \). Therefore

\[
    r_i = R \left(1 - e^{-ri}\right)e^{-pi} \\
    = R\left(e^{-ri} - e^{-ri+pi}\right)
\]

which is Eq. (4). Thus the infectious period \( r_i \) for the lesion as a whole is also \( r_i \) for each of the lesion's constituent elements.

I. An Analysis of Fungicidal Action

To return to Fig. 8, copper fungicides reduced \( r \) from 0.42 to 0.082 per day. One can take \( r \) to represent \( r_i \). (Values would not be greatly altered by keeping to the disease limits discussed in Section III,D). The potato variety Bintje, on which the blight records used in Fig. 8 were taken, is very susceptible, and \( i = 1 \) day is a suitable estimate. If the available information in the literature of sporulation and growth of blight lesions is combined, \( p = 6.7 \) days for unsprayed fields and 7.1 days for sprayed fields seem to be suitable estimates. With these estimates, Eq. (4) gives \( R = 20.4 \) per day for the unsprayed and 1.86 per day for the sprayed fields.

Spraying reduced \( R \) to roughly one-tenth of its value in unsprayed fields. It seems to be generally accepted that copper fungicides are primarily protectant: they reduce the proportion of spores that can germinate and start lesions. Therefore spraying, in this example, reduced the proportion of spores that germinated and started lesions to roughly one-tenth. This differs from our earlier estimate (Van der Plank, 1963) that the proportion was reduced to roughly one-fifth, this early estimate being based on a value \( p = 4 \) days that misinterpreted the data on sporulation (see Section III,F). The new estimate agrees well with the results of Björling and Sellgren (1957) cited in Section III,C. Using detached leaves, these workers found that bordeaux mixture reduced the proportion to roughly one-tenth. (This figure, one-tenth, is just an average of many experiments in which the proportions varied with varying experimental conditions.) When other sets of data (Van der Plank, 1963) similar to those used in Fig. 8 are reanalyzed with improved estimates of \( p \), answers in the general range of Björling and Sellgren's findings are obtained. One should not attach too much weight to this agreement; but at least one feels that the
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analyses are realistic and that one is beginning to understand the process of fungicidal action against epidemics.

J. The Threshold Theorem in Relation to Fungicidal Action

In Fig. 8 fungicides reduced \( r \) for potato blight from 0.42 to 0.082 per day. To do this they reduced \( R \) to, roughly, one-tenth. By how much would \( R \) have had to be reduced to stop the epidemic from developing at all; that is, by how much would \( R \) have had to be reduced to make \( r = 0 \)?

From Eq. (4) one finds that \( r > 0 \) only if \( iR > 1 \). This defines the threshold condition for an epidemic to develop. Interpreted, it means that in the long run an epidemic will develop only if each lesion, while it is infectious, brings about the infection of more than one daughter lesion (or, for systemic disease, if each systemically infected plant brings about the infection of more than one other plant).

To return to our question and use values from the previous Section, if \( i = 1 \) day and \( R = 20.4 \) per day, \( iR = 20.4 \). To make \( iR = 1 \) one must have \( R = 1 \) per day. To control the blight epidemic illustrated in Fig. 8 completely it would have been necessary for the copper fungicide to reduce the proportion of spores that germinated and started lesions to roughly one-twentieth.

It is common experience that potato blight epidemics can be kept completely in check, especially now that the dithiocarbamate fungicides are available. That is, the fungicides keep \( iR < 1 \). With the dithiocarbamates \( R \) is reduced in two ways: by reducing the proportion of spores that germinate and infect healthy tissue, and by reducing the number of viable spores (or sporangia) produced in the lesions.

Although one may admire the efficacy of these fungicides, however, one ought to temper any admiration with the thought that potato blight is not a very difficult disease to control, because \( i \) is short. With stem rust of wheat \( i \) is usually long, \( R \) often great, and \( iR \) therefore often very great. One expects, and finds, the disease to be difficult to control by fungicides in conditions (of weather favorable to disease or of varieties very susceptible to disease) that make \( R \) large.

With apple scab \( i \) is long, but \( R \) is usually relatively small for the stage of infection by conidia; and scab can usually be well controlled during this stage. Note that the threshold condition applies only to infection by conidia, i.e., to that stage of the epidemic when conidia released by old lesions start new lesions. During the period of asco-
spore discharge from dead leaves there is no threshold; and in general during this period the number of new lesions will be proportional to the number of ascospores, however low this number may be. For additional comments about apple scab, see Section III,K.

K. The Range of Models of Fungicidal Action

Opposite extremes are discussed on Sections II and III. In one section the discussion is about what to expect when fungicides are used to control a single generation of the fungus; in the other, what to expect when fungicides are applied to an established epidemic running through several generations.

Consider a disease discussed in Section II, wheat bunt, the subject of Fig. 3. Spores of the bunt fungus germinate and infect the young seedling. Thereafter the fungus spreads through the plant and eventually reaches the inflorescence. During the course of the season it does not spread from plant to plant, however; it is a “simple interest” disease, to adopt a term used earlier (Van der Plank, 1963). Fungicides are used to protect the young seedling against infection and (to consider only the events during a single season) our concern with fungicides ends there. Disease, or to be precise \(-\log(1 - y)\), is proportional to the number of surviving spores. There is no threshold of infection; and disease can occur as long as some spores, even only a few, survive, although the danger is necessarily smaller the fewer that survive.

In Section III a potato blight epidemic was discussed. It was implicitly assumed that fungicides were used against an epidemic already started, and that during the period in which fields were covered with fungicides the fungus increased through several generations, with parent lesions forming daughter lesions, and these in turn forming other lesions. For this discussion it was necessary to introduce concepts of latent periods and infectious periods, and to think not only in terms of protecting healthy tissue with fungicides but also of possibly reducing the number of viable spores produced in lesions. A finite infectious period automatically brings in a threshold condition for an epidemic to proceed.

In between these two extremes all gradations exist. Consider scab of apples caused by \(V. inaequalis\). In some climates of the world infection is mainly by ascospores, and the farmer can stop using fungicides as soon as the danger of ascospore discharge has passed. In these
climates protection against apple scab is essentially similar to protection against wheat bunt, and one can apply the concepts of Section II, with some minor differences in detail. (One difference is that apple scab is not systemic, and one must change from $y$ to $x$ for the reasons given in Section III,A. This difference is normally trivial, because in sprayed orchards lesions are usually too few to compete with one another for space. Another difference is that against apple scab one can use curative fungicides, such as phenyl mercury chloride, as well as straight protectants, but the difference is unimportant, because in any case one attempts to stop lesions from forming.) In other climates infection by conidia is important, and the fungus passes through several generations. In these climates fungicidal action against apple scab has much that is similar to action against potato blight; and one must apply many of the concepts of Section III.

In general, one must think of a wide range of models when one tries to analyze how fungicides control epidemics; the models may range anywhere between the extremes chosen in this chapter as examples.

IV. THE FUNGICIDE SQUARE: PATHOGEN, HOST, ENVIRONMENT, AND FUNGICIDE

Some data on the production of sporangia by *P. infestans* in lesions of potato blight were given in Table I. They dealt with the effect of maneb and of the resistance of the potato variety. In the very susceptible variety Green Mountain, maneb reduced the number of sporangia from 33,000 to 1200/cm$^3$ of washings from leaflets of similar size. In the absence of maneb, changing the variety from susceptible Green Mountain to more resistant Libertas reduced the number from 33,000 to 2000/cm$^3$. A combination of maneb and resistance reduced the number from 33,000 in Green Mountain without maneb to 300 in Libertas with maneb. If the effects were similar in the field during a blight epidemic, then maneb on Green Mountain would reduce $R$ to 42/330 of its value without maneb; changing from Green Mountain to Libertas, both untreated with maneb, would reduce $R$ to 2/33 of its value with Green Mountain; and changing from Green Mountain untreated with maneb to Libertas treated with maneb would reduce $R$ to 3/330 of its value with untreated Green Mountain. (All this considers only the effect on sporangia formation; any other effects of maneb or resistance on $R$—for example, an effect on the proportion of spores that germinate and start lesions—would be additional.)
The first conclusion from these data is that one does not distinguish between maneb and resistance in the kind of reduction of \( R \) they bring about. Both reduce \( R \) (from which, if one knows \( i \) and \( p \), one can measure their effect on \( r_0 \)). So it also is with other factors. Fewer sporangia are formed in dry air; dryness of air would reduce the formation of sporangia and hence reduce \( R \) proportionately; and the effect on \( R \) would not differ in kind from the effect of maneb or resistance. So it also is with other parameters: for \( i \) and \( p \) as well as \( R \). All factors, whether they come from the pathogen, host plant, environment, or fungicide, may affect \( R \), \( i \) or \( p \); and one cannot distinguish between the factors in the kind of effect they have on any particular parameter. There is no special epidemiology for mycologists or for plant breeders or for climatologists or for specialists in fungicides. There is one epidemiology for all concerned with plant disease.

The other conclusion to be drawn from Table I is that the effects of maneb and resistance were supplementary. Maneb by itself was effective; resistance by itself was effective; maneb plus resistance was doubly effective. Supplementary effects are general. It is easier to control disease with fungicides when the host plants have some resistance or when the weather (or other environmental condition) does not contribute to the development of disease. In practice this is well recognized; in theory one can easily see that this is true because all factors that curb the progress of an epidemic do so by reducing \( R \) or \( i \) or by increasing \( p \), and their effects on these parameters are cumulative.

The aim of chemical manufacturers is clear: to produce the best and safest fungicide. The aim of directors of agricultural research is equally clear: to establish methods for the reduction of disease in the best and safest way. Fungicides, the breeding of resistant varieties, and environmental control must not be thought of as divergent and mutually exclusive methods; they are convergent and mutually helpful. With crops that give a high return of money per acre it may be feasible or convenient to concentrate on fungicides; but even with these crops a modicum of resistance in the host plants or of environmental control will reduce the costs. With crops that give a small return of money per acre, it has been necessary to emphasize resistance in the host plants and to choose relatively safe environments. There are still losses from disease, however, and if fungicides must be used they are best used in addition to, not instead of, disease resistance and environmental control.
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CHAPTER 4

Determination and Measurement of Fungitoxicity

D. C. TORGESON

I. Introduction

The cost involved in the evaluation during a single year of thousands of chemicals for fungicidal activity (see Wellman, Chapter 5) makes the use of simple but discerning bioassay techniques obligatory. Since the empirical approach must still be used in discovering new fungicides, a great many techniques have of necessity been developed. The techniques and approaches involved in the evaluation of fungicides have been reviewed previously by Hamilton (1959), Horsfall (1945a, b, 1956), and McCullan (1947a, 1959, 1966). In the present discussion no attempt has been made to cover all of the literature on the subject, but to mention selectively procedures and approaches which may be useful to those interested in this aspect of contemporary fungicide development.

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The bioassay techniques discussed have been broken down into basic and practical types (Block, 1959). Basic methods are in vitro techniques used to determine whether or not a compound has any intrinsic fungitoxicity but with little consideration of its ultimate use, whereas, practical methods, by simulating the conditions of use, are designed to determine the possible practical usefulness of a compound. Practical tests are varied in nature because of the many types of intended application. Primary attention in this chapter is focused on practical tests for agricultural fungicides since this aspect of industrial and wood preservation is discussed in Chapters 10 and 11, respectively. Only laboratory techniques—the greenhouse being considered an extension of the laboratory—and their interpretation are discussed since field evaluation methods belong primarily in the realm of statistical design and are beyond the scope of this treatise.

II. Basic Fungitoxicity Tests

Basic fungitoxicity tests are designed mainly to measure the effects of test chemicals on germination, growth, or respiration of fungi. The first two approaches have been widely used in the primary evaluation of chemicals for fungicidal activity. Recently, however, there has been a tendency to use a battery of practical-type tests designed to detect compounds having characteristics suitable for specific potential fungicide use. In spite of this trend the basic fungitoxicity tests are still widely used when precise measurements of innate fungitoxicity are desired, such as in the determination of the most fungitoxic member of a homologous series of compounds or in quality control determinations.

A. Spore Germination

The classic technique for determining fungitoxicity is the spore germination method of which there are a great many variations. Prévost (1807), one of the fathers of modern plant pathology, demonstrated that copper sulfate solutions prevented germination of wheat bunt spores, thereby laying the scientific foundations for the control of certain plant diseases by chemical seed treatment. Carleton (1893) used the spore germination method in studying the effects of various chemicals on the germination of cereal rust uredospores and it was used by Swingle (1896) in his pioneering study on the fungitoxic action of bordeaux mixture.
1. Slide Germination

Reddick and Wallace (1910) are usually credited with introducing the slide germination method for use in the modern fungicide era. Their technique consisted of spraying glass microscope slides with the material to be tested, placing a drop of the test spores in a water suspension on the treated surface after the deposit had dried, and after a suitable incubation period observing the spore germination obtained. Subsequently, modifications of the technique were also used by Schmidt (1924a,b, 1925) in Germany.

A lack of understanding of the factors contributing to variation in results obtained in the so-called glass-slide spore germination test prevented the method from being accepted as a reliable means for measuring toxicity. McCallan published a paper from Cornell University in 1930 in which he pointed out a number of factors which must be carefully controlled if reproducible results are to be obtained. This and subsequent papers published by McCallan and co-workers at Boyce Thompson Institute (McCallan et al., 1941; McCallan and Wellman, 1942, 1943; McCallan and Wilcoxon, 1932, 1939; Wellman and McCallan, 1942; Wilcoxon and McCallan, 1939) led to the development of a standardized test-tube dilution technique for use with the slide germination method by the American Phytopathological Society, Committee on Standardization of Fungicide Tests (1947). In this method the toxicant and spores are mixed together, usually with an added nutrient, drops of the resulting suspension pipetted onto glass slides, and the percentage of germination subsequently determined. Microbreakers have been substituted for glass slides by Shafer (1952) and Spencer (1962), and petri dishes by Tamura (1954).

A modification of the original Reddick and Wallace (1910) technique was developed by Montgomery and Moore (1938) in which the fungicide was pipetted onto the slide and allowed to dry before the spore suspension was added. This procedure was also used, with slight modifications to improve its precision, by Peterson (1941), Young (1941), H. J. Miller (1949), Kirby and Frick (1953), and Průšová (1962). Frick (1964), in a later study on the variability inherent in this method, concluded that the method of testing fungicides by suspending them in solution with the spores was superior. Rather than applying the test chemical suspension to the slide with a pipette, a number of elaborate methods employing other means of application, such as a horizontal sprayer (Horsfall et al., 1940; McCallan and Wilcoxon, 1940; Ciferri and Baldacci, 1943; Blumer and Kundert, 1950), vertical sprayer (Ten Houten and Knik, 1948), pendulum
sprayer (Rabe, 1956), or settling tower (McCallan and Wilcoxson, 1940) have been described. In spite of the complexity of these means of attempting to obtain uniform and reproducible deposits of the fungicide on glass slides the results frequently have been variable (Frick, 1964) and the simpler method of pipetting is usually used in routine glass-slide spore germination assays for fungitoxicity. Various spray and settling tower methods of application have been widely used in studies concerned with artificial weathering of the fungicide deposit (Barratt, 1946; Blumer and Kundert, 1950; Chapman et al., 1950; Ciferri et al., 1944; Heuberger, 1940; Nisikado et al., 1951a) and although at present weathering tests are usually performed on plants these methods are still used occasionally. Since consistent high germination in the controls is essential for reproducible results in any spore germination method (McCallan, 1930), the number of species which can be used is limited. Historically, Alternaria oleracea, Glomerella cingulata, Monilinia fructicola, and Stemphylium sarcinaeforme have been the most commonly used species in slide germination tests. However, in order to test for fungitoxicity using the particular species for which a fungicide was sought, suitable methods have also been devised for Botrytis cinerea (Kirby and Frick, 1953), Physophthora infestans (M. J. Miller, 1949; Bohnen, 1962), Plasmopora viticola (Chancogne and Viel, 1953), and Helminthosporium oryzae, Piricularia oryzae and Gibberella saubinettii (Nisikado et al., 1951a). Zaracovitis (1964) has recently published a spore germination method for the powdery mildews which heretofore could not be satisfactorily handled. No satisfactory procedure has been developed for the rusts. Basically the various slide germination techniques are designed to determine fungicidal activity in the broadest sense, since it is usually not of importance whether or not a compound used to control a fungus is fungicidal in the restricted sense (capable of killing the fungus) or fungistatic (capable of inhibiting the development of the fungus). Effective postharvest fungicides must usually be fungicidal in the restricted sense, however, for they must destroy the fungus spores or mycelia present on the fruit or other plant part being treated during a relatively short period of contact. McCallan and Wellman (1942) used a centrifuge method to remove the treated spores from the fungicide solution at different intervals and then determined their viability. Greene (1961) described a simpler technique utilizing syringes with filter disks which is more rapid and efficient for short contact periods. Fungicidal activity of gaseous compounds is not usually determined.
4. DETERMINATION AND MEASUREMENT OF FUNGITOXICITY

by spore germination techniques because of problems of substrate penetration. Sampson and Ludwig (1956) avoided this problem by exposing dried spores of Alternaria solani on filter paper or sintered glass disks to varying dosages followed by determination of viability. Standard dosage-response curves were obtained using this method.

2. Agar Plate Germination

Water agar plates containing different amounts of chemical have been used in the determination of fungicidal activity. Gattani (1954) used such a technique and reported that germination of spores on the surface of the water agar was less variable than in the standard slide germination method. Gottlieb (1947) compared the results obtained using agar plate and slide germination methods and concluded that since different EDR90 values were obtained it may be important to use more than one bioassay procedure in the primary evaluation of chemicals as fungicides.

3. Shaker Flask Germination

Davies et al. (1948) and Darby (1960) studied the germination of spores in liquid culture in shaker flasks and found this to be a rapid method for determining the effect of chemicals on spore germination. Darby recommended that the method was suitable for routine studies of fungicides, in view of the speed and ease with which results were obtained and the better control of conditions such as pH, aeration, and nutrition afforded by shaker flasks in comparison with agar plate or slide germination techniques.

4. Spore Volume

Spores of Myrothecium verrucaria swell rapidly and germinate in the presence of sucrose and yeast extract. Mandels and Darby (1953) developed a rapid assay for fungitoxicity based on this spore-swelling phenomenon. Cell volume increases about five times in 3 hours at 30° C, but is inhibited by fungitoxic compounds. The cell volume is readily determined by centrifugation in hematocrit tubes.

B. Fungus Growth

One of the widely used fungicidal assay tests is that of mixing the test chemical with liquid agar medium which is then usually poured into a petri dish. Fungus mycelia or spores are introduced on the sur-
face of the hardened medium and the growth obtained is used to assess the test compound's fungitoxicity. Since the radial growth of a fungus in petri dishes is linear with respect to time and the presence of a toxicant in the agar does not alter the relationship, the toxicity of a compound can be determined by measuring the time required for the fungal colony to reach a particular diameter, or the size of colony attained in a certain period of time (Bateman, 1933; Brancato and Golding, 1953). Frequently, however, relative fungitoxicity is measured as the minimum concentration at which no growth is obtained. These tests are widely used by industrial microbiologists and the variations in techniques are multitudinous. Probably the one single advantage they have over the simpler and more precise slide germination technique is that nonsporulating fungi can be employed as test organisms. In the writer's experience there has been poor correlation between the activity found in agar plate tests and that found in greenhouse foliage or soil fungicide tests.

Manten et al. (1950) have described a roll culture technique in which molten nutrient agar containing the fungicide and inoculum is revolved in small bottles until it solidifies on the sides. Other rather ingenious modifications of the petri plate method which may be useful in specific cases are those of Mason and Powell (1947), Grosser and Friedrich (1947), Teshner (1955), Forberg (1949), Bomar (1962), and Latham and Linn (1965).

Leben and Keitt (1950), Thornberry (1950), and Richardson (1953) used paper disk methods in which the test chemical is added to a paper disk on a seeded agar plate. Effectiveness is determined by measuring the zone of inhibition around the paper disk. Results are of course complicated by differences in solubility of test compounds and hence their rate of diffusion in the agar medium.

C. Respiration

Mandels and Siu (1950) have described a method using respiration during growth in the presence of test chemicals as a means of rapidly determining fungistatic activity. Although respiration was found to be useful by Klöpping (1951), McCallan et al. (1954), Walker (1955), McCallan and Miller (1957), and Torgeson (1963) in studying the effects of fungicides on oxygen consumption, it does not appear to be a particularly useful means of determining fungitoxicity. The concentration of fungicide necessary to inhibit respiration is usually in excess of that required to inhibit growth.
Chemical seed treatment to disinfect or disinfest seeds or to protect seedlings from the ravages of damping-off organisms has been practiced, at least to a limited extent, for 300 years (Miles, 1946). With the discovery of a number of highly effective materials and the development of economical application methods in the twentieth century, seed treatments came into wide use. Currently nearly all seed sown in the agriculturally advanced countries is treated. This state of affairs was of course not reached by accident but rather as the result of evaluations of thousands of compounds by a great many workers. Early laboratory and greenhouse methods employed in the evaluation of chemicals as seed treatment fungicides have been reviewed by Horsfall (1938), Kadlow and Anderson (1937), and Taylor and Rupert (1946). Bioassays for seed treatment fungicidal activity are of two basic types. Either (1) the seed is infected or infested with a fungus pathogen, i.e., the smuts, and the purpose is to discover chemicals that will disinfect or disinfest the seed so that a healthy plant can be produced or (2) the seed is pathogen-free but is treated to determine whether or not the test chemical will protect the seed and developing seedling from fungus pathogens that cause damping-off in the soil. In both cases the chemical is usually applied to the surface of the seeds by rolling or shaking to ensure thorough coverage. Liquid compounds are frequently adsorbed on tale or another diluent to simplify the treating process.

It should be pointed out that few primary screening programs currently include tests for detecting chemicals useful as seed treatments. The relatively small size of the market and the effectiveness of materials now in use have caused the efforts formerly expended in this area to be diverted to the search for soil fungicides, a use generally viewed as being of greater potential size marketwise and at present exploited only to a limited extent. Compounds found active in other fungicide tests and in particular soil fungicide tests are, however, usually evaluated as seed treatment fungicides.

1. Seed Disinfestation and Disinfection

Early work in this area was principally concerned with the discovery and development of chemicals to control the cereal smuts. Laboratory bioassays using smut chlamydoospores have been used to a limited extent (Benloch, 1946; Detroux, 1948; Gassner, 1943; Krauss, 1928; Tornow, 1930), but Zade (1940) and Zogg (1964) have designed labora-
tory methods involving seeds. Most techniques involve the use of nat­
urally or artificially inoculated seed which is treated with the test
chemical and planted in the greenhouse, with control being assessed by
subsequently determining the percentage of infected heads. The Ameri­
can Phytopathological Society, Committee on Standardization of Fungi­
cidal Tests (1944) has detailed methods found suitable for determin­
ing the effectiveness of chemicals under greenhouse conditions to con­
trol bunt of wheat (*Tilletia tritici* and *Tilletia levis*), loose smut of
oats (*Ustilago avenae*) as well as covered smut (*Ustilago nigra*) of barley. Benloch
(1946) has also described a method using bunt of wheat. Unfortunately
these tests require considerable time and greenhouse space.

Muskett (1938) designed a laboratory method for evaluating fungi­
cides to control *Helminthosporium avenae* on oats. The test chemical
is applied to infested oat seeds and the seeds placed on moistened
filter paper in petri dishes. Subsequent irradiation of the seed induces
sporulation which permits identification of effective treatments. Effec­
tive compounds are then further evaluated in greenhouse tests. Results
obtained in the laboratory and greenhouse correlated well with those
obtained in the field. Similar methods were used (Muskett and Col­
bourn, 1942) for the evaluation of seed disinfectants for the prevention
of seed-borne diseases of flax caused by *Polyspora lini* and *Colletotri­
chum lini*. Arndt (1948) planted treated cottonseed infested with *Col­
letotrichum gossypii* in sand with the number and health of emerged
seedlings serving as an indication of effectiveness.

2. Seed Protectants

Although chemicals had been evaluated as seed protectants by a
number of workers in the 1930's and 1940's (Horsfall, 1938; Kadow
and Anderson, 1937; Taylor and Rupert, 1946), it was not until
McCallan (1948b) published a greenhouse method using peas and
other seeds in *Pythium*-infested soil that a truly precise method was
available. Modifications and adaptations of this method using other
fungi and seeds are widely used today.

B. Soil Fungicides

Fungicides can be used to control soil-borne diseases by eradicating
the pathogen from the soil or reducing the inoculum level, by fungi­
static action, or by systemic action within the host plant. Until recently
candidates for evaluation as soil fungicides were selected primarily
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because they were active fungicidally in spore germination tests or agar plate tests. But Zentmyer (1955) has pointed out that such an approach proved inadequate for detecting effective soil fungicides. The absence of soil was probably the confounding factor. Recently with the advent of increased interest in discovering new soil fungicides a number of laboratory and greenhouse methods designed specifically for this purpose have been developed. They are discussed below.

1. Soil-Fungus Techniques

The primary evaluation of hundreds or several thousands of chemicals per year for soil fungicidal activity necessitates that the method used be simple but accurate. The problem is further complicated by the specificity of chemicals for certain classes of soil-borne pathogens. This requires that a spectrum of test organisms be employed if the results obtained are to be indicative of the test chemicals' true soil fungicidal potentials. As was pointed out above, standard laboratory bioassays using inhibition of spore germination or mycelial growth in a nutrient medium are poor predictors of field performance. Since a successful soil fungicide must, in addition to being fungitoxic, be able to act in a complex soil medium, soil is probably a necessary component of any laboratory bioassay system. Many of the methods discussed below are not suitable for large-scale screening for soil fungicidal activity but nevertheless may be exceedingly useful in obtaining information on other factors which will be important in determining the possible usefulness of a compound in the field.

Zentmyer (1955) has described a technique which he found useful in evaluating compounds for activity against Phytophthora cinnamomi. Disks cut from fungal cultures are placed in autoclaved soil and the chemicals applied to the soil either as a drench or by dry-mixing. After an incubation period the fungal disks are removed by washing and placed on a nutrient medium to determine the viability of the fungus. Kendrick and Middleton (1954) and Domsch (1968c, 1962) extended the use of this method to a number of other plant pathogens. Corden and Young (1962) have also reported using an adaptation of the method in which they wet-mixed rather than dry-mixed the test chemicals with the soil to ensure better contact between chemicals and test fungi. The Zentmyer (1955) method and its modifications have been tried and found satisfactory for evaluating compounds in our laboratories but because of the many steps involved it was found to be a bit cumbersome when a large number of compounds must be evaluated. A primary test method was developed (Torgeson, 1969)
in which artificially infested autoclaved soil in small (4-oz) paper cups is drenched with the test chemical and effectiveness is determined after a 2- to 3-day incubation period by rating the mycelial growth on the soil surface. Four test fungi, *Fusarium oxysporum* f. *cubense*, *Pythium* sp., *Rhizoctonia solani*, and *Sclerotium rolfsii*, are used. Fungistatic and fungitoxic effects cannot be separated and although Domsch (1958a) has emphasized the importance of both types of activity this factor is not of great concern in a primary test. Results obtained appear to correlate well with those obtained in greenhouse evaluation tests.

Chinn and Ledingham (1962) have described a laboratory method for determining the direct effect of chemicals in natural soils. Chemicals are mixed with soil or are used as fumigants. Spores of *Helminthosporium sativum*, *Fusarium culmorum*, and *Alternaria tenuis* are cultured on straw, exposed 4 days to treated soil, recovered, and tested for viability by a modification of the flotation-viability method (Chinn et al., 1960). Nearly perfect correlation was obtained between this method and a test using seedlings as the assay organism. Ezekiel (1938) evaluated compounds for activity against *Phymatotrichum omnivorum* by observing the extent of mycelial growth from sclerotia buried in soil mixed with the test chemical. Carden and Young (1962) described a method for artificially infesting nonsterile field soil with *F. oxysporum* f. *cubense* without adding culture substrates to the soil. A soil dilution technique is then used to evaluate the effectiveness of the test chemicals. Thayer and Wehlberg (1963) successfully used bean stem sections as fungus traps for determining the toxicity of chemicals to *Pythium* and *Rhizoctonia*.

A number of assays have been described for determining the penetration of soil by fungicides. In general, autoclaved soil is packed into columns and the test chemical drenched on the soil surface. Efficacy may be determined by one of the following methods: counting the number of fungal colonies developing on nutrient media from samples collected from the column (Cetas and Whidden, 1960; Carden and Young, 1962), determining the viability of spores and mycelia on infested paper disks (Thomas, 1957), assaying the viability of *Phytophthora parasitica* at various levels in the column using citrus seedlings (Klotz et al., 1959), determining the viability of buried sclerotia (Ezekiel, 1938; Latham, 1962), determining the viability of a test fungus on agar disks (Cetas and Whidden 1960; Carden and Young, 1962; Newhall, 1968; Zentmyer, 1956), assaying the leachings for presence of the fungicide (Pote and Thomas, 1954) and measuring the zones of inhibition.
around samples taken at various depths and placed on a nutrient agar plate inoculated with fungus spores (Munnecke, 1954, 1958). Methods for evaluating the fungitoxicity of volatile compounds have been reported (Cordon and Young, 1962; Latham and Lien, 1965; P. M. Miller and Stothard, 1957; Oserkowski, 1954; Pryor and Walker, 1939; Richardson and Munnecke, 1964) in which glass jars, petri dishes, or other small containers are used with a variety of test organisms and growth media. The ability of chemicals to penetrate and kill fungi within plant tissues has been studied by Klotz et al. (1959), Ezekiel (1938), and Cordon and Young (1962). Rushdi and Jeffers (1956) have utilized a technique for measuring linear growth of fungi in soil and have demonstrated that adsorption of fungicides by soil is an important factor controlling activity. Evans (1954) designed a soil tube for studying the recolonization of sterilized soil by microorganisms which has been useful in following the survival and recolonization of fungi in soil treated with fungicides (Evans, 1955). Oxygen consumption by treated soils has been used by Domsch (1964) to study the effect of soil fungicides on the respiration of soil microorganisms.

2. Soil-Fungus-Host Technique

Conventional pot or similar greenhouse tests in which plants are used as indicators of the test chemical's fungicidal effectiveness are reliable but exceedingly cumbersome and expensive to conduct. The writer is of the opinion that economical primary evaluation of a large number of compounds is better accomplished by the use of a simple, broad-spectrum and accurate laboratory method and that greenhouse tests are more suited for use as an intermediate stage of evaluation of compounds found active in primary laboratory tests. However, for those who do not share the writer's views there is no doubt that greenhouse tests such as are described below can be used or adapted for use very satisfactorily—albeit expensively—in the primary screening of fungicides.

Since most soil-fungicide evaluation work has centered on those organisms causing diseases of seeds and seedlings it is not surprising that there are more published methods for determining efficacy of materials for the control of *R. solani* than for any other pathogen. Arndt (1953) described a sand-nutrient culture technique using aluminum dishes in which the test chemical is applied only to the sand immediately around cottonseed. The pathogen is inoculated into the center of the dish and the number of healthy seedlings remaining after 12 days is used as an indicator of chemical effectiveness. Plots of
infested soil treated by dusting and mixing or by drenching followed by a sowing of cottonseed have been successfully used by Sinclair (1957, 1958) and Brinkerhoff et al. (1954). Eissa and Sinclair (1962) used flats but attempted to simulate field conditions by placing infested soil on the edge of treated soil and measuring the progress of disease development. We have found this to be a very useful method in our laboratories. Ranney and Bird (1956), using a series of constant temperature tanks (Ranney, 1956), demonstrated that soil temperature may be an important factor in determining effectiveness and pointed out that this factor should not be overlooked in determining the possible field effectiveness of a potential soil fungicide. Activity of compounds against *R. solani* has been determined in our laboratories in both clay and plastic pots and in flats using cotton, peas and garden beans as indicator plants. Domsch (1958b) has reported a method using peas.

It has been pointed out in a number of the papers cited above that because a chemical is effective against *R. solani* in greenhouse tests it does not necessarily follow that good damping-off control will be obtained in the field. Other organisms in the damping-off complex, such as *Fusarium* and *Pythium*, against which the chemical is not active, may negate its field performance. Therefore, it is of the utmost importance to learn as much as possible about a compound's spectrum of activity in greenhouse tests prior to field testing. Once known, the problems associated with a narrow-spectrum fungicide may be circumvented by using it in combination with another fungicide possessing a different activity spectrum.

Fungicidal effectiveness for *Pythium* damping-off control can be readily determined in the greenhouse by the use of peas or other hosts (Domsch, 1958b; Torgeson et al., 1963). Greenhouse tests using such representative soil-borne plant diseases as tobacco black shank caused by *Phytophthora parasitica* var. *nicoiana*, tomato wilt caused by *Fusarium oxysporum* f. *solani*, and southern blight of peas or peanuts caused by *Sclerotium rolfsii* have also been found useful in our laboratories (Torgeson, 1959). In similar tests Davison and Vaughn (1957) used a *Fusarium* sp. causing a bean root rot, Domsch (1962) used *Fusarium orthoceros* and *F. solani* var. *martii* and peas, and Reinhart (1960) used *Fusarium oxysporum* f. *cucumerinum* causing a wilt of cucumber. Domsch (1962) also used *Thielavopsis basicola* with lupines and tobacco as test plants. An elaborate procedure has been described by Colhoun (1954) for the determination of fungicidal efficacy in the control of cabbage clubroot caused by *Plasmodiophora brassicae*. Since
the fundamental aspects of the environmental factors which influence the development of those soil-borne diseases causing major crop losses have been determined and reported, it is usually relatively simple to design a soil-fungus-host test system for greenhouse evaluation of fungicides for those diseases for which published methods are not available. The effect of factors such as inoculum potential, soil temperature, soil moisture, soil type, residual effectiveness, method of application, formulation, etc., which may have an important bearing on how well a potential soil fungicide will perform under field conditions can usually likewise be determined by suitable modifications of standard greenhouse evaluation methods.

C. Foliage Fungicides

Procedures for evaluating chemicals as protectant or eradicant fungicides on foliage of potted plants were initially designed to serve as an intermediate step between in vitro laboratory methods and field tests. With the recent trend toward the elimination of in vitro methods, however, practical-type foliage tests have come into use in primary evaluation programs. Unfortunately the specificity of fungicidal activity makes it necessary to use more than one test disease in a well-balanced fungicidal bioassay scheme. Tomato early and late blights and bean rust and powdery mildew are used in our primary evaluation program at Boyce Thompson Institute. The spectrum of activity of active compounds is then further explored using other foliage diseases.

McCallan and Wellman (1943a), in their now classic paper on tomato foliage disease methods, outlined the requirements of a desirable method of testing fungicides on the foliage of potted plants as follows:

Host plant. The host plant should be grown with ease and rapidity, should develop uniformly, be of a fairly open habit of growth suitable for spraying and robust enough to withstand spray pressure; the surface of the leaves should allow adequate wetting by typical fungicides; the plant should not be unduly sensitive to spray injury; preferably the plant should also represent a crop of importance, slow-growing plants occupy valuable greenhouse space and are frequently subject to attack by other plant diseases and insect pests.

Fungus. The fungus should be readily cultured and produce an abundance of pathogenic spores in a relatively short time. It should be representative of one of the major groups of plant disease fungi.

Disease. The disease should be readily obtained following simple procedures of inoculation and incubation; it should be reproducible; the lesions should develop rapidly, be seen with ease, and be suitable for quantitative measurement. The disease should not spread under normal greenhouse conditions. Finally, it is preferable that the disease be of economic importance.
Unfortunately these requirements cannot always be met except in a few cases. The fruit tree foliage fungicide market has, for example, always been a prime target of any fungicide program, but the methods developed for determining activity against the pathogens involved lack the rapidity and preciseness desired. They have thus, because of the cost factor, been relegated to use in the more advanced stages of foliage fungicide evaluation programs.

The foliage test methods discussed below have been broken down into those which employ diseases caused by nonobligate ascomycetes and imperfect fungi, phycomycetes, rusts, and powdery mildews. Although most of the methods were developed for use in searching for protectant fungicides they can be readily adapted for the evaluation of eradicant fungicides by merely inoculating the test plants at a suitable interval prior to application of the test chemical. Most laboratories utilize equipment similar to that described by McCallan and Wellman (1943a), McCallan (1948a), and Ehlers (1953). Equipment for applying dusts has also been described by McCallan (1950).

1. Diseases Caused by Nonobligate Ascomycetes and Fungi Imperfecti

Certainly the most extensively used test method in this category is the tomato early blight (Alternaria solani) foliage method developed by McCallan and Wellman (1943a). A similar method was later described by Stubbs (1953). McCallan and Wellman (1943a) also used Septoria lycopersici on tomato but found it a less satisfactory organism than either the tomato late or early blight organisms. Drummond (1949) has also described a method using S. lycopersici on tomato. Chocolate spot of broad bean caused by Botrytis fabae has been extensively used as a test disease in England (Crowdy and Wain, 1951; Morgan, 1953; Carter et al., 1964). Botrytis cinerea on tomato has been used by Brook (1957). Strawberry fruits rather than foliage were found useful by Moore and Tew (1955) in evaluating compounds for fungitoxicity against B. cinerea. The method of Atkins and Horn (1952) for evaluating compounds for the control of cucumber anthracnose caused by Colletotrichum lagenarium has been adopted for use in a number of laboratories. Colletotrichum truncatum on lima beans and Colletotrichum lindemuthianum on pinto beans were used by Pridham et al. (1956). Methods for evaluating compounds for effectiveness in controlling rice blast caused by Piricularia oryzae (Kulik and Arai, 1961; Asakawa et al., 1962) and Helminthosporium oryzae on rice (Nishikado et al., 1951b) have been reported.
4. Determination and Measurement of Fungitoxicity

Hamilton and co-workers pioneered in the development of methods employing potted apple trees in evaluating compounds for apple scab (Venturia inaequalis) control (Hamilton, 1931; Hamilton and Weaver, 1940; Hamilton and Mack, 1942, 1951; Hamilton and Szkolnik, 1957) and more recently (Hamilton et al., 1964) have described the use of cherry leaf spot (Gecomyces haimalis), cherry blossom blight (Botrytis cinerea), and peach brown rot (Monilinia fructicola) in greenhouse testing. Apple scab tests which are somewhat simpler to conduct have been devised by A. E. Rich and Richards (1959) and Moore (1960).

2. Diseases Caused by Phycomycetes

The method of McCallan and Wellman (1943a) using the late blight organism (Phytophthora infestans) on tomato is generally accepted as the standard test for evaluating chemicals for the control of this group of fungi. A modified but less precise method has been described by Chancogne and Viel (1957). Techniques using P. phaseoli on lima beans (Pridham et al., 1956) and P. palmivora on caeno pods (Hislop and Park, 1962) have also been developed. A novel method for evaluating the effectiveness of volatile compounds in controlling Peronospora tabacina on tobacco is described by Pinckard et al. (1940). Methods for grape downy mildew (Plasmopora viticola) using potted plants or detached leaves have been published (Baldacci and Ciferri, 1944; Lalon, 1946; Morel, 1947; Casarini, 1957). Like most methods requiring woody plants they are more suitable for the intermediate stages of foliage fungicide development than for the primary level.

3. Rust Diseases

Since the rusts are responsible for a number of important plant diseases it is desirable to include a representative in a fungicide evaluation program. A test utilizing snapdragon plants with Puccinia antirrhini as the test organism was developed by McCallan (1944) for this purpose. Although snapdragons meet most of the requirements for an ideal greenhouse method, the length of time required to grow the plants is a disadvantage and the method has been generally superseded by a test using faster growing bean plants and Uromyces phaseoli. McCallan and Zingerman (1962) studied the bean rust method and found it to be less precise than that of tomato late blight, but more precise than that of tomato early blight or snapdragon rust. Carter et al. (1964) and Evans and Saggars (1962) have also reported using bean rust. Broad bean rust (U. fabae) has been used similarly by Jacks (1954).

Although they are slightly more difficult to handle, the cereal rusts
are frequently used in primary or advanced stages of fungicide evaluation programs because of their great economic importance. Leaf rust (Puccinia rubigo-vera tritici (Puccinia recondita)) and stem rust (Puccinia graminis tritici) of wheat were satisfactorily used by Livingston (1953) and Forayth and Peturson (1959) in the evaluation of rust fungicides. The latter workers also used crown rust of oats (Puccinia coronata avenae). Wheat stem rust was also used by Hoteson (1953) and Pridham et al. (1956), and leaf rust by Jaworski and Hoffman (1963). Keil et al. (1958) found rye leaf rust (Puccinia rubigo-vera secalis) easier to handle than wheat rust and used it in their evaluation studies.

Hamilton and co-workers, in addition to their early work on the use of potted apple trees in apple scab control studies, also developed techniques for cedar apple rust (Gymnosporangium juniperi-virginianae) (Hamilton and Weaver, 1940; Hamilton and Mack, 1942, 1951; Hamilton and Sokolink, 1957; Hamilton, 1959). They demonstrated the usefulness of these methods in evaluating the fungitoxicity of compounds and in studying the influence of environmental factors on their effectiveness.

4. Powdery Mildew Diseases

Bean powdery mildew (Erysiphe polygoni) is the disease used for the primary evaluation of compounds active against the powdery mildews in many laboratories. Unfortunately, details of the methods have not been published, but in general a procedure similar to that used by Pridham et al. (1956) is followed. Detached bean leaves were used by Chancogne (1962). Methods employing cucumber powdery mildew (Erysiphe cichoracearum) have been described by Dekker (1961), Smith and Read (1961), and Chancogne (1962). Erysiphe graminis on wheat (Wain et al., 1963; Carter et al., 1964), blue grass (Pridham et al., 1956), and barley (Kirby and Frick, 1963a) is also a useful test organism.

The increased importance of apple powdery mildew (Podosphaera leucotricha) has prompted a number of workers to develop suitable greenhouse-evaluation test methods. Hamilton (1959) and Hamilton et al. (1964) have used apple powdery mildew but have not given an adequate description of their method. Moore (1960) used apple rootstocks as a source of uniform test plants. Apple seedlings were used by Torgeson and Lindberg (1961) in an attempt to reduce the time and space required when using larger plants. Kirby and Frick (1963a,b) and Zaracovitis (1963), as the result of a detailed study on the factors
affecting infection, devised a test using potted apple rootstocks. This appears to be the most precise method available at present.

5. Weatherability of Fungicide Deposits

It has long been recognized that an important characteristic of an effective fungicide in the field is persistence of the fungicide deposit on the leaf surfaces. Hamilton (1931) early established the feasibility of conducting studies on the retention of deposits when exposed to artificial rainfall on potted plants in the laboratory. These methods were further refined (Hamilton and Weaver, 1940; Hamilton and Mace, 1942, 1951) and found useful in predicting the field performance of fungicidal compounds by studying the importance of redistribution and the influence of particle size and formulation on deposit retention. Subsequently, many types of equipment for producing simulated rain were devised (Wellman and McCallan, 1944; Scholdt, 1954; Rabie, 1955; Somers and Thomas, 1956). The most elaborate and probably the most precise is that of Burchfield and Goenaga (1957a). The usefulness of this equipment has been demonstrated by Burchfield and Goenaga (1957b) and McCallan (1957).

Many chemicals found to be effective in the laboratory have failed under field conditions because the compound was degraded by ultraviolet light. Most fungicide evaluation laboratories, therefore, determine the stability of fungicidal compounds prior to their being field-tested by measuring the effectiveness of deposits of the fungicide following exposure to ultraviolet light. Unfortunately, no standardized methods have been published for studying the effect of ultraviolet irradiation on fungicide deposits on foliage.

Other factors are doubtless of importance in determining the efficacy of a foliage fungicide in the field but until they are better understood their effects on fungicidal effectiveness cannot be determined in the laboratory.

D. Systemic Fungicides

Even though the possibilities of achieving plant disease control through the use of systemic fungicides have been considered for quite a number of years (Norton, 1916), major interest in evaluating compounds for systemic activity has not developed until recently. *In vitro* tests have not played a role in this development since they are of no value in indicating the ability of a chemical to enter and move in a plant or of its activity within the living plant. Evidence of transforma-
tion within the plant of inactive chemicals into effective fungicides and changes in resistance makes the use of plants in any systemic fungicide evaluation system necessary.

With the outstanding exception of the systemic fungicide assay method using *Fusarium* wilt of tomato developed by Dimond et al. (1952), nearly all systemic fungicide test methods have been designed to detect activity against foliar diseases. Carter and Wain (1964) used a broad-leaf disk method in determining the systemic activity of chemicals against *Botrytis fabae* but most tests involve intact plants. Since control of a foliar disease obtained by applying a chemical post-infection may be due to systemic action, a number of the eradicant tests described in Section III,C may properly be considered systemic fungicide tests. Stubbs (1952) has, however, pointed out that root absorption appears to be the most effective means of evaluating compounds for systemic fungicidal activity.

Stubbs (1952) has described a systemic fungicide bioassay technique using *A. solani* on tomato plants growing in sand containing nutrient solution. Chemicals were applied principally in the solution to the roots, but movement from leaf to leaf was also studied by applying the test chemical to portions of the foliage and assessing control on the unsprayed leaves. *Alternaria solani* was also used by McNev and Sundholm (1949). A similar technique utilizing *R. fabae* on broad beans was used by Pawlett et al. (1955). Cucumbers have been used as host plants with *Erysiphe polygoni* (Dekker, 1961) and *Cladosporium cucumerinum* and *Colletotrichum lagenarium* (van Andel, 1962).

Because of their refractiveness to economic control by traditional foliar fungicides the rusts have been used by a number of workers in systemic fungicide evaluation studies. Bean rust has been used by Baldacci and Betto (1958), Betto and Bonola (1960), Evans and Sagers (1962), and Allen and Freiberg (1964), and wheat rust by Hotson (1953) and Allen and Freiberg (1964). Most of the work involving cereal rusts has utilized eradicant sprays which were discussed in Section III,C.

### IV. ASSESSMENT OF RESULTS

Toxicity experiments may be classified as (1) those in which the response of the organism to the toxicant is measured quantitatively, for example, the measurement of fungal colonies on agar plates or spore germ tube length; and (2) those in which the organisms being tested are divided into two groups based upon whether or not the or-
organisms have responded to the toxicant, for example, germinated or nongerminated spores in spore germination tests. The latter is called a quantal or all-or-none response. Since individual organisms differ in their reaction to the chemical, more organisms fall in the nongerminated or dead group as the concentration of the toxic chemical increases. Gaddum (1933) and Bliss (1935a) found that the logarithm of the individual lethal dose is normally distributed. Bliss (1935a,b, 1937) and Bliss and Marks (1939) showed that by converting the percentage response units to units of the standard deviation of the normal distribution (which were called probits) and plotting against the logarithm of doses, a straight line dosage-response curve was usually obtained. Procedures were developed for determining the relative potency of chemicals at any level of response as well as the parallelism of the curves. Wilcoxon and McCallan (1939) introduced these methods into the fungicide field and they have since become widely used. In spore germination tests, potency comparisons are usually made at the 50% response level which is the most precise point of the curve, but in foliage disease tests the 95% point is precise and is usually used (McCallan, 1943, 1944). Since the commonly used symbol LD for lethal dose is relatively restricted, the more general symbol ED for effective dose was introduced by McCallan (1948b) as being more appropriate for use in fungicide studies in which the percentage response measured is nongermination of spores, disease control, etc. Determination of ED50 and ED95 values has become commonplace and is extensively used in describing the potency of a fungicide.

The log-probit transformation does not always give a straight response line (Dimond et al., 1941; McCallan et al., 1941). The transformations necessary to straighten some of these curves have been discussed by Parker-Rhodes (1942) and McCallan (1947b).

Frequently, it is not necessary to undertake the calculations required to determine the precise ED value. If so, the results may be plotted on logarithmic probability graph paper and the ED value estimated (Wilcoxon and McCallan, 1939; McCallan et al., 1941; Dimond et al., 1941). The use of various graphic and other rapid statistical methods in determining the significance of differences between treatments has been reviewed by McIntosh (1961).

McCallan and Wellman (1943b) have pointed out that due to variation a group of chemicals may differ in their effectiveness from test to test, in which case it is preferable to use the “chemical X test” interaction rather than the “within test” error in determining significant differences between responses to different chemicals. Under these cir-
cumstances efficiency can be improved by running more tests with fewer replicates per test than by running fewer tests with more replicates. Because two chemicals may have dosage-response curves with different slopes one may be more toxic at a lower response level and the other at a higher response level. Dimond et al. (1941) have considered this crossing over of curves as an explanation of reversals of effectiveness in laboratory and field results. It is obvious then that the response of a fungus to a single dose of a fungicide cannot be used in comparing the fungitoxicity of a group of chemicals if a precise answer is desired. In a program in which many compounds are being evaluated for fungitoxicity it is fortunately not necessary to be this precise. A primary dosage level, several-fold higher than that at which known fungicides will give a 100% response, is usually selected. Those compounds not active at this level can be safely eliminated and the others can then be evaluated in a dosage series. A standard fungicide should be included in such tests to assure that the test organism is responding normally. Comparisons of EFs, EDs, or other ED values with the standard and test chemicals may also be made in giving an approximation of relative performance. The importance of slope of the dosage-response curve should of course not be neglected. Spore germination tests tend to yield steep curves, whereas, even with the same organism, foliage disease tests give much flatter curves (McCallan and Wellman, 1943b) as do seed germination tests (McCallan, 1947a) and field tests. Dimond et al. (1941) and Horsfall (1956) have been proponents of the idea that if the slope of the dosage-response curve differs for different fungicides their mode of action is dissimilar. This idea has been questioned by McCallan et al. (1959) who found that the relationships between uptake and external concentration of various fungicides are represented by three different types of curves and concluded that, since the mathematical relationship between uptake and applied concentration is not predictable, slope based on applied concentration does not indicate mode of action.

V. Correlation Among Basic, Practical, and Field Tests Results

Aside from their usefulness in studies on the nature of fungicidal action, laboratory test methods, basic and practical, are primarily designed to predict field performance. The usefulness of laboratory methods is dependent upon how well the results obtained correlate with those obtained in the field. The rather limited published reports on this subject have been well reviewed by McCallan (1969).
4. DETERMINATION AND MEASUREMENT OF FUNGITOXICITY

H. J. Miller (1943), Keil et al. (1952), and S. Rich et al. (1953) have reported good correlation between in vitro laboratory methods and field results if the importance of resistance to wash-off in rain and other factors are considered. Since the work involved in determining the importance of these factors even in in vitro studies is considerable, there has been a trend toward the use of the less precise practical type of test in screening compounds for use in the field. Hamilton (1959) has pointed out that the results of such tests correlate well with performance in the field. One might predict that as more and more specific fungicides are sought, the trend toward the use of practical-type tests will continue. Nevertheless the final evaluations must be made in the field under a variety of environments before the true potential of a fungicide can be determined.

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4. DETERMINATION AND MEASUREMENT OF FUNGITOXICITY


4. DETERMINATION AND MEASUREMENT OF FUNGICIDE POTENCY


4. DETERMINATION AND MEASUREMENT OF FUNGITOXICITY


CHAPTER 5

Commercial Fungicide Development

R. H. WELLMAN

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Highly organized industrial research laboratories are a prolific source of new chemicals that show fungicidal activities. Company biologists, accustomed to screening techniques, rapidly sort out those new chemicals that appear promising for agricultural uses. Few of these chemicals ever reach the commercial market as fungicides. The ones that do make it represent millions of dollars in development expense and years of widespread field testing.

A. Importance of Teamwork

Today, the emphasis must be on teamwork in order to discover and launch a successful commercial fungicide, or any other pesticide. Research teams, development teams, and marketing teams all must pull together—with each team working out particular phases of mutual problems. There must be a leader, of course, who is responsible for coordinating such phases of complicated team effort. He must see that the work proceeds in unison.

_Emphasizing team effort is not to de-emphasize the contribution of the individual._ Any effective team must be composed of superior individuals. Frequently one man has faith in a compound and by convincing his fellow team members that his faith is justified, he protects the survival of a promising new fungicide.

Teamwork was not always so highly organized in the search for agricultural chemicals. During the past two decades, however, attitudes toward the research, development, and marketing of agricultural chemicals have undergone drastic change. The industry has had to learn to think differently in order to meet the challenge of a business that continually has been growing more complex.

B. How It Used to Be

In the early 1930's, as we have reported previously (Wellman, 1959), most of the search for new agricultural chemicals was done by isolated workers in U.S. Department of Agriculture Experiment Stations. Syn-
thesis facilities were limited to some extent, or nonexistent. Experimentation was confined to chemicals that were available from chemical supply houses. This often created a dilemma. Even if an effective chemical was found, it frequently was difficult to interest a chemical manufacturer in producing the compound in large quantity.

Few studies were made beyond the initial searching for chemicals with fungicidal properties. Manufacturers knew little about available markets or what the farmer could afford to pay for a fungicide. With such a system, there was no patent protection to aid the manufacturer in recovering his expenses of developing large-scale production processes and, hence, very little incentive for the commercial production of promising fungicides.

The early-day approach of searching for commercial fungicides in many cases was about as effective as a shot in the dark. The evolution of an organized teamwork effort over the past 25 years, in finding, developing, and marketing commercial fungicides, has been much more successful than was the somewhat disorganized approach of the 1930's.

C. How It Is Today

Chemical manufacturers now form integrated teams for the purpose of developing fungicides and other agricultural chemicals. Chemical company personnel maintain close contact with their counterparts in government—scientists and agricultural experts at state and federal experiment stations. They also have close liaison with grower organizations, food processors, and, in some instances, with large growers themselves. So it is clear that teamwork exists beyond the confines of the company organization. It reaches throughout our agricultural society. Persons with diverse agricultural interests have found a way to pull together to bring better commercial fungicides and other agricultural tools to the farmer.

Members of a chemical company's portion of a fungicide development team include organic, formulation, and analytical chemists; plant pathologists; toxicologists; patent attorneys; market survey specialists; and the large group of people of varied training who are concerned with production and marketing. All these people have individual responsibilities but their activities must be considered as a group function. Some of the duties that exist throughout this large group include: Synthesizing new chemicals; finding which member of a given series of compounds is the most effective; comparing the activities of the new chemical with existing standard materials; investigating the economics of production;
making market surveys to determine the potential volume of sales; obtaining patent protection; working out analytical procedures and making analyses of the residue left on the crop; tracing the metabolic breakdown of the residues by both plants and animals; conducting toxicological studies; finding how to make a usable formulation; investigating the effect on the crop that is being protected and on the ecosystem in which the crop is grown; plant design and construction; market volume studies; providing first experimental and then commercial quantities of the product; and creating a demand for the product by telling farmers and their advisors what it will do for them.

D. Why the Search Is More Complicated

Yet, with this organized modern approach, the search for successful fungicides is becoming infinitely more complicated. Why? One of the most frustrating problems in developing modern commercial fungicides is that it is difficult to find a compound that performs better than those materials already on the market at a price that makes it economical for the farmer to use. We have some very outstanding pesticides available today, and it is getting harder to improve on these good products. In other words, it is more difficult to develop these second and third generation pesticides than it was to discover their forefathers. Companies now must screen many more compounds than they did 25 years ago to discover one that will outperform those that are presently on dealers' shelves. Federal and state experiment station personnel also have grown more experienced, and at the same time more sophisticated, because they have worked with so many candidate fungicides. To introduce a new compound, you must have a really outstanding chemical to interest agricultural experiment station people in conducting the necessary testing prior to commercial marketing.

E. Why It Is More Costly

It stands to reason, then, that the price tag for research and development on a fungicide certainly has gone up over the past two decades. Everything costs more today, and with the increased effort necessary to find fungicides that are better than the ones currently on the market, it is logical to assume that a chemical company attempting to launch a fungicide is going to incur some extremely high costs.
Getting into the fungicide business is a long and tedious process. In fact, developing any new farm chemical is no job for the timid or the impatient. It tests a manufacturer's mettle and challenges his gambling spirit. A firm must begin with a high degree of chemical and biological know-how and then invest at least two million dollars plus anywhere from 5 to 15 years of careful research and development.

Recommendations by the President's Science Advisory Committee have made testing and labeling procedures more difficult, time-consuming, and expensive. Many more tests are required today than in former years. Newly established safety requirements set up by government agencies have resulted in it taking least a year longer of research and development, on the average, to get a commercial label for a successful fungicide.

The developing trend in criticism of pesticides and the agricultural chemicals industry has accelerated the demand for increased testing of pesticides before marketing. Many writers and speakers—some of them crackpots, but some of them sincere—have found this industry a fertile pasture for publicity, and as a result much of the public is critical of the chemical manufacturers. Testing of agricultural fungicides prior to marketing will continue to be even more strict and comprehensive in the future. Industry always has believed in necessary testing, but it can be expected that future testing will be more complex, lengthy, and costly. Yet, in spite of these high costs, the industry must find a way to survive and continue. New fungicides are vital to agriculture, and because of this need the industry must provide them in the future.

With all of the time, effort, and costs in mind, it is obvious that a short delay to organize a sound program at the beginning of the development of a candidate fungicide will pay off in more rapid progress with less irritation for all concerned, and with surer prospects for rewards in the end.

**F. Do You Really Have a Product?**

This is the time for the development team to sit down collectively and ask themselves, "Do we really have a product?" This is the time for the scientists on the team to make sure they have made all practical information available to the market analysts and business members of the team—and the time for the business analysts to ask the scientists everything they need to know. Also, this is the time to determine if the market is worthwhile—before large sums of money are committed.
II. SOME FACTORS TO STUDY

It may seem obvious that a market study is necessary before the market development of any chemical is undertaken. Nevertheless, it is surprising how often the natural enthusiasm of a sales group or a persuasive research man may cause a large number of people to waste their time and effort on a project that prior careful consideration would have doomed from the start. For example, suppose our screening process and initial development work have turned up a fungicide that is specific to control stem rust on wheat. Think of the number of acres of wheat in this country and overseas! Perhaps there would be several applications over a season. The chemical looks promising from a production and profit standpoint. The sales department has been begging for a fungicide to sell on small grains. The candidate fungicide just happens to fit into the regional sales pattern very nicely. Yes, enthusiasm is riding high.

However, in spite of the large acreage to be treated and apparent large profit, company management must be told that stem rust of wheat may only hit about once every 5 years! Is there really a market?

Suppose a compound has been found that is specific for the control of mint rust and for that disease alone. The hypothetical chemical is effective at the rate of one pound per acre used once. Such a product would be valuable indeed to the growers of mint. There are approximately 37,000 acres of spearmint and 19,000 acres of peppermint grown in the United States. Conceivably every acre in the country could be treated and perhaps the chemical might sell for a gross profit of 50 cents a pound, although this is a very optimistic margin. When the development and selling expenses are considered, and when a comparison is made between spending the time and energy of technically trained people on this development, rather than elsewhere, it is immediately obvious that it could never be economically feasible to develop such a compound for the maximum gross profit of $25,000 a year. Or suppose a new chemical would lose money if it sold for less than 50 cents a pound and it had to compete with chemicals selling for 35 cents a pound that were equally efficient and already established for the use.

These cases are extremes, but they clearly highlight the financial facts of life as applied to developing a market for agricultural chemicals. It must also be realized that the total market potential, as measured by the total acres planted to the crop, multiplied by the number of pounds of chemical necessary per acre per year, is by no means a valid measure of the amount of a given fungicide that can be sold. In some areas, the disease problem may not exist; in others it may be so minor as to cause
no commercial damage. Certain farmers may not treat their acreage even where the disease is serious. Where competing fungicides are already in use, they will continue to hold a share of the market. Inevitably, the actual anticipated sales will be only a fraction of the total market potential.

A. The Market

Another aspect of the market study that must be examined is the rapidity with which sales can be expected to grow. Usually it takes longer to develop a market where a new practice is being introduced than where only a new product is being introduced. If the period between initial and substantial sales is too long and the effort too great, marketing a new fungicide may not be worthwhile. Not only must the actual extent of market available be determined, but also the necessary selling price, the cost of manufacture, raw material availabilities, and capital investment required to build facilities for the production must be considered. At least a qualitative and careful estimate of these factors should be made before reaching the decision to undertake the serious development of any agricultural chemical.

It is true that such a procedure may, in isolated cases, overlook hidden potentialities, but the efforts will be made considerably more efficient and the general success of the program will be much more likely if this procedure is followed. Many of the failures of individual products and even some of the failures of entire programs in the agricultural chemicals field could have been avoided had an adequate market survey been conducted early. Here is the place to be coldly objective first. Of course, objectivity should not be slanted toward the negative. Remember that there are other markets in addition to the domestic agricultural market. Evaluate your product for all types of fungicidal activity in all possible markets.

B. International Sales

If it appears that one has an effective chemical fungicide for United States crops, it should not be forgotten that the world crop acreage is four times that of this country. The fungicide market may be two or three times greater overseas than it is in the United States. In the process of developing fungicides for our own farmers, a company should continually be looking at the international market, too, if it is interested in marketing products overseas.
No different techniques of development are required, in most cases. Many fungus diseases are the same the world over. Late blight of potatoes is a European scourge as well as a domestic one. Apple scab is found around the world in fruit-growing areas.

It becomes necessary to take a closer look at a candidate fungicide's potential, when one considers that there are major crops in the world that are not grown in the United States. For example, banana leaf spot offers a considerable market for promising fungicides. Agricultural scientists need to examine materials in the early stages of laboratory screening and determine their possibilities for overseas applications. One must be aware of all market potentialities for candidate fungicides.

C. Industrial Fungicides

The various industrial fungicide applications should be kept in mind during the screening for agricultural activity. One may not have an agricultural fungicide, but one might discover an effective new product for industry. The requirements of industrial materials are different, in some respects, than agricultural chemicals. They do not have to be non-injurious to host plants, so scientists can be less selective in the screening process. However, the fungicidal action must persist longer. Some companies are specific in the development of industrial fungicides, but they do not fill all of industry's requirements. Most agricultural fungicides sooner or later get tested for industrial purposes. Wood preservation is the principal use for industrial fungicides, according to Johnson et al. (1963). Other large applications are in paints, and some fungicides are used in plastics or for the protection of outdoor fabrics.

After all preliminary reports are in on a candidate fungicide, and an objective evaluation has been made, the development team must finally decide if they really have a product. If the answer is "yes," then it is time to get down to some further careful planning.

D. Process Development

Process development is necessary to find out how the product can be made most economically, first in the pilot plant and finally in the large-scale plant. This study should begin as soon as a new fungicide shows promise. First, samples of the product must be produced for early field testing. Then the product is moved into the pilot plant, which satisfies two major requirements in the early stages of the development of a fungicide. It supplies quantities of the product for sales development
purposes as field testing is stepped up, and operating data for design of a large-scale plant.

E. Plant Engineering

Engineers must be brought into the picture early in the development of a new pesticide, when it appears to the biologists that the product has promise of reaching the commercial market. Plans must be made for the construction of an economical and efficient plant, and top engineering talent is necessary for the design, construction, and initial operation of these manufacturing facilities.

It is necessary for the engineers to work up rough estimates of costs and return on investment at estimated sales prices and volumes. These calculations are invaluable in determining whether the chemical can be made at reasonable cost and priced at a level that is economically feasible for the farmer. It would be folly for a chemical manufacturer to continue pouring money into a prospective fungicide when the economic outlook is hopeless.

However, even moderately good fungicides are hard to find. So before a product is discarded because of economics, attention must be given to such questions as whether the manufacturing process can be improved, the dosage lessened, or if additives can increase the product's value. Creativity in answering such questions on the part of the researchers really is the lifeblood of the entire team. Without it, the team simply could not function.

As development of the new agricultural chemical continues, proper engineering gives more accurate cost estimates through more detailed design work in successive steps. When it is time for management to decide on the appropriation for the multimillion dollar plant required to manufacture the new fungicide, the economics will have been defined within narrow limits and this necessitates very detailed engineering studies.

F. Introducing the Fungicide

Market development must progress concurrently with the later stages of research and the construction of suitable production facilities. Coordination of these efforts is important. Indeed, the essence of success is to have all necessary efforts proceed simultaneously so that all steps are complete when you are ready to introduce your new fungicide to the commercial market on a full-scale basis.
A chemical manufacturer should market a new fungicide as soon as possible. When effectiveness and safety are established and the formal requirements of the Miller Act and the Federal Insecticide Act have been satisfied, one should not delay in bringing the product to the commercial market place. By this time, of course, experiment station personnel have had the opportunity to evaluate the material under a variety of conditions. Yet it is unfair for experiment station scientists to continue to spend their time evaluating a new material unless there is reasonable assurance that it will be made available to growers if successful as a product.

If one has an effective fungicide, farmers will need it as soon as possible to improve their growing operations. And the sooner the marketing of a successful product begins, the sooner management will realize a return on its large investment.

No large-scale promotion or advertising should be undertaken until sufficient evidence is available to show that the product can be produced at a low enough cost to meet the requirements of intended use. Common sense dictates that the introduction of a new chemical cannot precede the ability to supply such chemical in reasonable quantities. Sufficient facts must be amassed to give intelligent suggestions for use and to avoid toxicological hazards to people making trial applications.

Proper timing of the introduction of a new fungicide for commercial sales must take into consideration all of the steps enumerated. The objective is to reach the farmer at the earliest possible moment consistent with these criteria. Each year that can be cut off the time necessary for development of a fungicide is really important. The interest on a $1,000,000 investment for one year is $60,000. Getting a fungicide to market a year earlier not only saves this sum of money for company stockholders, but starts a return on the total investment sooner. Companies that can conduct agricultural chemicals development programs swiftly and efficiently have a great advantage over those that cannot.

G. Physical and Chemical Properties

Let's discuss some of the individual steps that must proceed concurrently to form the fabric of the total fungicide development program. As soon as it has been determined that a development program is desirable, the physical and chemical properties of the new fungicide must be studied to the maximum extent consistent with economy. In particular, every property that affects the safety of workers or handlers, such as toxicity, vapor pressure, flash point, and explosive limits, should be determined.
A thorough literature survey must be made, and this should be combined with information developed in the laboratory. Light stability, water solubility, and rate of hydrolysis under acidic and basic conditions are particularly important. The more significant chemical properties are selected for inclusion in technical literature subsequently prepared.

Product specifications must be set for a new fungicide before introduction to the commercial market. These specifications are limits that guarantee that the physical and chemical properties of the product will be consistent from one shipment to another. They must be set to exclude any impurities undesirable from a residue standpoint and to guarantee uniform performance. They must also be lenient enough to permit economic production of the fungicide. They can be made more rigid as experience is gained in production. While a certain amount of flexibility is necessary in the development phase of the new product, the final goal of realistic product specifications must be kept firmly in sight.

Other factors that must be considered are toxicological data and residues. Studies in these areas are vital and will be discussed more thoroughly in a later passage on responsibilities under the law.

II. Formulation

For agricultural chemicals, a proper formulation often determines the success or failure of a candidate material. It may be a wettable powder, an emulsifiable concentrate, or some other form. The material must be easy to use, concentrated enough to be economical, and designed to get the chemical to the site of action most efficiently.

A fungicide must be stable in storage for periods of a year or longer, must not be affected by extremes of temperature from below 0°F to above 100°F, and must neither separate nor aggregate during storage. Conceivably, as a result of inadequate investigation of bulk density and its variability, 2 lb of material may not fit into a container designed to hold 5 lb.

I. Biological Data

Detailed data must be compiled to determine exactly how the fungicide is to be used. In addition to effectiveness on specific diseases, safety on recommended crops, and dosage and timing of applications previously discussed, it is necessary to know the effects of temperature, light, rain, other climatic conditions, and soil type and fertility on the chemical's performance. What are its compatibilities with other pesticides that
may be used in conjunction with it? What are its limitations? All these facts must go in the technical information sheet and will be the basis of all subsequent development.

J. Labeling

Proper labeling also is an ultimate objective. All claims made about the new fungicide must be very carefully handled. First, they must be the truth, then they must be all the pertinent truth, and finally they must be the truth in language the farmer understands. It is in this last phase that industry frequently is found wanting. Too often, label language is composed to satisfy scientists, lawyers, and government officials. Labels should be plain talk. The claims must be simple, clear, concise, and forceful.

Companies have a serious responsibility in labeling. Farmers should be able to follow directions so that they will not harvest a crop containing a residue in excess of what is legally allowed.

K. Patent Considerations

Patent considerations are all important. After spending millions of dollars in money, time, and effort in fungicide development, a company must be protected. If a fungicide team fails in this regard, management will not be likely to be willing to invest large sums in future fungicide development.

Good patent protection provides a few years of exclusive sales, during which time process know-how can be fully developed and the company name and trademark can become closely associated with the product. In a field where the preliminary investment is as high as it is in agricultural chemicals, strict attention to obtaining patent protection is vital in recouping the research costs. Obtaining adequate patent protection can itself be time-consuming and should be started as early as possible so that the further development of the fungicide will not be held up.

L. Marketing and Management

While all these important developmental and technical efforts are under way, other practical considerations must be kept in mind. A new fungicide has not finished its development when the technical problems
of its use are solved. It will not be available to the farmer until solutions also are found for its marketing problems.

An adequate sales force must either be in existence or be built. This force must be supported by such sales service organizations as credit, order, and traffic departments as well as by technical service departments.

Package design is an important adjunct to the successful introduction of a fungicide. Containers and container weights must be determined early in the development program. This would include the size and type of container, whether glass, paper, stainless steel, plain iron, or resin-lined. The package should be consistent with grower needs. Not many 100-lb packages will be sold if the average farm uses only 2 lb of product per season.

Obviously some careful studies are in order for proper packaging of a new product before launching it into the commercial world. A fungicide should be convenient to use—a liquid should be packaged to pour easily; a wettable powder may be packaged in units that will give the correct dosage when added to 100 gal of water in the spray tank. The package can and must be an attractive, efficient sales agent itself.

Publicity and advertising must be planned to help build the market for the new fungicide. As soon as one is sure that they have a product, technical articles about it should appear in the key scientific journals. Then the agricultural trade journals should receive product publicity releases at the time of commercial introduction. This will advise the trade on basic facts concerning your product. Publicity aimed at farm magazines, newspaper farm editors and radio-TV farm commentators also is a help. Remember, a new product is newsworthy. Let farmers know about its development and availability for use. A series of publicity releases can be planned to describe the following: What does the pesticide do? What are the production facilities? How is it different or better than other compounds on the market? Case history success stories also should be publicized during the initial years that the fungicide is on the market. Independent farm experts and editors should be urged to write articles about the product for farm journals.

Advertising must communicate basic facts about the product and urge growers to buy. Literature also must teach growers how to use the product. A product information program should be directed toward county agents, extension specialists, and experiment station workers because the farmer looks to them for recommendations.

Of course, all publicity information and advertising activities really pave the way for the technically trained salesman. He must be on the
spot to render technical service and ask for the order. A hard-hitting promotion program is no substitute for a good sales force. However, it is vital supplementation to salesmen. Promotion and sales should work closely together. They have a synergistic effect in creating a more effective marketing program.

Still another essential part of merchandising is developing an adequate distribution system. The chemical must be readily available to the farmer when he needs it. Distributors, dealers, and their salesmen should be educated so that they are prepared to tell the farmer what the material will do and are confident enough to convince him that he should use it. It is not until the grower knows that the right material at the right price is consistently available at the right places at the right times that the successful commercial development of a fungicide has been completed.

Along with all this discussion of research, product development, marketing, and promotion, it must be remembered that a sound business cannot be built on unsound business management. A technically oriented organization, such as an agricultural chemicals development team, can easily lose sight of business practicality in a search for scientific perfection. Before a company spends millions of dollars to get a new fungicide ready for market, management must be shown a way of getting a return on the investment. This must be done first in order to be able to eventually render necessary service to the farmer.

III. DEVELOPMENT COSTS

Let's take a closer look at today's fungicide development costs. Through a more comprehensive understanding of costs that can be anticipated, we will see more clearly why company management must have a realistic opportunity of realizing a return on its investment in a new product. The cost of bringing a new pesticide to the market place is something the chemical manufacturer can never stop thinking about, if he wants to operate at a profit.

If the supplier does not operate at a profit he cannot long afford to bring new products through the lengthy “growing up” period prior to the time he sells his first pound. These costs today are so large and burdensome that they may well consume the entire profit earned from the marketing of older, more established chemicals.

The industry faces an unusual problem in that the cost of its research and development programs is becoming so high that many companies are seriously thinking about discontinuing the expenditures necessary
5. COMMERCIAL FUNGICIDE DEVELOPMENT

to develop new compounds. We recently studied the agricultural chemicals that have shown really favorable sales potential over the past several years. As reported by Field (1964), we selected 45 promising new pesticide compounds developed for commercial marketing by the agricultural chemicals industry in a 5-year period of time. The cost of bringing these products to the point of marketing was found to be $150 million. The average cost, then, to develop each compound was more than $3 million before the first large-scale use by the farmer occurred. In a 10-year period, the price tag for developing new pesticides had risen from $1.2 million to $3 million! Our comparative figures were based on our own experiences and discussions with other concerns engaged in the same type of search.

A. The Cost of Failures

Perhaps one of the most interesting facts evolving from our early studies and discussions with other companies was that representatives from these different concerns agreed that to obtain one successful agricultural chemical, about 1800 compounds must be synthesized and run through biological screens. Each successful "1 in 1800" must bear all the cost of those compounds which fall by the wayside on the way to the top. In comparison to this experience, today the probability of discovering and developing a successful pesticide is about 1 in 3600.

B. Calculating Total Cost of a Successful Fungicide

The differences in costs of developing agricultural chemicals to the point where commercial sales can be considered between 1955 and 1965 are compared in Table 1. Although these estimated costs are based on pesticides in general, they also hold true for fungicides in particular. While the estimates in the table include cost of failures in research and development, it should be pointed out that they do not include costs of process development, manufacturing costs of field samples, and test marketing for the "successful" compound to be brought to the commercial market.

C. Drilling Dry Holes

Many people have written about the cost of developing new pesticides and fungicides over the past ten years, and their estimates range from $750,000 all the way up to $3.25 million. Any one of these estimates can
be correct, for a single company's experiences for developing pesticides is like exploration in the oil business. Most agricultural chemical manufacturers have drilled plenty of "dry holes" looking for new fungicides. And each company if it expects to remain in business continues to screen thousands of chemicals each year. Manufacturers have not lost their enthusiasm for agricultural chemicals, but they must be allowed—in the

### TABLE 1

**Cost of Developing an Agricultural Chemical**

<table>
<thead>
<tr>
<th></th>
<th>1955</th>
<th>1965</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival rate</td>
<td>Cost</td>
<td>Survival rate</td>
</tr>
<tr>
<td><strong>Initial screening:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synthesis</td>
<td>$150</td>
<td>$150</td>
</tr>
<tr>
<td>Biological screens</td>
<td>$200</td>
<td>$50</td>
</tr>
<tr>
<td>(1 in 30)</td>
<td>×30</td>
<td>(1 in 100)</td>
</tr>
<tr>
<td>Investment in each remaining chemical</td>
<td>$10,000</td>
<td>$20,000</td>
</tr>
<tr>
<td>Further laboratory, greenhouse work</td>
<td>1,000 each</td>
<td>3,000 each</td>
</tr>
<tr>
<td>(1 in 10)</td>
<td>×10</td>
<td>(1 in 9)</td>
</tr>
<tr>
<td>Investment in each remaining chemical</td>
<td>$115,000</td>
<td>$207,000</td>
</tr>
<tr>
<td>Cost of initial field tests</td>
<td>$3,000 each</td>
<td>$5,000 each</td>
</tr>
<tr>
<td>(1 in 3)</td>
<td>×3</td>
<td>(1 in 2)</td>
</tr>
<tr>
<td>Investment in each remaining chemical</td>
<td>$348,000</td>
<td>$421,000</td>
</tr>
<tr>
<td>Development costs</td>
<td>(field studies, analytical methods, residue and toxicological work, patent costs)</td>
<td>$250,000 each</td>
</tr>
<tr>
<td></td>
<td>$588,000 each</td>
<td>$1,459,000 each</td>
</tr>
<tr>
<td>(1 in 2)</td>
<td>×2</td>
<td>(1 in 2)</td>
</tr>
<tr>
<td>Overall survival</td>
<td>$1,196,000</td>
<td>$2,918,000</td>
</tr>
<tr>
<td>(1 in 1800)</td>
<td></td>
<td>(1 in 3600)</td>
</tr>
</tbody>
</table>
5. COMMERCIAL FUNGICIDE DEVELOPMENT

face of rising development costs—to earn a reasonable return on their investments. If they do not, what kind of company management would it be that would let them stay in business? They are in business to make a profit, and so is every other businessman, including the farmer.

We might well ask why does it cost more to develop a fungicide today than it did 10 years ago? What has changed? Several answers to these questions were given earlier in this chapter. But, no matter how much we ask or analyze, it is a fact of agricultural chemical life that the development costs have gone up sharply in recent times. As an example, we have broken down the costs of just one phase of fungicide development. Listed below (Table II) is our current estimate of what average toxicological studies would cost on a promising new fungicide. It is easy to see why these toxicological costs, together with many other expenses, soon add up to a development price tag of several million dollars.

Despite the fact that we have effective fungicides on the market today, many different types still will be needed for farmers in the future. It will take a lot of research and development to solve specific problems in agriculture. But suppose the chemical industry does solve these problems. How long will it take to get their money back if they develop a given fungicide for a specific problem?

In accordance with today’s management practices, we made a cash flow study designed to show how much of a certain fungicide a producer would have to sell to regain his research and development expense, and how long that company would have to sell such a product to earn a 6% return on its investment.

Imagine that the XYZ chemical company developed a fungicide for a given crop. There are 1.2 million acres of this crop grown in the United

<table>
<thead>
<tr>
<th>TABLE II</th>
<th>COST ESTIMATE TYPICAL OF TOXICOLOGICAL STUDIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 range-finding studies at $400 each</td>
<td>$ 40,000</td>
</tr>
<tr>
<td>10 90-day studies at $5,500 each species</td>
<td>55,000</td>
</tr>
<tr>
<td>2 2-year rat-feeding studies at $50,000 each</td>
<td>100,000</td>
</tr>
<tr>
<td>2 2-year dog-feeding studies at $50,000 each</td>
<td>100,000</td>
</tr>
<tr>
<td>Complete metabolism studies (biochemistry) on one agricultural chemical</td>
<td>50,000</td>
</tr>
<tr>
<td>Reproduction studies for 3 generations (rat)</td>
<td>3,000</td>
</tr>
<tr>
<td>Cancer study on mice</td>
<td>1,800</td>
</tr>
<tr>
<td>Continued toxicological work during first 5 years of marketing</td>
<td>119,300</td>
</tr>
<tr>
<td>Total</td>
<td>$469,100</td>
</tr>
</tbody>
</table>
States. For one reason or another, overseas marketing is impractical. At an estimated rate of one pound per acre, and at a grower cost of $2 per pound, the cost of using the fungicide is $2 per acre multiplied by the number of total acres (1.2 million) we arrive at a total market potential of $2.4 million if one application was applied to all acres. But, some acres will receive more than one application, some will receive no applications, and we know that this product will not be the only one used. Therefore, it was assumed that the attainable share of the market for this product was 20% of the total, or approximately $500,000. However, this is at grower prices. Sales dollars at manufacturer level were estimated to be 65% of grower level or $340,000.

Using these estimated market conditions and standard cost factors for research and development, production, and marketing expenses, let us see how the cash flow for this new product looks over a 20-year period and how much money it returns to the XYZ chemical company. In Fig. 1, note that after 10 years of sales, and 20 years from the start of the product’s development, XYZ chemical company still has not made any money, and has not even recovered its investment! Sales of the product did not begin until the sixth year. During this time there was a large annual outlay of cash. This cash flow, the cost of doing business, continued to a lesser extent throughout the 20-year period, but at the end of 20 years the “Cumulative Present Value” curve still

![Fig. 1. Discounted cash flow in the development of a fungicide by the XYZ company.](image-url)
had not risen above the "0" line. If it had reached that point, the XYZ company at least would have its money back.

One might ask why the "Cumulative Present Value" curve did not rise faster after initial fungicide development was over and sales began. Money, of course, has a "time" or interest value. So the interest that XYZ chemical company paid on its 20-year fungicide investment also had to be factored into "Cumulative Present Value."

It becomes readily apparent there is no guarantee that a compound with a reasonable chance of giving a profit per pound actually will be profitable to a company over the long haul. Chemicals are unpredictable. One cannot pick a winning horse by looking into his mouth. Neither can one pick a successful fungicide by just looking at the formulas of the many thousands of compounds that companies screen each year in search of those that might have beneficial and economic value. Agricultural chemical research is a mixture of much dogged determination to complete routine tests on thousands of candidate chemicals and an equally important portion of imagination and logical thought.

IV. FUNGICIDES AND THE LAW

There must be a great deal of research. A star performer in laboratory and greenhouse tests may flop in the field. This is why companies must prove what a promising fungicide can do under actual field conditions before it can be considered by government agencies for registration.

A. Toxicological Research

To prove that it is thorough, here are a few of the multitude of activities a manufacturer carries on in the development of a single, typical pesticide.

The toxicological work alone on 1900 experimental animals includes 2200 biochemical determinations and 2500 pathological slide examinations, according to Stone (1960). All totaled, 17 professional men spent 1200 man days on investigating the potential hazard to warm-blooded animals, and paper work (based on 4100 pages of research data and laboratory records) piles up in 15 intracompany reports totaling 280 pages of valuable scientific information.

Safe-use studies include such important areas of chemical pesticide investigation as skin penetration, skin and eye irritation, and inhalation examinations. Short- and long-term feeding trials of the material are undertaken. Rats are fed three different levels of the compound for
2 years and larger animals are fed in the same manner for one or more years. Metabolism studies in plants and animals are important. Long-term effects also are carefully sought out in other special studies. New pesticides are also tested on experimental animals to determine whether small amounts taken daily throughout a lifetime may gradually accumulate in body fat, liver tissues, or other organs. If there is ever an indication of such a buildup, wide safety margins are set to avoid any hazard. These thorough tests also determine whether the chemicals, in any amounts whatever, are capable of causing cancer. No chemical causing cancer is permitted to be applied to any food product. Extensive tests to determine the effects of the chemical on reproduction and many other tests vital to human safety are carried out.

This meticulous ferreting of information must take place even before the product is tested in the field. And the lengthy, methodical procedure, from first synthesis in the laboratory until it is marketed under a commercial label, accounts for large portions of the time that makes up the 5 or more years it takes to develop a new compound.

The outcome enables the manufacturer to instruct the consumer in the proper application and safe use of the product. Acting under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA), the U.S. Department of Agriculture can and does refuse registration unless the labeling does so instruct the user. That is only part of the story. It also refuses to register a pesticide whose toxicity has not been thoroughly studied, even though there is no evidence that it is unsafe. This way, the public rather than the pesticide manufacturer reaps the benefit of any doubt. The purpose of this kind of regulation is to lock the barn door before harm's horse gallops out of the stable. Moreover, it is this kind of regulation that even the public health experts often seek, but rarely get, some people feel. The FIFRA is well ahead of most other regulatory laws in this respect.

Company scientists in the field double-check scientists in the laboratories. Scientists in government agencies and in state experiment stations double-check the discoveries of the company scientists. When this is done, the company must prove to the U.S.D.A. scientists that the new product will be useful in agriculture and that it will be safe to use. The company also has to prove to the Food and Drug Administration (F.D.A.) scientists that it can be used without any hazard to consumers on each food crop for which it is to be registered. And then the F.D.A. double-checks to make sure before registration is granted.

Pesticides, like all chemicals, have some toxic properties, but this does not necessarily mean that their use will create a hazard to public
health. Toxicity and hazard are not synonymous terms. Toxicity is the capability of a substance to produce injury. While hazard is the probability that injury will result from the use of the substance in the quantity and manner proposed. As an example, the most toxic material will not present a hazard if it evaporates or becomes nontoxic by decomposition before the crop is used for food, or is present only in minute, harmless proportions.

Proof of efficiency is another major requisite to marketing a new pesticide compound under current laws. The efficiency tests in company laboratories and on experimental farms determine whether a new product shows promise of meeting all of government’s rigid standards of effectiveness and efficiency in pest control. If it does not, there is no hope for it.

The U.S.D.A. will not take the undocumented word of the developer that a new fungicide can be used safely and is effective. We have discussed in some detail the depth of the toxicological studies. Another parallel stage in developing a fungicide is residue testing on food crops.

B. Residue Research

Residue analyses determine the small amounts of the pesticide left on plants or animals or in the soil. This is called finding the parts of pesticide per million or billion parts of the object analyzed. The residue analyst is challenged to establish the quantities and locations of only a few micrograms (1 μg equals 1/1,000,000 gm) of a compound in an orange, ear of corn, sugar beet, liver of a cow, part of an insect, or other such complexity. This normally is very difficult because most substances will invariably yield traces of solvent, extractable materials in varying amounts and combinations of organic acids, hydrocarbons, waxes, oils, pigments, aldehydes, proteins, amino acids, and many other interfering molecules. The pesticide chemical must be separable from these contaminations before a reliable residue determination is possible. This becomes increasingly more difficult because of the number of variables that must be contended with. Also the amount of residue varies depending upon the deposit-retaining abilities of the crop surfaces to be treated, such as the differences in the surface textures of apples and peaches.

Differences between formulations may affect the amount of deposit, the rate of degradation, persistence, and penetration. The type of application equipment must also be considered as a possible variable factor. Environment also is an outside influence on residue testing that makes
the analyst's job more difficult. The amount of residue may vary on	hose portions of plants exposed directly to sunlight, rainfall, and wind.
In sampling, sufficient materials must be collected to compensate for
the variability in background influences contributed by the matter in­
olved. Proper sample collection has been a difficult analytical residue
problem to solve, but is now a standardized procedure.
Residue determination is an intricate task. Therefore, uniform proce­
dures have been established to assure reliability of results. Despite the
difficulty, the chemical laboratory is charged with developing a method,
and frequently several methods, to determine the infinitesimal amounts
of chemical residue that may remain in or on a wide variety of food
crops. Detecting complicated chemicals such as the common pesticides
and measuring accurately these minute amounts (sometimes as little as
one part per billion), calls for great skill. But this analytical method
must be perfected before any pesticide can be approved, recommended
for use, or put on the market.
Once it has been determined that a harmless residue remains on the
harvested product, a tolerance is set by the F.D.A. officials which pro­
vides for a very wide safety margin. For example, any tolerance is usu­
ally based on the amounts of residue that are at least 100 times less
than the amounts proved safe in toxicological studies, according to
Decker (1962). That is, if toxicological studies prove that 100 ppm of a
chemical residue are safe in the diet of experimental animals, the
F.D.A. might set a tolerance of 1 ppm on the raw agricultural product.
It is not generally understood that the tolerance or permissible residue
determined by F.D.A. authorities is in no instance set at a higher level
than actually found on crops, when the material is properly used. In
fact, the safety factor is frequently 200 to one or even 300 to one.
The tolerances established by the F.D.A. fit the U.S.D.A. label re­
quirements much as a hand fits a glove. They are based on the residues
found when the product is used as directed, and thus the residues left
on the food will be within the F.D.A. tolerances and therefore safe for
the consumer. Every farmer is on notice that if he violates the label
directions and exceeds allowable tolerances, his whole crop may be
seized by F.D.A.
When the F.D.A. finds that use according to directions leaves no resi­
dues, it announces a zero tolerance, i.e., no residue allowed. On some
special foods, such as milk, no residue of any chemical has been per­
mitted. In this case, any detectable residue whatever makes the food
product liable to seizure.
The fungicide manufacturer applies to the U.S.D.A. for registration of a label that permits sale of its product for use on each crop, and the firm must submit a proposed label. Specific label instructions tell when to use the material and the amounts and timing for best results. The label also states adequate safety warnings backed by the voluminous toxicological tests made by the company. A proposal for labeling contains research data on effectiveness, based on state and federal researchers' reports; documents proving a safe residue, results of toxicological tests and other vital information. An important aspect is that the burden of proof is on the chemical manufacturer. As a result, the words printed on pesticide labels probably constitute some of the most expensive lines in all of literature. And they should be read carefully.

D. Effectiveness of Product

Proof of performance and safety is not a new practice! Long before today's laws required extensive testing, companies made it a common practice to give every new product exhaustive analysis and frequently to discuss that analysis with government authorities. However, now there are laws that require what was informal practice in the past. These laws represent recognition by the state and federal governments of the economic necessity of pesticides in production of more, better and cheaper agricultural products, and the potential hazard of using them unwisely. They also set the standards by which manufacturers must prove that a new pesticide is effective and that the warnings on the label will protect the user.

E. Satisfying the Government

Regulations have been passed and gradually strengthened over the years to protect both the public and the user. Today the protection machinery takes the form of the following acts of Congress:

The Federal Insecticide, Fungicide, and Rodenticide Act of 1947 is the law that requires manufacturers to prove to the U.S.D.A. that pesticides are effective. This law also requires labeling to indicate which pests the chemical will control, the crops or livestock on which it can be used and in what amounts, and to give adequate warnings of hazards to users.

Public Law 518, or the Miller Pesticide Residue Amendment (July
22, 1954) to the Federal Food, Drug, and Cosmetic Act of 1938, is the statute that requires the manufacturer to prove how much residue, if any, will remain on each crop and that it will be safe in food. These are the data used by the F.D.A. to establish its tolerances.

The U.S.D.A. has been assigned the responsibility by Congress for registration of safe, effective farm chemicals including all necessary safe warnings. "Our job is to see that no one gets hurt, physically or economically, insofar as sound labeling can insure such results," said Justus Ward, chief of the Pesticide Regulation Division of the Agricultural Research Service, as reported by Strohm (1962). The Food and Drug Administration continues its surveillance of chemicals used on the farm. It spot-checks food on a regular basis, analyzing for residue in excess of legal tolerances. If above-tolerance amounts are found, the food is seized and may be destroyed. No other products are more thoroughly tested before use than are agricultural chemicals. Congress has made sure of this.

V. COORDINATION WITH GOVERNMENT RESEARCH

After carrying out extensive company-supervised testing, and as a means toward satisfying the government, an agricultural chemical manufacturer must then work out a coordinated program of testing with government research scientists. The first question the U.S.D.A. asks when a company applies for federal registration for a new fungicide is, "Where has it been tested, and what are the results?" This agency won't take the unsupported word of the developer, for it also wants evidence of effectiveness and safety from impartial experts.

A. Broad-Scale Testing through Experiment Stations

To measure the effectiveness of a new fungicide, a manufacturer may ask more than 100 researchers in state and U.S.D.A. Experiment Stations to give it careful tests. The scientists who conduct these tests are dedicated to better plant disease control. They search for new fungicides that are safe, yet can deliver more effective control for less money. These men are choosy in that they do not test every chemical that comes along. They only take those that show promise of being better in some way other than those already available on the market. Perhaps to some members of private industry they appear to be troublesome obstacles. But actually their important work is supported in part from research grants from private industry itself. However, be-
cause they work for the government, their test results are scrupulously objective.

In order to get the true broad-spectrum picture of the market potential for a new fungicide it might be tested on vegetables in New Jersey, cotton in Texas, grapes in California, apples in Washington, and for many other uses in other states. Researchers would try it in various amounts over all of the vast differences in climate and soil from coast to coast. It is easy to see why a tremendous amount of teamwork between private industry and the government experimenters is necessary.

**B. Final Goals**

Final goals are realistic determination of the best way of treating various crops, the right amounts to use, the best formulations and the timing for maximum control. Another goal is to see if the new material is compatible with others already in use. The government researchers keep a close eye on costs, too. A fungicide must not only control disease better than others already recommended, but it must do the job at a comparable price.

The work of these state, university, and U.S.D.A. scientists is essential in developing the capabilities of a candidate fungicide. They test over widespread conditions and against many problems that company researchers cannot possibly test and evaluate.

Coming into the home stretch of development is the application of the fungicide just as it would be used in big acreage farming. This full-scale testing turns up many facts that cannot be learned in laboratory or small-plot work.

If we were as adequately protected against highway accidents, fire, radiation, and the unsanitary conditions that permit the spread of insects and disease as we are currently protected against the hazards of agricultural pesticides, ours would be a far safer and healthier world.

A major problem is that few people realize the exacting research, the amount of time and the gigantic expense involved in preparing a new, effective and safe agricultural fungicide for introduction onto the farm market for use on food and feed crops.

**VI. THE NEED TO SUCCEED**

However, it is the chemical manufacturers’ responsibility to satisfy the government in detail on each new pesticide product. When these
responsibilities have been met in full, your new fungicide can be labeled and marketing may commence.

When farmers complain about the high cost of fungicides, they have little idea of the time, effort, and millions of dollars that went into providing them with the answer to their problem. In the future, we are going to have to let growers know more about these high costs. Of course, we in turn must recognize that no pesticide can be useful to the farmer unless it provides him with an advantage over alternate courses of action.

A. Industry's Role in Agriculture

It is essential that new fungicides continue to appear on the market if the farmer is to go on improving his crops both in quality and quantity, and our agricultural chemical industry is to be kept dynamic. Agriculture, in spite of, or maybe because of, vast surpluses, is intensely competitive. The individual farmer must grow the maximum possible yield per acre of the best quality crop at the lowest possible cost to avoid sinking into debt. Fungicides help him achieve all three of these aims by protecting his crops and by cutting his labor costs. Just as the farmer must have good business management, so must the chemical industry which produces and distributes the chemicals which the farmer uses so well.

B. Reasonable Profits Are Necessary

With the high cost of developing fungicides, no company can remain long in this field if it cannot plan or execute its merchandising so that it will not only recapture its research investment, but also obtain the rewards in profits that its risk and enterprise justify.

But in spite of all the pitfalls and difficulties described, fungicide development teams should face their futures optimistically. Though they are confronted with tremendous tasks, they also strive for rewarding goals. Developing economical and effective fungicides is a big challenge to the pesticide industry, for the ravage of crops by plant diseases is taking more than a $3 billion per year total in the United States alone. Half this loss is on corn, cotton, oats, and wheat, relatively low-value-per-acre crops that command little attention for control measures.

In the final analysis, new fungicides will grow into full maturity only if: (1) they give superior performance and fill real needs; (2) their
markets are realistically analyzed; (3) they are intelligently developed; (4) vigorous advertising and publicity campaigns are pursued; (5) they are merchandized in an orderly manner; and (6) the products are sold vigorously and honestly. If these requirements are fulfilled, the years ahead will continue to bring new fungicides that will serve the farmer still better than the excellent materials now on the market.

REFERENCES


CHAPTER 6

Formulation

E. Somers

I. Introduction

"Cookery is become an art, a noble science: cooks are gentlemen."
—Robert Burton (1577–1640)

Formulation is a sort of cookery. It is concerned with the methods of presenting the active ingredient, in this case a fungicide, in the most effective physical form—effective, that is, with regard to storage, application, and ultimate biological activity. Like cookery the basic science has lagged behind the technology and even today rule-of-thumb techniques make formulation something of an art. This is partly
because the nature of industrial life is such that a manufacturer may not wish to publicly divulge the exact nature of a pesticide formulation, and partly because pesticide workers have traditionally concentrated their energies on developing new and more potent chemicals rather than on improving existing ones. In addition it is often very difficult to define the biological specifications of a formulation. The situation is changing, however, as the toxicity of residues becomes so all-important, and there is now a real interest in making the best use of available materials and in exploiting the techniques of modern surface chemistry.

The amount of fungicide that is biologically effective at the relevant surface—be it living plant, foodstuff, textile or paper fiber—is so minute that for economic use the chemical must be greatly diluted before application. Nearly all the subsequent discussion will be devoted to agricultural fungicides—these may be diluted either with a solid and applied as a dust, or with a liquid and sprayed on to the plant target. Water provides a cheap and effective dilution medium and, with few exceptions, is used as the carrier for agricultural sprays. As fungicides are of very low water solubility they must be formulated to make them compatible with water, hence surface-active agents are required to prepare water-dispersible powders, stock emulsions, or emulsifiable concentrates. In addition, surface-active agents may be needed to improve the suspending, spreading, and wetting properties of the spray. Other supplements that may be incorporated with sprays includes stickers to improve the weather resistance of the deposit, and materials to improve spray deposition and penetration.

The type of formulation chosen for a fungicide is determined by a variety of factors of which cost and biological efficiency are the most important. Dusts are often the simplest and cheapest way of applying a pesticide but climatic conditions limit their use as compared with sprays. They are, however, often suitable for soil application and seed treatment. Most agricultural fungicide sprays are formulated from water-dispersible powders rather than emulsions. This is partly because emulsions are more expensive and less easy to transport, especially under tropical conditions, but also because fungicides are often phytotoxic compounds and are obviously less active in the solid than liquid state. Suspensions of solids are rarely marketed, since they are bulky and tend to settle out to a hard deposit difficult to re-disperse. Colloidal dispersions such as those of bordeaux and Burgundy mixtures are prepared by precipitation in situ. Solutions are used for soil fungicides when penetrating properties are required and for many
nonagricultural uses such as the treatment of timber, textiles, paper, and so forth.

II. TYPES OF FORMULATION

A. Dusts

Fungicide dusts, with certain exceptions, contain only a small proportion of the active ingredient, generally less than 10%. The other components are known as diluents, or carriers, and their function is to dilute economically a relatively expensive chemical and to improve the application properties of the formulation. A dust should flow easily, possess good crop-covering properties (i.e., dustability) and form an adherent deposit on the plant surface. In addition, the dust formulation should not cake either on storage or during application and should eliminate any possible phytotoxicity of the active fungicide.

The fungicide and the diluent may be formulated by simply mixing or, more commonly, grinding the ingredients in a mill (hammer, impact, vertical roller, or fluid-energy types). Another method is to impregnate the diluent with a solution of the fungicide in a volatile solvent or, if the fungicide is of sufficiently low melting point, with the liquid fungicide itself. Thus the dust may consist of a mixture of independent particles, or the active ingredient may coat the diluent particles, or a complex conglomerate of fungicide and diluent may be formed. One of the chief disadvantages of a dust formulation, particularly for airplane application, is that the particles fractionate in the air—the “fines” formed by grinding can separate from the carrier—and this effect may be prevented by the use of dusts prepared by impregnation (Eaton, 1959).

Dusts are often prepared in two stages. First, a concentrate of more than 25% active ingredient is formed by mixing the fungicide (by impregnation or grinding techniques) with a highly sorptive diluent. This dust concentrate is convenient to transport and store subsequent to final blending and dilution to a field strength dust. A confusion in terminology has arisen with these concentrates: the fungicide is first mixed with a “carrier” and later diluted with a “diluent” (Anonymous, 1956). However, as it is often the same material (attapulgite, and kaolin, for example) which fulfills each function it would seem preferable to use the terms diluent and carrier as synonyms.

The diluents most widely used are the silicate minerals attapulgite, kaolin, pyrophyllite, tale, Bentonite, and diatomaceous earth. Of lesser
importance are calcium and magnesium, carbonate, gypsum, lime, and the plant flours. In recent years synthetic diluents such as calcium silicate, hydrated silica, and hydrated sodium silicoaluminates with high adsorptive properties have been developed; because of their cost these are only suitable for concentrate formulations. The classification and description of diluents has been the subject of many papers (Watkins and Norton, 1947; David and Gardiner, 1950; Miller, 1950; Anonymous, 1956; Ebeling and Wagner, 1961).

The physicochemical properties of a diluent which can affect ultimate field performance are particle size and shape, compatibility, surface acid strength, hygroscopicity, sorptive capacity, flowability, grindability, electric charge, and bulk density (Miller, 1950; Eaton and McKone, 1960). Eaton and McKone (1960) considered that diluents for dust concentrates should possess good flowability, medium bulk density, high sorptive capacity, and good grindability while for field strength dusts a lower sorptive capacity is adequate. Particle size is an important determinant of dustability: Andreasen et al. (1939) showed that dusts dispersed best when freed from the smallest particles—these caused the larger particles to aggregate and "clumped" the dust. Further discussion of the physical properties of dusts is given in Section IV.B.

Sulfur is a fungicide which possesses in its own right many of the attributes of a successful diluent. Sulfur dusts, therefore, only require the addition of a small quantity of a conditioning agent—such as talc or magnesium carbonate (Hoskins, 1962)—which aids flowability by preventing agglomeration, or "balling," of the particles.

The use of granular formulations of pesticides has developed rapidly in recent years (Krause, 1959; Hoskins, 1962; B. Sumner, 1963). Because of their size granules do not drift when applied, as do dusts, and they can be formulated to release their active component at a controlled rate. The granules are usually based on mineral carriers such as attapulgite clay or vermiculite although vegetable residues from tobacco and coffee have been used. With fungicides, granules have limited application, being confined to soil and seed treatments.

B. Water-Dispersible Powders

The most popular formulation for an agricultural fungicide spray is a water-dispersible powder—also termed, although less exactly, a wettable powder. Essentially these powders are composed of the fungicide, diluent, surface-active agents to ensure suspending and wetting prop-
6. FORMULATION

Properties and, possibly, adjuvants such as stickers to improve deposition characteristics. A typical formulation would be: fungicide (5–50%, nonionic wetter (e.g., 1–10% polyethylene oxide condensate), thickening agent (e.g., carboxymethyl cellulose or an alginate), and a hydrophilic diluent such as bentonite (F. R. Bradbury and Campbell, 1962). With fungicides which are surface-active themselves, such as the alkyl guanidine salts, the addition of a silicone-type antifoaming agent may be necessary (Marks and Frederick, 1964).

Water-dispersible powders usually contain 25–50% active ingredient and the techniques of blending fungicide with diluent are those already described for dust concentrates. The requirements for the diluent are also similar to those for a dust concentrate except that the specifications for bulk density and flowability are not as stringent (Eaton and McKone, 1960). The powders are ground to a low particle size, below 10 µ, and this process is aided by the fact that many fungicides such as copper oxychloride, copper oxinate, zineb, maneb, and thiram are manufactured by precipitation in a water phase. The dithiocarbamates tend to decompose in suspension and on storage and several methods of chemical stabilization have been described in patents (Thorn and Ludwig, 1962).

The suspending agent in the formulation has two functions: to deflocculate the individual particles so that they form a smooth dispersion; and to act as a protective colloid which stabilizes the disperse system and reduces sedimentation in the spray tank. Materials such as sodium lignin sulfonate (sulfite lye), methyl celluloses, polyvinyl acetate, naphthalene sulfonic acid–formaldehyde condensation products, and aluminium silicates (Hintzmann, 1952) are used as suspending agents. These agents have very little wetting power and must be supplemented with more powerful wetting agents (Stanley, 1958).

A wide variety of wetting agents, of anionic and nonionic type, is now available (Schwartz et al., 1958; Moilliet et al., 1961) and their function in agricultural sprays is discussed below (Section V,B,2,b). The choice of a wetting agent should be governed by its behavior with the complete formulation so that incompatibility can be avoided. In addition, entrained air in the powders can lead to excessive foaming in the spray tank with some wetting agents (Howes, 1966) and anionic agents can be precipitated in hard water. For a water-dispersible powder the wetter should be a solid, rather than a liquid or low melting-point wax, without the hygroscopicity that will lead to caking of the particles.

Some of the anionic materials used are sodium laurel sulfate, alkyl-
ated naphthalene, and benzene sulfonates, and the powerfully wetting sodium dialkyl sulfosuccinates. Nonionic agents, such as the polyethylene glycol esters and ethers, are often preferred in spite of their expense for their unreactive chemical nature means that they are unaffected by hard water, are very stable under acid and alkaline conditions, and show little phytotoxicity. The relative oil and water solubility of these materials can be adjusted by altering the length of the polyethylene glycol chain, so producing an extremely versatile range of wetting agents. Stanley (1958) has suggested that the non-ionic fatty acid esters of sucrose, which are crystalline solids, and the solid urea complexes formed with liquid nonionic agents, may be suitable for water-dispersible powders.

C. Emulsions
Fungicides applied as foliar sprays are rarely formulated as emulsions, although there are exceptions: dinitrophenylcrotonate, bis(dimethylaminom)-3-amino-5-phenyl-triazolyl phosphine oxide, and some antibiotics (Asakawa et al., 1961). Mixed fungicide–oil emulsion sprays are applied to control Cercoспора leaf spot of banana (Klein, 1961). Emulsions are used for soil fungicides such as chloronitropropane, and trichlorodinitrobenzene; for wood preservatives such as copper naphthenate, and copper 3-phenylsalicylate; and for textiles, leather, and paper preservatives such as the triazine derivatives (Wolf and Ligett, 1959), N-phenylmaleamic acid esters (Ligett et al., 1959), and phenolic derivatives of quaternary ammonium compounds (Mangney and Pechmez, 1959).

The fungicide is dissolved in a water-immiscible solvent such as a petroleum fraction and, with the aid of a surface-active agent, this can be dispersed in water to form a stable emulsion. The theory and technology of emulsions has been well documented by C. G. Summer (1954). The choice of a solvent is important for it should be non-phytotoxic and have sufficient solution power to prevent the fungicide precipitating out on storage (Selz, 1953). Formerly stock emulsions stabilized by such hydrophilic colloids as carboxymethyl cellulose or gelatin were prepared, but these have been largely replaced by emulsifiable concentrates, or water-dispersible oils. Although stock emulsions are cheaper to prepare, their use is limited by variable shelf life, difficult handling, and the need to incorporate a wetting agent because of the poor wetting properties of the stabilizing agents.

Emulsifiable concentrates require a high concentration of an oil-soluble emulsifying agent, often more than 5% of their total weight,
to give instantaneous formation of a homogeneous emulsion on dilution in water. At these concentrations the highly surface-active emulsifiers confer good wetting properties to the spray. The efficiency of an emulsifier is related to the balance between the hydrophobic group of the molecule, which is contained in the oil phase, and the hydrophilic portion in the aqueous phase. This has led to the concept of the hydrophilic-lipophilic balance (HLB) which can be used as a guide in choosing an emulsifier for a specific formulation (Griffin, 1949; Davies, 1957). Originally single nonionic emulsifiers were used, but more effective emulsification with lower concentrations of materials has been found with combinations of two or more nonionics (Mayhew and Nunn, 1958; Kaeukemeyer and Amand, 1966) and with anionic/nonionic mixtures (Seltz and Linsley, 1959). These latter mixtures are now very popular and are usually composed of a nonionic polyethylene glycol ester or ether with an anionic agent such as sodium dioctyl sulfosuccinate or calcium dodecylbenzenesulfonate (Sandem et al., 1959; Altscher and Groll, 1963).

It has been known for many years that unstable emulsions of the "quick-breaking" type will give preferential retention of the oil phase on the spray target (Ebeling, 1963). However, these formulations are not economically viable because of application and storage problems. A more fruitful approach is to consider emulsions that are stable in the spray tank but break on the crop surface due to reaction between emulsifier and leaf. Cationic emulsifiers for this purpose have been proposed by Maxwell (1947), and Furmidge (1962a) has shown that preferential retention of oil can occur with emulsions stabilized by anionic and nonionic/cationic agents as well as by cationic emulsifiers alone.

Recent developments in spray machinery have made it possible to apply invert emulsions, i.e., water in oil, which are less susceptible to drift than normal emulsions (Gill, 1964; Colthurst et al., 1966). These viscous emulsions are formed in situ in a mixing chamber adjacent to the spray nozzle and require hydrophobic type emulsifiers; as yet their value in fungicide application has not been exploited.

D. Solutions

The application of fungicides as solutions presents few formulation problems unless the solubility of the compound is so low that it is necessary to solubilize it with surface-active agents. Of course surface-active agents may also be required to improve the wetting and penetration properties.
Foliar fungicides are of too low water solubility to be applied as solutions, except for the eradicant phenylmercuric acetate; concentrated aqueous solutions of this compound may be prepared by solubilization with monoethanolamine and polyoxyethylene sorbitan monolaurate (Rebold, 1960). However, soil fungicides such as napham, sodium methylthiocarbamate, and Cheshunt compound (copper sulfate and ammonium carbonate) are used as solution drenches, and seeds can be treated with liquid formulations of methylmercuric dicyandiamide or methoxyethyl mercuric chloride, whose solubility can be increased by the addition of a sequestering agent like EDTA (Prochim, 1958). For aqueous concentrates which precipitate out on dilution with hard water, the incorporation of a vinylpyrrolidinone copolymer has been proposed (Barker, 1960).

An interesting formulation technique for a fungicide solution has been developed by Tomlinson (1958) for the control of crook root of watercress. A finely powdered glass frit containing zinc oxide was applied to the watercress beds; this liberated sufficient soluble zinc in the CO\textsubscript{2}-containing water surrounding the plants for the disease to be controlled.

Many examples may be given of the formulation of nonagricultural fungicides as solutions: sorbic acid for food preservation (Gooding et al., 1955); sodium o-phenylphenate for the treatment of fruit wraps; phenylmercury triethanolammonium lactate; the monosodium salt of dichlorophene, and copper oxinate in acid solution (Marcou and Guillemaille, 1955) for textiles; and sodium pentachlorophenate for timber. Phenolic fungicides—having uses that cover a wide range of industry (Gruenhagen et al., 1951)—are often applied as solutions of the sodium salt.

III. Compatibility

Fungicides are often applied in conjunction with other pesticides, particularly insecticides, or with plant nutrients and it is important that the separate ingredients of these multicomponent sprays or dusts do not reduce the biological efficiency of any of the other components, i.e., that they are compatible. Incompatibility may be the result of chemical reaction between the individual components of the mixture—either the chemicals themselves or their formulating agents—or it may be caused physically, as in the flocculation of suspensions by oil emulsions and in the preferential adsorption of one toxicant on the carrier of the other. There are many examples of chemical interaction:
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Pyrethrum, rotenone, and—to a lesser extent—DDT, are inactivated by lime sulfur; the organophosphorus insecticides such as parathion, malathion, and Phosdrin are rapidly hydrolyzed by the alkaline bordeaux mixture; a cationic fungicide such as dodine can react with anionic wetting agents. To aid practical spray application numerous compatibility charts have been prepared by official and commercial organizations (see Henriet, 1952; Sharvelle, 1961). These charts are invaluable for determining gross compatibility characteristics but it must be remembered that the rate, and extent, of any chemical reaction is a function of the concentration of reactants and that the properties of the formulation supplements, such as wetting and emulsifying agents, are not usually considered in the construction of these tables.

Compatibility reactions between different fungicides, and between insecticides and fungicides, have been investigated by many workers. Incompatibility has been noted with the following combinations: lindane with dichlone and thiram on corn seed germination (Arnold and Apple, 1957); maneb with streptomycin (Palm and Young, 1957); parathion and lead arsenate with calcium polysulfide (Grou and Bontea, 1958); copper fungicides containing lime and 1-naphthyl methylcarbamate (Taschenberg et al. 1960); captan and didecyldimethylammonium bromide (Kirby and Frick, 1963); and reduction of streptomycin absorption in apple shoots by captan, dodine, zineb, and endrin (Shaffer and Goodman, 1964).

Reactions—either chemical or physical adsorption displacement—involving the surface-active agents which stabilize the suspensions or emulsions in the tank mixture can destroy the formulation properties of the pesticides. Thus, Thompson (1958) found that lime sulfur and magnesium sulfate precipitated anionic surface-active agents out of solution with a consequent reduction in wetting properties. The reactions of diluents are considered in detail later (Section V,B,1).

The addition of a cationic agent to an emulsion stabilized by an anionic agent will lead to emulsion breakdown due to the precipitation of an anion-cation complex. This effect may also occur with nonionic agents: Furmidge (1962a) showed that the oil phase of a nonionic emulsion was preferentially retained by apple leaves after the addition of didecyldimethylammonium bromide. A nonionic wetter was also found to increase oil phase retention from anionic and cationic emulsions. In general, however, incompatibility reactions are less frequent with the nonionic agents and these are often recommended when the ionic type have been shown to reduce the potency of a fungicide (Ishii, 1962).
IV. EVALUATION OF PHYSICAL PROPERTIES OF FORMULATIONS

A. Particle Size Analysis

Knowledge of the particle size of fungicide particles is essential for the correct formulation of a dust or spray suspension. The particles are usually irregular in shape and often far from spherical so that it is difficult to define the single parameter required for practical sizing. Parameters often used are the maximum chord, the projected area, the distance between two parallel tangents, and the maximum chord in a given direction. In many cases where the free-falling velocity in air or water is determined, the diameter of the “equivalent sphere” is taken as a measure of size; if calculated from Stokes’s law this is termed the Stokes’s diameter. The size distribution of the polydisperse particle system is given in terms of the number or weight of particles within a given size range. Size frequency data are often presented as the percentage in each size range or as the percentage under a series of given sizes. The average particle size of a given fraction can be calculated as the arithmetic mean diameter but, as the weight of fungicide is a parameter of toxicity and tenacity, it is preferable to use the diameter of the particle of mean weight. Hamilton et al. (1943) considered that the surface average diameter was the most appropriate measure of a fungicide’s toxicity but this suggestion has not been taken up by later workers.

The methods available for the determination of particle size have been extensively reviewed elsewhere (Orr and Dallavalle, 1959; Hergan, 1960; Irani and Callis, 1963; Analytical Methods Committee, 1963) and a detailed discussion of this topic would be inappropriate. The major techniques for sizing are sedimentation, microscopy, and sieving but the last method has been of little value for fungicide powders as these are invariably in the sub-sieve range for woven-wire meshes, i.e., below 55–75 μ. However, the recently developed micro-mesh sieves extending the range to 10 μ may prove useful (Irani and Callis, 1963). Very fine sieves have been used by Zieeh (1960) to determine, by wet-sieving, the proportion of large particles in a number of fungicide formulations; wettable powders were dispersed in water and dusts in petroleum ether.

The sizing technique adopted must be determined by the information required. Thus, for a water-dispersible powder, size distribution in suspension should be measured by sedimentation techniques while the sizing of particles in spray deposits may best be determined with a microscope. Similarly, dusts can be sized by air elutriation (Mathieu and
Henriet, 1962) and permeability (Hamilton et al., 1943) techniques or, if the properties of the deposited powder are of interest, by microscopy.

Sedimentation analysis is most commonly applied for the determination of the size distribution of fungicide powders. The rate of sedimentation under gravitational acceleration of freely falling homogeneous spherical particles is given by Stokes's law,

\[ d_e = \frac{175}{\sqrt{\frac{\eta u}{(u - \rho)}}} \] (1)

where \( d_e \) is the equivalent Stokes's diameter (μ), \( \eta \) is the viscosity of the fluid (poises), \( \sigma \) and \( \rho \) are the densities of the particles and fluid (grams per cubic centimeter) respectively, and \( u \) is the velocity (centimeters per minute). Stokes's equation holds strictly only at extremely slow motion; corrections must be applied as \( u \) increases (Rose, 1953). It is essential that the sedimenting particles are effectively dispersed and that there is no flocculation; lists of suitable dispersing agents have been given by Herdan (1960) and Irani and Callis (1963). The Andreasen pipet method, which samples the suspension at a fixed depth, has been widely applied to fungicide size analysis (Hamilton et al., 1943; Gullstrom and Burchfield, 1948; J. R. Gray, 1956; Somers and Thomas, 1956; Henriet and Mathieu, 1962) and a simplified version of this apparatus has been described by Thomas (1956). When even simpler techniques are required, fractional decantation from beakers has been recommended. This has been used for determining the particle size distribution of sulfur and of copper compounds (Collaborative Pesticides Analytical Committee, 1961, 1963), but the results of pipette and decantation methods are not always comparable (Henriet and Mathieu, 1962).

The interpretation of sedimentation data obtained with dispersible powders is complicated by the presence of the diluent, or carrier, in addition to the active ingredient. The Stokes's diameter of the sedimenting particle may be that of the fungicide in association with the carrier rather than that of the fungicide alone. Microscopic examination is required to determine the true constitution of the particles. Microscopy is, of course, the most direct method for particle size analysis but its use has been limited by the slowness and tedium of the operation. Recent developments in automatic sizing and counting have removed these disadvantages and automated instruments may be expected to become increasingly widespread, particularly when the demand is for rapid routine analysis (Furmidge, 1963; Winchester and Heritage, 1966).
The required physical characteristics of a dust have already been given (Section II,A) and the all-important size analysis is described above. The moisture content is usually determined gravimetrically or else by distillation with xylene (David and Gardiner, 1950); this latter method estimates only the free water present. The increase in weight of air-dried powders after equilibration in an atmosphere maintained at 95% relative humidity (20°C) has been taken by Eaton and McKone (1960) as a measure of hygroscopicity.

Essentially the dustability of a dust is related to the strength of the cohesive forces between the particles and these forces will be affected—to an extent yet barely understood—by such properties as particle size and shape, moisture content and hygroscopicity. A settling tower was used by Andreasen et al. (1939) to measure dustability in terms of the percentage dust still airborne after a defined time interval.

The bulk density of a powder, determined by tapping tests or by surface dredging (David and Gardiner, 1950; J. R. Gray, 1956; Eaton and McKone, 1960; World Health Organization, 1961), is certainly influenced by the interparticle adhesion for this makes it more difficult for the particles to roll over one another into positions of minimum potential energy (Gregg, 1951). However, Eaton and McKone (1960) could not correlate bulk density with any formulation requirement other than the obvious one of packing volume. The cohesion of a powder may also be measured by determining the force required to break a thick layer (1 cm) of the deposit (J. R. Gray, 1956).

The flowability of dusts has been related by Wilcoxon and McCallan (1931) to the angle of slope of a cone of dust carefully built up on a circular base. They found that sulfur dusts with small angles of slope both flowed freely and dusted well. Petraseu and Ilie (1957) could find no correlation between the talus angle and other physical properties, including porosity, of a number of mineral diluents. A different type of flowability test, related more to the manufacturing blending processes, has been used by Eaton and McKone (1960). This is based on the weight of silica sand that must be added to the powder to make it flow continuously through a small orifice. Rates of flow of this type are known to be closely related to the particle size of the powder (Irani and Callis, 1963).

C. Physical Stability

The physical stability of suspensions is an essential requirement of effective spray application and is usually determined by sedimentation
under gravity; early techniques have been reviewed by Gooden and Ringel (1956), Milčević and Mišić (1960), and Martens (1960). The suspension is thoroughly mixed and then allowed to stand undisturbed at a constant temperature. After a defined time interval the amount of active ingredient in suspension is determined by pipette sampling (Collaborative Pesticides Analytical Committee, 1962). Similar suspensibility tests have been recommended for other pesticides (World Health Organization, 1961) although Howes (1966) regarded them as not sufficiently stringent for field practice. In all these tests with water-dispersible powders it has been found that careful definition of dispersion technique is very important, for it can profoundly affect the rate of settling.

The suspensibility of powders can change after storage primarily because of agglomeration of the particles, although deterioration of the surface-active supplements may also occur: this is particularly important under tropical conditions. Pearce et al. (1959) have shown that high initial suspensibility is not a criterion of "shelf life" and have suggested subjecting the powders to high temperatures for a short period, probably at 70°C for 4 hours (Miles et al., 1959), as a test for tropical storage.

Emulsion stability is a term often used loosely in the literature (see C. G. Sumner, 1954). Rigorously defined, an emulsion is unstable when it "breaks," i.e., the dispersion process is reversed, but in formulation technology stability is usually measured by observing the rate of creaming or settling—which simply represents a local change in the dispersed phase/volume ratio and is not itself a sign that the dispersion is unstable. Creaming rates, and possible phase separation, are generally measured in graduated cylinders (Selz, 1953; Behrens and Griffin, 1953) and official specifications have been established for emulsions of pesticides other than fungicides by the World Health Organization (1961). Apparatus to determine the sedimentation of heavier-than-water emulsions has been described by Sparr and Bowen (1954).

D. Wetting Ability

The wetting properties of a liquid are determined by the ability of that liquid to form a stable liquid-solid interface when excess of the liquid is drained from the surface (A. C. Evans and Martin, 1935). This is an important consideration with high-volume sprays which are formulated to completely wet the target. Surface-active agents are added to reduce the air-liquid and liquid-solid interfacial energies until the receding contact angle is reduced to zero. Therefore wetting ability
cannot be determined unless both these interfacial energies are assessed—a fact which was understood by Cooper and Nuttall (1915), but has not prevented many subsequent workers from needlessly measuring surface tensions.

The technique of leaf-dipping tests has been described by Ashworth and Lloyd (1961) and Conibear and Furmidge (1965). Practically, it is easier to determine the concentration of wetter required to give 100% wetting than to assess intermediate degrees of wetting. At complete wetting, when impaction is no longer important, the results of dipping tests were found to be comparable with those from spraying (Ashworth and Lloyd, 1961).

A. C. Evans and Martin (1935) found that determination of the receding contact angle by the plate method provided a quantitative measure of the wetting properties of solutions of surface-active agents. Recent measurements of the receding contact angle of moving drops for a wide range of wetters have shown good correlation with wetting ability only when the wetters are compared in terms of the concentration required to reduce the angle to zero (Furmidge, 1965). Practically, a simpler technique is needed and in the past this has been based on the observed wetting of a waxed card dipped into the spray fluid. More sophisticated tests, derived from the textile industry, are now used. Initially, a Draves test was proposed (Thompson, 1968), but this has been replaced by a modified Shapiro test based on the sinking time of a standard cotton tape (Ashworth and Lloyd, 1961). The concentration of anionic or nonionic wetter which gave a sinking time not greater than 15 seconds satisfactorily wetted cabbage leaves. In more comprehensive tests Furmidge (1965) showed that this method was not suitable for cationic agents or for predicting the order of efficiency of wetters on black currant and banana leaves: it did, however, provide a rough guide to the overall efficiency of many wetters on leaf surfaces.

E. Tenacity

The tenacity of a protective fungicide—defined as the ratio of the amount of residue at a given time to its initial deposit (Fajans and Martin, 1937)—is determined by the physicochemical properties of the fungicide and plant surface and by the nature and type of weathering to which the deposit is exposed. In the field the weathering of wind, rain, dew, and sun is augmented by the growth of the plant, which can detach a brittle deposit, and by the abrasion of one leaf against
another. The generally accepted, but little investigated, view is that rain is the most important weathering agent although Somers and Thomas (1956) found that wind alone could reduce deposits of copper fungicides on broad beans by more than 50%. Laboratory evaluations of fungicide tenacity have therefore been largely confined to simulating the action of rain, although dry adherence tests suitable for dusts have been described (Henriet, 1960; Martens, 1960).

Rain will remove fungicide deposits by two processes: by dissolving or redispersing the active ingredient so that it washes off the surface; and by the mechanical beating action of the raindrops. Basically all the workers who have determined fungicide tenacity have used either an immersion test, which corresponds to the first rain process, or a spray test which more closely simulates the total rain action. In the immersion test slides or leaves are agitated in water (Montgomery and Moore, 1937; Heuberger, 1940) or else immersed in circulating water (Blumer and Kundert, 1951). A variant of this technique is to subject the deposits to a slow continuous trickle of water (Stellmach, 1959). In the spraying method the fungicide deposit is washed with an atomized jet of water (Fajans and Martin, 1937; Zich, 1947; Harry, 1948; Green, 1950; Somers, 1956); these sprays are often fiercely applied giving a "rainfall" of more than 1 inch per minute. Apparatus to give a "rainfall" more equivalent to that in nature, e.g., 1 inch per hour, has been described by Rabe (1956) and Burchfield and Goenaga (1957a).

In tenacity determinations it is important to realize that the tenacity index is generally related to the level of the initial deposit (Somers and Thomas, 1956) hence comparisons of different formulations should be made at the same surface concentration of active ingredient.

V. EFFECT OF FORMULATION ON FUNGICIDE PERFORMANCE

A. Physical Properties of the Fungicide

Because agricultural fungicides are usually applied in the solid state, either as dusts or suspensions, it is particularly important to know the optimum size and shape of fungicide particles required for biological control. Little consideration has been given to the influence of particle shape on fungicidal activity probably because of the inherent technical difficulties in preparing fractions of differently shaped particles with the same size distribution. Recently, however, Somers and Pring (1967) have shown that precipitated needle-shaped crystals of captan show essentially the same tenacity as irregular, broken particles of the same
In studies with sulfur allotropes, Feichtmeir (1949) reported that for 1-2 µ fractions the carbon disulfide-insoluble form was three times as toxic to *Sclerotinia fructicola* and *Erysiphe graminis hordei* as was carbon disulfide-soluble sulfur. The higher toxicity of the amorphous, carbon disulfide-insoluble form as compared with crystalline sulfur has been attributed to the higher vapor pressure of the former (Feichtmeir, 1952). The importance of particle size in formulating fungicides is, however, clearly recognized and has been the subject of many investigations.

Inevitably, the fungicides applied in the solid state are only sparingly soluble in water and their solubility will increase as the particle size decreases in accord with the classic Ostwald-Freundlich equation and its later modifications—provided, of course, that the particles maintain a reasonably fixed morphology (Fischer, 1962). Similarly, as Burchfield (1960) has pointed out, the rate of solution of the particles and their rate of sublimation are also dependent on the size of the particles. In practice the larger number of particles per unit weight of the fine particles, as compared with the coarse, and their greater surface energy means that they are more available for toxic action and form more tenacious deposits. The superior tenacity of small particles may also be due to the fact that they are more deformable than large particles and hence stick more firmly to each other and to the underlying plant surface (Gregg, 1951).

Some of the earliest work on particle size was carried out with the sulfur fungicides; Thatcher and Streeter (1925) considered that the fineness of sulfur might well be the controlling factor governing its adherence to foliage. The most important of the early studies is that by Wilcoxen and McCallan (1931) who showed that as the particle size of sulfur dusts was decreased so both the toxicity to conidia of *S. fructicola*, and tenacity of deposits on different types of leaf surface increased. These workers gave a clear illustration of the effect of particle size on coverage: the toxicity of three sulfur dusts of different mean particle diameter differed significantly when compared on an equal weight basis, but there was no difference in toxicity when the dusts were compared on the basis of an equal number of particles per unit area of treated slide. The inverse relationship between particle size and fungicidal effectiveness was also established by Hamilton et al. (1943) with sprays of wettable sulfur formulations in orchard trials against apple scab, greenhouse experiments against cedar-apple rust, and the *in vitro* spore germination of *S. fructicola*.

A more rigorous experimental technique was adopted by Feichtmeir
(1949), who separated suspensions of sulfur by fractional sedimentation into narrower particle size ranges, namely: 1–2 \( \mu \), 5–6 \( \mu \), 10–11 \( \mu \), and 15–16 \( \mu \) diameter. The ED\(_{50}\) of these fractions, for carbon disulfide-soluble sulfur, against \( S.\ fructicola \) decreased directly as the surface area per unit weight increased. Rabe (1956) assayed six fractions of sulfur, the largest being 3–8 \( \mu \) in diameter, against \( V.\ inaequalis \) and found that the ED\(_{50}\) decreased with particle size only down to the <2 \( \mu \) fraction; reducing the particle diameter below 2 \( \mu \) gave no further increase in toxicity—the surface was now saturated with sulfur. Artificial rain tests showed the <2 \( \mu \) fraction to be the most tenacious.

McNew and Burchfield (1950) separated dichlone into seven closely defined size fractions and found that particles of mean radius 0.45 \( \mu \) were 40 times as effective as those of 21.5 \( \mu \) in protecting tomato foliage from infection by \( A.\ solani \) in greenhouse tests. Although particle size can determine the effectiveness of protectant fungicides it seems to be less important for seed treatments. Burchfield and McNew (1950) treated pea seed with four dichlone dust formulations having average particle radii ranging from 2.3 to 9.5 \( \mu \); there was no significant difference in the percent emergence of seedlings after the seeds were planted in infested soil. Similar results were reported for tetrachlorop-\( \mu \)-benzoquinone, except with very large particles (35 \( \mu \)). Heuberger and Horsfall (1939), however, have reported that the seed-protective value of cuprous oxide increases with diminishing particle size. Discrepancies may be expected in experiments of this type where the fungicide dose per seed is uncontrolled but it is probable that particle size is important for seed protection only insofar as it leads to good adhesion of fungicide to the seed and good dispersibility of the formulation. Mathieu and Henriet (1962), for example, found that thiram dusts of low particle size gave the best adhesion (after shaking) to flax seeds.

Although diminution of particle size leads to increased biological effectiveness, a limit to this process is reached when the surface becomes completely saturated with the fungicide, i.e., at maximum coverage, as has been already noted from the work of Wilcoxon and McCallan (1931) and Rabe (1956). Burchfield and McNew (1950) expressed this relationship quantitatively in the form:

\[
\frac{1}{G} = m \ln \frac{3G}{4 \pi \rho r^3} + q
\]  

(2)

where \( G \) is the ED\(_{50}\) of the fungicide in grams per square centimeter, \( \rho \) its density, \( r \) the mean particle radius (the particles are assumed
to be spherical), and \( m \) and \( q \) constants. This equation states that the amount of material required to control a disease is inversely proportional to the logarithm of the number of particles into which it is subdivided and was found to hold for the control of \( A. \) solani by dichlone. As the particle size is decreased below the limiting value other factors may come into play which adversely affect the biological control. Thus the increase in rate of oxidation, photochemical degradation, solubility, etc. with decreasing particle size may mean that the very small particles are less effective than those in the intermediate range. Feichtmeir (1949) ascribed the poor control of \( Erysiphe polygoni \) by 1–2 \( \mu \) sulfur, as compared with larger size fractions, to the increased rate of sublimation of the smaller particles. Calculations by Burchfield and McNew (1950), based on their dichlone work, gave the value for the radius \( r \) at which 95% disease control is attained with minimum total surface as:

\[
r = \left( \frac{3}{8m'q'\pi} \right)^{1/3} = 4.9 \mu
\]

where the constants are the same as those of Eq. (2). The optimum radius of dichlone calculated from Eq. (3) was only slightly higher than that found experimentally.

Results with copper fungicides conform to the same general pattern. Heuberger and Horsfall (1939) found that the fungitoxicity of cuprous oxide increased as the particle size was decreased; Hamilton et al. (1943) have given similar results for copper ammonium zeolite; Hyre (1942) reported that both the toxicity and tenacity of basic copper carbonate and a basic copper sulfate preparation were inversely proportional to particle size; and Nitsche and Vigel (1958) confirmed in the field that the rain-resistance of cuprous oxide and copper oxychloride increased with decreasing particle size. Fractions of cupric oxide when assayed against \( Alternaria tenuis \) showed no increase in toxicity when the particle diameter was reduced below the <6 \( \mu \) fraction indicating that the saturation limit had been reached with this fraction (Somers and Thomas, 1956). Stellmach (1959) separated copper oxychloride and cuprous oxide into closely defined particle size ranges and showed the fractions of smallest diameter (<1 \( \mu \)) to be the most toxic to \( A. \) tenuis and the most tenacious when subjected to an artificial rain test. However, when the deposits were continuously eluted with water the <1 \( \mu \) fraction of cuprous oxide was less tenacious than the 1–3 \( \mu \) fraction—in opposition to the results with copper
oxychloride—perhaps due to the greater solubility of the smaller particles.

Samples of zineb ground to a finer size showed the expected increase in toxicity to *Stemphylium sarcoicaforme* (Corte et al., 1958). The finer particles did not lose their biological activity at a significantly greater rate than did the original powder.

The biological effectiveness of bordeaux mixture, the gelatinous precipitate formed by copper sulfate and calcium hydroxide, is determined to an appreciable extent by its physical characteristics. Variation in the physical state of its components and in their method of mixing can affect both the tenacity and fungitoxicity of bordeaux mixture, and presumably of the related Burgundy mixture. Somers (1959b) compared seven different methods of preparing bordeaux and found that the addition of concentrated lime paste to dilute copper sulfate gave the most tenacious and fungitoxic spray deposits probably because of the slow rate of crystallization of the hydrogel produced by this method. Wenetz and Krexner (1958) recommended adding solid copper sulfate "snow" to the calcium hydroxide suspension.

On aging, the bordeaux hydrogel is converted to spherocrystals of much lower tenacity (Burchfield and Goenaga, 1957b) and toxicity (Tanaka, 1951). McCallan (1957), however, found no significant difference between the control of *A. solani* on tomato given by 24-hour aged, and fresh 10 : 10 : 100 bordeaux mixture until the plants were subjected to artificial rain when the aged preparation gave markedly lower disease control. Bordeaux mixtures with a much lower proportion of lime age to form microcrystals which are more tenacious than the large spherocrystals (Burchfield and Goenaga, 1957b) and hence give better disease control (McCallan, 1957).

Two fungicides—glyodin (2-heptadecyl-2-imidazoline acetate) and dodine (n-dodecylguanidine acetate)—each possess a hydrophilic ionizing group and a hydrophobic hydrocarbon group in the same molecule. The amphipathic nature of these molecules endows them with surface activity in their own right. Both fungicides, for example, reduce the surface tension of water and can improve the wetting properties of spray formulations. Kretchman and Mitchell (1957) showed that glyodin was comparable to a proprietary surface-active agent in improving the wetting properties of sprays applied to the difficultly wettable broccoli plant.

As clouds of fungicide dusts are discharged, electrification will occur which can influence the retention and adhesion of the deposit on the plant surface. In a recent review Hoskins (1962) considers that the
presence of electrostatic charge on the particles is more likely to affect the adhesion of the dust than its initial deposition, although the latter may be increased by suitable charging devices (Shreeve, 1960). The charge on the particles varies with their size (H. F. Wilson et al., 1944) and it may be surmised—in the absence of experimental evidence—that the nature and distribution of this charge will affect the biological control achieved by the dust.

B. Effect of Supplements

The formulation of a fungicide for field application entails the addition of supplements whose purpose may be to improve the distribution, retention, coverage, and tenacity of the final deposit. Improvement in the physical properties of a dust or spray does not necessarily lead to increased disease control and the influence of these supplements on biological effectiveness is now considered.

1. Diluents

It has been seen that in the formulation of a dust or dispersible powder the active ingredient is extended with a diluent which functions both to dilute and to improve the application properties of the fungicide. These diluents, generally of mineral composition, although considered chemically inert can affect the biological activity of a fungicide by reacting with the fungicide or with any of the other adjuvants. The literature on diluents for fungicides is, however, small compared with that on their use with insecticides—probably because diluents can kill insects if sufficiently abrasive (Somers, 1960).

A clear example of reaction between diluent and fungicide has been given by Daines et al. (1957). Captan decomposes in aqueous solution to form hydrogen and chloride ions and its phytotoxicity to bean (Phaseolus vulgaris L.) was found to be increased by the diluents attapulgite, kaolinite, and talc while magnesium oxide and calcium carbonate reduced leaf damage. Kaolinite, which caused the greatest phytotoxicity, has a much lower cation-exchange capacity than the other diluents and so cannot easily absorb the hydrogen ions released during captan hydrolysis. Attapulgite has the greatest potential for reaction with hydrogen ions but also the greatest capacity to absorb moisture and hence prolong the decomposition of captan. The intermediate properties of talc appear to make it the most suitable diluent for wettable powders of captan. The rate of captan hydrolysis increases rapidly with increasing pH so that the alkaline “safeners” could pos-
sibly reduce the fungitoxicity of captan as well as its phytotoxicity; this effect has not, however, been investigated under field conditions with commercial formulations containing a calcium carbonate "safener" (Lukens, 1965). Burchfield (1960) has reported that the phytotoxicity of 2,4-dichloro-6-(o-chloronitro)-s-triazine which also produced hydrochloric acid on hydrolysis can be reduced with calcium carbonate. In addition he noted that the formulation of dichlone with some attapulgite clays gave reaction products which were phytotoxic. Fillers with highly acidic surfaces are known to be capable of catalytically degrading insecticides (Eaton and McKone, 1960) and a similar effect can be expected with organic fungicides. Ullrich (1964) used the color change of a series of indicator bases to determine the surface acidity, in terms of the pK value, of a number of mineral fillers. Diluents may also act with, or be physically absorbed by, wetting agents and so reduce the latter's surface activity. The surface activity of the nonionic polyoxyethylene nonyl phenols was found to be reduced by some diluents, particularly an acid clay, while dinitrolite had no effect and seemed unable to absorb the wetting agent (Hirotu and Udagawa, 1957).

Ark and Wilson (1956) have shown that pyrophyllites, micronized sulfur, hydrated lime, and calcium and magnesium carbonates are excellent dust carriers for streptomycin, releasing it readily when in contact with water and not impairing its activity after storage. Other diluents such as tale, fuller's earth, and an attapulgite clay bind the streptomycin so that it is no longer available when mixed with water. Assays against Erwinia amylovora suggested that the gradual release of streptomycin with a pyrophyllite was more effective than the more rapid loss given by gypsum (Ark and Thompson, 1959).

Sometimes mixtures of diluents prove more effective than a single filler. A zineb dust with three parts of pyrophyllite to one of tale gave better control of Pseudomonas tabaci than did formulations with either of the fillers alone (Rui, 1963). Diluents can also influence the tenacity of a deposit. Sato and Ishiyama (1961) found that with organomericuric dusts the tenacity decreased in the filler order: calcium hydroxide > tale > diatomaceous earth.

2. Surface-Active Agents

a. Toxicity to Fungi and Plants. It is generally accepted that ionic surface-active compounds can be toxic to microorganisms, acting either by physically disrupting the cell membrane or by inhibiting enzymes located there (Newton, 1960). Cationic surface-active agents have
been used for many years as bactericides and are known to be effective fungicides, particularly against the powdery mildews (Kirby and Frick, 1963), but their value in the field is restricted by high water solubility and phytotoxicity (Furmidge, 1959a,b). In fact, because of their cost, limited wetting-out ability, and phytotoxicity, cationic surface-active agents have little place in fungicide formulation; their use as fungicides is considered elsewhere (Volume II, Chapter 12).

The fungitoxicity of other classes of surface-active agents has been demonstrated in in vitro tests by a number of workers. Kirby and Frick (1953) assayed commercial wetters against V. inaequalis and found the anionic compounds to be much more toxic than the nonionic—as would be expected from the greater charge, and hence reaction potential, of the former. Slight toxicity was also reported for the protective colloid methyl cellulose. In tests against Monilinia fructicola, A. solani, Puccinia coronata, and Puccinia recondita, Forsyth (1964) also found that anionic surface-active agents were more toxic than nonionic although the latter were surprisingly effective against the Puccinia uredospores. The activity of nonionic agents against a range of fungal species has been reported by Steiner (1964). Amphoteric surface-active agents of betaine structure inhibited the germination of Cochliobolus miyabeanus, their toxicity increasing with increasing ability to lower surface tension (Hirota et al., 1958).

In vivo greenhouse tests on barley seedlings have shown that Ergisiphe graminis infection can be controlled by anionic and nonionic surface-active agents (Parmentier, 1956). Similar experiments by Kirby and Frick (1963) corroborated the toxicity of anionic agents to E. graminis but no significant effect was found for two nonionic compounds.

Surface-active agents have been demonstrated, by in vitro assay, to act as synergists for fungicides, possibly through their effect on cell permeability. Thus, sodium dodecyl sulfate at nontoxic concentrations enhanced the activity of phenylmercuric borate and hydroxyquinoline sulfate against Aspergillus niger (Thoest et al., 1955); amphoterie surface-active agents of betaine structure increased the toxicity of phenylmercuric acetate to C. miyabeanus (Hirota et al., 1958); the fungistatic activity of thiram against A. tenuis was improved by two anionic agents (Kalbe and Vogel, 1960); proprietary anionic and nonionic agents gave synergistic action with antifungal drugs against Trichophyton spp. (Enomoto, 1961); and a quaternary ammonium cationic agent synergized the fungitoxicity of phenylmercuric acetate and copper sulfate (Janke and Kraus, 1958).
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The opposite effect—a loss of fungitoxicity following the incorporation of a surface-active agent—has been reported for preservatives when formulated with nonionic emulsifiers (De Navarre, 1957, 1959). The explanation may be that the fungicides are being solubilized by the high concentrations of emulsifiers, for Aoki et al. (1967) have shown that it is the concentration of toxicant in the aqueous phase, not in the micelles of the surface-active agent, that determines antifungal potency. Similarly, the toxicity of zinc in Stemphylium sp. was antagonized by anionic and nonionic agents, especially at high concentrations of the latter (Corte, 1961).

Surface-active agents, particularly ionic compounds, at sufficiently high concentration can injure plant surfaces, probably by disrupting the cell membranes following entry through the cuticle. The phytotoxicity of many proprietary surface-active agents, often of undivulged composition, has been determined in screening trials by Gast and Early (1956) and by Jansen et al. (1961). Nakata and Tsuchida (1961) found that both anionic and nonionic commercial wetting agents could injure lychee leaves, a nonionic isooctyl phenoxypolyethoxy ethanol being the least damaging. Nonionic wetters have also been reported to increase the phytotoxicity of fungicides probably by increasing the penetration, deposition, or distribution of the fungicide (Swales and Williams, 1956; Daines et al., 1957).

The most thorough investigation of phytotoxicity is that by Furmidge (1959a,b) who used only surface-active agents of known chemical structure, generally of high purity. Tests on apple and plum leaves showed that ionic agents could be appreciably phytotoxic even at concentrations of 0.05% but that nonionic materials were almost invariably safe. Furmidge regarded the physical disorganization of cell structure produced by surface-active agents as being governed by their penetration into the leaf and by their degree of adsorption on cell membranes and walls. Increasing the size of the molecule or ion would be expected to decrease the rate of penetration but, in a homologous series, will increase surface activity and hence adsorption. The interaction of these opposing effects will give maximum phytotoxicity with the material having the largest molecule or ion able to penetrate the plant cuticle. The large molecular size of nonionic agents may be as important as their slight chemical reactivity in determining their low phytotoxicity. Recent tests, however, have produced no evidence that either ionic or nonionic surface-active agents affect the permeability of a hydrated leaf cuticle to rapidly or slowly penetrating model compounds (Darlington and Barry, 1955). This may seem paradoxical until it is
realized that Darlington and Barry only used defect-free cuticles while Furmidge clearly regarded the penetration of surface-active agents to proceed via cracks or faults in the leaf cuticle.

b. Effect on Spray Deposition and Tenacity. The addition of a wetting agent to a fungicide spray has often been reported to increase disease control in the field or greenhouse (Eyre and Salmon, 1916; Parmentier, 1966; Swales and Williams, 1966; Forsyth and Jursic, 1961; Palmer and Henneberry, 1961). This is usually interpreted as being a result of improved distribution or deposition of the fungicide rather than of the toxicity of the surface-active agent, for the biological efficiency of a spray deposit is dependent not only on the level of deposit but also on the distribution and coverage achieved (Somers, 1959c). Physical factors in spraying are fully considered elsewhere (Chapter 12) and here it is proposed to discuss only the influence of surface-active agents.

The function of a surface-active agent in a spray formulation depends on the type of application proposed. Low-volume spraying is designed to produce a deposit of discrete droplets without runoff; surface-active agents will tend to lower the contact angle between the plant surface and droplet and so increase its spread. With high-volume spraying the aim is to drench the target surface completely so that a continuous film of liquid remains after the excess fluid has drained off; this spray requires both spreading and wetting properties. Almost all the work with surface-active agents has been confined to their use as wetting agents for high-volume sprays and it is well established that efficient wetting gives a lower deposit, although more uniform, than that from a spray with an intermediate degree of wetting-out (Mollinet et al., 1961). Maximum initial retention occurs at the point of incipient runoff and the level of this deposit decreases with increasing wetting ability of the spray (Martin, 1964).

The value of a wetting agent for runoff sprays has to be appraised for each specific use. When the surface is virtually unwettable, as with leaves of cabbage or banana, the droplets will bounce and roll off the surface is a wetting agent is not incorporated, while with a wettable leaf such as potato the retention will be lowered by the addition of a surface-active agent to the spray fluid. Kearns and Martin (1957) found that the addition of a nonionic wetter to bordeaux mixture increased the deposit on banana leaves by a factor of three. Blackman et al. (1958) reported that the introduction of a wetting agent increased the retention by barley and pea but decreased that of sunflower and white mustard. Thomas and Potter (1966) have shown that
the incorporation of an ionic or nonionic wetter decreased the retention of zineb by leaf surfaces, on some leaves an increased concentration of agent gave dramatic reductions in deposition. These results were obtained with sprays; leaf immersion tests often gave different results depending on the chemical and on the leaf treated.

The most comprehensive treatment of the retention of surface-active agent solutions by leaves has been given by Furmidge (1962b). He has derived a theoretical retention factor that is calculated from the values of the dynamic advancing and receding contact angles of the spray liquid on the solid surfaces and from the surface tension of the spray fluid. Experimental results using a range of leaf types have shown that the retention factor has a real validity in predicting both maximum retention and the levels of retention after runoff. The decrease in retention after runoff was very dependent on the roughness of the leaf surface, there being a much greater loss with smooth than rough leaves. In a later paper Furmidge (1964) reported the wetting ability of some 65 surface-active agents as determined by leaf and slide immersion tests.

Even when the surface is completely wetted, the type and concentration of wetting agent used can profoundly affect the level of runoff deposit. Somers (1957a) found that the retention of Burgundy mixture by leaf surfaces was greater with a nonionic than a cationic wetter. Anionic sulfosuccinate wetters gave a marked reduction in deposit level at concentrations above 0.05%—an effect noted with different types of leaf surface and also with a noncolloidal cuprous oxide suspension. To a lesser extent another anionic agent, sodium dodecyl sulfate, gave the same effect (Somers, 1957b). These results have recently been confirmed by Thomas and Potter (1966) but the explanation of this phenomenon is still uncertain. It could be due to the ability of the wetting agent, above its critical micelle concentration, to strongly deflocculate the fungicide particles and so reduce their adhesion to the leaf surface (Molliet et al., 1961), or the intense wetting-out of the sulfosuccinates may produce an excessively thin and streaky drainage film (Stanley and Radley, 1960).

The surface-active agents that are most efficient at wetting a surface can be expected to be equally effective in removing the fungicide deposit after rainfall for they will dissolve in the superficial water and redisperse the fungicide (Fajaus and Martin, 1937; Somers, 1956). Surface-active agents are essential ingredients of fungicide formulations but the deleterious effect they exert on tenacity can be overcome, to some extent, by the incorporation of stickers as is discussed below.
3. Stickers

These supplements are added to sprays or, less commonly, dusts to improve the tenacity of the deposits against the combined weathering action of sun, wind, and rain. It has already been noted (Section IV,E) that for the laboratory evaluation of tenacity rain is regarded as the most important weathering factor. Since the introduction of protectant fungicides in the last century a very large number of possible stickers have been incorporated in sprays; their use being particularly stimulated as bordeaux mixture has been replaced by the less tenacious "fixed copper" and dithiocarbamates. In general, stickers have been chosen empirically from materials such as plant flours and gums, casein derivatives, clays, natural resins, and oil emulsions that are general purpose adhesives. Only in recent years have more specific formulations been developed. An extensive review of the early literature has been made by Harry (1948). It is unfortunate that many of these papers are concerned with proprietary products of unspecified composition and there has often been little consideration of the effects of the supplements on the biological efficiency of the fungicides.

Of the early papers the most important is that by Fajans and Martin (1937); in this work the effect of supplements on the initial retention and on the tenacity of spray deposits was clearly distinguished. A petroleum oil emulsion and those supplements which gave insoluble residues, e.g., gelatin and lime casein, gave improved tenacity of cuprous oxide and iodide and the effective stickers were found to improve control of Phytophthora infestans in a field trial. Later work (Fajans and Martin, 1938) showed cottonseed oil to be a better sticker than refined petroleum oil, possibly because of the semidrying character of the former; high concentrations of the emulsifier, sulfite lye, reduced the adhesive properties of the oils. E. E. Wilson (1942) found that petroleum oil emulsions reduced the in vitro fungitoxicity of bordeaux mixture but in the field the increase in tenacity gave increased control of Coryneum Beijerinckii on peach.

Many workers have recommended such proteinaceous materials as flours and casein derivatives as stickers—their adhesive value is probably related to the gelatinous nature of the spray deposit formed. However, Heuberger and Horsfall (1942) established that these materials reduce the fungitoxicity of copper compounds; copper–protein complexes are formed and the concentration of toxic cupric ion thereby reduced. In field trials of a number of copper sprays against P. infestans
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on potato Large (1945) found that only a cuprous oxide-bentonite formulation was comparable to the invincible bordeaux mixture. The comprehensive testing by Harry (1948) of 100 supplements with a basic copper sulfate yielded disappointing results. Although many of the compounds increased the tenacity of the fungicide in both laboratory and field trials, none gave any real improvement in disease control. Garman (1950), however, reported that soybean oil, and bentonite-skim milk increased the tenacity and biological effectiveness of lead arsenate-sulfur spray deposits.

Polyethylene polysulfides have been proposed as stickers which are also fungicides in their own right (Stewart and Standen, 1946). They have been found to render sulfur deposits very resistant to leaching by water (Green, 1950) and to increase the biological effectiveness of captan, as assayed against Helminthosporium oryzae (Chaves, 1954) and Cercospora nicotianae (Davide and Orillo, 1959). Polyethylene polysulfide has also been proposed as a sticker for zineb or captan applied to control coffee rust (Reddy and Davide, 1959; Valdez et al., 1959). The preparation of polymeric polysulfides has been described in a patent by Fettes and Berenbaum (1957).

Oils have often been recommended to improve the adhesion of dusts. Takita and Fukunaga (1954) found nondrying vegetable oils to be more effective than mineral oils but advised a mixture of the two types as a practical treatment; the oil content of the dusts should not exceed 2%.

Somers (1956) tested nearly 50 representative stickers with a cupric oxide spray and selected rubber and polyvinyl acetate latices, coumarone resin, and a linseed oil emulsion as the most promising supplements. In a field trial these materials increased the tenacity of the fungicide but little, if any, improved control of P. infestans was observed. In vitro bioassay of the spray deposits against A. tenuis explained these results for with the exception of polyvinyl acetate and chloride all the other supplements markedly reduced the toxicity of the cupric oxide. This loss in toxicity could be due both to the stickers physically sealing-off the fungicide (Green and Goldsworthy, 1937) and to the observed flocculation of the cupric oxide suspension giving larger, and hence less toxic, particles. Polyvinyl acetate latex is an effective dispersing agent for copper fungicides and further work showed its potential as a sticker which does not inhibit toxicity, but its value in the field is still unproved (Somers, 1959a). However, phenylmercuric chloride formulated as a point in polyvinyl acetate has been found to be effective against stem cankers of Gloeosporium peren-
nanae (Sharples and Somers, 1959) and a patented mixed salt fungicide has incorporated this synthetic latex (Fahlberg-List, 1959).

Although oil emulsions may lower the toxicity of a fungicide, this effect is often compensated for by the increased tenacity of the deposit. Thus improved biological control has been reported for a summer white-oil emulsion with bordeaux mixture (Glasscock and English, 1956) and for a linseed oil emulsion with phenymercuric chloride (Sharples and Somers, 1959). Recently J. D. Wilson and Hedden (1963) have shown that the tenacity of a copper fungicide on sugar beet foliage increased with increasing concentration and viscosity of the added oil.

Formulations have been introduced by Hartley and Howes (1961, 1963) for ultra-low-volume spraying in which the fungicide is dispersed in a liquid amine-stearate mixture; the amine evaporates off leaving a monolayer of the fatty acid coating the spray droplet. The deposits formed are reported to retain their full biological activity and certainly possess a very high degree of rain resistance. Unfortunately the formulations cannot be diluted down to the more conventional higher volumes. Mixtures of magnesium salts of fatty acids with esters or ethers of polyglycols have been proposed as stickers for copper compounds (Telle and Grewe, 1957) and compositions based on butadiene-styrene copolymers have been patented as supplements which retard evaporation in addition to conferring increased adhesion and toxicity to the fungicide (Yowell and Gleason, 1957; Flueck et al., 1962). The adhesive properties of the polybutene emulsions, well-known as acaricides and powdery mildew fungicides (J. F. Bradbury and Fisher, 1963), may well serve as a basis for future stickers.

The stickers described above act, as their name implies, by improving the adherence of the fungicide deposit to the plant surface. A different approach has been suggested by McIntosh (1963, 1966) in which the deposits are made water-repellent to improve their rainfastness. The supplements used have included paraffin wax emulsions and compounds such as sodium thiobenzoate and sodium methyl silicinate that are converted to insoluble residues on drying. McIntosh found that all these additives increased both the tenacity and water-repellency (determined by contact-angle measurements) or copper oxychloride deposits and in some instances improved control of *P. infestans* was observed; however, the silicone deposits were brittle and tended to peel from the leaf. A similar principle is the basis of a patent in which oxidized polyethylene waxes are applied as dilute emulsions to give water resistant deposits (Cosby and Darley, 1961).
It must be concluded that in spite of extensive investigations stickers have not conclusively proved their value in the field. Many supplements can increase the tenacity of a fungicide deposit, but the result is often that little or no improvement in biological efficiency occurs. There are many reasons for this phenomenon: the sticker may interact chemically with the fungicide, as do the proteins with copper; it may physically seal off the fungicide and agglomerate the particles, as do the oil emulsions; and it may reduce the redistribution and the rate of solution of the fungicide so that a toxic concentration is not maintained in the film of moisture covering the plant surface. Finally, the rapid growth of new foliage requiring further spraying will reduce the economic value of a sticker. It would seem that stickers will only be needed for specific formulations of protectant fungicides when high tenacity is of overriding importance.

4. Miscellaneous Supplements

The formulation of chemotherapeutants, which act within the plant, should be designed to increase their rate of absorption and translocation rather than their deposition or tenacity (Dimond, 1962). Surface-active agents, particularly of the nonionic type, are known to increase these rates for growth regulators and herbicides (Carrier and Dybing, 1959; Somers, 1960) and recently Ford et al. (1965) have found that a proprietary wetter at a nonfungicidal, nonphytotoxic concentration improved the eradicant action of streptomycin on cucumber powdery mildew, presumably by the same mechanism. R. A. Gray (1956), however, reported that wetting agents had little influence on the absorption of streptomycin by bean leaves but found glycerol to be extremely effective in increasing antibiotic absorption; to a lesser extent sorbitol, diethylene glycol, and other polyhydroxy alcohols were also effective. Formulation with glycerol increased the control of common bacterial blight of beans given by streptomycin sprays. Hygroscopic materials such as glycerol will reduce the evaporation rate of the antibiotic solution and so prolong the period available for penetration, for it is known that absorption occurs readily only while the spray remains wet (Mitchell et al., 1960); they do not necessarily need to increase the rate of penetration. It is also possible that glycerol may modify the permeability of the leaf cuticle (Sivadjian, 1956). Rich (1956) found that the chemotherapeutic activity of zineb against Cladosporium cucumerinum was significantly improved by the addition of glycerol.

Supplements can be added to high-volume sprays to increase the fungicide deposit retained by the plant surface after runoff. Oil emul-
sions are the most effective deposit-builders; they react with the fungusicide suspension to give oil-wetted agglomerates which are preferentially retained by the sprayed surface (Fajans and Martin, 1938; Somers, 1956). The flocculation may result from chemical reaction between emulsifier and solid (Fajans and Martin, 1938), but the increase of free surface area alone on the addition of the solid could so denude the emulsion of surface-active agent that the emulsion breaks. Two factors limit the usefulness of combined oil emulsion-suspensions: the reduced fungitoxicity of the oily agglomerates already noted, and the practical difficulty of application. Insoluble metallic soaps, formed in situ, also act as deposit-builders; they are adsorbed on the fungicide, nonpolar groups outward, to give oily particles which are preferentially retained (Ebeling, 1963). E. Evans et al. (1962b) noted that repeated spraying of bordeaux mixture alternated with a simulated rain weathering gave a continuous buildup of the weathered residue. This pattern was not followed with copper oxychloride applications unless an aluminium hydroxide gel was added to the spray. Apparently residue buildup is related to the colloidal character of the irreversible gels formed by bordeaux mixture and aluminium hydroxide. Field trials against potato blight and black pod disease of cacao showed that aluminium hydroxide could improve the efficiency of copper oxychloride to the level of bordeaux mixture (E. Evans et al., 1962a).

With the increasing development of aerial spraying the need has arisen for supplements which will reduce the evaporation of the spray droplets. The problem is most acute with sprays of water-dispersible powders—these may arrive at the plant surface as a nonadhesive dust. Amine-stearates have proved to be very successful antievaporants; when added to fungicide suspensions evaporation under simulated tropical conditions was reduced to such an extent that drops as small as 80 µ showed no detectable evaporation (Hartley and Howes, 1961; Amsden, 1962).

VI. CONCLUSIONS

It is apparent that the inherent activity of a fungicide can be greatly improved—or, for that matter, diminished—by the formulation techniques adopted. The nature of the formulation should be determined by the proposed use of the fungicide. Thus, high-volume sprays should completely wet-out their target while for low-volume sprays spreading, not wetting-out, is important. A protectant fungicide deposit must be tenacious while a chemotherapeutant must be absorbed by the plant.
surface. A soil fungicide may be required to be released slowly at a controlled rate from a granular formulation while a seed protectant should adhere well to the seed coat. Sometimes there is a conflict between these requirements as when a tenacious deposit is required from a high-volume spray—here the formulation should be that with the minimum possible wetting ability—or when an emulsifier gives intense wetting-out and hence low deposition.

The nature of the biological substrate is also an important factor in formulation: a spray intended for potato foliage does not require the concentration of wetting agent as does that for banana; a fungicide drench for a coarse soil does not require the penetrating powers of one for a finely textured soil.

The key to effective formulation is therefore an exact knowledge of the biological requirements of the fungicide. Herein lies the manufacturer's greatest problem for the biological specifications are much more difficult to define than are the physicochemical ones governing dispersion, suspension, and storage. The result is that physical specifications for such properties as high stability of emulsions or suspensions may be quite unrelated to the biological efficiency of these products (Duyfjes, 1958). Only when a clearer definition of biological function is given will it be possible to achieve more effective formulation.

REFERENCES


6. FORMULATION


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CHAPTER 7

Application and Use of Soil- and Seed-Treatment Fungicides*

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I. Introduction

Applications of chemicals to the soil for pest control date back to the latter part of the nineteenth century. According to Newhall (1955), carbon disulfide (CS₂) was applied to soil about 1877 for the control of grape Phylloxera. Thus initiated the use of chemicals as soil treatments. Some of the principles established for the use of CS₂ in 1877 are

still in use today and are applicable to other fumigant-type soil treat­
ments. Goring (1962) attributed the initiation of fumigation of soil for 
pest control to Kühn in 1881. Various chemicals have been used for soil 
treatment, and each has a distinguished history and a fascinating 
story of development. Inclusion of such information about each chemi­
cal is beyond the scope of this chapter, inasmuch as several complete 
reviews are available in which the history, development, and various 
other facets of the field of soil fungicides are described (Newhall, 1955; 
Kendrick and Zentmeyer, 1957; Bollen, 1961; Vaartaja, 1964; Goring, 

The history and development of seed-treatment fungicides is replete 
with renowned names of plant pathologists. According to Miles (1946), 
Tillet in 1775 made the first recommendation for the chemical control 
of wheat bunt; it called for washing the seed with water, applying lye, 
and sprinkling it with lime. However, the first recorded chemical con­
trol of wheat bunt came about as a result of the wrecking of a shipload 
of wheat near Bristol, England, about 1670. Seed from the ship's cargo 
was salvaged and planted by farmers, and a crop free from bunt was 
produced. This then constituted the first chemical seed treatment for 
plant disease control, the use of salt brine, a practice that endured for 
100 years (Bayer-Semesan Company, 1939).

The classic work of Prévost (1807) established the basis for future 
chemical control of plant disease by seed treatment. Prévost's observa­
tion that copper sulfate was toxic to bunt spores led to the adoption 
and widespread use of the copper sulfate dip, a wet treatment. Toward 
the end of the nineteenth century, Geuther (1895) in Germany and 
Bolley (1897) in the United States recommended formaldehyde, also a 
seed treatment, as a seed-treatment chemical for bunt control. Formal­
dehyde and copper sulfate treatments were cumbersome to use, and at 
times reduced the germinability of the seed drastically.

According to Leukel (1936), the first intelligent use of copper sulfate 
dates back to 1761 (Schulthess, 1761). In spite of Prévost's (1807) 
experiments that demonstrated the activity of copper sulfate against 
wheat bunt spores, this chemical was not widely used for bunt control 
until Kühn (1873) showed its effectiveness when applied to seed. 

All seed applications of chemicals required wetting the seed, includ­
ing brining the seed, copper sulfate, and formaldehyde treatments. In 
addition, the hot-water treatment devised by Jensen (1888) involved 
the complete wetting of the seed. All of these treatments had a pro­
ounced tendency to reduce seed germination. To offset some of the 
disadvantages of the wet treatments, the use of dust applications of
copper carbonate was stressed after the effectiveness of this chemical against bunt was established by the work of Darnell-Smith (1915, 1917) and Darnell-Smith and Ross (1919) in Australia.

The widespread use of dust-type treatments exposed certain shortcomings of dusts, such as excessive dustiness in the immediate vicinity of the treater and a tendency for the chemical to "dust-off" the treated seed during subsequent handling. In an effort to overcome some of the objections to dust treatment, the slurry method was introduced by the du Pont Company in 1946 (Miles, 1946). During the 19 years since the development of the slurry method and slurry-type treating machinery, the seed-treating industry has made giant strides both from the standpoint of the chemicals available for application and in the machines that apply the chemicals to seeds.

Miles (1946) observed: "The growers of Tillet's time would look with amazement on modern day seed treaters (the slurry machine of 1946) with a 300-bushel-per-hour capacity. There are indications just coming over the horizon, however, that even within the next decade or two we may look back with a tolerant smile at the inadequacy and inefficiency of present day seed-treating chemicals and methods." Miles's prophecy was correct; the machines used to apply the new seed-treatment chemicals of 1966 are indeed a far cry from the slurry machine of 1946, both from the standpoint of increased capacity and efficiency. Nevertheless, it is altogether fitting that tribute be paid to the slurry machine, the development of which foreshadowed the excellent seed-treating machines of today.

This discussion of the products of a technological evolution both in chemical fungicides and in the machinery for their application as treatments of soil and seed will be approached mainly from the standpoint of the treatment method; to a lesser degree the discussion will involve also the chemical and a representative crop for which the method and chemical might be used.

II. SOIL TREATMENT

A. Fumigation

Fumigation for the control of soil-borne pests has a relatively short history, dating back about 80 years. According to Goring (1962), the intensive research that has been done only during the last 50 years has been reviewed frequently in some 26 publications. In the opinion of many researchers, the true soil fumigants are the highly volatile chemi-
cals such as carbon disulfide, chloropicrin, methyl bromide, etc. Methods of application vary according to the chemical used and the area of soil to be treated. Goring lists several types of application that have been employed with soil fumigants. Examples of these and certain other types of application will be discussed.

1. **Trench Method**

   The trench method of applying soil fumigants is simple, requiring only a glass jar fitted with a lid in which holes on opposite edges have been punched (Page, 1963). The fumigant chemical is placed in the jar and poured into the bottom of trenches 6 to 8 inches deep in the area of soil to be treated. The trenches are spaced about 9 inches apart and each one is closed as soon as the chemical has been applied, after which the next trench in line is opened.

   A modification of the trench method is the application of chemicals at the points of line intersection in a grid design established on the soil surface. The chemical is poured into holes in the soil through a funnel to the desired depth (Page, 1963). Some unpleasantness from the chemicals applied might be experienced by the operator. A slight breeze during the application period might tend to remove some of the volatiles from the vicinity of the operator.

2. **Multiple-Point Applications**

   Hand-operated applicators have been designed for single-point applications. One such device consists of the following parts: a reservoir tank, depth gauge, injection pump, delivery tube, and a metering device to vary the amount of material applied. The applicator is held by the T-shaped handle and pushed into the soil to the collar at the base, the depth gauge. The chemical is then ejected by the pump through the delivery tube into the soil. The pattern of application is established on the surface by a grid system with intersecting lines on 6 × 6- or 9 × 9-inch centers. The amount of material applied in milliliters per injection determines gallonage applied per acre. For example, 1 ml injected in a 6 × 6-inch grid will apply 46 gal per acre while the same amount injected on 9 × 9-inch centers will apply only 20 gal (Page, 1963). The maximum spacing allowed for effective control of soil-borne pests depends largely on the chemical used, the dosage applied, the depth of application, and the soil environment (Goring, 1962).

   Methods of applying soil fumigants are in reality modifications of the single-point application. Single-point applications are not generally adapted to field control but find a place in greenhouse operations where
individual containers of soil for individual plants must be treated. In addition, field testing of single-point applications permits the establishment of maximum spacing for multiple-point applications and also for the treatment of individual plants such as trees or shrubs.

Single- and multiple-line application of fumigants permits the more or less continuous treatment of large areas of soil. Single- and multiple-line systems consist of similar basic components that include a reservoir tank, pump, bypass valves, pressure regulator, strainer, release valve, pressure gauge, and tubing to carry the fumigant to the base of the chisel or plow. Multiple lines can be established from the single line through use of T-connectors, a manifold, or gang-type pump that meter the fumigant to the individual lines (Page, 1963). Blade and sweep applicators are established in a manner similar to multiple-line applicators, which indeed they are.

A blade applicator consists of a blade fitted with fan-type spray nozzles that are recessed in and beneath the blade that is drawn through the soil at the desired depth for application of the fumigant (Page, 1963). As the soil passes over the blade it is sprayed with the fumigant chemical; thus a more or less continuous band of treated soil is established. This type of application enhances lateral dispersion of the fumigant.

Sweeps function in a similar manner but each spray nozzle is separated and housed within the body of the sweep. Viewed from above, each sweep is triangular and fitted with a fan nozzle at a point 3 inches from the point of the sweep (Page, 1963). With proper spacing of the sweeps, the resulting band of treated soil is similar to that obtained with the blade applicator. Sweeps have the advantage that they generally clear themselves of most plant debris in the soil, while blade applicators tend to accumulate debris.

Systems without pumps are also used where the reservoir tank supplies the fumigant to the chisel or plow by gravity (Newhall and Lear, 1948). Such a system is adaptable to single or multiple lines, depending on the implements used for working the soil. Tractor-drawn equipment usually will accommodate a multiple-line system, while horse-drawn or small tractor equipment is adapted only to single lines (Todd and Ferguson, 1956).

Emulsifiable chemicals that decompose in the soil to produce volatile products toxic to fungi can be dispensed either with single or multiple lines, or by gravity; they are ideal for open trench-type applications.

Application of the fumigant in the same row where seed will be planted later is practical and more economical than treating the entire
soil area (Shurtleff et al., 1957). Care must be exercised to mark the treated rows so that planting at the later date can be done in treated soil.

Once the fumigant chemical is injected into the soil, some form of surface seal usually is attempted. Single- and multiple-point applications can be sealed by stepping on the point of application, when the hand-operated injector is used. Tractor-drawn equipment that applies the fumigant is often followed by a wooden float, harrow, culti-packer, or some other piece of equipment that will close the soil over the treated area.

Granules of other porous material impregnated with fumigant-type chemicals can be used where other equipment is not available or for specific purposes (Vaartaja, 1964). Almost any fertilizer applicator designed to apply granular fertilizer beneath the soil surface will be suitable for this type application. The granules are placed to the desired depth and then as with other methods of applying fumigants the soil should be closed over the cuts made by the application equipment.

3. Gaseous Fumigants

Certain highly volatile gases, such as methyl bromide, are employed for soil fumigation. Fumigants of this type are packaged in small or large containers appropriate for various fumigation practices. Small batches of soil can be treated in piles, drums, greenhouse benches, or other containers. Newhall and Lear (1948) outline the procedure for treating small batches of soil with various fumigants, and suggest that the soil temperature should be above 50°F when methyl bromide is used. For best results the soil moisture should be favorable for seed germination.

Directions for treating small areas of soil in the field, home garden, or lawn area point out that the plastic cover should be kept above the soil surface to allow free circulation of the gas applied (Hawks et al., 1961; Shurtleff et al., 1957). Straw-filled bags, pots, crumpled fertilizer bags, etc., can be used as cover supports. In making the application, tubing from the applicator can of methyl bromide is fastened securely to an evaporator pan located beneath the plastic cover. Each 1-lb can of methyl bromide is sufficient to treat 100 sq ft of soil surface area.

Large field-size areas can be treated with methyl bromide or other gaseous fumigants by means of tractor-mounted equipment. Large bottles of compressed fumigant are attached to the tractor, and lines from these are attached to pressure-regulating valves. Lines from the valves are attached to the chisel for injection into the soil. For gaseous-
type fumigants that are not compressed, a similar type arrangement employing a pump run from the power takeoff of the tractor, can be used (Sharvelle, 1961).

Tractor-mounted equipment used for gaseous soil fumigation also might include attachments to lay a plastic ground cover, and to move soil over the edge of this cover to make an almost gastight seal (Page, 1963; Shurtleff et al., 1957).

B. Surface Applications

The idea of fungicide applications to soil might suggest to the layman the indiscriminate broadcasting of chemicals to the soil surface. Such an image is incorrect and definite reasons dictate the manner of application. Soil-surface fungicide applications are basically of two types, those that are left undisturbed after application and those that are worked into the soil.

1. Undisturbed Surface Applications

The control of several plant pathogens can be achieved by applying a fungicide to the soil surface and leaving the surface undisturbed. Depending on the size of the surface area to be treated, equipment varies considerably. Small plots with only a few square feet of surface can be treated with wettable powders suspended in water and sprinkled onto the soil as a drench, or they may be applied as dusts through a hand-operated duster. Knapsack dusters and sprayers are practical for treating small plots and may be used for sizes up to a few acres.

Several other methods of applying fungicides to the soil surface are employed for both small and large plots. For example, Sprague (1961) mixed PCNB (pentachloronitrobenzene) with a pelleted fertilizer and applied the material with a fertilizer-sprayer for wheat snow mold control. Small spreaders of the type used for home lawn fertilization can be used to apply fungicides to small plots, with a similar mix of fungicide and fertilizer. The amount of fungicide and fertilizer should, of course, be less than phytotoxic doses. Small amounts of fungicide powders are difficult to mix uniformly with pelleted fertilizer. Sprague (1961) observed that many growers prefer to use a fungicide talc mix and apply the combination by airplane, rather than attempt mixing the fungicide with pellets to be applied with a spreader.

Aerial application of fertilizer, mixed with a mercury fungicide for wheat snow mold control, has been used and found quite satisfactory (McKay and Raeder, 1953). Not only was the fertilizer the best carrier
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for the fungicide, but it provided 20 lb per acre of nitrogen fertilization. Hexachlorobenzene (HCB) applied by airplane controlled dwarf bunt (Tilletia controversa Kuhn) in naturally infested fields near Kalispell, Montana (Purdy, 1963).

Almost all aerial applications of pesticides are made for insect control or, at best, for control of plant diseases that attack the above-ground parts of plants. Such applications usually are not made directly to the soil. The two cases cited above where aerial applications of fungicides have provided disease control the applications were aimed for the soil; obviously, however, some deposit on the growing plants was inevitable. But disease control was provided by the fungicide placed on the soil surface.

2. Fungicides Applied to the Surface and Worked into the Soil

Fungicides applied to the soil surface are later intimately mixed with the soil to a prescribed depth. This method has been used to control several diseases. The work of Menzies (1957) may best typify this approach.

Menzies (1957) applied PCNB to the soil surface at various rates per acre. He investigated the control of Rhizoctonia stem canker and tuber sclerotia on potatoes after mixing the fungicide with the upper few inches of the soil. He investigated different methods of mixing and obtained best results in plots where the soil and fungicide were mixed by a rotary-type tillage or by plowing. Menzies found the tilling operation preferable since the differential control obtained was not significantly less to warrant the increased cost of rotary tillage. Control of stem canker was obtained at 10 to 20 lb per acre of PCNB, but control of the tuber sclerotial stage and Streptomyces scab required 40 to 50 lb. For commercial operations, the fungicide can be applied to the soil surface by mechanical spreaders or similar equipment.

Applications of large amounts of sulfur to the soil for Streptomyces scab control typifies yet another soil application where the fungicide is worked into the soil. Astonishingly high rates of sulfur were needed to alter the pH of soils, according to Hooker and Kent (1950). Broadcast applications of 2000 lb per acre were needed to produce a certain alteration of pH in two types of soil, but 6000 lb were needed to produce similar effects in another soil type. Control of potato scab is obtained in soil when the pH value is near 5.0. Broadcasting of the sulfur was followed by tilling to mix the sulfur with the 4-inch surface layer of soil of the plot area treated. While a certain degree of scab con-
trol is obtained from sulfur applications, there has been little commercial acceptance of this method (Walker, 1952).

3. In-the-Row Applications

Several factors have prompted the development of in-the-row type of fungicide application. Where crop seed is planted in rows, broadcasting places the fungicide over the site of the row as well as between the row. The inefficiency of such applications and the desire to attain higher levels of disease control stimulated development of in-the-row applications. Accurate placement of fungicidal chemicals at the site where maximum protection might be obtained also increased the desire for the development of such applications.

Perhaps one of the first in-the-row applications was that of formaldehyde for the control of onion smut, *Urocystis colchici* (Schlecht.) Rab. (Walker, 1947). Application equipment was simple and consisted of a small tank containing 1 to 2 gal of a formaldehyde solution (1 pint of 37-40% formaldehyde to 16 gal water). The tank outlet at the bottom of the tank was attached through a cutoff valve to a pipe attached behind the seed spout. Formaldehyde solution was dripped into the open row after the seed was dropped and before the row was closed. The per acre application rate of the formaldehyde solution was 200 gal. Effectiveness of this control measure was demonstrated by the 545 bushels of onion per acre produced on treated plots compared with only 200 bushels on untreated ones. Adaptation of this gravity-fed system was made for use with tractor-drawn seeders.

Other types of in-the-row treatments have been developed including dusts applied directly into the row or through the drill box; sprays of suspensions of wettable powders or solutions of fungicides; granules from special applicators or through the drill box; and fungicides in transplant water.

a. In-the-Row Dust Applications. In-the-row dust applications might be considered the forerunner of other in-the-row application methods that employ tractor-mounted application equipment. An example of dusts applied in the row at planting is found in the results of experiments for dwarf bunt control as reported by Holton and Jackson (1952). A 4-row seeder was used in their experiments to which a duster powered with a gasoline engine was attached. The dust machine consisted of a hopper containing the dust and sufficient agitation to keep the dust flowable and free from packing; an auger to move the dust to the discharge opening; and a fan to develop the desired air velocity. Dust
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fell into the airstream created by the fan and was forcibly moved to the discharge end of the delivery tube. The delivery tube, attached to the seed spout, directed the dust stream into the opened row behind the point where the seed was dropped. Closing attachments were located immediately behind the dust delivery tube and closed the row while dust was swirling and mixing with the closing soil.

Holton and Jackson (1952) reported significant control of dwarf bunt by one fungicide containing hexachlorobenzene (HCB), and severe stand reductions from mercurial fungicides tested. They point out at least one prominent problem associated with in-the-row dust applications; namely, accurate control of the rate of application. In addition, their experience showed that dusts applied by power dusters often create unfavorable working conditions around the equipment. Winds during the time of application carry considerable amounts of the dust away from the desired site of application and reduce the effectiveness of the treatment.

Rotating drum-type dusters have been employed for modern day in-the-row dust applications for cotton disease control. Experiments conducted in New Mexico showed that dusts applied in the row at planting by powered dusters gave better yields of lint than planter-box applications (Maier and Staffeldt, 1963). Application rates of 15 to 20 lb per acre caused considerable drying of the soil, and these rates require more elaborate equipment than lower rates of application.

Various types of modifications have been developed to increase the efficiency of in-the-row dust applications. Multiple outlets for dust dispersion in the row permits a better distribution of the dust in the treated area. Instead of the exposed outlet of older machines, such as was used by Holton and Jackson (1952), the discharge end of the delivery tube can be designed with a cover to reduce the loss of dust in the air and to keep it in the soil where it will be most effective.

Some other desirable features for efficient dusters are high air velocity in the air hoses to keep the dust moving, reduced to low air velocity at the discharge end of the delivery tube to reduce losses due to drift. There are advantages to dust applications, one of which is that only the fungicide formulation need be moved during planting. In addition, the duster can be used for other purposes later as the crop develops. Among disadvantages, as noted previously, is the fact that high rates of application dry the soil, and that where soil moisture may be limited some troubles with stand development may be experienced.

b. Planter- or Drill-Box Applications. Fungicides formulated as dusts or wettable powders can be applied simultaneously with the seed when
the two are mixed in the seed hopper or planter box. Seed and fungicide fall together through the seed spout of the seeder and are placed together in the soil, thus affording good protection from certain pathogens. However, some problems associated with this type of application make it undesirable, while certain advantages tend to offset the disadvantages. Fungicide formulations designed for soil treatment can be used for planter-box applications, but certain formulations originally made for seed treatment purposes cannot be safely used in the planter box. Seed size also influences the desirability of applications of this type; small-seeded crops are less readily used with planter-box applications than large-seeded ones. A large-seeded crop such as cotton seems to be ideally suited for planter-box applications, particularly fuzzy or reginned seed. Fungicide dusts tend to stick to this type of seed more readily than to acid-delinted seed. Fungicide dusts tend to settle to the bottom of the planter box when acid-delinted seed is used (Maier and Staffeldt, 1963).

Additional drawbacks of planter-box applications are that the method is not suited when the planting depth is more than 1½ inches. The depth of planting is related to distribution of the fungicide which is usually less than uniform even when the seed is placed less than 1½ inches deep. The amount of fungicides needed to protect cotton seedlings from soil-borne fungi is about 3 to 5 lb per acre, a rate that might delay germination when the soil is dry (Maier and Staffeldt, 1963). Perhaps the greatest advantage is that no additional equipment is needed; only the planter, seed, and fungicide.

Less protection was obtained in New Mexico from planter-box applications than by in-the-row dust or spray applications with the same fungicides (Maier and Staffeldt, 1963). These results notwithstanding, many cotton growers in California have adopted the planter-box application as a standard practice (Leach, 1965). In California there are nearly 500,000 acres of cotton treated by the planter-box method (Leach, 1965). The standard treatment is 10 lb of dust containing 10% captan and 10% PCNB mixed with 100 lb of cottonseed and planted at the rate of 30 to 40 lb per acre. Good control of *Rhizoctonia solani* Kühn is obtained by this treatment. Amounts of fungicide mixed with seed vary with the seeding rate; higher rates of seeding require higher rates of fungicide in the planter box (Garrett and Goolsby, 1964).

c. In-the-row Spray Applications. In-the-row spray treatment began in California during the mid-1940's and was first reported in 1947 (Leach and Snyder, 1947). From this beginning, which gave rather poor control of certain bean diseases, the spray-type in-the-row treatment
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has developed into the most effective, but perhaps not the most widely used method of treatment for cotton-seedling disease control. In addition, in-the-row sprays have also controlled seedling diseases of sugar beets in greenhouse experiments (Hille and Leach, 1952).

In-the-row spray treatments have been developed and used mostly for the control of seedling diseases of cotton and sugar beet. NABAM reacted with zinc sulfate was the first commercially used spray fungicide for in-the-row treatment control of cotton seedling diseases (Leach, 1965). From a start of about 14,000 acres in 1954 the acreage treated by this method in 1964 has increased to almost 100,000. Additional greenhouse testing of in-the-row applications was carried out in Texas, where Ranney and Bird (1956) reported that captan-PCNB-zineb gave best results. Bird et al. (1957) reported that the mixing of fungicide with the covering soil during seeding operations reduced the incidence of seedling diseases in cotton. However, results suggested that additional evaluation of in-the-row sprays was needed to definitely establish the method and to locate the placement where fungicides were most effective.

Equipment used for in-the-row spray applications is relatively simple and consists of: a reservoir tank provided with an agitator in which the fungicide is suspended or diluted in water; a pump of some type run either from the power takeoff of the tractor or by a gasoline engine; a pressure gauge and regulator, and a bypass line return to the tank. A control valve for turning the system on and off is located beyond the pressure gauge and is connected with the hoses that lead to the distribution manifold. A manifold is of course not needed for single-line operations but is an essential part of multiline systems. The number of rows to be seeded and treated establishes the number of outlets needed in the distribution manifold.

Perhaps one of the most variable aspects of the spray systems used is the location of the nozzles from which the fungicide solution or suspension is applied to the soil. Nozzle placement depends to a certain degree on the crop for which the treatment is designed. For example, two nozzles are commonly used for treating cotton. The front nozzle, a cone type, is located behind the seed tube and in front of the seed press wheel. The second nozzle, a flat fan type, is located behind the seed press wheel between the closing disks and in front of the row press wheel. The second nozzle directs the spray onto the soil as the row is being closed (Luick et al., 1959; Maier and Staffeldt, 1963; Ranney and Hillis, 1958). Nozzle placement in a three-nozzle system for cotton disease control is described and illustrated by Aldred et al. (1961). In-
the-row sprays for potato-disease control use three nozzles: one located in front of furrow opening disks, another behind the seed-piece spout and between closing disks, and one behind the closing disks. The first and third nozzles are flat fan types and the second a cone type.

Spraying systems with single nozzles are also used and generally have a cone nozzle placed between the seed press wheel and the closing disks or closing press wheel.

Cotton seedling diseases are amenable to control by soil treatment utilizing combinations of fungicides. Fields infested with both *Rhizoctonia* and *Pythium* species present problems which at present are kept under control only by combination treatments. PCNB is highly toxic to *Rhizoctonia* but has no effect against *Pythium*. Captan, on the other hand, is toxic to *Pythium* but not to *Rhizoctonia* (Leach, 1965). The best performance of fungicides tested in New Mexico was by the combination of Dexon plus PCNB (Maier and Staffeldt, 1963), while PCNB plus captan was best in Texas (Bird et al., 1957). Seedling diseases of bean that involve *Rhizoctonia* sp. and *Pythium* sp. can also be controlled by PCNB plus captan sprays in the row.

d. Granule Application. In-the-row applications of granules impregnated with fungicides has been used for the control of certain diseases. For example, the *Rhizoctonia* diseases of potato, both the stem canker and sclerotial stages on tuber, can be controlled by applications of PCNB simultaneously with the planting operation. Complete and intimate mixing of the granules with the soil is essential for effective control. Granules are placed by the applicator delivery tubes in front of the row opening tools, behind the seed spout, and behind the row closing attachment. As the soil closes over the row it is mixed with the granules of fungicide.

Single fan-type granule nozzles can be aligned with the row to deliver granules directly into the row. By slightly inclining the fan nozzle, at a 45° angle, granules are distributed from the bottom of the row to the surface, increasing the efficiency of the treatment. Granules in the hopper are kept from packing tightly by some form of agitator that must be turned or operated fast enough to keep the granules flowing but not so fast that they are pulverized. The rate of application is adjusted by opening or closing the orifice of the hopper. Also the rate is dependent on the speed of the tractor and size of granule (Hansen and Meggitt, 1963). Calibration of this type of equipment is simple and involves the catching of granules delivered at the nozzle over a given distance at a certain speed. Recalibration is necessary when the granule size is altered.
Applications in Transplant Water. Although application of fungicide in transplant water is not, in the strictest sense, in-the-row treatment, it is nevertheless a type of application that fits well within this grouping. Plants grown in seed beds and later transplanted into the field by machinery are placed one plant at a time in rows. Certain diseases can be controlled when fungicides are mixed or diluted with the water metered into the dibble hole along with the seedling plant. Club root of crucifers is a disease that can be controlled in this way.

Walker et al. (1944) showed that mercuric chloride added to the transplant water reduced the severity of club root on cabbage and increased the yield significantly. Although this treatment failed to eliminate the disease it had a favorable effect on plant growth and development as reflected in the increased yield. They included several other fungicides in their experiments, and all other materials tested proved to be inferior to mercuric chloride.

4. Soil Drench Applications

Vaartaja (1964) has reviewed some of the literature dealing with this type of soil application. Weekly applications of chloranil at the rate of 35 lb per acre accompanied by an abundance of water reduced postemergence damping-off of coffee and cinchona (Crandall, 1950). The stem rot disease of begonia can be controlled by soil drenching with 1 tablespoon per gallon of captan, zineb, or Semesan, according to Pirone et al. (1960).

Application of soil drenches as a means of seedling disease control finds a place almost exclusively in seed beds or greenhouse-grown crops. Equipment needs are few, including a sprinkle can or small capacity spray equipment. Most commonly the fungicide is diluted with water, and the dilution is applied directly to the soil before or after disease symptoms appear.

Ark and Sibray (1964) reported that a drench of methyl mercury dicyandiamide effectively controlled damping-off in many species of ornamentals seeded in leaf mold soil. Other fungicides have been applied to control seedling disease problems of various crops or plant species, including captan, PCNB, thiram, zineb, etc.

In addition to diseases of seed plants that can be controlled by soil drenches, certain diseases of mushrooms can also be controlled by drenching the beds with effective fungicides. Sinden and Yoder (1949) reported that Verticillium spot and bubble diseases of mushrooms could be controlled by zinc ethylene bisdithiocarbamate applied at the rate of 0.5 lb/100 gal of water applied at the rate of 1 quart of solution.
per 10 sq ft. Three applications were necessary to obtain control: (1) soon after the casing soil is applied to the bed, (2) between the first and second break of mushrooms, and (3) between the second and third breaks.

Many other examples of soil drenching as a means of plant disease control occur throughout the literature but these few examples serve adequately to illustrate the method.

5. Systemics Applied to the Soil

A generalization can be made that might be an axiom, "Plant diseases of a systemic nature are not affected significantly by foliage applications of heavy metal fungicides." To a lesser degree, perhaps, a similar generalization can be made with regard to many nonmetallic organic fungicides. There are possible exceptions such as the report by Kinney (1891) that potato plants sprayed with bordeaux mixture produced tubers that were relatively free of scab when compared with tubers on nonsprayed plants. Copper applied to seed apparently affected the susceptibility of tomato plants to some soil-borne fungus pathogens, according to Horsfall (1930). Thus, some systemic activity of heavy metals, such as copper, might occur to reduce the incidence or severity of certain diseases. These are, however, exceptions rather than the rule.

Most serious plant diseases are not affected appreciably by plant surface applications of fungicides, once the disease is well established in the plant. Certain antibiotic-type materials might be excepted from this generalization.

Various plant diseases have been investigated with regard to their control by systemic-type chemicals. Vascular diseases that become systemic have occupied a large share of these investigations. Classic among these is the work with Dutch elm disease. Horsfall and Zentmeyer (1941, 1942) reported that the toxin of Dutch elm disease could be antidoted to a certain extent by 8-quinolinol sulfate. This early work was later confirmed and embellished as reported in 1946 by Zentmeyer et al. (1946).

According to Horsfall and Dimond (1951), results with 8-quinolinol sulfate were erratic and several reports were contradictory. An explanation of the behavior of this chemical can be found in the work of Dimond (1947), Dimond et al. (1949), and Stoddard (1946). Their results, according to Horsfall and Dimond (1951), showed that the action of the sulfate was of short duration while that of 8-quinolinol benzoate persisted for a longer period of time. In addition, results that showed that 8-quinolinol benzoate had to be in the tree before infection
occurred, in order to be effective. Thus, it had a systemic protective action rather than a therapeutic activity against the Dutch elm disease. According to Horsfall and Dimond (1951), 8-quinoledin benzoate is more uniformly distributed in the elm tree from applications made to the soil than from injection directly into the tree. Dimond (1948) suggested that the most efficient form of application was injection into the soil in the vicinity of feeding roots.

Stoddard (1946) reported that soil drenched with oxyquinoline sulfate controlled Fusarium black rot of geranium cuttings. A commercial scale test with cuttings in the greenhouse showed that when oxyquinoline sulfate was applied as a soil drench at the rate of 1.6 oz/50 gal of water in three successive applications at weekly intervals a high percentage of previously infected plants recovered.

C. Theoretical Principles of Soil Treatment

1. Principles of Soil Fumigation

Fumigation of soil by definition is the exposure of the soil to fumes of chemicals for the purpose of killing pests or sterilizing the soil. The principles that govern the effectiveness of such chemical fumes are many and complex. Goring (1962) reviewed the literature pertaining to the theoretical principles of soil fumigation from various types of applications, and included in the review the mathematical theories that govern the movement of chemicals in the soil. A complete review of this type is beyond the scope of this chapter, but certain principles mentioned by Goring are necessary to any discussion of soil fumigation principles.

Single-point injections of fumigants are perhaps the simplest form of fumigation and the principles that pertain to them also apply to a certain extent at least to other types of soil injection. According to Goring (1962), diffusion of a liquid fumigant applied at a point in a porous isotopic soil mass, infinite in three dimensions, would be spherical in three dimensions if gravity or convection did not influence the system. Gravity will, however, influence the ultimate distribution of fumigant, since percolation of the liquid phase of the fumigant will occur before volatilisation takes place. Goring (1962) pointed out that for practical rates of application percolation is negligible. Movement of the fumigant in soil takes place primarily by diffusion. Diffusion is rapid through air spaces but is impeded considerably by water that fills the pores of the soil, since diffusion through water is quite slow. The ultimate distribution is affected by many factors, including the nature of the chemical applied and the soil environment. Goring states that the distribution of
the fumigant between the soil-air and soil-water will be governed by Henry's law, which for this system, can be interpreted to mean that "for dilute solutions and a given set of soil conditions, the ratio of the concentration of the fumigant in the soil water to that in the soil air is constant."

Distribution and pest control patterns of fumigants in the soil tend to be skewed downward from the point of injection (Goring, 1962). As the distance from the point of injection increases, concentration of the fumigant decreases to the point where pest control is no longer obtained. Lateral distribution is related to time and to dosage of fumigant applied. Obviously, the concentration of the fumigant in the soil air spaces or in the soil water governs control of pests located at these sites.

The ultimate destiny of fumigants applied to the soil is loss into the atmosphere in the form of volatiles from the chemical applied or the decomposition in the soil. The time needed for both of these phenomena governs the duration of the waiting period until the treated area can be seeded if the chemical applied is toxic to plants. (Most fumigants are highly phytotoxic.) Concentration gradients and pest control patterns for various types of application of fumigants are shown by Goring's illustrations to be identical (Goring, 1962).

The various facets of the soil environment influence, to a great extent, the effectiveness of any fumigant applied. Natural as well as introduced conditions are functionally effective, and include such factors as soil temperature, soil moisture, soil porosity, soil texture, organic matter, and reflecting boundaries such as plastic covers. Little can be done to control or alter the soil temperature, soil texture, or organic matter in the soil. However, soil moisture can be altered to such an extent that the porosity of the soil is affected. In addition, water-filled soil pores near the soil surface function as a reflecting boundary. Soil compaction tends to close pores near the surface and it too develops into a type of reflecting boundary. The role of a reflecting boundary in relation to the performance of the fumigant is brought out by Goring (1962). Highly volatile fumigants such as methyl bromide perform more effectively under a cover than in uncovered areas. Likewise, less volatile fumigants also perform more efficiently under a cover.

Simultaneous applications of fumigant and cover offer the best opportunity for efficient fumigation. According to Goring (1962), simultaneous operations are not always possible; and, depending on the chemical and the manner of application, some time can elapse between the applications of the fumigant and the cover. For example, there can be
no time lapse between the application and covering when methyl bromide is placed on the soil surface, but when injected to 3 inches deep an interval of 1 to 15 minutes can occur. Injection to a 6-inch depth permits an interval of more than 15 minutes between application and cover.

Thus, there are many factors that influence the behavior of fumigants applied to soil for pest control. Mathematical relations of fumigant-soil systems are illustrated by 18 equations in the review prepared by Goering (1962) and a complete insight into the operation and behavior of fumigants in the soil can be obtained by consulting it.

2. Soil-Surface Applications

The primary concern in connection with any soil application is placement of the fungicide where its toxicity to the pathogen will be most effective. That certain diseases can be controlled by surface applications attests to the desirability of this as the most effective placement for control of those diseases. The efficiency of any surface application is perhaps measured more easily than any other type of application. In spray applications the distribution pattern is evident in the deposits of fungicide particles, water droplets, or the colors of dyes applied in association with the fungicide. Concentrations can be determined by placing a paper of contrasting color to measure the fungicide or dye deposited by its distribution on the paper. Similarly, water droplets can be observed on nonabsorbent paper or other similar surfaces such as plant leaves. If faulty distribution is detected, corrective measures can then be taken.

3. In-the-Row Application Principles

The attractiveness of in-the-row applications can be explained from several viewpoints: accurate placement of the fungicide, effective control from reduced rates of application, and reduced tendency to develop fungicide-resistant strains of fungi.

Fungicide formulations, placed in the row with the seed, release their fungitoxic effect in a restricted zone of soil. This type of treatment permits the placement of the fungicide at sites where most effective disease control can be obtained for certain diseases. Furthermore, the rate of application per unit of soil can be increased in the treated zone to achieve better disease control without increasing the per-acre application rate. For example, by widening the row spacing and keeping the rate per acre constant, the effective rate of application within the row in parts per million increases. When applied at 4 lb per acre the fungicide present in a 2 × 2-inch treated zone of soil is 67 ppm for a 20-inch
row spacing, but is doubled when rows are spaced 40 inches apart (Leach, 1965). Of course, the phytotoxic threshold still should not be exceeded.

The distribution of fungicides incorporated in the soil cannot be observed directly, as it can in surface applications. Garber and Leach (1957) evaluated other methods to determine the distribution of fungicides in soil. Radioautograms made from treated soil profiles showed that when one spray nozzle was directed either toward the open furrow or the soil as the row was being closed, gave poor distribution. When the opened furrow was sprayed, the main concentration was in the bottom of the row; the other nozzle placement tended to concentrate the material at the top of the row near the soil surface. A two-nozzle arrangement gave better distribution than either alone. Luck et al. (1959) confirmed the usefulness of radioautograms for evaluating the fungicide distribution in soil.

Garber and Leach (1957) sprayed safranin into the row and then made soil profiles, measuring $2\frac{1}{2} \times 2\frac{1}{2} \times \frac{1}{2}$ inches, and were divided into 25 cubic half-inches. Safranin was removed from each cube with xylene and measured colorimetrically. Results were similar to those obtained with radioactive rubidium. Fluorescent dyes were suggested as a possible rapid means to evaluate distribution.

A considerable reduction in the per-acre rate of application without reduced disease control can be realized from in-the-row treatment. Alteration of row spacing, planting depth, and application rates permit satisfactory seeding-disease control in cotton, with as little as $\frac{1}{2}$ to 2 lb of material per acre (Leach, 1965). Treatment of a $1 \times 1$-inch soil profile at 1 lb per acre provides 67 ppm of the fungicide in the treated area, whereas 1 lb per acre applied to treat a $2 \times 2$-inch profile provides only 16.5 ppm. The planting depth in these examples would be 1 and 2 inches, respectively (Leach, 1965). Thus, the actual rate of fungicide applied per acre can be reduced significantly without a reduction in disease control. Under these conditions, fungicide efficiency is increased considerably and economic advantages are obvious.

Soil-surface applications of fungicides at high rates per acre conceivably can establish a selection pressure that might select forms from the pathogen population resistant to the action of the fungicide. Adaptations of fungi to certain toxic materials have been demonstrated with several pathogens. Gattani (1951) reported that cultures of a species of *Alternaria* became adapted to thiram and tolylmercury acetate after successive transfers to media containing the chemicals investigated. He noted that cultures adapted to thiram behaved as unadapted...
cultures to tolymercury acetate, and vice versa. Other fungi are known
to have developed resistance to certain toxic chemicals (Christiansen,
1946; Stakman et al., 1946). In addition to adaptations for high resist­
ance, the combining ability or sexual processes of Ustilago maydis
(DC.) Cda. were affected by sodium arsenite (Hirschborn and Mun­
necke, 1950).

Thus, adaptation does occur and could influence the pathogen popu­
lation in a soil to which a fungicide was applied at frequent intervals.
Where the amount of fungicide can be reduced (as with in-the-row
applications) the opportunity for selection of a resistant or tolerant
type is reduced considerably and might even be prevented. Fungicidal
action of the materials applied to the soil would determine to a great
extent the possibility of the pathogen circumventing its toxicity.

Some specificity is indicated in the fungicides used for cotton seedling
disease control. Thus, PCNB exerts a toxic action on Rhizoctonia but
not on Pythium, whereas captan and other materials used in conjunc­
tion with PCNB have a greater influence on Pythium than on Rhizoc­
tonia. Adaptation and selection of resistant types within the species
Rhizoctonia solani to the normally toxic fungicide PCNB has been ob­
served and reported by Shatla and Sinclair (1962, 1963) and Thomas
(1962). Acquired resistance to various fungicides has been observed for
Maier (1962) observed that selected isolates of R. solani behaved dif­
erently when exposed to several fungicides, but there apparently was
no relation between the reaction to the fungicides and the pathogenic­
ity of the isolates.

According to Domasz (1964), certain pathogens might increase in
the soil as the result of the specific action of the wrong chemical, elimi­
nation of antagonists, or the stimulatory effect of low concentrations of
the toxicant applied. Thus, many of the soil fungicides used are broad­
spectrum types, and have high levels of potency against many fungus
species.

III. SEED TREATMENT

Many seed-borne diseases are amenable to control when the proper
chemical is applied to seed. Treatment of crop seed dates back to
around 1670 when wheat seed was accidently submerged in seawater
near Bristol, England, and subsequently produced a crop free from
bunt. Intelligent use of chemical seed treatments did not occur until
about 1870 when, according to Leukel (1936), Kühn (1873) recom
7. USE OF SOIL- AND SEED-TREATMENT FUNGICIDES

mended that wheat seed be treated with copper sulfate. This is considered the first definite recommendation of a seed treatment chemical based on experimental evidence.

When copper sulfate was first recommended for bunt control, only a bag of seed, a bucket or similar container, and the chemical dissolved in water were needed to treat seed. Since that time the development of seed-treating machinery has been commensurate with the technological advances of the twentieth century, and it has been influenced greatly by the requirements of cereal seed treatment. Certain seed-treating devices, while not readily adaptable to large-scale treating operations are, nonetheless, important for applications of chemicals to seed of various crops. The technological evolution of present-day seed-treating machinery will be traced from the standpoint of cereal seed treatment, ranging from batch-type to high-capacity continuous treaters.

A. Batch-Type Treaters

By far the simplest of the batch-type treaters is the shovel. Specific instructions for the shovel method of treating seed (in this case grain seed) state to begin by placing 1 bushel of seed on a grain tight floor, then scatter the recommended amount of dust over it (Bayer-Semesan Company, 1939). Build the pile to any size by adding 1 bushel of seed at a time with the proper amount of dust. Build a new cone of seed with the shovel and repeat twice until no streaks of the dust are visible. The instructions indicate that only volatile fungicides should be applied by the shovel method; but it is obvious that nonvolatile materials could also be applied if care to assure complete coverage of the seed through repeated shoveling were taken. Instructions for the shovel method of application of formaldehyde to oat seed are outlined by Koehler (1935) and include directions for a spray or sprinkle application.

The rotary batch treater, made from a metal oil drum or similar container, is a machine with which many agriculturists are familiar. The diagonal barrel is another name used for this treater. Construction of the rotary treater is simple, requiring only a barrel, either metal or wood, 1-inch pipe, two hinges, and two sawhorses. For construction details see Bayer-Semesan Company (1939), Brentzel (1952), Koehler (1935), Leukel and Tapke (1954, 1955), Sharvelle (1961), and Machacek et al. (1950).

According to Leukel and Tapke (1954, 1955), the rotary treater operated by two men has a treating capacity of 20 to 40 bushels of grain.
seed per hour. The rotary barrel should be turned slowly for about 25 revolutions for the seed to tumble from one end of the barrel to the other.

The rotary barrel treaters or similar type machines are suitable for small lots of seed of small grains, corn, cotton, or almost any seed that is not easily injured. For seeds sensitive to damage, such as some large-seeded legumes, this type of application might be unsatisfactory. Almost any dust fungicide can be effectively applied in the barrel-type treaters; if the proper effective chemical is used, control of surface seed-borne diseases can be obtained. For example, seed-borne wheat bunt (Tilletia caries (DC.) Tul. and Tilletia foetida (Wallr.) Liro) can be controlled when seed is treated with mercurial dusts or wettable powders or with hexachlorobenzene, any of which can be applied to the seed in the rotary treaters.

Onion smut (U. chlorici) has been effectively controlled when onion seeds were pelleted with 80% hexachlorobenzene (HCB) at the rate of 1:1 (Duran and Fischer, 1959). Onion growers in the Walla Walla area of Washington apply HCB to the seed by the batch method, using any type of container available, jars, buckets, and small metal drums. Onion seed and sufficient formulation are placed in the container and mixed, but the actual rate of application seldom reaches the desired 1:1 ratio. Good control of smut is nonetheless obtained from this type of application.

Small batches of vegetable seed can be treated with fungicides by using a glass quart jar as the mixing container (Haskell and Doolittle, 1942). Seed and fungicide are put in the jar and mixed by vigorously shaking the jar to achieve thorough mixing. Many seed-borne diseases of vegetable crops are amenable to control with seed-treatment fungicides. McNew et al. (1951) list various diseases and fungicides shown to provide control when applied to seed.

B. Gravity Treaters

Several types of gravity treaters have been used for applications of chemicals to small grain seed. One type consisted of one or two sets of cones over which the seed and dust fungicide passed and were mixed. The upper cone of each set was perforated to allow the dust to pass through. The point of this cone faced upward to let the cone function as a seed divider. As the seed passed over the perforated cone it was collected in the lower cone while mixing with the fungicide. After passing through all the sets of cones the seed was discharged at the base of the
treater and bagged or placed in bulk storage. The exterior of the treater usually was a 55-gal metal oil drum or some similar type container. The capacity of this cone-type gravity treater is 30 to 60 bushels per hour. Construction details are shown by Lenkel and Tapke (1954, 1955), Bayer-Semesan Company (1939), Mackeck et al. (1950), and Sharvelle (1961).

Another rather simple gravity treater with no moving parts was the Minnesota seed grain treater developed by Moore (1937). This treater is constructed of wood and consists of a proportioning trough 9 1/2 X 11 1/2 X 68 inches, in which 2 bushels of seed with the correct amount of dust fungicide is placed to form a layer about 7 inches deep. Each bushel of seed is added separately with the fungicide and mixed before the second bushel of seed is added. Mixing should be done with a board, but never by hand. When the seed and fungicide are mixed, the proportioning trough is elevated at its free end, and the seed is dumped into the mixing chute in which five wooden baffles are placed. As the seed falls through the mixing chute and over the baffles, it is mixed with fungicide dust. The seed is caught in a bag at the bottom discharge end of the treater to reduce the loss of the dust. The capacity of this treater is in the neighborhood of 40 bushels per hour. Construction details are shown by Moore (1937), Holton and Heald (1941), Bayer-Semesan Company (1939), and Sharvelle (1961).

C. Continuous-Flow Dust Treaters

Continuous-flow dust treaters were developed and used to apply copper carbonate to wheat seed after this chemical was shown to be effective against bunt (Darnell-Smith, 1915, 1917; Darnell-Smith and Ross, 1919). The principal features of such a machine were a means to meter the seed into the mixing chamber and a device to meter the copper carbonate formulation and bring it into contact with the seed. The seed and dust were mixed in various types of chambers that tumbled the seed in rotating drums, augers equipped with metal or brush paddles, screw-type augers, or by gravity through chutes in which baffles enhanced the mixing of seed and fungicide (Hurst et al., 1936).

According to Hurst et al. (1936), the demand for accuracy in the metering of dust fungicides into the mixing chamber with seed was not great when copper carbonate was applied at 2 to 3 oz per bushel. They pointed out that a glance at the discharge end of the treater, to observe the coverage of seed with the chemical, alerted the operator if underdosages were being applied. Excessive dustiness around the machine in-
dicated an overdosage. Proper adjustment of the machine could be made to remedy either condition. Wheat seed can hold only so much copper carbonate dust. Excess dust will slough off and become deposited in the bottom of the sack or bulk bin. Thus, accurate metering of the fungicide into the treater is not needed so long as complete coverage of the seed is obtained; this is generally not a problem, since an excess of dust is usually applied.

The introduction of organic mercurial dusts in the form of ethyl mercury chloride, and later ethyl mercury phosphate, soon brought to the forefront the need for accuracy in metering of fungicide dusts. Because of the relatively low rates of application of these materials, underdosage cannot be readily seen at the treater discharge; sometimes becoming apparent later, by lack of disease control. Likewise, overtreatment is not easily detected at the treater, unless there is excessive dustiness around the machine. Often overdosage is detected from varying degrees of phytotoxicity (Hurst et al., 1936). Thus, to attain satisfactory disease control and seed germination, accurate metering is essential (although not always available) in machines designed for copper carbonate application.

Hurst et al. (1936) developed an accurate metering device suitable for organic mercurials, formulated for application at the rate of \( \frac{1}{2} \) oz per bushel. The essentials of this fungicide feeder or metering device are a rectangular sheet-metal hopper with a channel iron and reciprocating slide attached at the bottom. The slide moves back and forth and pushes the dust to the discharge openings. The amount of dust, and, thereby, the rate of application for a given flow of seed, can be adjusted. A centrally located partition forms two compartments in the hopper, one immediately above the discharge plate and the other to one side and with a slanted bottom. This construction permits a somewhat uniform depth of dust above the discharge plate. Wire loops attach to the slide and provide enough agitation in the dust reservoir to prevent bridging above the openings in the discharge plate that might impede the flow of dust.

This metering principle is employed for the development of a gravity-type treater, in which seed to be treated is fed from a bin or hopper above the opening to the treater. A deflector plate at the inlet opening deflects seed into one of two compartments. This deflector is attached to a shaft that is in turn attached to the shaft of plates at the bottom of the treater. This attachment is so designed that the seed accumulated in the chamber of the treater is dumped before the next measured amount of seed is introduced into the treater. Fungicide metering is
accomplished by the device (already described) that is attached to, and operated by, the shaft to which the deflector is attached. This machine represents the first accurately calibrated seed-treating machine.

D. Slurry Treater

While they solved some of the irritating problems of the wet treatments, dust fungicides had certain disadvantages. Dustiness around the treater and dusting-off of fungicides in the normal handling of seed were of primary concern. These objectionable characteristics were partially offset when the slurry method of application was developed, and when fungicide formulations were altered to adapt to the new application technique (Miles, 1946). Fungicide formulations for slurry applications containing wetting agents are referred to as wettable powders. The basic principle underlying the slurry application is the incorporation of the wettable powder in a water carrier to form a thick suspension. This suspension is metered into the treating machine to apply the required amount of fungicide to the seed for disease control. In general, all slurry machines have an adjustable hopper to control the flow of seed into the machine; a slurry tank with an agitator to keep the wettable powder in suspension; positive seed and slurry metering devices; and an auger to mix the seed and fungicide, and move it to the discharge spout of the treater. Slurry machines are available with capacities near 600 bushels of grain seed treated per hour. Most machines can be modified to treat cottonseed; and, without modification, seeds of small grains, legumes, grass, and vegetables can be treated (Purdy et al., 1961). Several brands of slurry machines that differ slightly in their construction are available.

E. Drum Treater

The introduction of the liquid formulations of organic mercurials for seed application in the late 1940’s brought with it the introduction of the drum-type seed treater. Like the slurry machine, it has an adjustable hopper to control seed flow, a positive seed and fungicide metering device, and an inclined drum with baffles at the discharge end to control the time the seed is retained in the rotating drum mixing chamber. The fungicide is introduced into the mixing chamber through tubing attached to metal fingers at the inlet end of the drum. The liquid fungicide flows down the outside of the fingers, and as the seed enters the mixing chamber some remove the fungicide from the fingers. The fungicide is
then distributed over the remaining seed by the tumbling action of the seed in the mixing chamber. Machines of this type have capacities near 600 bushels per hour. Several models of this type of treater that can be used to treat seed of small grains, legumes, grasses, and vegetables are produced. Special models are produced for cotton and peanut seed treatment; and sold under the trade name of Panogen Seed Treaters (Panogen Inc., 1957).

**F. Mist Treater**

The need for further refinement of seed-treating machinery became apparent with the demand for complete and uniform coverage of treated seed by the user, the farmer who paid for the treating job. While the drum-type treater was a decided improvement over the slurry machine, coverage was still not complete and some seeds appeared to be poorly treated (Purdy, 1958). The direct application of small volumes of fungicide to large volumes of seed presented a terrific problem of distribution. The Mist-o-matic Treater is one of the machines thus far developed that has apparently solved the problem of complete coverage. Initially, this machine consisted of an adjustable hopper to regulate seed flow; a positive seed and fungicide metering device; and one rapidly spinning disk that broke the fungicide solution into small droplets. The seed passed through the zone of droplets by gravity, and then was discharged from the machine. Wheat seed treated in this manner appeared to be freckled. Coverage was not complete (Purdy, 1957). The efficiency of the machine was greatly increased with the addition of two additional spinning disks and a short mixing auger at the bottom of the machine. Coverage provided by this triple treater is complete and uniform when the machine is adjusted and properly used.

Drum-type and mist-type treaters can be modified for the simultaneous application of two or more chemical formulations. Combinations of fungicides or fungicide plus insecticide can be applied in both treaters.

**G. Spray Treaters**

A continuous spray-type seed treater that employs the use of spray nozzles to introduce the fungicide suspension into the mixing chamber with the seed was developed for treatment of sugarbeet seed by Kepner and Leach (1949). The corky nature of the beet seed hull quickly absorbs any liquid applied to it; poor coverage in slurry applications prompted the investigation of this type of treating process. Armor
(1948) assembled the first spray-type treater for treating sugarbeet seed that paved the way for the evaluation and use of the spray method of treating seed. Leach (1948) compared several methods of treating sugarbeet seed with the spray method, and reported that spraying seed with the fungicide suspension was effective as dust applications. Development by Kepner and Leach (1949) of a continuous-type treater employed the spray-treatment method proved to be effective for sugar beet seed treatment.

This continuous-type spray treater has an adjustable hopper to control the flow of seed to the treater; a tank containing the treating solution or suspension that is equipped with a signal device to show when the mechanical agitator is operating; pressure gauges that show the pressure in pounds per square inch at the tank and nozzle ends of the pressure system; one flat fan nozzle; a monitoring system by which control of the flow of the treating solution or suspension is controlled; a rotating cylinder about 6 ft long and big enough to permit good distribution of the fungicide; and a corrugated liner for the mixing drum. The treating solution or suspension is placed under pressure in the reservoir tank and passes through lines of tubing to the nozzle and is sprayed onto the seed. Redistribution of the fungicide is brought about by the tumbling action of the seed in the mixing drum. The capacity of this machine is 2500 to 3500 lb of decorticated sugarbeet seed per hour, and coverage obtained ranges from 96 to 100% (Kepner and Leach, 1949).

The spray application method of applying fungicides to seed has been employed in another treater for applications to seed of small grains. The treater is slightly different from others. Seeds are metered into the mixing chamber by a screw-type auger rather than by gravity that is common to many treaters. Perhaps the greatest divergence from other machines is the 12-ft-long mixing drum with a diameter of 2 ft. The interior surface of the drum has four equally spaced, half-round ribs that enhance the mixing action provided by the rotation of the slightly inclined drum. At least three types of applications are made with this machine. They include direct applications of undiluted liquid formulations, suspensions of wettable powders, and dust applications in association with application of water to aid the retention of dust by the seed. The liquids, suspensions, and water applied in the treater are under pressure and are sprayed from two nozzles onto the seed in the mixing drum. Compressed air provided by an air compressor aids the distribution of powdered chemicals when introduced into the mixing drum.
The capacity of this type of treater is near 600 bushels of small grain seed per hour. Coverage is good and approaches 100% when one of the spray nozzles is perpendicular to the long axis of the drum and the other parallel with the axis. The entire 12-ft length of the drum is needed to obtain complete coverage (Purdy and Gregg, 1964).

Special equipment is available to convert various treaters for application of specific chemical formulations. Dust machines can be so converted that undiluted liquid formulations can be applied effectively. Other types of treating can be accomplished when certain types of equipment are used. A simple device is a bottle equipped with a top designed to apply liquid formulations a drop at a time to grain being loaded or elevated by a screw-type auger. The chemical is applied to the seed at the bottom of the auger, and as the seed is moved by the auger it becomes mixed with the fungicide (Panogen Inc., 1957).

Small cement mixers can be used for batch treating seed as well as almost any other type of container that can be handled in such a way to distribute the fungicide applied over the seed surfaces.

H. Bulb and Corm Treatment

Large fleshy storage organs of many plant species are subject to attack by various fungi that seriously affect the plants produced. Fungicides have been employed to control certain pests of bulbs, corms, and roots. Only a few examples of such usage will be described since methods and equipment used are similar for many of the diseases.

Basal rot of narcissus caused by *Fusarium oxysporum* f. *narcissi* (Cke. and Mass.) Sny. and Hans. is worldwide and certain control measures have been developed that include fungicide dips for bulbs (Gould, 1957). Mercury base fungicides are used for bulb dapping, and phenyl mercury acetate (PMA) is the commonest form used in western Washington. The general recommendation is to dip bulbs for 5 minutes in a solution of PMA containing 1 lb PMA/500 gal water. Specific directions are available for the replenishment of the PMA in the treating solution to maintain an effective concentration of PMA. Replenishment rate is related to the soil type in which the bulbs were grown. Treating solutions for bulbs grown in sandy soil require less frequent replenishment than solutions for bulbs grown in peat soils. The cleaner the bulbs before treatment the longer the treating solution will remain effective. Organic matter in the form of plant parts or peat from peat soils, as well as soil itself, react with and render inactive the mercury in the treating solution; thus the need for replenishment.
When corrosive chemicals like mercury are used for dipping bulbs, plastic liners or plastic based paints must be used to protect the interior of the treating tank.

Injury from use of certain forms of mercury may occur if the bulbs are immature, or if they are not dried rapidly. Of course injury will occur if the treating solution is too strong. According to Gould et al. (1949), Ceresan, New Improved Ceresan, and Ceresan M all caused some injury to bulbs when used for the control of basal rot. Apparently, the condition of the bulbs influenced the amount of injury, because the greatest amounts of injury were observed when bulbs were treated within 3 days from the time they were dug.

The use of more than one fungicide applied at different times has resulted in better control of Colletotrichum lili Plakidas, the cause of black scale of Easter lily. Bulbs are dipped in a solution of phenyl mercury triethanol ammonium lactate, 1:500 to 1:2000, for 24 hours, dried for 18 hours, and then dusted with Arasan to prevent reinfection after being planted (McWhorter, 1957; LeBeau, 1946, 1947; LeBeau and Reynolds, 1947).

Gladiolus corms can be treated in a solution of mercuric chloride 1:1000 for 6 to 8 hours to control hard rot (Septoria gladioli Pass.). Other diseases can also be controlled by dipping corms in solutions of organic mercurials (Pirone et al., 1960).

Dipping the true root that is used as a seed piece of sweet potato for the propagation of the crop in solutions of fungicides has provided control of black rot [Ceratostomella fimbriata (Ell. and Halst.) J. A. Elliott]. Standard treating solutions have been mercuric chloride (1:1000), Semesan Bel, 1 lb/7.5 gal, and borax, 1 lb/5 gal (Jeffers and Cox, 1946; Walker, 1952). Jeffers and Cox (1946) reported that Spergon (1 lb/4 gal) and Phygon (0.5 lb/4 gal) were as effective as the above-described treatments, and in addition, caused less damage to the roots as measured by the delay in and number of sprouts produced.

Other examples could be cited but these few will serve to demonstrate the dip-type treatment of fleshy storage organs as a technique for the control of certain plant diseases. The equipment used is simple and easily modified to accommodate large quantities of material to be treated.

I. Potato Seed-Piece Treatment

Seed pieces of the potato (Solanum tuberosum L.) benefit from treatment with certain chemicals; control of black leg or seed-piece rot by Erwinia carotovora (Jones) Holland is a case in point. For this disease
the chemicals applied have a bactericidal action, but their use illustrates the effectiveness of potato seed-piece treatment as a control measure.

Bonde (1955) reported that the treatment of seed pieces in a solution of streptomycin 100 ppm had a favorable effect on potatoes, including control of seed-piece decay and a reduced incidence of black leg. He reported also that an instantaneous dip was as effective as the 30-minute dip on farms where tests were conducted. Some reduced emergence of plants was experienced in one test after seed pieces had been treated for 30 minutes. In contrast, Duncan and Gallegley (1963) reported that seed-piece treatment had little or no effect if done on the same day as cutting and planting. In addition, they reported that treatment with 100 ppm streptomycin 30 days before planting reduced the stand and yield of potatoes. This deleterious effect apparently could not be offset by captan, which when used alone gave yields and stands similar to those obtained when cutting, treating, and planting were done on the same day. Thus, the results appear to be controversial as to the benefits from seed-piece treatment with streptomycin, whereas treatment with captan was of definite benefit. Zineb and maneb were equal to captan in the investigations conducted by Duncan and Gallegley (1963).

Equipment needed for the treatment of potato seed pieces would be similar to that used for other dipping-type treatments. Tank size and quantity of treating solution vary with the quantity of material to be treated. The basic needs then are, in addition to the tank and treating solution, a means to bring the seed pieces in contact with the solution and a place to allow the treated seed pieces to drain and dry. For dust applications to seed pieces, various types of equipment can be used. Requirements for such equipment depend on the quantity of seed pieces to be treated.

Guthrie (1959) reported that seed-piece treatment with Semesan Bel, Agrimycin, and PCNB all increased stands and yields in his tests. Although treatment with Semesan Bel killed some surface cells, and saprophytic fungi grew profusely on these dead cells, certain benefits were still apparent from seed-piece treatment with Semesan Bel. In his studies, PCNB and Agrimycin controlled seed-piece decay when these materials were applied alone or in combination with one another.

**J. Hot- and Cold-Water Treatments**

Although hot water does not fit well within the framework of a treatise on chemical fungicides, its importance for the control of certain
seed-borne diseases can neither be ignored nor omitted from a comprehensive discussion of seed treatments. Jensen (1888) described and used a hot-water treatment for the control of smuts in oats and barley that was later used for the control of loose smuts of wheat and barley. This hot-water treatment is still in use today, but in general it is adapted only to small lots of seed. The need for precise control of temperature and time during the treating process are such that, when absolute control is lacking, control of the diseases may not be obtained, and reduced germination of the treated seed can result. Directions for this treatment are outlined by Sharvelle (1961).

Another method used for loose-smut control is the anaerobic or cold-water soak. Tyner (1953) has shown that the soaking of seed in water at 72 to 77°F for 56 to 64 hours controlled loose smut effectively. He noted the presence of an odoriferous decomposition product after this period of immersion. A suspension of Spergon in the soaking water reduced the period needed for disease control and prevented the development of the decomposition product. However, Machacek, and Wallace (1957) reported that the addition of Spergon reduced seed germination more than the water soak alone.

Since Tyner's report (1953), the method used for the cold-water soak treatment has been altered slightly, to include a presoak period of about 4 hours. Seeds are drip dried, then placed in a sealed container for certain periods of time ranging from about 41 to 70 hours. This depends to a certain degree on the temperature during the period (Sharvelle, 1961; Minz and Gavrielitch-Galmond, 1959; Morton et al., 1960). According to Bartos and Zemanek (1960), control of loose smut of oats was obtained by using an anaerobic treatment of infested seed. Seed was soaked 2 hours, then placed in airtight bottles for 2 days; soaking and storing of seed was done at 22°C.

The equipment for the cold-water soak treatment can be a drum, a barrel, or similar container of sufficient size to be filled to three-quarter capacity with the seed to be treated. A plastic sheet, or some other device to seal the container after the soaking solution has been drained from the container, is needed. Facilities for drying the seed are needed after the treating process has been completed.

K. Theoretical Principles of Seed Treatment

1. Coverage

Through coverage presented no problem in seed treatment until the introduction of organic mercury fungicides, formulated as dusts or wet-
table powders for application in continuous-type treaters. Where the entire seed lot is immersed in the treating solution, as with the dip method of treating seed, every seed is uniformly and completely covered, provided that seed remained in the solution long enough for the solution to wet the innermost seeds in the bag. The introduction of the copper carbonate treatment and its subsequent use still provided complete coverage of all seeds, because excess copper carbonate was used without concern for any phytotoxic aftereffects. The seed (in this case wheat seed) could hold only so much copper carbonate; and the maximum amount held was less than an injurious dose but often impaired the flow of seed in grain drills (Leukel, 1930). Coverage of seed with mercurial fungicides applied at the rate of ½ oz per bushel in dust machines was undoubtedly less than that obtained with copper carbonate applied at 2 oz per bushel. In all probability it was less than complete on every seed.

While solving some of the inherent problems associated with dust fungicide applications, the slurry method of seed treatment introduced other problems; the lack of complete coverage of treated seed was the most serious.

Slurry applications of organic mercurials in practice probably failed to cover or even place any of the fungicide on all seed. This deficiency, however, was not considered serious, because the volatile nature of the mercury fungicides theoretically would offset the lack of complete coverage. In 1958 I showed that, on the basis of color distribution, seeds treated by the slurry method of application were incompletely covered and that as much as 25.9% of so-called treated seed was inadequately covered (Purdy, 1958). Before 1958 the major fungicide used in the Pacific Northwest for the treatment of wheat seed was a mercurial and its presence could not readily be detected on the seed, since the dye incorporated in the formulation did not color the seed sufficiently.

The need for complete coverage of all treated seed is obvious since certain fungicides provide some measure of control of organisms in the soil when the chemical is applied to the seed. Without complete coverage, however, control is also incomplete. There is nothing associated with seed-treatment fungicides and their application that will offset the lack of complete and uniform coverage. The demand for uniformly complete coverage has precipitated and stimulated development of better seed-treating machines that meet this demand.

2. Vapor Action

Vapor action is that property of certain active ingredients in fungicide formulations that allows their distribution in a seed mass by the
formation of volatile products that diffuse throughout the seed mass in a vapor phase. Many organic mercurial fungicides produce vapors and are often referred to as volatile fungicides. The slurry method of application was designed to take advantage of this property of vapor action. In principle, a small amount of fungicide is applied to some seed and assumed to move to other non-contacted seed by vapor action, thus protecting non-contacted seed from invasion by disease organisms.

The role of vapor action in the control of wheat bunt in the Pacific Northwest was undetermined until experiments conducted in 1956 suggested a restricted role for the vapors of certain fungicides. Purdy and Holton (1956) showed that bunt spore germination was inhibited by certain organic mercurials but not by others when the fungicides were placed within sealed petri plates but not in direct contact with spores on an agar surface. In addition, spores on seeds were killed by some fungicides and not others. Results reported in 1960 suggested that vapor action is not effective in the control of wheat bunt by seed treatment in the Pacific Northwest (Purdy and Holton, 1960). Fungicides applied to seed mixed with untreated, inoculated seed showed that vapor action did not provide protection against infection by spores located on the untreated seed. These reports suggest that during the many years when wheat bunt was a major limiting factor in wheat production in the Pacific Northwest, seed applications of volatile fungicides failed to provide adequate control of seed-borne common bunt, due to inadequate coverage of the treated seed. This, together with spores in the soil, perpetuated the bunt problem annually in major proportions, except when environment intervened to suppress infection.

Arny (1962) holds the view that vapor action is effective in controlling wheat bunt. He reported that the vapors of methylmercury diicyandiamide reduced the infection percentages not only of wheat bunt, but also of Victoria blight of oats and Helminthosporium seedling blight of barley. These results suggested to Arny that the vapors do move from treated to untreated seed in such a manner as to effect control of these diseases. Arny and Leben (1954) reported that Panogen, and to a lesser extent Ceresan M, were taken up by oat seed when seed and fungicide were placed in closed glass containers. Their results showed that phenylmercury acetate and phenylmercury urea were relatively ineffective in the vapor phase. The amount of fungicide absorbed into the seed was measured by a Glomerella mycelial assay on agar.

Convincing evidence that the mercury from Panogen (methylmercury diicyandiamide) moves by vapor action has been presented by Lindstrom (1958, 1961, 1962). Radioautographs of seed treated with a radioactive mercury formulation and mixed with untreated seed show conclusively
that the tagged mercury moved from treated to nontreated seed. Un­
fortunately, the biological activity of the vapor-deposited mercury was
determined only with a species of *Fusarium* that caused a seedling
blight. A more convincing presentation could have been put forth if the
biological activity of the vapor-deposited mercury had been made with
ungerminated resting spores of certain other seed-borne fungi such as
wheat bunt. Fungitoxic properties of chemicals seem to be greater
against mycelial and conidial cultures than against heavy-walled spores
such as the teliospores of wheat bunt. The results and observations
presented by Lindstrom (1958, 1961, 1962) notwithstanding, the bio­
logical activity associated with movement of fungicides from seed to
seed by vapor action has not been conclusively demonstrated, at least
not so far as wheat bunt is concerned.

3. Bioassay of Fungicides Applied to Seed

Measuring the amount of fungicides on an individual seed is a prob­
lem that challenges the best chemical techniques and modern apparatus.
Perhaps the measurement of the small amount of chemical applied to
one seed when the rate of application is \( \frac{1}{2} \) oz per bushel (assuming
complete and uniform coverage of the treated seed) is beyond the scope
of chemical analysis. The exactness and sensitivity of chemical tests
must meet further obstacles, since formulations applied to seed gener­
ally contain low percentages of active ingredient. For example, Cer­
esan M contains 7.7% of active ingredient, 3.2% of which is metallic
mercury. Ceresan M is applied to wheat seed at the rate of \( \frac{1}{2} \) oz per
bushel (about 500,000 seeds). Thus, when the treatment is uniform and
coverage of all seeds is equal, each seed will receive \( \frac{1}{500,000} \) of the
total amount of chemical applied to the seed mass.

Detection of such small quantities of fungicidal chemicals on seed can
be done most effectively and efficiently with some type of bioassay. The
response of certain fungi to small amounts of chemicals constitutes a
sensitive and accurate measure of the amount of chemical on the seed.
Several bioassays have been developed for certain types of fungicides.
All fungicidal chemicals, however, cannot be assayed by the same test
fungus.

Certain fungicides, such as the mercurials on treated seeds, can be
measured quantitatively by an agar-sheet method. Machacek (1950)
used such a bioassay to evaluate the efficiency of treating machines.
Arny (1952) patterned a procedure for quantitating the amount of a
mercurial fungicide on treated seed after a method first used by Leben
and Keitt (1950) for determination of tetramethylthiuramdisulfide on
seed. The zone of inhibition around seed treated with the mercurial
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represented the area into which Glomerella cingulata (Stonem) Spauld. and v. Schrenk failed to grow. The diameter of the zone of inhibition was the measure of effectiveness when compared with the diameter of zones around seed treated in the laboratory at known rates of application. Lockwood et al. (1952) developed a technique to use large sheets of agar for a bioassay with G. cingulata as the test fungus. More samples of treated seed or paper discs impregnated with the fungitoxic chemical can be evaluated on the agar sheet than in petri plates.

Lindstrom (1962) sounded a note of caution when he observed that the agar sheet bioassay is applicable only for a comparison that involves but one fungicide. According to him it cannot be adapted for the simultaneous comparison of several fungicides with one another. He reasoned that the zones of inhibition around treated seed are related to the diffusion constant of the particular fungicide as well as the inhibition time; both of these are probably unique to each fungicide.

Some chemicals are not broad-spectrum fungicides and require special bioassay tests for their determination on treated seed. Hexachlorobenzene (HCB), specific for wheat bunt fungi, is an example of such a fungicide. A bioassay using G. cingulata or any fungus other than wheat bunt fungi would be entirely unsatisfactory and would fail completely to show the fungicidal nature of the chemical. For this reason, a modification of a procedure developed by Zogg (1954) was used by Purdy (1958) to determine the distribution of HCB on treated wheat seed. Finely screened soil was placed on a 4-cm layer of vermiculite in the bottom of a glass staining dish. A glass tube that almost touched the bottom of the dish protruded above the soil surface. Water was added through the tube to wet the soil from the bottom. Bunt spores were dusted through cheesecloth onto the surface of the soil. Spores germinated and produced a white growth that covered the soil surface after 8 days of incubation at 10°C. Treated seed were pressed into the soil surface; zones of inhibition formed around well-treated seed were visible as dark halos around the seed where spores failed to germinate. Standards were established by treating seed in the laboratory at known rates of application. This method is not quantitative but is reliable for qualitative determinations. Seed treated with PCNB (pentachloronitrobenzene) can also be used in this test since this fungicide is also toxic to bunt spores.

4. Chemical Injury of Seed

Damaged seedlings or complete elimination of germinability can result from overdosages of certain fungicides. When lethal doses of chemicals are applied to seed, all seed respond similarly by failing to germi-
nate. Less than lethal doses provoke various degrees of injury that are usually apparent by the production of abnormal structures in the seedling stages of plant development. The degree of apparent damage is related to the amount of the overdosage. For example, slight chemical injury from mercury is manifest in wheat seedlings by terminal swelling of the first three primary roots (Purdy, 1956a). The fourth and fifth primary roots often do not show any apparent injury. Plants affected in this manner survive and the subsequent development of the plant proceeds in a normal manner. In 1956 I illustrated various degrees of chemical injury to wheat seedlings, ranging from those with little damage to seedlings with spherical swellings in place of the coleoptile and first three primary roots (Purdy, 1956a).

Injury of the seed coat is reported to accentuate damage from seed-treatment fungicides. Crosier (1934) and Hoppe (1948) reported that increased seed coat damage intensified chemical injury in wheat and corn. Roane and Starling (1958) recorded greater reductions in emergence when seed coats of wheat were damaged and fungicides were applied at more than the recommended rates of application for wheat-seed treatment. Koehler and Bever (1956) observed that the fungicide as well as the temperature after treatment influenced the amount of damage from seed treatments after 10 months' storage.

Sass (1937) reported that hypertrophy of corn occurred from chemical injury and that the condition developed without an increase of cell numbers. Anatomical response of wheat plants to chemical overdosages also results in hypertrophy of the plumule and roots, without an increase in cell numbers but, with considerably increased cell size (Purdy, 1956a). The increased cell size was apparent even in the meristematic tissue of the shoot and root apexes.

5. Storage of Treated Seed

The effectiveness of small amounts of chemicals applied to seed is assumed to increase by storing the seed after treatment for various periods of time. Depending on the kind of seed treated and the chemical used, storage has been reported to enhance the effectiveness of certain treatments, particularly on oats and barley where the seed is covered by other plant parts referred to as hulls. For example, Michaeck (1954) reported storage of treated oat and barley seed for 4 to 8 days after treatment was needed for control of loose and covered smuts. He further reported that wheat seed treated with organic mercury should be stored for 4 days after treatment to achieve control of bunt, while seed treated with HCB or PCNB need not be stored at all. Storage for as
long as 14 days after treatment failed to increase the effectiveness of seven fungicides against wheat bunt over planting seed ½ hour after treatment (Purdy, 1956b). Lindstrom (1958, 1961) reported having observed that redistribution of mercury was reasonably uniform after 2 hours and that only a slight increase in uniformity resulted with additional storage. Heden and Ulfvarson (1963) confirmed the results of Lindstrom in their observations of wheat and oats made 20 minutes after treatment.

Another facet of storage after treatment is the question of how long treated seed can be stored without impairing their germinability. Koehler and Bever (1956) reported that germination of treated seed was affected by temperature during the after-treatment storage period as well as by the fungicide used. In general, there seems to be a notable lack of consistency in reports on the germination of stored treated seed. In his review of the literature, Leukel (1948) revealed that some reports show that treatment after given periods of storage was beneficial for certain varieties but not for others. Perhaps, as Roane and Starling (1958) pointed out, the condition of the seed is of prime importance in determining whether or not injury will result. It seems that seeds with undamaged seed coats, low in moisture content, stored at low temperatures, and treated at the recommended rate of application for the particular fungicide will still be viable after rather extended periods of storage.

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CHAPTER 8

Application and Use of Foliar Fungicides

R. J. COURSHEE

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I. INTRODUCTION

A. Good Spraying

It is customary to teach students of horticulture that spraying for crop protection is good when it leads to uniform and complete cover over the whole crop surface. Search as one may, however, it is rarely possible to find data which support this general thesis in any conclusive manner. Any set of results which shows that spraying which is physically good is also biologically and economically good, is usually countered by other data which show that spraying which is physically bad is nevertheless biologically adequate. There is, therefore, some doubt about the economic value of striving after physically good spraying in many plant protection tasks.

One would expect good spray cover to be more biologically successful than bad spray cover wherever both the pest and the pesticide are static because otherwise they may not contact one another. A weakly soluble, involatile fungicide acting outside the plant would, one might think, work best if the spray cover were good. Copper used against potato blight has been viewed in this way for many years and yet a wide variety of field trials has shown that poor spray cover is biologically successful.

During World War II, when back-garden food production was important, Martin (1940) showed that even a watering-can application of bordeaux mixture gave blight control. Large (Large et al., 1946) has suggested that available evidence shows that if approximately 2 μg of copper are present on a square centimeter of leaf, then it doesn't matter very much how it is distributed, this deposit controls blight in the field.

More recently, I was associated with some trials, conducted by Brenchley (1965), of aircraft spraying of copper fungicides against blight at about 20 liters/hectare (2 gal/acre). There cannot be many spraying methods in use which lead to a more sparse or erratically distributed deposit than 20 liters/hectare applied from the air with a spray-drop size near 300 μ. Yet it was not possible to show or even suggest that this poor physical cover was less capable of preventing blight than spraying methods which gave a good cover.

Redistribution is usually given the credit for such results because
it is widely believed that a water film is essential for blight spore germination and that the water permits redistribution of the fungicide to occur. A more complete explanation can now be advanced, but before doing this and before describing what I think to be the true importance of good cover, I should like to cite examples from other pesticides to show how powerful the factors involved in redistribution can be.

B. Redistribution Examples

It is not necessary to dwell for long on the part played by redistribution in the action of systemic pesticides—mainly insecticides and weed killers. It is well known that some features of good spraying are quite unimportant for such materials. With these pesticides uniformity is desirable to this extent: each plant can often be considered an entity, and provided the right amount of chemical is available to it in its ambit, the distribution within each ambit is unimportant. A few drops of spray on a few leaves are frequently, although not always, as successful as many drops on all leaves provided the amount of chemical on a plant is unaltered.

Good spraying for this purpose therefore requires the application of the same quantity of chemical to each plant and the retention of as much chemical as possible on it. It is not necessary to place pesticide on each leaf and it is unimportant whether 1% or 99% of the leaf is covered by one or a thousand drops. There are exceptions to all generalities. In this example, unwanted phytotoxic effects may differ with the degree of leaf cover so that cover is sometimes not completely unimportant for even a systemic pesticide. In general, however, the effect of a systemic insecticide particularly, is independent of the extent to which spray drops cover the leaves. Movement of pesticide from point of application to where it can be effective compensates for incomplete cover.

The pest may move equally well into the regions occupied by the pesticide. One of the most clear-cut and simple examples of this was described by the author (Courshee, 1955). The Sudan cotton jassid, Empoasca lybica, is observed in the bright sunshine of a Sudanese field to move from the underside of one leaf to the underside of another. Yet a DDT spray applied from the air which falls almost exclusively onto the upper side of the leaf is nevertheless effective. By good fortune work was avoided during the intense midday heat, and to make up time, work had to be done at night when the hoppers were found to
spend nearly as much time on the upper as the lower leaf surface. Underleaf spray cover appeared unimportant in the light of this knowledge until it was learned that underleaf deposits were more persistent even in a dry climate.

The desert locust hopper, *Schistocerca gregaria*, provides the most extreme example of mobility in a nonwinged insect pest (Courshee and MacDonald, 1964). In the most common desert breeding conditions the hoppers move consistently with the wind (that is, parallel to the fixed dunes which form during the wet season); distances of the order of a kilometer a day. This movement and feeding over wide stretches of country allows a very irregular distribution of insecticide to be fully effective. If insecticide is placed in strips across the line of march, the hoppers feed on contaminated and clean vegetation alternately. Therefore, with a sufficiently persistent and cumulative stomach poison which is palatable, they eventually consume a lethal dose.

The daily food intake of a nymph of this species of locust is 1 gram of vegetation per gram of insect (Ellis and Ashall, 1957). Since the medium lethal dose of dieldrin is only 2 ppm (under certain conditions) (MacCuaig, 1958), a very slight contamination of the vegetation is sufficient provided time is available for insecticide accumulation. The relationship indicating the importance of time is shown in Fig. 1. Clearly these treated strips can be a kilometer or more apart and even then, one treated grass blade out of every dozen is probably sufficient. Extremely erratic distribution is biologically effective and at the same time offers enormous advantages in the cost and speed of the work when areas of the order of tens of thousands of square kilometers have to be treated cheaply in a few days.

Such examples of highly mobile pesticides in the case of systemics and highly mobile insects seem at first glance to be a little irrelevant to a paper on fungicides. Later it will be suggested that the opposite is true for two reasons. First, it may be shown that these examples parallel in principle the results obtained with some fungi and fungicides. Second, one should not and in fact cannot yet consider the spraying of fungicides to be something separate from the spraying of weed killers. The changes in spraying technique at the farm end are extremely powerful forces. Because of high cost, very few farmers will put on 1000 liters/hectare (100 gil/acre) with high-pressure drop-leg machines these days. One has to accept economic merit as the sole measure of good spraying, and look carefully at results obtained with weed killers and insecticides to learn about spraying fungicides.

The need for spraying to certain physical standards is still undenia-
ble when mobile pests and pesticides are concerned, but the examples show that a physical measure of what constitutes economically good spraying is not constant. In general, pesticides are suitable for the type of spraying machinery which is commonplace. They have been tried in the field with conventional machines and are marketed because they are successful. Therefore, the uneven distribution of pesticides caused by spraying machines should not be criticized for its lack of biological effectiveness. It is possible for a very uneven deposit to be both effective and suitable economically.

![Diagram](image)

**Fig 1.** Effect of the level of dieldrin on vegetation on the time required to obtain mortality of locusts (*Schistocerca gregaria*).

However, the following example shows how an improvement in the physical performance of a machine can, under certain conditions, upgrade the economic performance of the combination, the pesticide and its application. This will suggest that the original aim of physically good spraying has some merit after all, but mainly for certain tasks. These may be only those which parallel the example closely. Quite different plant protection tasks should also be considered, however, and it should be remembered that present practice is no more than the result of a marriage of convenience between the type of machines which are conveniently available and pesticides which happen to suit
those machines. Physically good spraying, if it were attainable, might
result in significant improvements in plant protection practice even for
those tasks for which we should expect its value to be small.

C. A Fungicide Example

Several years ago a team of which I was a member at the National
Institute of Agricultural Engineering, looked at this problem of de­
fining economically good spraying in physical terms. Of the few ex­
amples we were permitted to work on, it was thought that a fixed
copper fungicide against potato late blight would demand the most
complete spray cover and so this combination of fungus and fungicide
was tested first.

Potatoes growing in polyethylene bags were sprayed in the labora­
tory with a machine giving drops of only one size at any setting. Three
drop sizes were used and three degrees of cover were attained at each
drop size by varying the dilution, leaving the dosage rate fixed at 2 µg
of copper per square centimeter. After the deposit had dried, a fine
mist of distilled water and fresh (24-hour-old) sporangia of Phytoph­
thora infestans was applied to the plants. These were then kept in a
chamber at 95% relative humidity and 20°C until blight lesions formed
5 days later.

The results of this early work have already been reported (Cour­
shee, 1960a). The protection attained, compared with plants infected
but not treated with fungicide, was equal to the degree of spray cover.
Maximum cover of 27% gave nearly 27% control, that is to say, 73
lesions formed on sprayed plants for every 100 lesions on unsprayed
ones. This was very encouraging and was in accord with photographs
which showed blight lesions growing underneath the bright blue specks
of copper deposit and suggested that infection, which was always subse­
quent to spraying, could occur very close to the fungicide and in any
part of the leaf area not covered by spray. Underneath the epidermis
the lesion could grow quite healthily out of reach of the fungicide.
(Measurements of the reproductive capacity of such lesions were not
made. It was suspected that this was lowered, but perhaps through
chance contamination of the daughter spores.)

Clearly, the next step was to investigate the role of redistribution
in changing the original cover to a more complete one. So sprayed
plants were placed either under a distilled water rain machine or in
rain in the open, and infected as before. Alarmingly, neither artificial
nor natural redistribution caused any improvement.
APPLICATION AND USE OF FOLIAR FUNGICIDES

In the field, spray cover rarely reaches 27%. If redistribution does not raise the level of control, then, it was thought that 27% control must in fact be useful. If one considers blight to develop in the manner of compound interest, then a decrease from a multiplication rate \( M \) in each of perhaps 10 generations in a season, to 0.73 \( M \) does in fact lead to a large change in the level of disease at the end of the season. Depending upon the multiplication rate per generation this could lead to the experimentally observed delay of about 10 days in blight development caused by spraying (Courshee, 1960a). At this stage of the investigation, redistribution appeared to be unnecessary for the success of antiblant sprays.

It seemed desirable to make some corresponding tests on the results attained with field spray machines and field conditions of infection. This was done by placing plants in pots in the path of a spray machine treating a field of potatoes. We were able in this way to obtain plants treated in the field under nearly field conditions. Some of these were exposed to rain before infection and some were not. Part of each group was infected in the laboratory and part infected by being placed for a period of 24 hours in a field in which blight was already widespread at about the stage where 1% of the leaves showed lesions. Again redistribution caused no change, and field and laboratory infections were equivalent. The laboratory infection was usually heavier than the field infection but the control given by any treatment was the same for both sources of infection.

Many machines were tested. Three of the results turned out to be important for the rest of the work. First, nearly all field machines gave very weak degrees of control—20% was a common figure. Second, in field infection, it was quite common for what Hirst (1958) calls pepperpot infection to occur. A leaf was covered with a few to perhaps a dozen lesions. Finally, one machine, a sprayer applying 1000 liters/hectare (100 gal/acre) of bordeaux at a pressure of 6 atmospheres (90 psi) gave consistently good results and as much as 70% protection against both laboratory and field infection.

The first two results together served to destroy the thesis that 20% protection was good enough if it was provided against each bout of infection. A leaf succumbs almost as quickly to one lesion as it does to ten. Therefore what good would it do to prevent two spores from causing infection if there were still eight left? The knowledge that the original theory was almost certainly wrong was an additional reason for pursuing the problem further.

The high degree of control achieved with the high-pressure sprayer
suggested that very good spray cover was in fact attainable. A visual check with fluorescent tracers showed that the spray cover was nearly complete without the intervention of any redistribution mechanism. This encouraged us to study the value of more nearly complete spray cover.

The next experiments were performed in the laboratory and plants were sprayed to give complete cover and, as one might expect, complete control of blight spores applied to them was obtained. The dose rate was then reduced from the standard 2 µg/cm² to find out what lower limits of dose would suffice if the cover remained complete, i.e., to find out how much copper had to be redistributed into the unsprayed areas.

Naturally as the dosage rate shrank to levels approaching that corresponding to the in vitro toxicity of copper to blight spores it was necessary to control the spore loading during an experiment. This could not be done readily with a sprayed-on suspension of spores. Instead the suspension of sporangia was applied as single drops drawn from a stirred suspension of spores with a platinum wire loop. Similar drops were placed on glass slides and the number of spores counted. By dilution of the suspension, the number of spores applied to a square millimeter of leaf surface could be controlled. Four such spore-laden drops were placed on an experimental leaf and the lesions arising—presumably from one or more surviving spores—were counted. This method has disadvantages in that it is very insensitive in comparison to what would have been achieved by placing single spores on the leaf.

The results of one series of trials are shown in Fig. 2. The control exerted by a given dosage rate is dependent upon the spore content of a drop and the area of leaf which it covers. The experiment was done with between one and five sporangia per square millimeter using drops covering ten square millimeters and the data given in Fig. 2 is extrapolated to the level of interest in the field, i.e., one sporangium per drop or a spore loading of one per ten square millimeters. (The reader will recognize that this extrapolation critically affects the practical interpretation of Fig. 2.)

This result is presented in the units we need but an attempt has been made to indicate practical units also on the assumption of 100% spray recovery on 5 hectares of leaf surface per hectare of land. This is actually the value of the leaf area ratio at about the time the rows close over as the haulm starts to lean. The figures speak for themselves. The conventional field application is 2 kg of copper per hectare of land.
Thus a minute amount of fungicide is sufficient provided the cover is good. It seems that all the previous failures to achieve control must be attributable to failure to achieve or maintain cover before or after redistribution. Clearly such tiny doses would have to be strongly retained. A correspondingly tiny loss would reduce the small deposit to a level below that at which it could maintain its effectiveness. We had already done a great deal of work on retention by chemical assessment. Now as a matter of course we added a biological assessment of the pesticide retained after treatment by a rain machine using this technique which allowed us to control the spore loading. Almost immediately the picture which had eluded us fell into place. Some spore-bearing drops placed on leaves inclined for exposure to rain rolled down to the lowest edge of the leaf. Chemically it had been found that this is exactly where all the copper runs to in retention experiments. When most of the leaf is clean enough for infection to occur, a large mass of copper is still present all along the lower edge of a leaf. Therefore, if spores are accompanied by rain this causes both the copper and the spores to run to the lower edge where they will become lodged together. Almost all our original series of experiments—several hundred of them—had been done with an airborne spray of spores which did not run to the same point as the copper after

![Fig. 2. Dosage response of potato late blight to copper.](image-url)

<table>
<thead>
<tr>
<th>Copper dose (gm/hectare of ground)</th>
<th>0.0001</th>
<th>0.001</th>
<th>0.01</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>(µg/cm² of leaf)</td>
<td>0.01</td>
<td>5</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Control of disease (%)</td>
<td>x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Mixture of races on King Edward potatoes</td>
<td>x x x</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
redistribution by rain. The most important lesson to come from this is that there are two extreme kinds of infection—dry and wet—with all positions between them. At first both our artificial and natural infection had always been nearly dry simply in order to avoid disturbing the cover which we were investigating originally, and because massive amounts of water are quite unnecessary for blight spore germination.

Hirst (1958) had already shown from aerial spore counts that rain reduces the number of airborne spores to zero. Therefore it must be correspondingly effective in delivering spores to the plants at ground level. Water (or rain) is necessary for spore arrival. This is in accord with the predominance of spores on the upper surfaces of leaves (Björling and Sellgren, 1955), and the occurrence of leaf edge infection in the early stages of blight. Later in the attack, pepper-pot infection occurs through the transfer of relatively dry spores from nearby fruiting lesions. These modes of infection have very important consequences for spraying machinery.

In retrospect this work seems very simple in that all the factors involved appear to be consistent with this explanation. However, the theory of the way in which an erratic deposit of weakly retained fungicide is effective against wet infection is as yet unproved, and this simple explanation still depends upon unchecked surmises about the bionomics of blight and its control.

This demonstration that minute doses of copper are sufficient if they are well distributed and strongly retained, would be valuable for commercial practice, among potatoes as well as other crops affected by diseases which are similar to potato blight, if it were possible to spray all parts of every leaf.

It provides a reason for attempting to obtain complete spray cover. It also provides the hope, or rather the expectation, that even in quite different plant-protection tasks, a comparable analysis of the whole process would permit a more frugal use of pesticide. The gain is very unlikely to be as large as it was in this example but could provide a valuable move toward reducing the amounts of chemical used and therefore the cost and the residue hazard.

The consideration which was given to examples from outside the sphere of fungicides may now seem justifiable. Conventional formulations of fixed copper salts are not by any means static. They redistribute very readily, but in a way which is perhaps different from the expected method of redistribution. Because they redistribute so readily they are also lost very readily and large amounts must be applied to leave a residue large enough to be effective. Furthermore, in one
type of infection, the sporangia are also very likely to be mobile, to an extent which is sufficient to compensate for sparse cover of the leaf. Both the pest and the pesticide are mobile in a limited way just as insects and systemic insecticides are on a larger scale.

This is a very brief summary of a considerable amount of work which is still to be fully reported. It is necessary, however, to present this extensive introduction in order to justify taking a reader's time in discussing the physical performance of sprayers with uniform distribution and complete cover in mind.

In this example there is a choice. Successful biological effects can be obtained with machines giving poor physical cover using large amounts of readily redistributed fungicide, but only against the wet type of infection (which is almost certainly the more important). Both the wet and dry types of infection can be effectively controlled using tiny amounts of fungicide provided it adheres well (because losses from such small deposits cannot be permitted). If it adheres thoroughly, it must be applied to cover all the leaf surfaces because redistribution can no longer be relied upon. This promises to be a much cheaper process and to avoid the phytotoxic effects met with heavier copper doses. Whether or not a sprayer performs well in a biological test depends upon many factors.

Thus it would be a mistake to consider the performance of a machine alone or just in conjunction with a chemical and pest. The whole process, including modes of infection, the surface chemistry of the formulations used, and the weather, should be examined and irrelevant factors discarded only when it is known that they are unimportant.

II. METHODS OF APPLICATION

The quantities of active ingredient applied to crops are of the order of 1 kilo/hectare (1 lb/acre). This amount is generally diluted with relatively inert carrier materials in any quantity up to 10,000 liters/hectare (100 gal/acre). More usually nowadays, the advantages formerly provided by extreme dilution of the active ingredient can be obtained by formulation and application so that more usual spray volumes in the majority of crops now are of the order of a few hundred liters per hectare (tens of gallons per acre). The active ingredient can also be applied as a dust mixed with a carrier in the range 5 kg/hectare (5 lb/acre) upward. The amount of carrier usually causes no change in effectiveness.

Clearly, spraying or dusting can be termed a lightweight agricultural
operation provided fairly concentrated materials are applied. Accord­
ingly, small portable or even manually powered machines are just as
capable of spreading such quantities of material as large mechanically
powered machines. The difference in the work rate of machines widely
different in size and power consumption is not nearly as great as might
be expected. This is because the process of actually distributing the
material requires so little power that the work rate tends to be in­
dependent of the power available. This is not to deny that big ma­
chines work faster than small ones. They usually do, but not only or
even mainly because they are bigger.

This small power requirement of spraying is one of the main differ­
ences in comparison to other agricultural tasks. It means that practi­
cally any machine is able to do the work at an acceptable speed and
cost and consequently a very wide range of styles, sizes, and methods
has evolved, from a smoke canister to twin-engined aircraft.

There may be a number of important differences between them but
these are masked by the more numerous unimportant differences. If
it is recognized that all sorts of sprayers and dusters are merely ve­
hicles for spreading a small amount of active ingredient over a large
area, much of the concern for the variety of machines and for the
methods of grouping them into categories may no longer be needed.
Time will be spent in discussing what a machine does, but very little
on whether it flies or is carried, or the sizes of the spray drops which
it forms. In fact, it is suggested that classification of machines usually
causes more confusion than enlightenment and hides the important fact
that a given machine can change its category and its function accord­
ing to the way in which it is used.

While dropping one traditional approach, another will be emphasized
as discreetly as possible. An applied subject like this can be considered
quite basically without losing sight of its objective. This can be de­
scribed thus: diseases are not controlled for the sake of science but
for a practical motive, and conventionally a single simple criterion—
money—is used to judge success. Without emphasizing this commer­
cial objective of the work, the reader will be reminded of its existence
occasionally, but only in a semiquantitative way since profit is a flexible
measure of success dependent upon circumstances quite beyond the
scope of this chapter. It is necessary to mention money, however, in
order to justify plant protection practices which are widespread and
yet which appear ill-advised unless viewed from this financial stand­
point. In England, dusting with fungicides which are intended to per­
sist through the damp summer seems quite unwarranted because of
the readiness with which the dust is lost from the surface of the crop. Yet its use is widespread because farmers believe that the combination of ease and speed with a moderate degree of effectiveness suits their requirements best. So we shall be concerned with principles and functions common to a variety of machines and, whenever it is informative, with some practical and economic interpretations of them.

A. Work Rates of Machines

The speed at which work can be done while actually spraying is limited by two factors either of which may predominate. The rate at which fungicide is emitted is one. A small output from the machine is sufficiently quick whenever concentrated materials are applied because the amounts of active ingredient needed are very small. Consequently, even small machines are not limited by their output of active ingredient if it is in concentrated form whereas they can apply dilute solutions only slowly. I once worked with a diminutive Indian laborer who treated 10 hectares of experimental plots with a portable sprayer during one working day. Twenty-five acres per man per day is a rapid rate of work.

The second factor limiting the work rate is the speed with which ground can be covered. This is the product of the forward speed and the swathe width. With a sprayer treating a band equal in width to the length of its boom, the speed is therefore proportional to the boom length and the machine with the longer boom is quicker. Spray and dust can and often are spread by the wind however, and the swathe width thus treated in each passage of the machine (tens of meters) is dependent on the wind and not on the machine.

These two simple features of spraying are mentioned to help counter a widespread tendency towards excessive size in machines. Large machines are practicable in certain circumstances and they can be cheaper than smaller machines which use more labor. But cheapness and high rates of work are not the prerogatives of big machines nowadays. Concentrated fungicides and small drops which drift to cover a wide swathe allow small machines to work almost as quickly as big machines and fast enough for the work rate to be of little importance when selecting a sprayer or duster.

B. Materials Applied

Just as a range of machine sizes and styles can all be used, so also the fungicides can be presented in a number of forms—as dilute or
concentrated solutions in solvents with and without additives for several purposes, as emulsions with one of many solvents in one phase and water as the other, as dusts with small or large amounts of inert material. As in the case of machinery, an explicit survey of all classes of materials will not be made. Instead, some of the particular characteristics which are of importance among different materials will be mentioned as they affect the processes, deposition, retention, and so on being discussed.

It is worthwhile to comment further on the approximate figures for the quantities of material which have to be applied. Fungicides are relatively nontoxic to fungi in contrast to the high degrees of activity of insecticides and mammalian toxins. Silver is among the most active of fungicides with an LD₅₀ in the range 90–560 ppm, i.e., 90–560 μg of silver per gram of spores. Other fungicides and particularly organic fungicides are far less powerful with LD₅₀ above 1000 ppm (Miller and McCallan, 1958). Nevertheless, despite this low toxicity, minute amounts of fungicide are needed for each individual spore weighing perhaps 10⁻⁹ gm. With a toxicity of 1000 ppm, one millionth of a microgram is sufficient for such a spore. Common application rates to a hectare are therefore around 10¹⁵ times the quantity needed for an individual spore. The work of application is to find out how to keep this margin to the minimum which is sufficient in practice.

III. PRODUCTION OF SMALL PARTICLES

A. Spray Nozzles

To propel and distribute the chemical on the crop, it is formed into spray or dust particles. Dust is milled in the factory and so we need not consider its production. Spray is produced by the use of nozzles on the machine. Fraser (1956) lists three nozzle categories—moving air, moving liquid, and centrifugal nozzles. These contain two mechanisms for forming spray: (1) interaction of air and liquid films and (2) internal disruption forces.

Disintegration of thin liquid films occurs under the action of either surface tension forces in the liquid or, at higher relative speeds, by the impact of the liquid film on the surrounding air. The liquid may move through still air—still relative to the observer—as in the pressure nozzles, or the air may move past the relatively still liquid as in most air blast nozzles. Many sprayers, particularly airplanes, have both the liquid and the air moving. Kruse et al. (1949) and later
Yeo (1961) pointed out that essentially it does not matter which moves, the relative velocity alone determines the breakup process. The basis of all nozzles of this type is the presentation of the liquid in the form of a thin film. It breaks up through striking the air at high speeds above 20 meters per second whereas at lower speeds it will first form a network of filaments which then collapse and form drops along their length under the action of surface tension. If the air is moving it is considerably more difficult to cause it to act on the liquid film efficiently and most air-blast nozzles are very inefficient and require considerably more energy than nozzles in which the liquid is made to move. However, they have certain advantages for many purposes which are mentioned below.

Internal disruption can occur in several ways but in practice the only mechanism used is centrifugal atomization in which in principle the outermost drops on a rotating disk are flung away from the body of the liquid. In the laboratory a flat circular disk is commonly used, but in the field where larger flow rates must be used, it is essential to make provision for accelerating the liquid, and radial vanes of some form are required or the liquid will slip across the surface of the disk. Usually this is done by replacing the disk with a cylindrical gauze. The vane disks common in the chemical industry for avoiding slip are not used because they have not been found necessary with agricultural spray liquids.

B. Film Formation

Fraser (1956) also describes a number of ways in which the liquid may be constrained to flow and form a thin film in the air. Two of the most common methods are those used for swirl nozzles (cone nozzles in American terminology) and fan nozzles. In a swirl nozzle the liquid enters the swirl chamber through tangential passages and so rotates. Its circular movement causes it to leave through the central circular aperture of the disk as a hollow, rapidly rotating cylinder. As soon as this reaches free air it spins out into a cone-shaped film which is then broken up. The throughput, liquid speed, and the cone angle determine the thickness of the sheet at any point and the corresponding drop size to a large extent.

Fan-shaped sheets are usually formed by the convergence of the liquid moving in a passage which rapidly narrows in one direction and opens up in a perpendicular plane—the plane of the fan. A sheet of similar triangular shape is also very conveniently formed by cutting
a thin slit across the wall of a small-diameter hollow cylinder so that the slit lies in a circular surface.

The relative merits of the main classes of nozzle—swirl, fan, air blast, and rotating—can be discussed in terms of these functions; forming drops, propelling them and distributing them, and practicability. In this section we are concerned with the first and the last properties.

C. Film Breakup

When a sheet breaks—as a bubble bursts when struck by moving air—an area of a sheet of $A$ square microns and a thickness of $T$ microns, gives a spray drop of volume $AT$ cubic microns. This can be considered a characteristic drop size determined by the sheet geometry and pattern of breakup. Even for a given sheet, however, the process is erratic so that a range of drop sizes forms. In addition, the sheet is very readily deflected from its plane by the air and there is then a tendency at low speeds for large areas of the sheet to roll up into filaments giving rise to large drops with diameters of the order of the filament diameter. A wide range of drop sizes therefore arises out of a given nozzle. The relative frequency of occurrence is called the drop spectrum. Drops below 50 $\mu$ and above 500 $\mu$ are uncommon in the drop spectrum from film breakup in conventional practice. Even this is a wide range with one 500-$\mu$ drop having the same volume as 1000 drops each 50 $\mu$ in diameter.

The spectra of dissimilar nozzles overlap almost completely. The fact that a fine spray has a surface-to-volume mean diameter (SMD) of 150 $\mu$ and a coarse spray of 300 $\mu$, means that the latter has only one-half the covering power of the former. The two sprays will contain more or less the same drop sizes, however, and in practice have not shown any difference in biological effect when applied as a placed spray. Figure 3 shows how the spray SMD varies with pressure and throughput when water is used in a conical film. A flat film of comparable dimensions generally gives a spray about 10% coarser and a drop size range (for example, the range which contains 95% of the spray) about 10% wider. The drop sizes from one-third to three times the SMD contain about 95% of the spray volume in a well-made nozzle. A roughly made nozzle gives a wider range of sizes.

Since the film of liquid formed and broken up in an air-blast nozzle is basically the same except that the air is moving instead of the liquid, the drop spectra of air-blast nozzles is similar to that of pre-
sure nozzles working at the same relative speed between the air and the liquid. Since the air can be made to move very quickly at low pressures, however, it is possible at pressures of only a few pounds per square inch (a fraction of 1 atmosphere) to form a very fine spray with air-blast nozzles. This is their chief characteristic as illustrated in Fig. 4. A very modest air speed of 50 meters/second gives, with water, the relative fine spray with $SMD = 120\mu$ at a pressure of about ½ of an atmosphere, provided the nozzle design is able to make full use of the relative speed. Fraser (1956) pointed out that if the quantity of air supplied is low, this performance will not be achieved, but in agricultural air-blast spraying we usually have very much more air than liquid, since a large amount of air is needed to propel the spray to the target. The only results quoted are for water or fluids of comparable consistency and somewhat different figures are obtained with other liquids which can be sprayed.

One of the advantages of an air-blast nozzle is that it can atomize both viscous and viscoelastic fluids far better than pressure nozzles because these materials do not form films very readily under pressure.
This may be important because small spray drops are absent from sprays of viscoelastic fluids (Courshee, 1959a) so that these provide a means of avoiding spray fine enough to be carried as drift with the wind. Another important advantage in practice is that large apertures are used in these nozzles and they do not block easily.

![Diagram](image)

Fig. 4. Mean drop size from air-blast nozzles.

Normally one would not advocate air-blast nozzles except on machines on which the air blast is present in order to carry the spray. If the copper example quoted in the introduction is typical of other fungicides, however, it may become worthwhile to use air-blast nozzles more widely in special machines or machine adapters for applying fungicides because of their special ability to give very fine spray and thus a complete spray cover with small volumes of liquid.

The spinning-disk nozzles have similar advantages with somewhat greater mechanical complexity. One feature, which is occasionally a disadvantage, is that spinning disks form an annular circle of spray. Such a distribution is difficult to control and use efficiently in many
circumstances. These nozzles have the advantage of providing very fine spray conveniently with only small amounts of energy. At very low throughputs—about 10 ml/minute off a conventional disk or 100 ml/minute off a spinning cage of the size commonly used (up to 20 cm in diameter)—the drops form individually, and they are all the same size. With water each characteristic drop is accompanied by two satellites each about 2% of its volume. If these are ignored, therefore, the spray consists of drops of one size only. Throughputs as low as this are valuable in the laboratory but are not useful in the field.

The drop size formed by a spinning disk under these conditions of flow is given in Eq. (1) (Walton and Prewett, 1949).

\[ SMD = \frac{4}{W} \sqrt{\frac{S}{PD}} \]  

(1)

where \( S \) = surface tension (dynes per centimeter of liquid); \( P \) = liquid density (grams per cubic centimeter); \( D \) = disk diameter (centimeters); \( W \) = disk angular velocity (radians per second); \( SMD \) = surface-to-volume mean diameter (centimeters).

As the throughput rises to practical levels, more and more satellites form and the breakup changes more and more to filament breakup, the spectrum approaches that of a good film-forming nozzle. It is a little more regular and finer, for a given relative speed, because filaments of excessive size cannot form easily. Also, of course, the high relative speed is formed with a very low power consumption because it is the liquid which is moving not the air at low pressures.

Yeo (1959) has shown how both film breakup and filament breakup from rotating cages (Yeo, 1961) can be correlated with the relative speed of the liquid through the air. This must include the speed of the nozzle if this is moving, as on an airplane. Figure 4 shows that for air-blast nozzles this is also true, and in fact all three main types of nozzle can be considered air-blast nozzles under practical conditions with the differences between them being limited to practicability and to somewhat different drop spectra. The overlap however is large and if we were to try to demonstrate biological differences arising from different drop spectra or spray nozzles we should start by working with extremes from the range.

IV. MOVEMENT TOWARD THE TARGET

These small particles of spray or dust can be shot at the target at high velocity through the air as spray is from a pressure nozzle. Dust
and fine spray are more usually blown toward the target on a column of air. If the direction of their path, with or without air, is determined by the machine, we say that they form a placed spray—the fungicide is placed on the target. In the field, however, many sprays and all dusts are strongly affected by any wind that moves past them so the machine cannot direct particles accurately in the open and a calibration under calm conditions does not quite represent practice. It is useful, however, to distinguish the attempt at placement from drift spraying when no attempt is made to direct the particles. In the latter method they are simply released in the wind and allowed to drift as they settle. Very wide swathes are covered in this way with both a reduction in uniformity and an increase in the scale on which non-uniformity occurs.

With ground crops at short range and a coarse spray, the movement to the target is under control and independent of moderate wind. With a fine spray or dust no projection is possible because the particles lose their inertia after a very few centimeters. They can be placed to the extent that a jet of air can carry them to a region of the crop, but then within that region where the jet breaks up, their further motion toward the crop depends largely upon the air movement.

With taller crops at longer range and using airplanes in still air, the spray can still be placed, but the longer the range the more sensitive is the movement to the wind. This applies especially to crops with a small surface area—deciduous trees treated early in the season, for example. Spray may be aimed at the tree, but 90% of it passes through the bare portions of the target tree and then hits trees beyond when it is being moved by the wind alone (Courshee et al., 1953). So to a large extent such spraying, particularly with fine spray below 50 μ, is drift spraying.

Clearly any position is possible between a placed spray and a drift spray. Two are mentioned at some length because the importance of the part of the spray which drifts has been underestimated even when spraying large volumes of dilute material (large-volume spraying). Particularly in the case of fungicides, small-drop, small-volume spraying is the more common method of application, and the fraction of the spray which drifts onto the target is becoming ever greater with considerable change in the quantity and pattern of the deposit and in its effect.

A. Airplane Spraying

Airplane spraying is a particular example of an important application method which usually lies between placement and drift spraying.
The nozzles on an aircraft usually point downward so that the initial projection of the spray when the aircraft is moving is downward and forward. A large spray drop, 200 μ, might travel half a meter before losing its horizontal velocity relative to the air. The greater part of the spray from an aircraft accordingly comes to rest in mid-air and forms a cloud which has no horizontal velocity relative to the air after a few hundredths of a second. Thereafter the spray goes where the air goes. Roughly speaking, the air movement caused by an airplane drives the spray symmetrically to either side to a distance of about two wing spans (Chamberlain et al., 1955). Some spray is caught in the upward air motion of the wing tip vortices on the outer leg of the spiral and becomes drift. The whole air motion is itself susceptible to displacement by the wind to an extent dependent mainly upon wind speed and flying height for a given aircraft.

Airplane spraying is included at the beginning of this section to introduce it and because it is in this respect—the way in which the spray moves to the target—that this method of application is special. In other respects it is better to regard the airplane as being just like many other spray machines with the difference that it moves more quickly and causes no wheel damage.

B. The Range of Small Particles Moving through Air

This has been studied for levels of speed and drop size of interest for this work. Typical results using water are shown in Fig. 5 (Courshee and Ireson, 1961). It is clear that small drops cannot be projected any distance which is significant and even large 500-μ drops can be projected only a meter or so. If large drops are propelled too quickly they simply break up instead of going further. This limits the coverage of a single spinning disk, for example, to a two-meter-wide swathe and disks on a boom sprayer should be less than a meter apart.

C. The Range of a Jet of Particles

Nordly (1965) has considered the range attainable with a jet of particles, e.g., the stream of spray from a nozzle. This depends upon the liquid throughput and the jet angle. Drop size is not so important as it appears to be. A fine spray results from a low throughput and a large jet angle and thus fine spray is associated with a short range but is not the main cause of it as it is when single drops penetrate the air alone. The range of a jet results from air movement generated by the loss of momentum of the liquid so that the spray has less air resistance to overcome.
Dimensional analysis coupled with the observed fact that the range attained by a moving sprayer is inversely proportional to the angle of the spray jet gives us this equation for range $R$.

$$R = \frac{KV^3U}{\theta s}$$

where $R =$ range (meters) ; $\theta =$ angle of spray jet (degrees) ; $V =$ spray output in liters per second at speed $U$ (meters per second) ; $s =$ sprayer speed (meters per second).

The constant of proportionality has the value 1.0 with these units and a plot of a few of these variables is shown in Fig. 6.

**D. The Range of an Air Jet in a General Airstream**

The range attainable by the spray alone is very limited unless a large liquid output is used. Therefore when small volumes are applied the spray must be carried to remote targets on a stream of air. The theoretical range attainable in this way can be deduced just as it was for a liquid jet. The presentation of the equation in a ready-to-use format
form is a little difficult because the sprayer speed through the air is compounded of its ground speed and the wind speed over the ground. Moreover, the distortion of the air jet by the air which appears to bend its tip downwind may weaken its coherence and ability to penetrate the air. At high wind speeds the jet appears to penetrate irregularly, at one moment reaching out a long way and the next failing to travel more than a short distance.

This must be expected because the air jet is a relatively large-scale disturbance to insert into the wind so that the wind itself is changed and the scale and degree of turbulence within the jet may be altered. Moreover, the range attained is the range relative to the wind not to the point of emission of the air jet, so that in the last slow stages of the jet the final position attained by it is moving with the wind and whereas a jet with a given ejection speed may travel a constant 10 meters through the air it may move only 6 meters away from the machine (in a given time) if the wind is toward it and conversely 14 meters if the wind is away from the machine. For this reason a proportionality constant of only 0.8 was taken to plot Eq. (1) in familiar
terms for air in Fig. 7 to indicate what may be called the effective range. This is the range at which the air jet appears to break up.

This is simply a set of design data showing how to push spray with or without air any required distance up to a practical limit in this work of about 20 meters. It is clear that there is no difficulty, but that a long range with liquid requires a lot of liquid and a long range with an air jet requires a lot of power since the necessary momentum must be obtained in one way or another.

The principal purpose of drift spraying is to cover a very wide band of crop in each passage of the machine in order to increase the work rate. When using a fairly coarse spray (>200 μ) drift spraying is adequately described by considering the movement of spray horizontally with the wind speed and vertically at the terminal velocity of the drops of various sizes. The terminal velocity in air of spray drops of water is shown in Fig. 8. The distance traveled horizontally before the spray reaches the ground from an emission height \( H \) is \( HU/V \) where \( U \) is the wind speed and \( V \) the terminal velocity of the drops. Fine
spray can therefore travel long distances when emitted high above the ground in a moderate wind.

However, this simple formula is not quite sufficient to describe the drift of spray which is so fine—100 μ or less—as to diffuse as though it were smoke. The mean angle of fall of such fine spray is given by $\tan^{-1} \frac{V}{U}$ below the horizontal (3°–12°). In addition, however, diffusion spreads the spray about 7° to either side of this mean fall angle (Courshoe, 1959b). Therefore drops of a single size are not found concentrated at one point downwind but are spread over a wide band. In fact, such fine sprays are normally used (SMD < 100 μ) that all sizes of drops in the spray follow almost identical paths and are found right across the treated swathe (Fig. 9).

The method is very powerful, and treated swathes can be hundreds of meters wide when spray is applied from aircraft. The distribution of a drifting spray is far less regular than that of a placed spray. Swathe spacings of a few tens of meters are therefore used for applying fungicides as a drifting spray so that numerous adjacent swathes overlap and so reduce the deposit irregularity. Even then the cover is usually sparse and erratic and confined to surfaces facing the wind and it is often unsuitable for a static fungicide. It is, however, important
to consider drift spraying itself even though its suitability for fungicides is doubtful, as it is so often a greater part of ordinary spraying than it appears to be.

**F. Drift Prevention**

The drift of obnoxious chemicals out of the treated field and onto a neighboring crop which is either sensitive to the spray or is ready for harvesting and consumption, has been a source of trouble in many countries, but more so with weed killers than fungicides. It is desirable to minimize contamination of crops, however, and therefore drift should not be permitted.

In a series of reports Courshee (1959a) showed how drift from ordinary ground crop sprayers could be reduced to very low levels, with over 99% of the spray landing within 3 meters. This is done by projecting the spray from the nozzles to the crop and avoiding the small drops which can stay airborne. To do this, the nozzles must be near (50 cm) the crop and the pressure (for watery liquids), little more than 1 atmosphere. The ensuing low projection velocity of 10 meters/second is less disadvantageous than the sudden change in the drop spectrum leading to much spray smaller than 100 μ which would occur at about this pressure (as the atomization changes from filament to sheet disruption), if the projection velocity were raised.
With tall dormant crops, drift is the major part of the spray and at present no remedy for this has been proposed so that it is essential to use nontoxic fungicides or to ensure that downwind contamination with toxic materials can cause no harm.

Spray drift from aircraft is a special problem. The principles developed for ground sprayers are applicable to aircraft, but not as successful (Courshie, 1960b). In particular, the spray tends to sheet breakup because the nozzle velocity through the stationary air is high even when the nozzles are aimed rearward. To change back to filament breakup at high nozzle speeds it is necessary to modify the physical properties of the spray liquids. One method is to form very viscous solutions, for example, by adding molasses. The invert emulsions in which the oil phase is the continuous one, appear to be in this class because they give rise to extremely coarse sprays with only a slight diminution in the amount of fine spray (Akeson and Yates, 1964).

Fluids which have an element of rigidity—jellylike fluids—appear much more successful in avoiding small drops while giving rise to a spray of moderate fineness. They have not been exploited yet because of formulation difficulties and because the mechanism of avoiding the formation of small drops—which depends upon the stiffness of the fluid which is just perceptible when the fluid is poured and upon the time constants of their apparent viscosity—is not fully understood or reliable yet.

G. Spray Evaporation

Drift will also arise if the coarse spray is allowed to evaporate enroute and become fine spray. Conditions favoring evaporation are also to be avoided when fine spray is used because important fractions of the pesticide may be lost through total evaporation (MacCuag and Watts, 1960). This is particularly important for drift spraying when the spray is airborne for long periods of time (Rainey, 1960), but it has also occurred in conventional aircraft spraying and tree spraying (Cunningham, Brann, and Fleming, 1962).

If very small volumes of spray are being applied, solvents or carriers can be used which are relatively nonvolatile. Their price becomes prohibitive above 20 liters/hectare. Most of the huge area of banana plantations throughout the world are sprayed with a copper-in-oil suspension in this way. When using larger volumes of water a material which prevents evaporation is added. Twenty percent of sugar molasses is fully effective and a proprietary compound Lovo, is also available.
Amsden (1964) has shown that the time a water drop survives before it evaporates completely is given by this equation.

\[ \text{Lifetime} = \frac{D^2}{80 \Delta T} \text{ seconds} \]

where \( D \) = drop size (microns); \( \Delta T \) = difference (°C) between the wet and dry bulb of a hygrometer.

Therefore the life of a 100-μ drop on a warm dry day is to be measured in seconds, far longer than the time it is moving quickly as a result of its momentum as this is only a fraction of a second. Therefore, negligible evaporation occurs in a placed spray and the evaporation which is measured must result during the time the spray is drifting, after it has lost most of its velocity relative to the machine.

V. Deposition on the Target

We now have the particles as far as the region containing the target. It now remains to cause them to land on it by sedimentation, impaction, interception, or attraction. Sedimentation is the process of settling at terminal velocity onto mainly horizontal surfaces. Impaction occurs when a particle is carried on an airstream to a surface of any inclination. Interception matters for only very small targets. It is the process of collision between a particle and a surface even though the path of the center of the particle does not meet the surface. A fourth mode of deposition may become important. This is impact at zero air speed past the target, through the momentum of the particle.

Small particles less than 100 μ moving with an airstream do not land on bluff targets because when the air is turned aside, they too are turned. No similar inability to land occurs on a measurable scale with particles moving without an accompanying airstream. Indeed there are few mechanisms, and these are on the whole very weak, which could turn them aside. The only factor which might prevent them from landing is a like electric charge but since most plants are very good conductors an approaching charged particle would actually generate an image charge of opposite polarity and be attracted.

This tendency of small particles to go around targets has been spoken of so often that we should expect incomplete deposition to be an important factor in practice. However it is suggested bluntly that for particle sizes greater than about 30 μ a deposition efficiency in the open air of above 50% can be assumed on targets of the size and micro-roughness of, for example, apple leaves and small particle sizes.
need not be a cause of inadequate deposition of sprays. Dusts and fogs are excluded from it because their particle sizes are much smaller than this limit. There are also other factors concerned with deposition which can have more importance than impaction and they must be considered in some detail also.

A. Impaction and Interception

A set of curves prepared by Yeo (1960) quoting Langmuir (Langmuir and Blodgett, 1946) are shown in Fig. 10. These indicate high impact efficiency, except at very low wind speeds of 0.5 meter/second which is very rare out of doors, on rather fine targets of all particles over 30 \( \mu \) in diameter. The choice of such fine targets, 0.1 and 1.0 cm in radius, is interesting and probably deliberate. Although leaves and twigs of many kinds are much larger and although the impact on them should be much less efficient, they are often rough to a degree which causes it to be as efficient as it is on these fine cylinders. So these curves are not atypical of the impact we should expect on leaves. On smooth artificial targets of larger sizes, impact does become appreciably less efficient (Jarman, 1959).

The impaction of dust is aided appreciably by placing electrical

![Fig. 10. Effect of wind speed and cylinder size on the impaction of fine spray on smooth cylinders (From Yeo, 1960; courtesy of the International Agricultural Aviation Centre.)](image-url)
charges on the particles and causing them to be attracted to the surface (Bowen et al., 1952) without apparently effecting the adherence of a given particle. However, another effect of the charge is to cause more of the small (≤10 μ) particles to land and these are more strongly retained than the large (>20 μ) particles. An indirect consequence of charging is, therefore, that the persistence of a deposit formed from charged particles would be higher but only if these smaller particles are available to contribute to it.

Interception of particles is also important mainly for dust. This occurs when the center of a particle misses the target but owing to its finite size an edge collides. This contributes only a small amount, of the order of a few percent even on hairy targets, but this is not negligible for uncharged dust for which deposition tends to be very low in practice.

B. The Geometry of Deposition

When coarse sprays and large spray volumes were used exclusively it was perhaps proper to neglect the effect of the shape of the plant and its leaves on the deposition of particles. Now that fine spray is used more, the angle of approach of the particle to the target tends to be close to the horizontal so that the deposition tends to occur preferentially on vertical surfaces. Generalizing one can say that, if impact is efficient, deposition will occur to the extent that the target blocks the paths of the spray particles. If the leaves all happen to lie horizontally and the spray is fine enough to travel horizontally then no deposition will occur in non-turbulent air. As the angle between the target surface and the line of approach of the particles increases, so the deposit rises in proportion to the sine of this angle.

In turbulent air some movement toward all surfaces occurs even if these are parallel to the mean drop path. Also of course all surfaces are rough and do not lie in one plane so that deposition is never zero. Nevertheless several-fold differences must be expected to occur between deposits on vertical and near-horizontal leaves, depending upon the angle of approach of the drops in the drifting stage—nearly vertical to the ground if the spray is coarse and the air still, and nearly horizontal for particles below 100 μ in a light wind. This is sometimes important for sprays applied with high speed air jets. These tend to turn leaves into a position where they lie parallel to the jet and present the minimum area on which the spray can land. This helps
spray to penetrate the outer layers of foliage, but could result in the deposit there being deficient.

C. Drop Bouncing

Some drops fail to wet a surface with which they, nominally, come into contact. It is possible that they do not contact it and there is a layer of air between them at all times. In any case, drops can rebound elastically, particularly from peas and Brassicaceae, in fact from any surface which is difficult to wet and especially from surfaces with a waxy bloom. The exact nature of the process is not understood, but Hartley and Brumskill (1958) showed that drop size and surface tension of the liquid are both important—a high surface tension inhibits wetting and the deformation of the sphere which is essential for contact to occur. The drop sizes which cause bouncing—about 300 μ—must be large enough for their momentum to be sufficient to cause them to rebound but not so large that the impact causes them to shatter. This is one of the factors which can be valuable in causing selective deposition of phytotoxic pesticides, but is only a disadvantage for fungicides. It is best avoided by using a fine spray and a wetting agent.

D. Initial Retention

A film of liquid over the surface of the crop will drain off if its thickness is excessive. The thickness retained depends upon the plant surface and the properties of the liquid on it. An excessive ability to flow over the leaf causes the thickness of the film retained to be undesirably thin. Commonly the film thickness is about 150 μ which corresponds to a deposit level of 1500 liters/hectare of leaf surface. Somers (1957) applied a copper-bearing spray and found runoff occurred when the deposit level reached between 5 and 10 mg of spray per square centimeter of leaf surface, i.e., when the deposit reached 1000–2000 liters/hectare of leaf. Clearly runoff is not commonplace in practice. If enough spray were applied for it to occur it would usually have the valuable effect of limiting the deposit density of a pesticide to 5x–10x mg/cm² where x is the concentration of pesticide in the spray (wt/wt). Sometimes, however, preferential deposition of the pesticide and the converse occurs. Rich (1954) has given figures suggesting that a fungicide deposit of bordeaux can reach a limiting level independent of the concentration.
of copper in the spray indicating that the maximum initial retention of a gelatious spray like Bordeaux is complexly dependent upon the circumstances.

E. Recovery

All of these factors, impact, interception, sedimentation, and in the case of large volumes of spray, initial retention, contribute to the recovery of the pesticide—the fraction of the material emitted which is found on the crop. A high recovery is not a complete index of machine performance, but it is a very important measure in that it determines the quantity of fungicide (and hence its cost) which has to pass through the machine in order to attain a given level of deposit.

The recovery is normally high on leafy crops that obscure the ground. On young row crops or dormant tree crops, recovery is low—of the order of 20%—the remainder landing on the ground. If, however, a fine spray is used, the movement toward the ground is kept to a minimum and the spray stays airborne until it lands on a near-vertical surface. An appreciably higher recovery on a sparse crop can thereby be obtained, but of course the deposit is now most dense on vertical surfaces facing the wind and this irregular distribution may be unsatisfactory for most fungicides.

VI. DISTRIBUTION ON THE TARGET

The effectiveness of a deposit is not determined by its density alone. How it is distributed so that it may become available and how the actual transfer takes place is also important.

The distribution on the target is partly controlled by the sprayer. It depends on the movement of the particles toward the target and their deposition on it and therefore it also depends greatly on the form of the target.

Complete uniformity over the whole of the plant surface is rarely needed because, for example, spores land preferentially on certain surfaces. Björing and Sellgren (1965) have shown how spores land mainly on the physical upper surfaces of potato leaves, but pointed out that conditions for germination are more favorable for the few spores landing on the undersurfaces.

The recovery which matters is that which places the fungicide in regions where it is potentially useful. We have to view uniformity in the same way. Equal and sufficient deposits are required on all sites susceptible to attack but none is needed on the others. So far, how-
ever, selective deposition on particular parts of a plant has not been achieved other than by a deposit which is restricted to the upper surfaces of leaves. To cover completely the parts that matter, it is usually necessary to cover the whole plant.

A lack of uniformity must, by definition, result either in underdosing of the lightly treated areas and a consequent lack of effect there, or overdosing in heavily treated areas with an accompanying higher cost. The latter is found most in practice as it is economical to raise the mean deposit density to very high levels—far higher than would be necessary with a uniform deposit—in order to ensure that the least dosed areas are not inadequately dosed.

It is probable that gaps in the defense caused by, for example, leaves bare of fungicide, are often responsible for its eventual collapse. The supposition is that a successful initial infection on an untreated leaf so raises the inoculum potential in the immediate vicinity that the protective spray there is not able to effect a control although it would have given an acceptable probability of protection against a weaker bout of infection coming from farther away. This seems a reasonable supposition but it needs thorough testing. It is to avoid these gaps that the mean deposit density must rise and with it the cost of the work.

A. Nonuniformity

Without being specific about the nature of the individual targets which must each receive an equal dose, presume that a cumulative distribution curve can be prepared. For example, the targets may be leaves, and a measure of the dose on each of them permits one to draw Fig. 11 which shows the percentage of targets which have a dose greater than a certain level, \(D_L\) (line A). If the application rate is doubled then it is feasible that curves B, C\(_1\), or C\(_2\) would be obtained. Curve B results from a doubling of the deposit density on each leaf. If \(D_L\) is the deposit density which must be exceeded on every leaf, then doubling the deposit on each will, in this example, change the number of underdosed leaves from \(N_L\) to zero. Unfortunately, in practice the usual effect of increasing the application rate is to produce the curve C. Each increment decreases the number of underdosed leaves but does not eliminate them, simply because underdosed leaves are often the concealed ones and they remain concealed and relatively underdosed however much spray is applied. This is the sort of result found for cotton by Courbois (1955).

One can see, at least in principle, that to treat the most elusive
Deposit density on targets

**Fig. 11.** Hypothetical deposit distribution curves. Curve A, typical distribution of doses on targets. Curve B, a distribution which would be obtained from A if the deposit on each target were doubled by doubling the application rate. Curves C₁ and C₂, distributions which are more likely to be obtained by doubling the application rate. The heavy deposits are increased and many of the leaves with inadequate deposits continue to receive insufficient spray chemical, D₀, hypothetical lower limit of a suitable range of deposit density.

leaves, many others are going to be very heavily dosed and gross contamination and high cost will arise. This is, however, mere theory. It is difficult to test the theory except in the rather artificial ways already described in the blight example in the introduction, because it is difficult in the field to make marked changes in the deposit distribution. Machines able to give a more regular distribution could be made so that no increase in application rate is needed to eliminate the underdosed leaves. At least this can be done on certain crops, but the machines developed for it are a little more expensive than simpler ones and an overall economic advantage from their use still has to be demonstrated. Tunstall (Tunstall and Mathews, 1961) has shown that valuable improvements can be made in this way against insect pests of cotton.

**B. Attenuation**

When a dense canopy is sprayed, the outermost exposed leaves receive a full dose. The leaves beyond them are treated by a spray which
is depleted by the deposit on the outermost leaves and so on through
the crop so that the farthest leaves may be only lightly dosed.

The distribution then follows an exponential law. If the deposition
efficiency on the leaves is $E$ and their area in the path of the spray
is $A$ square meters in a cubic meter of the target space, then a fraction
$EA$ of the spray arriving on a square meter will land in the first meter.
An equation for the fraction of the spray lost $dQ/Q$ in a small length
of the path $dx$ can thus be written.

\[
-\frac{dQ}{Q} = EA \, dx \tag{4a}
\]

\[
Q = Q_0 \exp (-EAx)
\]

where $Q =$ spray penetrating the canopy, and $Q_0 =$ spray arriving at
the canopy and since the deposit density is in general proportional to
the spray

\[
P = P_0 \exp (-EAx) \tag{4b}
\]

where $P =$ the deposit density on inner leaves (grams per milliliter)
and $P_0 =$ the deposit density on the outer exposed leaves; $EA$ is called
the attenuation factor per meter. A low impact efficiency allows the
spray to penetrate usually without attenuation. However, a spray fine
enough to fail to impact (<50 $\mu$) will inevitably be traveling in a
predominantly horizontal direction so that if the majority of the
leaves are substantially vertical $A$ will be large and the attenuation
factor $EA$ may increase at the same time as the impaction efficiency
falls and a decreased penetration will result.

C. Deposit Distribution from Drift Spraying

During the process of drift spraying distribution must be considered
carefully since the deposit density varies on a wide scale both greatly
and erratically. Adjacent swathes are usually made to overlap in order
to reduce the variability with distance from the spray run and in a
direction parallel to the spray runs. Also when drift spraying, pene-
tration of a canopy of foliage is strongly dependent upon drop size.

When an aircraft sprays trees, nearly all the deposit arises from
spray that drifts. The finer the drifting spray the more nearly hori-
zontal and long its path, so that attenuation increases. Correspondingly,
the amount of spray penetrating to the lower levels of the canopy is
less with fine spray (50 $\mu$) than with coarser spray, despite the lower
impact efficiency of the finer spray (Courshee, 1964). This work was
done in a tamarisk forest so that the vertical leaves tended to pick up drifting spray readily. In all it is clear that erratic distribution is a great weakness of drift spraying but perhaps not so great that fungicides cannot be applied successfully in this way. Some commercial operators claim that the distribution is not too erratic.

D. Geometric Control over Distribution

The spray from a nozzle can be directed so that the distribution on a nearby crop is also controlled. On low-growing ground crops, the nozzles are spaced regularly along a boom. Each should have the same output and the distribution within the band treated by each one should be such that the deposit density is uniform along the length of the boom when measured on a horizontal surface. To this end the nozzles are usually spaced so that each point on the ground is sprayed by two of them. A low-growing crop is thus uniformly treated. But this may have limited relevance for a three-dimensional crop.

The distribution of spray on taller crops is controlled in a similar way, by aiming and spacing the nozzles and if necessary using different spray outputs on different nozzles.

VII. Spray Cover

Although it is not usually possible to cover all of the crop surface at reasonable cost and therefore redistribution is essential to provide protection over areas originally bare of spray, the machine must play its part in making sure that the blank areas are sufficiently small and few in number for redistribution to be effective. The original cover of the surface before redistribution occurs, indicated by Eq. (5), may therefore be important.

\[
C = 15 \frac{V R K^2}{A (SMD)}
\]  

where \(C\) = cover (% of surface); \(V\) = volume (liters per hectare of ground); \(A\) = area of crop surface on this hectare (hectares); \(R\) = recovery (%); \((SMD)\) = drop size (surface-volume mean diameter, in microns); \(K\) = ratio of the diameter of a stain to the diameter of a drop which formed it—the spread factor.

Although a high degree of cover is obtainable by applying a large spray volume, an equivalent effect can be had by decreasing the spray-drop size or increasing the spread factor. The spread factor of most
water-based sprays is only between two and three even when a wetting agent is added. Sprays based on oil have a spread factor which is usually in excess of five providing the fungicide is fluid or soluble. Powders suspended in oil tend to settle out so that the spread factor of the fungicide is somewhat lower than that of the oil carrier.

When numerical examples for a fairly sparse crop are inserted into this equation it will be found that even a spray volume of 30 liters/hectare can give rise to spray cover approaching unity provided the spray is fine (<100 μ) and the spread factor large (>5). The spread factor is particularly important as it enters in the equation as a squared factor.

Although cover is a simple and convenient measure, it may not directly affect the degree of protection obtained against diseases. Clearly, a very sparse cover given by a multitude of very small particles would be entirely effective simply because the gaps between adjacent particles are too small to allow a spore to germinate there without being contacted by the fungicide. This seems to be the function of the conventional concentrated fungicide dusts which are applied at the rate of only 5 kg/hectare. The particle mean size is commonly 20 μ and the spread factor for dust is of course unity so that cover obtained on 1 hectare, without loss, is 3% (specific gravity = 1). At the same time, however, although only a slight cover is obtained, the number of particles per square centimeter is a little over 10,000.

The number of particles \( N \) per square centimeter is given in Eq. (6).

\[
N = \frac{2 \times 10^7 VR}{AD^2}
\]  

Thus, if \( R \) and \( A \) are unity then even with a medium fine spray of 70 μ, 6000 particles per square centimeter are obtained with an application of only 100 liters/hectare. Quite conventional spray practice can lead to a mean gap size of the order of 100 μ with unit spread factor and to zero gap size with an oil.

Cover seems likely to be the more relevant criterion when cover is high—that is, if a large spray volume is applied and a formulation with a high spread factor is used. If, on the other hand, only a small spray volume is used so that cover tends to be thin, the gap size may be a more important measure of the probable control over a disease. This is still all in the realm of theory, however. It is only mentioned in the hope of suggesting some lines of thought should further experiments be undertaken to relate biological effect to these features of application now that fungicides are being used more and more often.
in a concentrated form. The apparent size of the gaps between adjacent spray particles is probably very different from the effectively bare area. Short range redistribution may allow each individual particle to exert an effective control over an area much larger than that which it seems to occupy (Courshee et al., 1963).

VIII. PERSISTENCE AND REDISTRIBUTION

Most fungicides are used as preventive agents and are accordingly required to persist for prolonged periods on the foliage. Conversely, one is usually compelled to rely upon ready redistribution and weak persistence to compensate for deficiencies in the original distribution. Aside from chemical decay of the pesticide and losses in excess of those needed for suitable redistribution, it is clear that the two requirements of redistribution and persistence are sometimes incompatible. The mechanisms giving rise to the one which is desirable are also the cause of the other which is not. The greater the reliance that has to be placed on redistribution, the more probable premature loss becomes. The requirement for redistribution arises from the habit of the plant and the disease and from the deficiencies in the distribution achieved by the machines used to apply the chemical.

Fungicides commonly in use are not afflicted so much as insecticides are by loss which is not part of redistribution. Many insecticides have a short life on the plant through chemical breakdown or unnecessary volatilization. Fungicides, on the whole, can be more permanent provided they are not loosely attached. They often are, however, in order to encourage the sort of massive redistribution, and loss, which it is suggested, occur with the large applications of fixed coppers for potato-blight control.

Those concerned with application are therefore in a quandary. Their problem becomes nonexistent, however, as soon as the original distribution is made so good that short-range redistribution alone is sufficient for control to be achieved. Some final movement of the fungicide is obviously necessary. If copper is fixed well to the leaf then a spore landing nearby is in contact with a source of copper ions for as long as a water film exists between them. Therefore, the distribution necessarily depends on the kinetics of this transfer mechanism. Phenylmercury chloride and karathane are apple fungicides which are able to exert an appreciable action at a distance in the vapor phase (Moore, 1965). A too readily soluble salt would allow more ions than are needed

*For a more detailed review of this subject see Ebeling (1963).
to go into solution and be lost. An excessively volatile fungicide would cause a buildup of lethal concentrations of vapor over a greater range than is needed. There is no point in a deposit being so volatile that it can give rise to lethal vapor concentrations on a nearby leaf when that leaf carries its own quota of fungicide, and all the air between them is saturated with the fungicide vapor, probably without anything useful being achieved.

A. Methods of Fungicide Loss

Massive removal of the whole deposit appears to be the most common source of loss. It occurs very readily with excessively heavy deposits of dust although the lighter deposits which are in more intimate contact with the leaf are more strongly retained. Wettable powders are also frequently removed very readily en masse by rain especially if the wetting agent used is one which permits a dry deposit to redisperse in water. If this extremely powerful source of loss is avoided, then fungicide disappears by chemical decay, by solution in rain water, and by volatilization. Sorption into the foliage surface is rarely of importance as a source of loss.

Solution loss can be readily demonstrated with “insoluble” copper salts when they are formulated so as to be strongly retained on leaves of tomato seedlings. Under these conditions, the copper, for example, appears to dissolve to form a saturated solution in rain and the amount of copper removed is linearly proportional to the amount of rain which falls and quite independent of the amount of copper initially present, and of the intensity of the rain. Similar loss would be expected from a volatile deposit and has been demonstrated for materials other than fungicides. The rate at which vapor is removed is proportional to the vapor pressure of the material and to the Reynolds number of the air flowing over the surface. The mechanism appears to be that the air near the surface is saturated with vapor, but that transfer of this saturated layer to the free air depends upon the thickness of the boundary layer, so that loss is not directly proportional to the velocity of the air past the evaporating chemical nor is it affected by the amount of chemical present.

B. Loss Curves

Once again the treatment of this section must be theoretical but it is perhaps worthwhile despite this as it may help in providing a clearer picture than has been available elsewhere.
Loss may be considered adequately in two main categories: (1) where the rate is constant as in the case of solution and volatilization usually, and (2) where the rate of loss depends upon the amount of material present. If we call the deposit $P$ then either

$$-\frac{dP}{dt} = K_1 \quad \text{or} \quad (7a)$$

$$-\frac{dP}{dt} = K_2 P \quad (7b)$$

where $K_1$ and $K_2$ are constants.

Constant-loss curves (Eq. (7a)) are like those described for copper which is only slightly soluble and which adheres well (Fig. 12a). They have two typical characteristics. One is that the initial deposit density (I.D.D.) controls the deposit density after a fixed period of weathering, linearly (Fig. 12b), and the other is that if we plot the loss caused by a fixed period of weathering against the initial deposit.

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**Fig. 12.** Constant-loss lines.
density we should get a horizontal straight line (Fig. 12c). That is to say, initial deposit density does not affect the quantity lost but does affect correspondingly the proportion lost after a fixed period of weathering. My own results for bordeaux suggest a constant rate of loss of about 1 mg of copper per square centimeter for each 2 cm of rain which falls on the leaf. Rich’s curve for bordeaux (Rich, 1954) also tends to show a loss independent of the initial deposit.

The exponential type of decay typical of massive loss is commonly met with in wettable powders and dusts [Eq. (7b)]. Both the deposit residue line and the relationships between initial deposit density and the residue are curved (Fig. 13). With this type of loss it is difficult and expensive to ensure a large residue by simply increasing the initial deposit density to very high levels as the loss caused by a fixed period of weathering is then higher for a heavier initial deposit density. This loss curve is typical for a wide range of wettable powders and it may also be typical of Rich’s results for zineb also. The loss curves

![Figure 13. Higher order loss curves.](attachment:figure13.png)
arise because this mode of loss is for the most part a first-order reaction, that is, the amount of material present determines how quickly the loss occurs.

In practice, perfect constant-loss curves are rare. They can be demonstrated accurately but only for fungicide formulations which adhere very firmly—some stable insecticides provide the best examples, but insoluble fungicides can also adhere well enough to be lost at a constant rate. Losses which occur in this way are the smallest ones and first-order reactions lead to much heavier losses. Excessive deposits, especially of dust, usually decay more quickly even than the rate corresponding to a first-order reaction in the initial stages and rather more slowly eventually.

Probably there is more than one rate constant involved in the decay of a dust deposit leading to an initial very heavy loss, and perhaps also the final small residues adhere well and decay at a slower constant rate. Strong adherence (of the deposit to itself and to the leaf epicuticle and of this to the underlying leaf structure) is the main factor which causes a deposit to follow a constant-loss curve. Many wetting agents, glues, and waterproofing materials are used with varying success to hold a surface deposit in place.

One must note that strong adherence will usually cause an improvement in effectiveness if the cover is good but may tend to cause a decrease in effectiveness if the cover is bad when strong redistribution may be essential. As the two extreme examples described in the introduction show in principle, what should be done depends upon the requirements and the circumstances.

IX. TRANSFER OF THE FUNGICIDE

The speed at which toxic effects are achieved is an important part of plant protection which is influenced by application and which does not usually receive consideration. It is clear that because of several sources of variability, toxicity is not an all-or-nothing effect. Additionally it is not only partial but also slow or quick. Often it is partially effective quickly and more completely effective slowly. This may appear trite. Nevertheless a study of the kinetics of the processes of infection and its prevention would perhaps be valuable. Factors concerned with application would contribute to this study.

The entry of fungicide into the fungus is one step at which speed may be critical. Horsfall (1957) pointed out that toxic effect depends upon both innate toxicity and upon the ability of the fungicide to
penetrate in sufficient quantities. Penetration in turn depends not only upon this ability but also upon the speed at which penetration takes place and upon the time available for it to do so. The entry of water-soluble fungicides into a stirred suspension of spores from bulk solution is so quick as to be considered instantaneous for our practical purposes in the field (Miller and McCallan, 1958). The transfer is not as quick when protecting a leaf by means of a fungicide from fungus attack. A chemical applied before the spores land will normally be present as a dry patchy deposit composed of particles of a range of sizes. The spore is frequently exposed to the fungicide only as long as a water bridge persists between them. So the fungicide has to leave the deposit and enter the solution, traverse the gap, and effect an entry into the spore during some brief period before the transient water bridge ceases to exist.

Each of these steps may be the results of several physical events occurring at different speeds, sometimes competing and sometimes reinforcing. Despite its complexity the transfer process may still occur at the speed of a simple first-order reaction. In comparable work with contact and stomach insecticides, transfer can be linearly proportional to deposit density (Courshee, 1966).

In the example discussed in the introduction it was shown that very small deposits of fungicide were effective. If this result is reconsidered in terms of the speed at which the effect was achieved, however, it seems that these results may be valid only for the special conditions of the experiments. The potato or tomato plants were treated with fungicide which was allowed to dry before the spores were applied and then kept in a water-saturated atmosphere overnight. It was not realized at the time but it can now be seen that the spores were exposed to dilute fungicide solutions on the surface of the leaf for long periods of time. In effect the spores were subjected to conditions permitting the uptake of fungicide which were very nearly as favorable as if immersed in fungicide solution in a glass beaker. We were perhaps justified in being pleased with the results but perhaps ought not to have been surprised to find that, under these conditions, copper is just as toxic on a plant leaf as Miller and McCallan (1958) had found it to be in a glass beaker. Nevertheless it is perhaps surprising to find that competitive absorption of copper by the plant was not very great over the period of the experiment. During this time the copper appeared to transfer exclusively to the spores, suggesting that their affinity for copper is much higher than that of the leaf surface.

This does not mean that partition between them will necessarily be
similar during briefer time intervals. This would depend upon the speeds with which the patterns of distribution form. If, as is probable, copper is absorbed at the leaf surface rapidly, all that is available could be locked up in this site to be released more slowly to the spore. The expected equilibrium distribution of fungicide may not be attained until much later.

In practice, the time available for transfer to occur may be brief. For sufficient fungicide to be transferred the speed of transfer must rise as the time becomes shorter. This higher rate would have to be achieved by a greater driving force, i.e., a more soluble or a more quickly soluble fungicide, or possibly, a greater deposit density of it. This suggests another good reason for the customary use of quantities of active ingredients which are in excess of those which would, given time, result in the accumulation of a sufficient dose within the spore.

The process of transfer is probably complex. It is the end result of several steps each with its own rate and equilibrium constants and it may be rare for transfer to be analyzable in any simple way. However, a useful illustration of the sort of effects which might obtain in practice is given by a simple analysis based on the assumption that the rate of transfer is proportional to the deposit density $P$.

$$\frac{dp}{dt} = -K_t P$$  \hspace{1cm} (8)

This equation has the solution $P = P_0 \exp (-K_t t)$ where $P_0$ is the initial deposit density. From this one can find the time taken to transfer a lethal quantity of fungicide.

If the deposit available to a spore is well in excess of the quantity of fungicide needed to kill it, then an approximate relationship between the several factors is given by Eq. (9) providing no fungicide is excreted or decomposed.

$$K_d = \frac{1}{TP_0}$$ \hspace{1cm} (9)

$T$ is a measure of the toxicity of the fungicide—for example, the reciprocal of a median lethal dose. $K_d$ is another constant proportional to the rate constant $K_t$ for the transfer process.

This general reciprocal relationship provides only an indication of what might be expected for fungicides. It has been shown to hold partially for certain insecticide effects. In particular the relationship $Ct = \text{constant used for fumigation, where } C$ is the pesticide concentration, will be recognized as a special case of the more general Eq. (9).
Figure 14 shows the effect of dosage rate (applied topically) on the time taken to kill (Courshee, 1966).

The constant $K_2$ is a measure of the unspecified factors affecting the rate of transfer. The size of the particles within the spray stains, the solubility of the fungicide, or its volatility if it is transferred in the vapor phase for example, contribute to its value.

Some of the important effects of particle size which have been reported on the toxic effect of a given deposit of fungicide (Wilcoxen and McCallan, 1931) might result from an effect on the rate at which it is transferred. One has only to postulate a detoxifying mechanism to see intuitively that the equilibrium concentration of fungicide within the fungus results from a balance between the rates at which the fungicide arrives and is detoxified. Effectiveness also could well be determined by these two speeds as much as by the amount of fungicide outside the spores.

At present, poor distribution of fungicides over the foliage of crops is responsible for many a gap in the protective deposit and the breakdown of control. It will probably be worthwhile in the future to spray so that such gaps occur only very infrequently. There will be fungicide present almost wherever it is needed on the crop when better machines are more widely available. Then a breakdown may result more from a failure to transfer fungicide to the spores quickly enough and the next task will be to ensure the suitability of the factors which regulate this process.
X. Conclusions

This essay suggests that fungicides can be applied to foliage in much better ways than those widely used at present, that is, in ways such that adequate effectiveness can be obtained at a lower cost. But this has not yet been done except on an experimental scale and except for a small amount of commercial spraying done by specialist contractors. One hopes this delay is due simply to a time lag. At present a deficiency in a fungicide program is normally countered by an increased application rate, usually with negligible penalties. The success of this way of working has perhaps been too great. It has allowed us to remain indifferent to the defects of our machines and application techniques. So the present commercial practice probably goes too far in the direction of using a lot of fungicide and relatively poor machines. A better balance point would be obtained by using machines which are more closely matched to the properties of slightly soluble fungicides which adhere to the leaves strongly.

Spraying and dusting machines are merely the tools used to apply the fungicides in ways which are determined by the biology of the pest and its host and by the physical chemistry of the fungicide. All the factors which contribute to the successful control of a disease, or indeed to an economic gain from the operation, have to be viewed objectively and quantitatively for an optimum method of application to be selected. Small particles have to be formed easily and deposited efficiently on the surface to be treated. There they should be suitably distributed and should provide a sufficient degree of cover. They should not be so loosely attached that they may be lost wastefully nor so strongly bound that they are released to the spores too slowly.

The application of fungicides to crops is simply a matter of getting sufficient quantities of fungicide to the right place at the right time and then ensuring that these stay there but also transfer readily to the fungus spores. The procedures for doing this well are widely variable and sometimes complex so that the choice of best ways of working—fungicide, formulation, and machine—can be a difficult but fascinating task.

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8. APPLICATION AND USE OF FOLIAR FUNGICIDES


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CHAPTER 9

Application and Use of Postharvest Fungicides

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I. INTRODUCTION

The serious economic nature of postharvest diseases is evident from the fact that the cost of processing and marketing most fruits and vegetables greatly exceeds the value of the raw commodity itself. Decay losses of only a few percent often turn an otherwise successful agricultural venture into an economic failure despite months of the most meticulous farming practices. Overall losses due to spoilage after harvest are staggering to the imagination. Current losses of fresh fruits and vegetables in the United States during shipment and retail marketing are estimated at approximately $200 million annually (U.S. Dept. Agr., Agr. Res. Serv., 1965). A major portion of this loss is attributed to diseases caused by fungi and bacteria.

The most serious postharvest diseases are those which cause rapid and extensive breakdown of high moisture commodities, often soiling the entire package and causing secondary infections in the advanced stages of the disease. This condition is exemplified by the attack of *Rhizopus* spp. on stone fruits and strawberries, *Penicillium* spp. on citrus and pome fruits (blue and green mold decays), and *Erwinia carotovora* on leafy vegetables and potatoes (bacterial soft rots). It is estimated that 30% of all fruit decay is caused by species of *Penicillium* and 36% of all vegetable decay by soft rot bacteria (Wiant and Bratley, 1948).

The stem end rots and brown rots are another group of postharvest diseases which are quite serious on specific commodities, but typically the pathogen is restricted to the inner tissues of the host and there is less tendency for the disease to spread after harvest. Examples of economically important diseases in this category are *Monilinia fructicola* on stone fruits (brown rot), *Phytophthora* spp. on citrus fruits (brown rot), and stem end rots of citrus fruits caused by *Diplodia natalensis*, *Phomopsis citri*, and *Alternaria citri*.

The diseases cited above are responsible for the preponderance of postharvest disease losses, and hence, considerable effort has been directed toward the development of efficient methods for their control. Major emphasis in this chapter will be placed on the application of fungicides after harvest to control these and other postharvest diseases. However, other control measures will be briefly discussed where these are more efficient than, or supplementary to, postharvest chemical treatments. Certain aspects of the postharvest disease problem have been considered in detail elsewhere, and the reader is urged to consult these reviews for a more comprehensive evaluation of these specific subjects (Charley, 1959; Friedlan, 1960; J. M. Harvey and Pentzer, 1953;...
9. APPLICATION AND USE OF POSTHARVEST FUNGICIDES


The terms "fungicide" and "bactericide" will be used in this chapter to denote agents that inactivate or inhibit fungi and bacteria, respectively. No attempt will be made to differentiate lethal and static action except in those cases where a distinct effect is specified. Patent literature will be reviewed only where it is desirable to establish priority or in those instances in which individual patent descriptions contribute substantially to the development of a subject.

II. ETIOLOGY OF POSTHARVEST DISEASES IN RELATION TO CONTROL

The successful formulation of a treatment for control of a postharvest disease requires an accurate evaluation of the mode and time of infection as well as of the subsequent development of the pathogen in the host tissues. Such information is essential because the effectiveness of a given treatment depends both on the number of pathogen cells and their depth in the host tissue. All fungicides currently in use for control of postharvest diseases are rather limited in their ability to penetrate the commodity being treated and therefore it is quite possible for a rapidly developing pathogen to be beyond the reach of an effective fungicide within 24 hours after inoculation. Furthermore, it is reasonable to assume that the number of cells of the pathogen exhibiting resistance to the treatment increases as a function of the total population within the host tissue (Sommer et al., 196b). For these reasons it is desirable that the control measure be applied as soon after infection as possible, although the maximum tolerated delay will depend upon a number of factors which are considered in Section IV,D.

A. Infection by the Pathogen Before Harvest

The initiation of some postharvest diseases can be traced to infection by the pathogen in the field—days, weeks, or even months before harvest. Botrytis disease of strawberries and grapes, brown rot (M. fructicola) of stone fruits, and brown rot (Phytophthora spp.) of citrus fruits may all be initiated in the field as the crop approaches maturity. A high level of inoculum and an adequate moisture regime are responsible for a serious degree of field infection. Environmental conditions permitting, the pathogen will become established beyond the reach of most chemical fungicides within a matter of a few days. The progress of the disease after incipient infection may be arrested by unfavorable en-
environmental conditions, resulting in infected, but symptomless, fruit at the time of harvest. The ability of a postharvest treatment to inactivate these infections depends upon the time of infection and the weather leading up to harvest. A more satisfactory control for such postharvest diseases, however, lies in the application of protectant or eradicant sprays in the field, often in combination with a postharvest treatment. Field sprays of copper (bordeaux mixture) and captan have been suggested for control of *Phytophthora* on citrus fruits (Klotz et al., 1961), captan and thiram for control of *Botrytis* on strawberries (Edney, 1964; Horn, 1961; P. M. Miller and Stoddard, 1959; Pownson, 1960), captan for the control of *Botrytis* on grapes (J. M. Harvey, 1959), and liquid lime sulfur for control of *M. fructicola* on peaches. Field sprays of captan, bordeaux mixture, and lime sulfur immediately before harvest are feasible because of the relatively high residue tolerances for these fungicides in the United States.

In contrast to the more or less active preharvest attack in the field by the pathogens described above, some postharvest diseases result from latent infections or by entrapment of propagules of the pathogen in the fruit at a very early stage of its development. Progress of these diseases is prevented by host resistance and may not develop until the fruit reaches maturity and is harvested. Examples of such diseases are *Gloeosporium musarum* on bananas (Meredith, 1961a), *M. fructicola* on apricots (Wade, 1956), *Gloeosporium* spp. on apples (Edney, 1958), and *Phomopsis, Diplodia, and Alternaria* stem end rots of citrus fruits (Bartholomew, 1926; Brooks, 1944; Minz, 1946). The postharvest diseases caused by *Gloeosporium* and *Monilinia* are true latent infections, whereas spores of *Diplodia* and *Alternaria* are merely entrapped in the fruit tissue during its development on the tree. Since *Gloeosporium* rot of apples cannot be controlled by chemical treatments applied after harvest (Edney, 1958), control measures have been directed toward the field application of phenylmercuric acetate to reduce the inoculum level in the orchard (Burchill and Edney, 1963) and captan to prevent infection of young fruit (Marsh and Edney, 1957). Protectant spray of thiram are reported to control latent infections of *Monilinia* on apricots (Wade, 1956). Although fungicide treatments applied after harvest are usually ineffective for the control of diseases arising from true latent infections, promising results have been obtained in recent years with heat and irradiation therapy, both of which have the advantage of deeper penetration into the host tissues (see Section III,B).

The stem end rots of citrus fruits caused by *Diplodia, Phomopsis,* and
Alternaria differ from the diseases described above in several important respects. The propagules of the stem end rot pathogens are restricted to the calyx, receptacle, and stem of the fruit prior to harvest. Spores or hyphae of these pathogens are entrapped in these fruit tissues during growth of the fruit in the field. Further development of the pathogen during the growing season is checked by resistance of the host tissues. After harvest the stem tissues undergo normal senescence, the pathogen is free to move into the fruit proper, and decay develops rapidly thereafter. Field sprays are of little value in controlling these stem end rots due to the abundant source of inoculum in the field and the long period over which infestation of the stem end parts may take place. Fortunately, infestations of Diplodia and Phomopsis appear to be localized since decay due to these fungi can be controlled by application of fungicides after harvest (see Section VI,E). Alternaria stem end rot of lemons is not controlled by any known fungicide treatment, but may be held in check by postharvest application of 2,4-D (2,4-dichlorophenoxyacetic acid) which delays the senescence of the calyx and receptacle of the fruit (see Section VI,J,1).

B. Infection by the Pathogen During and After Harvest

A major loss in fresh fruits and vegetables after harvest is due to diseases that originate in mechanical and physiological injuries to the fruit surface during harvesting and marketing (Friedman, 1960). Fungal pathogens such as Penicillium, Rhizopus, Geotrichum, and Trichoderma attack susceptible hosts only through mechanical injuries or by contact with a diseased individual. Other pathogens such as M. fructicola and G. musarum may invade peaches and bananas, respectively, by infection through the intact epidermis while the fruit are still on the plant but entry of these pathogens through mechanical injuries is of major importance in the development of postharvest disease (Haller, 1952; Meredith, 1961a). E. carotovora may enter potato tubers before digging, but the major infection takes place through lenticels and particularly injuries after harvest (Ramsey et al., 1949). Soft rot of leafy vegetables is almost entirely initiated at injuries (Ramsey et al., 1959).

Postharvest diseases that can be traced to wound infection during harvest are most amenable to control by postharvest treatment. This will be assumed to be the situation in the ensuing discussion unless specified to the contrary.
III. OBJECTIVES OF POSTHARVEST FUNGICIDE TREATMENT

Postharvest disease losses fall into two categories, which, although related, can be treated separately by postharvest fungicides. The most obvious consequence of postharvest disease is that a unit of the commodity attacked by the pathogen is unfit for consumption. The second aspect of the problem is that the overall value of the commodity may be seriously impaired by unsightly superficial contamination even though its palatability is not affected. Soilage of citrus fruits by spores of *Penicillium digitatum* and the growth of saprophytic fungi on melon rinds are examples of the latter situation. Soilage of citrus fruits causes major economic losses even though only a few percent of the fruit are actually decayed (Eckert, 1959a; Rygg et al., 1962).

A. Prevention of Injection

Since the major overall cause of postharvest disease is invasion by the pathogen after harvest through wounds or natural openings in the surface of the host, a reduction of initial infection can be accomplished by a lessening of the number of harvest injuries and by a reduction in the amount of available inoculum. Measures to reduce harvest injuries are beyond the scope of this review and have been considered in detail elsewhere (e.g., H. S. Fawcett, 1936; Haller, 1952); therefore, only fungicide treatments to reduce the inoculum level will be discussed here.

1. Use of Fungicides to Reduce the Inoculum Level

Major sources of pathogen inoculum are picking and storage boxes, the atmosphere in packing houses and storage rooms, cleaning baths and hydrocoolers, and commodity processing equipment. Adams and Tamburo (1959) isolated common decay fungi from apple- and peach-picking boxes and found that postharvest decay of these fruits could be substantially reduced by spraying the boxes with protectant fungicides. Decay of strawberries and figs have likewise been reduced by treating the picking boxes with solutions of sodium o-phenylphenate (J. M. Harvey, 1956b) and 8-hydroxyquinoline (Jarvis, 1960), respectively. Barta et al. (1951) found that impregnating wood berry boxes with paraffin wax greatly reduced contamination and that no obvious advantage was obtained by the use of a fungicide in the wax. Treatment of fruit containers with captan, copper 8-hydroxyquinoline, copper naphthenate, sodium o-phenylphenate, or tributylin succinate is permitted in the United States (U.S. Dept. Agr., Pesticides
Regulation Division, 1964, 1965). Since the wetting of wood boxes is not always desirable, fumigation treatments have been widely used for disinfecting fruit containers. Picking and storage boxes for citrus fruits are frequently fumigated with formaldehyde after use and sulfur dioxide gas has been recommended for picking boxes for figs (J. M. Harvey, 1956b).

Fruit-conveying belts are often disinfected with quaternary ammonium salts, sodium hypochlorite, or sodium o-phenylphenate. Water in soaking tanks and hydrocoolers may be charged with 100-150 ppm active chlorine to prevent the buildup of pathogenic microorganisms which could efficiently inoculate each fruit or vegetable passing through the contaminated water.

2. Interrupting the Infection Process

After inoculation has taken place it is possible to prevent disease by interrupting the infection process with certain fungicide treatments, provided that development of the pathogen is still quite limited. Since only a short period of contact with a solution of an efficient fungicide is sufficient to inactivate an inoculation, it is often assumed that postharvest fungicides are lethal to the pathogen during the period of contact (McCallan and Wellman, 1942). This does not appear to be the general situation, however, since most treatments that are highly effective in preventing decay of inoculated fruit are not lethal to the pathogen in vitro under the same conditions. Examples which may be cited are sodium o-phenylphenate, sodium tetraborate, sodium carbonate, 2-aminobutane, and biphenyl. Conversely, some treatments (e.g., hypochlorous acid) that are lethal to the pathogen in vitro are completely ineffective in preventing decay of inoculated fruit (see specific fungicides, Section VI). In the author's experience, most effective fungicide treatments prevent decay of inoculated fruit through a fungistatic, rather than by a lethal, action.

To prevent the development of a pathogen present in an injury in the fruit surface, it is only necessary that an inhibitory concentration of the fungicide be present as long as conditions in the injured tissue are conducive to growth of the pathogen. The deposition of a persistent fungistat in a susceptible injury on the surface of the fruit is most desirable, but not essential for prevention of decay. Superficial injuries in the peel of apples and oranges remain susceptible to infection by Penicillium spp. for only a few days (Eckert and Kolberen, 1968; Miyakawa, 1968; Wright and Smith, 1954). Prevention of infection during this period either by a chemical inhibitor or by an unfavorable envi-
ronment (see Section IV, C) will result in some degree of disease control. It is believed that aliphatic amines and ammonia prevent decay of oranges by temporarily increasing the pH of injuries on the surface of the fruit, thereby preventing infection by *P. digitatum* (Eckert and Kolberen, 1963b). Treatment of oranges with solutions of sodium o-phenylphenate results in selective accumulation of o-phenylphenol in injuries on the fruit surface, presumably exerting a chronic inhibition to a pathogen situated there (Eckert, 1962).

B. Eradication or Attenuation of an Established Pathogen

To the writer's knowledge, no one has been successful in eradicating the pathogen, without injuring the host, after the symptoms of a postharvest disease were detectable. It has been possible, however, to eradicate and attenuate deep-seated infections by heat treatment and gamma irradiation, and in a few instances to retard the development of a pathogen within the host by fungicide treatment.

1. Chemical Treatments

Most fungicides which have been applied after harvest are very limited in their ability to penetrate host tissues and thus are ineffective against established decay lesions. However, the development of established lesions of *Rhizopus* in peaches and *Botrytis* in grapes have been reported to be retarded by 2,6-dichloro-4-nitroaniline and high concentrations of sulfur dioxide, respectively (Nelson, 1958; Ogawa et al., 1963c). While inhibition of the development of a pathogen within a diseased host may be of questionable significance per se, the important consequence of such action is that the pathogen will be delayed in its spread to adjacent fruits.

No concentrated effort seems to have been directed to the development of local systemic fungicides for postharvest use. Such agents would be of great value in increasing the flexibility of fruit handling operations, since they presumably would increase the period of time during which a postharvest treatment could be effectively applied. Furthermore, a systemic fungicide distributed throughout the outer tissues of the fruit could conceivably retard the development of the pathogen and thereby reduce spread of the disease by contact, as well as spore soilage of adjacent fruits.

2. Heat Therapy

Heat treatment appears to be the best and most convenient means for therapy of postharvest diseases. Hot water has usually been used as
the heat transfer medium for this purpose owing to its availability, heat capacity, and lack of residue problem. However, when it is undesirable to transfer the commodity from the picking container or subject it to liquid water, low-pressure steam or hot air might be a more appropriate means for applying the heat treatment (Kushman and Cooley, 1949; W. L. Smith and Bassett, 1964).

H. S. Fawcett (1922) appears to have been the first to point out the advantages of heated water for the therapy of a postharvest disease that was beyond control by fungicide treatment. He reported that infections of *Phytophthora citrophthora* could be eradicated from lemons after 30-hours incubation by submerging the fruit in water at 120°F for 2 minutes. The standard treatment at that time, copper sulfate solution, was not effective after 4-hours incubation. Water at 115°F for 2 minutes eradicated *Phytophthora* infections from fruit inoculated 8 hours prior to treatment. This hot-water treatment of 115-118°F for 2 to 4 minutes has been the standard method for postharvest control of *Phytophthora* on citrus fruits for many years in California. Klotz and DeWolfe (1961) found that when lemon fruits were submerged for 4 minutes in 118°F water the temperature at a depth of 318 μ beneath the surface of the fruit reached 111°F. They further reported that both zoospores and hyphae of *Phytophthora* were killed by 1-minute exposure to 111°F. In recent years, a number of reports have been published indicating the feasibility of using heated water for the control of several postharvest diseases; namely, *Rhizopus* and *Monilinia* on peaches (W. L. Smith, Jr., 1962b; W. L. Smith and Bassett, 1964), *Penicillium* and *Diplodia* on oranges (Smoot and Melvin, 1964), *Colletotrichum gloeosporioides* on mangoes (Pennock and Maldonaldo, 1962; Smoot and Segall, 1963) and on papayas (Akamine and Arisumi, 1953), and *Gloeosporium* on apples (Burchill, 1964). Most of the recommendations for effective disease control have fallen in the range of 120-130°F for 2 to 5 minutes. Although it is reasonable to assume that these treatments were effective due to a direct effect of the heat on the pathogen (Klotz and DeWolfe, 1961; W. L. Smith and Bassett, 1964), other explanations involving the host-parasite relationship are also possible (Hopkins and Lotz, 1948; McClure, 1959). One wishes that data were reported relating to the thermal death points of the pathogens with the actual temperature of the fruit tissue as was done by Klotz and DeWolfe (1961).

Despite the obvious advantages of low cost and lack of chemical residues on treated commodities, the hot-water treatment has several shortcomings. First, due to the relatively poor heat conductivity of host
tissue, the water temperatures required in conjunction with a feasible treatment time are rather close to the injury threshold for the commodity. On the other hand, low heat transfer prevents a large rise in fruit pulp temperature which would increase the refrigeration load during shipment (W. L. Smith, 1962b). Second, experience with the hot-water treatment of lemons in California has shown that many lots of fruit will not tolerate the temperatures required for control of Phytophthora without first preconditioning the fruit. Even though fruit do not exhibit visible injury symptoms, the adverse effect of the hot water may show later as an increase in decay (Harding and Savage, 1964).

3. Ionizing Radiation

Gamma irradiation is the only true therapeutic treatment now available for control of postharvest diseases. Due to the great penetrating powers of high-energy gamma rays, propagules of pathogenic organisms may be inactivated regardless of their depth in host tissues if the administered radiation dosages are sufficiently high. This fact coupled with the possibility of treating packaged commodities has encouraged intensive research to apply the gamma-irradiation treatment for the control of postharvest diseases. Although attractive in theory, the eradication of deep-seated infections by gamma irradiation has two serious limitations. First, deep-seated infections usually are associated with relatively large populations of cells of the pathogen. Radiation dosages necessary to inactivate the most resistant individuals of the population generally exceed the tolerance of the host cells (Beraha et al., 1959; Sommer et al., 1964a,b). Second, species of pathogens that are present in the fruit as "latent" (deep-seated) infections, e.g., Diplodia and Alternaria on citrus fruits, are relatively resistant to gamma irradiation while fungi such as P. digitatum and Penicillium italicum which invade the host superficially through wounds after harvest are relatively sensitive to irradiation (Sommer et al., 1964b).

The overall success of radiation treatments apparently depends upon the sensitivity and size of population of the invading pathogen as well as upon the radiation resistance and physiological life expectancy of the host commodity. At radiation dosage tolerated by the commodity, the principal beneficial effect likely to be realized is a delay in the onset of decay rather than complete eradication of the pathogen (Beraha et al., 1959; Maxie et al., 1964; Sommer et al., 1964a,b). Such a retarding effect would be of great value in the control of a postharvest disease such as Botrytis on strawberries where the pathogen is beyond the reach of fungicide treatment at the time of harvest and the physi-
ological life of the commodity is recognized to be only 2 to 3 weeks under the most ideal storage conditions. In contrast, citrus fruits, grapes, and apples can be stored under refrigeration for many weeks and then held up well for a week or more at room temperature. It is not likely that the commercial handling of such commodities will be benefited by radiation treatments that inhibit, but do not eradicate the pathogen. On the other hand, it is well known that pasteurizing dosages of radiation constitute a physiological stress to the commodity which is manifest as an increase in respiration, loss in ascorbic acid, and increased susceptibility to weak pathogens even though the radiation treatment causes no visible injury to the fruit (Beraha et al., 1959; Sommer and Fortlage, 1966; Maxie et al., 1964). Beraha et al. (1959) irradiated lemons inoculated with P. digitatum and P. italicum and found that dosages tolerated by the fruit would delay the onset of decay for about 12 days at 75°F, but that treatment predisposed the lemons to attack by A. citri. An effective fungicide treatment, such as sodium o-phenylphenate, protects lemons for at least 2 weeks under these same conditions.

Gamma irradiation appears to offer considerable promise for certain commodities, such as strawberries, which demand minimum handling after harvest, are recognized to have a short physiological life expectancy, and are infected by the pathogen prior to harvest (Maxie et al., 1964). The commercial treatment of strawberries by irradiation is considered to be economically feasible (Droge, 1965).

4. Attenuation of Established Infections by Refrigeration

It is axiomatic that postharvest fungicide treatments are complementary to all other practices, including refrigeration, that maintain the quality of a living commodity. The use of fungicides as a substitute for refrigeration is justifiable only in those rare instances in which facilities for the latter are unavailable.

Refrigeration alone, however, does not provide adequate protection against postharvest diseases of many fruits and vegetables. Low temperatures are most effective in retarding the development of diseases caused by microorganisms which grow well only at moderately high temperatures, and for commodities which tolerate low temperatures and are recognized to have a short physiological life-span. Thus, Rhizopus rot of strawberries is fully controlled by 40°F shipping temperatures because Rhizopus stolonifer grows poorly at these temperatures and the berries are marketed without delay in recognition of their ephemeral nature. Unfortunately, Botrytis cinerea is considerably more
difficult to keep in check at practical shipping temperatures, and an effective fungicide treatment for control of this disease on strawberries would be a desirable adjunct to refrigeration. Refrigeration is least efficient for long-lived commodities which are injured at temperatures which effectively retard growth of the pathogen. For example, lemons are usually stored for 1 to 4 months at 58°F, the optimum for development of high quality. The growth of most fungi responsible for lemon decay are only slightly retarded at this temperature. Even apples and grapes which are stored at near freezing temperatures are decayed by *Penicillium* and *Botrytis*, respectively, which develop slowly but surely during the long storage period normal for these commodities.

Although certain diseases such as *Penicillium* decay of oranges and brown rot of peaches can be completely suppressed by low temperatures, it is usually not practical, nor even desirable, to rely entirely upon refrigeration as the only control measure. Rapid cooling facilities, which are not always available, and sustained low temperatures are necessary to assure that infected fruit will be free from disease symptoms on arrival at the market. The temperature at which the fruit are held thereafter is indeterminable, and the fruit are destined to decay. This sequence of events, if regularly repeated, can seriously depress the demand for the "decay-prone" commodity. It is only rarely reported that low-temperature treatment reduces the ultimate level of decay (McClure and Smith, 1959; Smith and McClure, 1960).

Refrigeration is of indisputable value in prolonging the marketable life of perishable commodities after harvest, but is not the ultimate solution to the problem of microbial decay. An effective fungicide treatment makes it possible to realize the full benefits of refrigeration.

**C. Moderation of the Disease Symptoms**

It was pointed out earlier that contamination of a commodity by fungus spores, superficial mycelial growth, or debris from diseased individuals is often as serious from an economic standpoint as loss due to primary attack by the pathogens. Often a postharvest fungicide treatment will incidentally reduce such losses, and in rarer cases, may be the sole purpose of the treatment.

1. **Preventing Surface Growth and Sporulation of the Pathogen on the Diseased Host**

In the first report on the potential of biphenyl as a fungicide for citrus fruits after harvest, Tomkins (1936) observed that biphenyl
vapors inhibited sporulation of *P. digitatum* on the surface of decayed oranges (Fig. 1). This ability of biphenyl to control sporulation of *Penicillium*, and thus the spoilage of adjacent fruit by fungus spores, has made it possible to successfully market unwrapped citrus fruits from California (Bekert, 1959a; Rygg et al., 1961). Lemons are fumigated repeatedly with nitrogen trichloride during storage to control *Penicillium* sporulation as well as for a reduction of decay. By use of this treatment, fewer fruit are rejected after storage because of obvious spore contamination.

Christ (1959) reported that the development of symptoms (pycnidia) of "black spot" disease (*Phoma citricarpa* McAl.) on oranges during shipment could be partially controlled by dipping the fruit in a 5% solution of sodium carbonate after harvest. This disease arises from latent infections in the field and does not result in decay, but the black pycnidia on the rind seriously disfigure the fruit. The superficial growth of *Cladosporium* and *Alternaria* on the rind of melons and on buttons of citrus fruits during storage under high-humidity conditions significa-
cantly detracts from the appearance of these fruits. Saprophytic growth of *Alternaria* on the buttons of oranges can be prevented by the vapors of dibromotetrachloroethane (Eckert and Kolbezen, 1963a) but not by biphenyl or o-phenylphenol (Van der Plank et al., 1940). Dithiocarbamates, tetraiodoethylene, and sodium o-phenylphenate are used on melons to control the growth of *Alternaria* on the rinds (Lipton and Stewart, 1961).

2. Preventing Spread of the Pathogen from Diseased to Healthy Fruits

Fungi such as *R. stolonifera*, *B. cinerea*, and *Trichoderma viride* can spread by contact from diseased to sound fruit under conditions encountered in storage rooms and during shipment. In fact, contact spread frequently accounts for the major losses by these pathogens. One fruit infected with *Rhizopus* may result in a nest involving 10 to 20 decayed peaches within 2 to 3 days at room temperature (Haller, 1952). Diseases caused by *Botrytis* on grapes and pears and *Trichoderma* on lemons develop similarly during long-term storage, even at low temperatures.

The spread of *Botrytis* on pears can be prevented by oiled paper wraps containing copper sulfate (Cooley and Crenshaw, 1931). Such wraps are widely used in commercial pear shipments today. Although copper impregnated wraps have not proved successful for control of *Rhizopus* on tomatoes (Butler, 1937), various active halogen compounds have shown promise for this purpose (Tomkins, 1934; Eckert and Kolbezen, 1962b). The effectiveness of wrappers impregnated with dibromodimethylhydantoin is illustrated in Fig. 2. Recently, Leupschen (1964) has shown that wraps impregnated with 2,6-dichloro-4-nitroaniline were 90-98% effective in preventing the spread of *Rhizopus* on peaches. Spread of *Trichoderma* on lemons was prevented by wrapping individual fruits in paper tissue containing 9 mg/sq ft of o-phenylphenol (Van der Plank et al., 1940). It should be stressed that wrappers impregnated with a fungicide usually do not prevent disease of the originally infected fruit but rather prevent secondary infection of adjacent fruits.

It is the standard commercial practice to fumigate stored grapes at 10-day intervals with 0.25% sulfur dioxide to prevent the spread of *Botrytis* (J. M. Harvey, 1956a; J. M. Harvey and Pentzer, 1960). Sodium bisulfite is added to grape packages to prevent spread of *Botrytis* during storage and long distance shipment (Pentzer, 1939; Van der Plank, 1939; Van der Plank and Van Wyk, 1940), and more recently dibromotetrachloroethane has shown considerable promise for this purpose (Chiarappa et al., 1962; Nelson et al., 1963).
IV. Biological and Environmental Factors Influencing the Effectiveness of the Treatment

Conflicting reports regarding the effectiveness of a given fungicide treatment can often be accounted for by differences in the environment during application of the treatment, inoculum level or age of the infection, or susceptibility of the host to infection. Fungicide formulations and methods of application are usually specified in published reports but other conditions which may have a pronounced influence on the test results are often inadequately described.

A. Host Interactions

Fresh fruits and vegetables tend to become more prone to decay as they mature. This may be due to an increase in the susceptibility of the commodity to mechanical injury (Wright and Smith, 1954) and, in many cases, to physiological changes in the host tissues which make them a more suitable substrate for rapid development of the pathogen (Edney, 1958; Sitterly and Shay, 1960; Tonkins, 1951; Mine, 1946).

Fig. 2. Spread of Rhizopus stolonifer in a box of packed tomatoes A, fruit wrapped in untreated tissue papers; B, fruit wrapped in tissue papers each impregnated with 174 mg dibromochlorphthalein.
This latter situation is very significant in the case of postharvest diseases arising from latent field infections.

In the writer's experience, the influence of fruit maturity on the effectiveness of postharvest fungicides is very noticeable in the case of *Penicillium expansum* on apples and *Geotrichum* and *Penicillium* on lemons. Others have commented on the relation between maturity and control of stem end rots of citrus fruits (DeWolfe et al., 1959; Winston, 1935) and brown rot of peaches (Cardinell and Barr, 1952). Optimum performance of a postharvest fungicide can be expected in the treatment of fruit which have reached physiological maturity (marketability), but which have not become so mature as to be very susceptible to invasion by decay-inciting microorganisms. For example, it is a common observation that solutions of sodium o-phenylphenate are quite effective in preventing decay of recently harvested apples inoculated with *P. expansum*. However, it is much more difficult to control this same disease on apples which have been stored for several months prior to treatment. Harding and Savage (1964) observed that 4% sodium carbonate solution at 118°F, a treatment often recommended for control of both *Penicillium* and *Phytophthora* decays of lemons, caused an increase in decay of mature (yellow) lemons over the same treatment applied at 90°F. The 118°F treatment did not cause an increase in decay of immature (green) lemons.

The host tissues may interact directly with the applied fungicide in several ways. For example, immature grapes absorb more sulfur dioxide than mature grapes (Winkler and Jacob, 1925) and the level of sulfur dioxide in the berries has a definite effect upon the control of Botrytis decay (Nelson, 1958; Postzer et al., 1932). The host may increase or decrease the activity of a fungicide by direct chemical or physical interaction. Biphenyl dissolves in the oil of the rind of citrus fruits, thereby nullifying its fungistatic properties (Rajzman, 1965). Ammonia and aliphatic amines, which depend upon their basicity for fungicidal action, are neutralized by acid constituents of the peel of citrus fruits (Eckert and Kolbeczen, 1963b). Sulfur dioxide is both neutralized and reduced by components of grape tissue (Joslyn and Braverman, 1954; Nelson, 1958). Highly reactive fungicides, such as positive halogen compounds, reactive alkylhalides (e.g., dibromotetraethyl ether) and ethylene oxide are rapidly inactivated after absorption by fruit tissues.

It is also possible for the effectiveness of a fungicide to be improved by interaction with the host. This phenomenon would be anticipated with weakly acidic fungicides such as phenols (sodium o-phenylphenate and sodium salicylanilide), organic acids (potassium sorbate), and salts
of weak inorganic acids such as sodium bisulfite and sodium hypochlorite. The undissociated form of these acids is recognized to be more active than the anionic form (Long and Roberts, 1958; Bell et al., 1959; Cruess et al., 1931; Van der Plank, 1945) and the salts of these acids would be at least partially hydrolyzed in the acidic tissues of most fruits (Beneke and Fabian, 1955). Nelson (1958) has speculated that cultural and environmental conditions that tend to depress the total acidity of grapes may be significant factors in determining the efficiency of sulfur dioxide fumigation for control of Botrytis decay.

The development of resistance to infection by injured tissues of the host also may influence the effectiveness of a fungicide treatment. This phenomenon is well documented for sweet potatoes in relation to black rot (Endoconidiophora) and Rhizopus decay (Kushman and Cooley, 1949; McClure, 1959) and has been reported also for apples and citrus fruits (Wright and Smith, 1954; Miyakawa, 1958; Eckert and Kolbezen, 1963b; Hopkins and Loucks, 1948). The combination of a postharvest fungicide treatment together with methods of developing host resistance to attack by the pathogen should be more thoroughly explored.

B. Sensitivity of the Pathogen to the Fungicide Treatment

The observation that some postharvest fungicides are more effective if the inoculated fruit are incubated for a number of hours before treatment (Section IV,D) has led to speculation that the susceptibility of the pathogen increases during the early stages of infection. The most obvious explanation for this phenomenon is that the sensitivity of the pathogen increases with a certain degree of hydration or with increased physiological activity. Couey and Uota (1961; Couey, 1965) reported that the toxicity of sulfur dioxide to spores of Botrytis and Alternaria was greatly increased if the spores were hydrated prior to the exposure period or if the relative humidity of the fumigation atmosphere was increased. The same general relationship has been reported for Alternaria solani spores fumigated with chloropicrin, ethylene oxide, or methyl bromide (Simpson and Ludwig, 1956; Munnecke et al., 1959).

There is some data to support the hypothesis that physiological activity per se of a fungal pathogen increases its sensitivity to a fungicide. The clearest example of this phenomenon had been provided by Yanagita and Yamagishi (1958) who showed that spores of Aspergillus niger lost resistance to both pentachlorophenol and heat in the early stages of germination before the appearance of germ tubes. Marloth
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(1931) reported that sodium tetaborate and sodium bicarbonate solutions were slightly more effective in inactivating germinated spores than ungerminated spores of *P. digitatum* and *P. italicum*, but expressed the opinion that control of fruit decay by these fungicides was not due to its lethal action on the pathogen. Yanai et al. (1964) observed that hyphae of *Penicillium ictaloides* were somewhat more susceptible to ethylene oxide than were spores of the same organism, but the differences in dosages required for inactivation of the two stages were not greatly different. Spores and young mycelial cells of *B. cinerea*, *P. digitatum*, and *P. italicum* have been shown by Sommer and associates (1964a,b) to have about the same tolerance to gamma irradiation.

The observed increase in sensitivity of *Alternaria* spores to methyl bromide and chloropicrin is probably related to an increase in physiological activity of the spores, but in the case of the water-soluble sulfur dioxide it can be argued that hydration of the spores results in an increase in the quantity of the fungicide absorbed. From a standpoint of practical decay control the sensitivity of the pathogen at the time of treatment should be important only in the case of those fungicides which exert their principle effect on the pathogen during a relatively short treatment period (e.g., hypochlorous acid). With 2-aminobutane salts and other agents which are purely fungistatic, the hydration or physiological activity of the pathogen at the time of application should not be a factor in determining the effectiveness of the treatment.

C. Environment

1. Temperature

The efficiency of a fungicide is often improved by increasing the temperature at which it is applied. This effect is commonly observed in solution treatments (e.g., Eckert and Kolbeman, 1964), especially when the fungicide concentration is low, the fruit are rinsed after treatment, or if the fungicide exerts its action on the pathogen only during the period of treatment. The temperature effect is much less pronounced, if at all detectable, when the fungicide owes its effectiveness to fungistatic residues which remain on the commodity after treatment. Figure 3 illustrates the influence of temperature on the effectiveness of 2-aminobutane, both when the fruit are rinsed after treatment and when fungistatic residues of this fungicide are intentionally left on the fruit. Fumigation treatments generally are affected by temperature (Section V,A), although accounts of this phenomenon are rarely encountered in the fungicide literature. The role of temperature in in-
creasing the efficiency of a fungicide treatment can be attributed to several factors acting alone or in combination: (1) Improved penetration of the fungicide into the host tissues (Hayward and Grierson, 1960), and in the case of vapor treatments, increased diffusion of the gas into the bulk of the commodity (Sinclair and Lindgren, 1958); (2) improved penetration of the fungicide into the pathogen cells; (3) increased physiological activity of the pathogen which may increase

![Graph A](image1)

**FIG. 3.** Effect of concentration, contact time, pH, and temperature on the effectiveness of solutions of 2-aminobutane hydrochloride against Penicillium digitatum on oranges. Unless specified otherwise, the oranges were submerged for 4 minutes in solutions at pH 8.5 and 23°C. In plot B the 2-aminobutane concentration was 0.5% whereas in C and D the concentration was 0.25%.

![Graph B](image2)
its susceptibility to lethal agents (Section IV,B); (4) possible increase in resistance of the host to infection (Section IV,A); (5) increase in the rate of reaction of those fungicides that chemically alter cell constituents of the pathogen, e.g., positive chlorine compounds (Van der Plank, 1945).

2. Humidity

A high relative humidity generally improves the fungicidal effectiveness of fumigants by increasing the degree of hydration of the pathogen (Section IV,B). On the other hand, high humidity may reduce the practical efficiency of water-soluble fumigants such as sulfur dioxide and ammonia because they are strongly sorbed by surfaces in the fumigation chamber under high humidity conditions. Large increases in the applied dosage of the fumigant are necessary to counteract the effect of sorption at high humidities (Cant and Nelson, 1967; Nelson and Richardson, 1961; Nelson et al., 1964).

High-humidity and relatively high-temperature conditions favor the development of resistance of sweet potatoes and oranges to infection by pathogenic fungi (Section IV,A), but more commonly, high humidity favors the development of decay in fresh fruits (Loucks and Hopkins, 1946; Nelson et al., 1964).

D. Timing of the Fungicide Treatment

The duration of the postinoculation period during which a fungicide may be effectively applied depends upon the depth of inoculation, growth rate of the pathogen, susceptibility of the host to infection, humidity and temperature, and the depth to which inhibitory concentrations of the fungicide may penetrate into host tissues.

With rapidly developing diseases, such as *Rhizopus* on stone fruits at temperatures prevailing during the harvest season, best results are obtained if the fungicide treatment is applied within 12 hours after inoculation (Luvisi and Sommer, 1960; Ogawa et al., 1963c). *P. digitatum* and *P. expansum* infect citrus fruits and apples, respectively, at a much slower rate, and an effective fungicide will satisfactorily control decay of these fruits if applied within 24–36 hours (at room temperature) after inoculation (Long and Roberts, 1958). If the prevailing temperature is below optimum for the pathogen, the incubation period may be increased considerably without adversely affecting the efficiency of the fungicide treatment.
The specific fungicide as well as the concentration and conditions of application can have a dramatic effect upon the permissible delay between inoculation and treatment. Long and Roberts (1958) reported that oranges inoculated with *P. digitatum* could be held for at least 48 hours at 60°F without adverse effect upon a sodium o-phenylphenate solution treatment, whereas sodium tetraborate had to be applied within a few hours after inoculation for maximum effectiveness. Luvisi and Sommer (1960) found that treatment with dehydroacetic acid for control of *M. fructicola* on peaches could be safely delayed for 12 hours after inoculation, but sodium o-phenylphenate lost much of its effectiveness if it was not applied within 6 hours of inoculation. Meredith (1960c) observed that sodium salicylanilide would effectively control decay of bananas inoculated with *G. maximum* 6 hours prior to treatment, whereas nystatin had to be applied immediately after inoculation for the same degree of effectiveness. Several cases have been reported in which the effectiveness of a postharvest fungicide is improved if the treatment is delayed for a certain critical number of hours after inoculation (Littauer, 1947; Luvisi and Sommer, 1960; Ogawa et al., 1963c; Roistacher et al., 1958). Possible explanations for this phenomenon have been considered in Section IV,B.

V. METHODS OF APPLYING POSTHARVEST FUNGICIDE TREATMENTS

Postharvest fungicides may be applied in several ways, the most appropriate being determined by intrinsic effectiveness, compatibility with commodity handling practices, and in most instances by the physical, chemical, and biological properties of the fungicide itself. Some fungicides are lethal to the pathogen during the time of treatment and should be applied in a manner which results in minimum residue of the fungicide remaining on the commodity. More frequently, the application method is designed to leave residues of the fungicide in specific areas on the commodity, e.g., superficial injuries, where they inhibit development of a potential pathogen. The ideal method of applying a fungicide is the one which selectively inactivates the pathogen with minimum expenditure of the fungicide, least incidental residues, and minimum disturbance of the normal flow of commodity to the consumer. It must be borne in mind that the treated commodity may be consumed within days after application of the treatment. Thus adverse effects of a postharvest fungicide on the appearance or palatability of the product cannot be tolerated.
1. Fumigation Treatments

Fumigation is the most efficient means of applying a fungicide to a commodity after harvest. A fumigant can be applied to fruits and vegetables in the field containers immediately after harvest, thereby minimizing handling of a delicate commodity. Citrus fruits are normally treated with a fungicide solution, but often the lengthy delay between harvest and application of the treatment greatly reduces the effectiveness of the fungicide. A fumigant applied soon after harvest would inactivate or inhibit the development of a pathogen, thereby increasing the effectiveness of a solution treatment applied subsequently. A fumigation treatment is far superior to a solution treatment for strawberries and other commodities which are seldom treated with water for any reason for fear of greatly increasing decay. Relatively few fumigants have been developed for control of postharvest diseases. The reason for this is evident when one considers that a suitable material must be reasonably volatile, possess the desired antimicrobial properties, and either have an inherent low order of phytotoxicity or not be able to penetrate the cells of the host. Theoretically, the required degree of volatility would depend upon the activity of the fumigant against the pathogen. However, in practice, a reasonably high volatility is required for efficient distribution of the fumigant and to compensate for losses due to sorption by the commodity.

The sole objective of the fumigation treatment is to deliver an effective concentration of the active agent to potential infection sites on the commodity. Any of the fumigant that does not reach this site is not only wasted, but may appear as undesirable residues on the commodity. Thus, the ideal fumigant is one which is accumulated at potential infection sites, e.g., fresh injuries, but which is barred from penetrating the intact surface of the commodity. Highly polar gases, such as sulfur dioxide and amines, fall into this category, and have been shown to accumulate in injured areas of the fumigated commodity (Eckert and Kolbezen, 1963b; Gunther et al., 1959b; Nelson, 1958). Polar fumigants offer the possibility of applying low vapor concentrations with reasonable expectation that rather high concentrations will accumulate at potential infection sites, with little extraneous absorption by commodities which are covered with a waxy cuticle. Unfortunately, water-soluble fumigants are also absorbed by most other moist or hydrated surfaces in the fumigation chamber.

Two factors determine the penetration of a fumigant into the bulk
of a commodity in a static atmosphere—diffusion and sorption of the
gas by the commodity. The rate of diffusion is directly proportional to
temperature and inversely proportional to the square root of the molec­
ular weight of the gas. Thus a relatively light gas such as ammonia will
diffuse into narrow interstices between fruits at a rate about 2.1 times
that of 2-aminobutane. Sorption of the fumigant by the commodity
tends to reduce the concentration in interstitial spaces. This effect be­
comes very pronounced where the ratio of exposed surface area to gas
volume is high. The amount of fumigant lost by sorption is dependent
upon the chemical nature of the fumigant, the chemical and physical
properties of the commodity and fumigation chamber, and the tempera­
ture and humidity prevailing during the fumigation. Sorption loss may
be categorized as physical adsorption due to molecular attraction be­
tween the fumigant and exposed surfaces, chemical interaction between
the fumigant and materials in the chamber, and solution of the fumi­
gant in water and lipid constituents of the commodity. These phenom­
eena have been considered in detail for insecticide fumigants (Monro,
1960; Sinclair and Lindgren, 1958).

All fumigants exhibit physical adsorption to a greater and lesser de­
gree. Fumigants with higher boiling points tend to be more highly
adsorbed than the more volatile fumigants (Monro, 1960). Sorption
losses due to chemical interaction between the fumigant and materials
in the fumigation chamber are very important in the case of several
fumigants used for postharvest disease control. Compounds such as
ethylene oxide, nitrogen trichloride, and dibromotetrachloroethane re­
act chemically with the commodity and other substances in the cham­
ber, whereas acidic and basic gases, such as sulfur dioxide and amines
form nonvolatile salts with complementary functions in the fumigation
chamber. Losses of the fumigant by such interactions are irreversible
and show up as residues, principally in the commodity itself. The most
serious loss of water-soluble fumigants is due to their solution in free
or bound water. The effect of free moisture and humidity on sorption
losses has been intensively evaluated for sulfur dioxide fumigation of
grapes (Canç and Nelson, 1957; Nelson and Baker, 1963; Nelson et al.,
1964; Nelson and Richardson, 1961). Sulfur dioxide, as well as other
highly polar fumigants, are strongly sorbed by the hydrated cellulose of
wood boxes and packing materials, necessitating an increase in dosage
of the applied fumigant as the relative humidity in the chamber is
increased.

Temperature influences the sorption of fumigants in two ways. The
rate of physical adsorption increases with temperature, but when equi-
Equilibrium is reached the quantity of fumigant adsorbed is less at the higher temperatures. Second, the solubility of a gas in water decreases with an increase in temperature. This second factor would be of practical significance for fumigants which are only slightly soluble in water.

For fumigation of fresh fruits and vegetables after harvest it would appear desirable to hold as high a temperature and as low a relative humidity as possible with due consideration to maintaining the quality of the commodity. Higher temperatures increase the activity of the fumigant towards the pathogen (Coney and Uota, 1961), increase the rate of diffusion, and decrease the total quantity of fumigant (residues) sorbed by the commodity. A high relative humidity causes large losses of water-soluble fumigants by sorption, increases the quantity of fumigant required for a given level of effectiveness, increases overall decay, and accentuates the corrosion problem associated with fumigants such as sulfur dioxide and nitrogen trichloride (Nelson and Tomlinson, 1958; Nelson et al., 1964).

Many fresh fruits and vegetables, e.g., grapes, must be stored at low temperatures and high relative humidities to maintain their quality. In these cases, the dosage of the fumigant must be increased to compensate for its lower efficiency under these conditions. On the other hand, some commodities (e.g., oranges) are “ripened” or de-greened at relatively high temperatures before being stored or transported under refrigeration. It appears desirable to apply the fumigation treatment at the higher temperatures when a choice of environmental conditions is available.

The distribution of fumigants in large chambers can be greatly improved by fans and other mechanical devices that distribute and agitate the atmosphere during the fumigation treatment. However, molecular diffusion is still the principal means whereby the fumigant finally moves into interstices between units of the commodity and penetrates to the potential infection site. Moreover, the effectiveness of in-package fumigation treatments during transit depends almost entirely upon distribution by molecular diffusion (Section V,A,1,a).

Fumigants now in commercial use for control of postharvest diseases are sulfur dioxide, nitrogen trichloride, ammonia, tetrachloroethylene, and dichloromethane. Ethylene oxide is used for dried fruits only. Formaldehyde is frequently used as a disinfectant for storage rooms and fruit containers but is injurious to fresh fruits and vegetables. Specific details of these treatments are taken up in Section VI.

a. Fumigation in Individual Commodity Containers. It is advantageous to fumigate some fresh fruits after packaging in order to inacti-
9. APPLICATION AND USE OF POSTHARVEST FUNGICIDES

Volatile recent inoculations, and, if possible, to prevent later spread of the pathogen. In some instances the treatment can be carried out effectively by creating a mass movement of the fumigation atmosphere through the packed containers. This method is employed to fumigate grapes in railroad cars with sulfur dioxide (Uota and Harvey, 1964), and a variety of fruits and vegetables with nitrogen trichloride (Frey, 1950).

This procedure is limited to the fumigation of commodities in well-ventilated and specially stacked containers. Many of the fiberboard containers currently used for shipping fresh commodities are difficult to fumigate in this manner. Another method of fumigating packaged commodities is to generate the fumigant gas within each container, which then functions as a miniature fumatorium. This concept has attracted considerable attention over the past 30 years because it offers a means of fumigating tightly closed packages, and also maintaining an atmosphere around the commodity which would inhibit pathogenic organisms for the duration of a long transit or storage period.

Volatile in-package treatments that have been proposed fall into two general categories. One type consists of a chemical or a combination of chemicals which react within the container to yield a volatile fungicide. This type of system is usually activated by moisture absorbed from the atmosphere inside the container. Examples of such systems are the generation of amines from mixtures of amine salts and alkaline salts (Eckert et al., 1963; Eckert and Kolbezen, 1963b; Gunther et al., 1964a), sulfur dioxide by hydrolysis of sodium bisulfite (Penzler, 1933; Van der Plank and Van Wyk, 1940), and nitrogen trichloride by hydrolysis of dichloroisocyanuric acid (Eckert et al., 1969). The alternate means of generating a fumigant in the commodity container is by direct vaporization of a solid or liquid fungicide which has significant, but not excessive, vapor pressure at moderate temperatures (ca. 0.01-0.1 mm Hg at 20°C). In examples which have been suggested, the volatile fungicide is impregnated into paper which is introduced into the container with the fruit. The impregnated paper may be used as a fruit wrap or merely inserted between layers of fruit. Examples of volatile fungicides that have been proposed are biphenyl (Tomkins, 1936), o-phenylphenol and its esters (Tomkins, 1937b, 1963; Van der Plank et al., 1940), iodine (Tomkins, 1934), dibromotetrachloroethane (Eckert and Kolbezen, 1963a), and 2,6-dichloro-4-nitroaniline (Luepschen, 1964).

Ideally, the fungicide vapors should reach an effective concentration in the atmosphere within a few hours after the container is closed so that incipient infections will be inactivated before the pathogen be-
comes irreversibly established. The rate of evolution should be sufficient to deliver the minimum effective dosage to the site of infection and to compensate for losses due to sorption and leakage through the walls of the container. It may not be necessary to transfer a lethal dosage of the volatile fungicide to the infection site nor continue its evolution indefinitely, since superficial injuries may develop resistance to infection in a few days (Section IV.A). Thus temporary inhibition of a pathogen may greatly reduce the probability of decay. Furthermore, some fungicides (e.g., sulfur dioxide and 2-aminobutane) applied in the vapor phase are absorbed at potential infection sites and may inhibit development of pathogens long after the vapors have dissipated from the atmosphere (Eckert and Kolbezen, 1963b; McCallan and Weedon, 1940; Nelson, 1958). However, if the purpose of the in-package fumigation is to prevent sporulation and spread of the pathogen, it is then necessary that the volatile fungicide be continuously evolved in order to maintain a vapor phase concentration that is fungistatic to the aerial hyphae of the pathogen.

Irrespective of the purpose of the treatment, the rate of evolution of the fungicide should be controlled to deliver the minimum effective dosage, since an excessively high rate of volatilization will not only waste the fungicide but may also be detrimental to fruit quality, result in excessive residues, and lead to offensive levels of the fungicide vapors in the storage room. Ideally, the rate of volatilization of the fungicide should be negligible at low temperatures which do not permit growth of the pathogen and should have a temperature coefficient similar to that for the growth of the pathogen. This goal is very difficult to approach in practice. Some control over the rate of evolution of water-activated systems is possible by choice of the reactants and by regulating the ingress of water vapor into the formulation (Eckert et al., 1963; Van der Plank and Van Wyk, 1940). The control of evolution in water-activated systems is very difficult owing to variations in relative humidity which occur in closed containers of fruit during cooling (Harding, 1969b; Kolbezen and Eckert, 1963). Formulations based upon the direct evaporation of slightly volatile organic fungicides are more reliable and easier to control than systems that depend upon water activation. Although the maximum rate of volatilization of a fungicide under a fixed set of conditions will be determined by its vapor pressure curve, a lesser rate may be achieved by reducing the surface area of the formulation or covering the formulation with some material that impedes the diffusion of the fungicide vapors.

Most of the earlier research on in-package fumigation centered
around the use of individual fruit wrappers as the carrier for the volatile fungicide. This method of application resulted in excellent distribution of the vapors in the package but was rather limited in the selection of fungicides that could be formulated in this manner. Due to changes in packaging of fresh fruits as well as cost considerations, the individual fruit wrapper is less frequently used in the United States today than in former times. Most of the current research on in-package fungicides is directed towards the use of one or two impregnated papers placed between layers of fruit. The latter method of application is clearly less efficient than the use of treated wrappers, due to the lack of uniformity of the fungicide concentration in the atmosphere within the container. Poor distribution leads to a compromise whereby fruit adjacent to the formulation receive excessive dosages of the fumigant, leading to high residues and possibly injury to the fruit, while fruit further removed may receive inadequate concentrations of the fungicide. The distribution of biphenyl and ammonia vapors in containers of citrus fruit have been investigated in detail (Rygg et al., 1964; Eckert et al., 1963).

2. **Smokes, Fogs, and Dusts**

The application of a postharvest fungicide in the form of airborne particulates is a rather inefficient means of transferring the fungicide to the commodity. Smoke, aerosol, and dust formulations should be considered only under special circumstances such as when solution treatment of the commodity is impractical or undesirable and an effective fumigant is not available. Owing to their particulate nature such treatments cannot approach the efficiency of a true gas in penetrating into the commodity.

A smoke containing o-phenylphenol has been suggested for control of *Penicillium* on oranges in storage (Kitajima and Yamada, 1957), but references to such treatments are rare. Aerosols and fogs have not been used to any extent for application of postharvest fungicides since they offer no obvious advantage over fumigation or solution treatments for the available fungicides. In an aerosol, a pesticide in a volatile solvent is atomized into the atmosphere. As the solvent evaporates, very fine particles of the pesticide are dispersed in the air and settle out on the commodity being treated. One method currently used to apply o-phenylphenol to citrus fruits could be classified as an aerosol. A mixture of o-phenylphenol and cumarone-indene resins in a hydrocarbon solvent is atomized onto citrus fruits as they are being transported on a roller conveyor. Most of the hydrocarbon solvent is evaporated before or im-
mediately after the aerosol comes in contact with the fruit, thereby leaving a fungicide coating on the fruits.

In "fogs" the liquid pesticide or pesticide in solution is atomized to a very fine droplet size, which remains dispersed in the air for long periods of time. This is a convenient and efficient method of decontaminating fruit storage rooms and equipment with solutions of formaldehyde. Solutions of a boric acid-lactic acid complex have also been applied to storage areas as a "fog" (Section VI.A.1).

With the exception of sulfur dust which was formerly used to control \textit{M. fructicola} on peaches (Section VI.C), postharvest treatments with dust formulations have never received widespread usage. The difficulty of obtaining uniform distribution on the commodity together with the very poor penetrating power of a dust makes such application a poor alternative to a fumigation or solution treatment.

### B. Solutions, Dispersions, and Emulsions

Postharvest fungicides are more often applied in the form of aqueous solutions than by any other method. The popularity of solution treatment rests upon the fact that they are easy to prepare and apply uniformly, possess penetrating powers approaching or exceeding those of fumigants, and are compatible, in many instances, with mechanical fruit-handling operations. Solution treatments are conveniently applied to commodities that are wetted for other purposes; for example, cleaning of apples and citrus fruits, removing latex from bananas, and hydrocooling a variety of fruits and vegetables. Often the fungicide treatment can be combined with another operation. However, when the commodity is not normally treated with water, the application of a fungicide in solution should be approached with caution, since fruits and vegetables tend to develop decay after treatment with water (Becker et al., 1958a; DiMarco and Davis, 1957a; Grierson and Hayward, 1959; Magie, 1951). In fact, it is frequently necessary to add fungicides and bactericides to water used for washing and hydrocooling in order to prevent excessive decay due to these processes. The probable role of water in increasing decay is in the hydration of injuries, water soaking of stomates and lenticles, and its function as a source and means of inoculation if the water is recirculated.

True solutions have a decided advantage over dispersions (wettable powders) in that they do not require vigorous agitation to maintain uniformity and may be pumped for spray, flood, or foam applications. Dispersions of wettable powders are difficult to handle in such applica-
9. APPLICATION AND USE OF POSTHARVEST FUNGICIDES

Solution treatments are usually applied by floating the commodity through a tank containing the fungicide solution or by flooding, spraying, or foaming the solution onto the fruit as they are being transported on a roller conveyor or transverse brushes. It is not advisable to rinse or soak the commodity prior to application of the fungicide solution because small breaks and punctures in the surface will be filled with the first solution, thereby impeding the penetration of the fungicide into these potential infection sites. Solution treatments are invariably followed by a spray rinse of fresh water to remove excess fungicide and loose dirt from the surface of the commodity.

Application of a fungicide solution by immersion of the commodity in a tank filled with the fungicide solution has several advantages over other methods of applying solutions. Firstly, the temperature of the solution can be accurately controlled at levels required to control diseases such as *Phytophthora* brown rot of citrus fruits which cannot be controlled by the fungicide itself (Section III,B,2). Furthermore, the tank treatment provides the maximum opportunity for the fungicide to penetrate to potential infection sites since the commodity is totally submerged in the solution for 1 to 4 minutes. Since the commodity is usually rinsed with water after application of the fungicide solution, all factors which improve penetration and retention of the fungicide will improve the effectiveness of the treatment (see Fig. 3). The principal disadvantage of the tank treatment is that it requires a relatively large volume of the fungicide solution which must be used over a period of several months to reduce the unit cost of the treatment. The tank treatment method of application is best suited for fungicides which are inexpensive or stable, and preferably possess bacteriostatic properties. Since the tank solution is used over an extended period of time, additional quantities of the fungicide must be added periodically to compensate for that carried out of the tank by the fruit. Also, the pH of the solution must be frequently adjusted in the case of fungicides such as sodium o-phenylphenate which are readily hydrolyzed by weakly acidic materials brought into the solution along with the fruit (Section VI,E).

In Florida, recirculated solutions of sodium o-phenylphenate are flooded onto citrus fruits as they are carried along on a roller conveyor or transverse brushes (Hopkins and McCormack, 1956b). This method eliminates the tank and reduces the volume of solution required, but the pH and temperature of the solution are more difficult to control accurately. However, a rather low temperature is recommended (90°F) for this treatment and pH control is less critical because hexamethylene-
tetramine is added to the formulation to prevent phytotoxicity (Section VI,E).

The most recent innovation in applying solutions of fungicides is to whip the solution into a foam which is applied as a curtain through which the fruit are transported on transverse brushes (Cunning, 1955; Harding and Savage, 1965). The rate of application of the fungicide foam is adjusted to match the rate at which the fruit carry out the foam. Since the solution is not recirculated, there is no problem of pH control or contamination. This method appears to offer an efficient means for applying wettable powders, emulsions, and fungicides that are not sufficiently stable to be applied by other methods of handling solution treatments. The foam application is poorly suited for fungicides which show maximum efficiency at elevated temperatures, since it would be difficult to control the temperature of the foam. Tests comparing the different methods of applying solutions of sodium o-phenylphenate indicate that all of the methods described above provide adequate control of decay of citrus fruits (Harding and Savage, 1965; Hopkins and McCormack, 1959b).

Sodium o-phenylphenate has been applied to fruit in water-emulsion wax formulations. Although the primary purpose of this treatment is to retard water loss and to impart a gloss to the fruit, it has been hoped that the coating would also provide some measure of decay control. This method of applying sodium o-phenylphenate is inferior to solution applications, although a combination of the two is superior to either by itself (Hopkins and McCormack, 1959b). Some fruits, such as grapefruit and melons, do not always receive solution treatments and a formulation of a fungicide in a coating formulation might be the only available method for applying a postharvest fungicide.

Certain water-insoluble fungicides have shown promise in controlling postharvest disease when applied as solutions in organic solvents (Siegler and Childs, 1947; Smoot et al., 1960). The use of an organic solvent solely for the purpose of applying a fungicide does not seem justifiable, but water-insoluble fungicides might be incorporated in formulations of coating resins which are dissolved in petroleum solvents and widely used on a number of fruits today.

C. Barriers to Impede the Spread of the Disease

Post-harvest diseases caused by B. cinerea, Trichoderma viride, and P. italicum spread from diseased to healthy fruit by contact even at optimum storage temperatures for the commodity. Contact spread of
these diseases can be reduced by wrapping the individual fruits in plain tissue paper or better, a paper impregnated with a fungistatic chemical (Fig. 2). Other barriers such as waxed paper cups and paper dividers between fruit layers have been used with some degree of success.

Fungicides suitable as barriers to impede the spread of the pathogen should be reasonably stable, low in volatility, and should be formulated in such a way that they are not transferred readily to the commodity. If no residues of the fungicide are found on the commodity, a tolerance is obviously not required. Bis(tributyltin) succinate is registered for use in cellophane wrappers for tomatoes in the United States, on the basis that none of this compound is transferred to the fruit (U.S. Dept. Agr., Pesticides Regulation Division, 1964). Other chemicals which have shown promise as barriers to the spread of pathogens have been discussed in Section III,C.

VI. PROPERTIES AND APPLICATIONS OF SPECIFIC FUNGICIDES

A. Borates and other Alkaline Salts

1. Borates

Borax (sodium tetraborate, \(\text{Na}_2\text{B}_4\text{O}_7\cdot 10\text{H}_2\text{O}\)) was the first postharvest fungicide used on a commercial basis for control of citrus fruit decay. Since its introduction in the mid-1920's (Fulton and Bowman, 1924; Barger and Hawkins, 1925) this treatment has been used extensively in all citrus-producing regions of the world. More recently, borax has been reported to be effective in reducing decay of melons (Barger et al., 1948) and sweet potatoes (Martin et al., 1949; Person et al., 1948).

Borax is soluble in water to the extent of approximately 5.1% at 68°F, 6.3% at 77°F, 7.9% at 86°F, and 12.8% at 104°F on a weight/weight basis (Seidell and Linke, 1952). At solution temperatures below 100°F, a 6% solution of borax is only weakly fungicidal to spores of \(P. \text{digitatum} \) in vitro, but at 110°F is lethal after 5 minutes contact (Hwang and Klotz, 1938; Marloth, 1931). These same concentrations are quite fungistatic to the colonial growth of this organism \(P. \text{digitatum} \) in vitro and the antifungal action is more pronounced in alkaline solution (Moreau, 1961; Tomkins, 197a).

Early experiments with oranges revealed that 6-8% borax was necessary for effective control of \(P. \text{digitatum} \) decay and stem end rots of oranges, and that the efficiency of the treatment was improved by increasing the temperature of the solution to 110°F or by allowing the borax residues to dry on the fruit (Barger and Hawkins, 1925; Fulton,
and Bowman, 1924; Winston, 1935). It was clearly recognized at this time that the borax treatment was not lethal to the stem end rot fungi during the period the fruit were in the bath, but rather that residues remaining on the fruit inhibited the development of these organisms. The work of Hwang and Klotz (1938) similarly showed that the control of *Penicillium* by borax solutions at temperatures less than 110°F (Barger and Hawkins, 1925) must also be due to the fungistatic effect of residual borax on the fruit.

In commercial treatment of fruit it is highly undesirable to leave gross residues of borax on the surface of the fruit. Not only would it impair the appearance of the fruit, but it is also illegal to do so in the United States. It might, however, be possible to deposit small quantities of borax at potential infection sites on the fruit, which would result in rather high concentrations in the microenvironment of the pathogen when the solution evaporated. This could be accomplished by increasing the concentration and temperatures of the borax solution or the time the fruit were exposed to the solution. All of these factors would increase the residue of borax remaining in injuries on the fruit surface and under the calyx after the fruit were lightly rinsed with fresh water to remove the bulk of the borax solution.

The parameters of borax concentration and period of treatment are fixed by practical considerations. The time of treatment cannot exceed 4 minutes in a commercial citrus-packing house. The concentration of borax could be increased by increasing the temperature, but would crystallize out on the equipment when the solution was left unheated for a short time. The best means to increase the efficiency of the borax treatment is by an increase in temperature which not only increases the penetration of the solution but, at temperatures above 110°F, is lethal to *Penicillium* spores which are exposed on the fruit.

Within a few years after the borax treatment was introduced it became obvious that it was not an ideal treatment in that concentrations of 6–8% which effectively controlled decay would begin to crystallize within a short time after the processing line was closed down. Since the borate ion is the active constituent of the borax treatment (Winston, 1935; Morcan, 1961) several borate salts of greater water solubility and higher boron content have been suggested as replacements for borax. These salts or mixtures of salts are usually applied at a concentration equivalent to a 5–8% borax solution, and are generally about equal in effectiveness to a comparable borax solution. Slight differences in performance are probably due to differences in alkalinity of solutions of the different salts (Fidler and Tomkine, 1939).
Borax is appreciably more soluble in water in the presence of boric acid. A mixture of 4% borax and 2% boric acid has been used for treatment of citrus fruit in California for several decades. This solution contains approximately as much boron as a 7% solution of borax. The fruit are submerged for 2–4 minutes in the solution at 110–115°F and thoroughly rinsed immediately after treatment. Boric acid is not used alone because it may be injurious to some fruit (Winston, 1935, 1948).

Sodium metaborate (NaBO₂·4H₂O) contains less boron than borax but is soluble in water to the extent of 74 gm/100 ml water at 68°F. Its solutions are more alkaline than those of borax due to its high sodium content. Solutions of sodium metaborate have been found effective for treatment of melons and citrus fruits (Barger et al., 1948; Godfrey and Ryall, 1948; Lauriol, 1954), although this compound is not as effective against Penicillium spp. in vitro as is borax (Hwang and Klotz, 1938).

Sodium penta borate (Na₂B₄O₆·10H₂O) contains 1.6 times more boron than borax and is soluble in water to the extent of 18 gm/100 ml at 68°F. Its solutions are less alkaline than those of borax. This compound has been tested on citrus fruits and found to be more effective than borax (Cuillut, 1956; Lauriol, 1954).

More recently, interest has been shown in addition compounds comprised of boric acid and lactic acid and aliphatic amines (Moreau, 1956, 1961). These compounds are soluble in water in all proportions and are as effective as a borax solution containing an equivalent concentration of borate ions. These compounds do not crystallize on the surface of treated fruit as do inorganic borates, and their residues prevent the spread of Penicillium from decayed to sound fruit.

Over the years borates have been used extensively for treatment of oranges, but rarely for lemons. Borates are more effective in controlling P. digitatum than P. italicum (Barger, 1928; H. S. Fawcett, 1936). Penicillium italicum can cause a severe decay problem on lemons during storage since it spreads rapidly by contact whereas P. digitatum is usually confined to a single fruit. In mixed infections of the two fungi, the faster growing P. digitatum overruns P. italicum, and contact spread of decay is not so serious a problem as when P. italicum is present alone. For this reason, California lemons are treated with sodium carbonate (see next section) rather than borax for decay control.

The treatment of oranges with borates has rapidly been losing popularity in recent years. The principal reason in California is that the large volume of rinse water contaminated with boron creates a serious disposal problem since there is a danger of phytotoxicity to growing
plants if it finds its way into the soil. For maximum effectiveness against the stem end rot problem in Florida citrus fruits, the borate treatment should not be rinsed immediately from the fruit (Winston, 1935, 1948). However, it has been shown that such treatments increase the boron content of the fruit peel (Furlong, 1948) and are therefore not permissible in the United States. Sodium o-phenylphenate is effective in the control of both stem end rot and *Penicillium* decay, even when the fruit are rinsed after treatment, and does not constitute so serious a disposal problem as borate salts. For these reasons, sodium o-phenylphenate has largely replaced the borates as a treatment for oranges in the United States (Section VI,E).

2. Sodium Carbonate

Shortly after the introduction of borax, sodium carbonate and sodium bicarbonate were suggested for the control of *Penicillium* spp. on citrus fruit (Barger, 1928; Dodge, 1929). Both carbonates are less toxic to *Penicillium* spores *in vitro* than are borates, and their effectiveness in fruit treatment appears to be due to the accumulation of alkaline residues in potential infection sites on the fruit (Hwang and Klotz, 1938; Marloth, 1931). Sodium carbonate is superior to sodium bicarbonate for control of *Penicillium* on citrus fruits, but both are inferior to borax. Solutions of sodium carbonate at concentrations of 3-5% and temperatures of 110-118°F are the standard treatment for lemons in California. Harding and Savage (1964) have pointed out that sodium carbonate solutions at 118°F may predispose mature fruit to decay. The superiority of sodium carbonate over borax as a treatment for lemons was discussed in the previous section.

Weak solutions of sodium hydroxide (1-3%) have been demonstrated to control *Penicillium* spp. on citrus fruits (Fidler and Tomkins, 1939; Fidler et al., 1949; Tomkins, 1935), but such solutions are too corrosive to fruit as evidenced by the finding that the incidence of *Colletotrichum* was increased on fruit so treated (Fidler and Tomkins, 1939).

B. Metals

Metal salts and complexes (except dithiocarbamates) are rarely used as postharvest fungicides because solutions of these compounds are ineffective in preventing decay of inoculated fruit despite their high *in vitro* activity against the fungi responsible for decay. For example, a few parts per million of tributyltin oxide or copper 8-hydroxyquinoline in agar medium completely arrests the growth of *P. digitatum,*
yet solutions containing several thousand parts per million of these compounds have little effect upon the subsequent decay of inoculated fruit dipped in these solutions. These powerful fungicides apparently are unable to penetrate or accumulate in injured fruit tissue. It has been reported that Phytophthora brown rot of citrus fruit cannot be prevented by dipping fruit in an 0.01% solution of CuSO₄·5H₂O 4 hours after inoculation despite the fact that zoospores of P. citrophthora are completely inactivated in a solution containing a few parts per million of copper ion (H. S. Fawcett, 1922; Klotz and DeWolfe, 1961).

Copper compounds may have some applications as postharvest treatments to prevent infection and to retard the spread of the pathogen. Parris (1952) recommended that the freshly cut stems of watermelons be treated with a paste of copper sulfate and starch to prevent infection by Diplodia at that site. The successful use of copper impregnated wrappers to retard the spread of Botrytis on pears was discussed in Section III,C,2.

Storage diseases of flower bulbs and corms have been effectively controlled by postharvest treatment with solutions of organomercury and organotin compounds (Gould et al., 1961; Hassis, 1954; V. L. Miller and Gould, 1963), and Diplodia stem end rot of oranges was prevented by application of 0.1% sodium ethylmercurithiosalicylate to the stem of the fruit (Godfrey and Friend, 1940). The use of organomercury and organotin compounds for postharvest treatment of edible commodities would appear to be restricted by the toxicity of these compounds.

C. Sulfur Compounds

1. Elemental Sulfur and Polysulfides

Sulfur dust has been used commercially on peaches after harvest to reduce infection by M. fructicola. It is recommended that the treatment be applied within 3 hours after harvest (Cardinell and Barr, 1952), indicating that the sulfur functions mainly as a protectant fungicide. Reports indicate that postharvest treatment with sulfur dust is moderately effective for control of brown rot (Cardinell, 1952; Haller, 1952; M. A. Smith, 1930; W. L. Smith et al., 1956), but the treatment is rarely used today because it is not effective against Rhizopus rot, the dust treatment is not compatible with modern packing house operations, and a better fungicide (viz. sodium a-phenylphenate) is now available.

Liquid lime sulfur (solution of calcium polysulfides) is used to some extent as a field spray on peaches after late season rains to eradicate incipient infections of M. fructicola. Liquid lime sulfur has also been
evaluated as a postharvest treatment for peach brown rot and the effectiveness of this treatment is comparable with sulfur dust (Haller, 1932; Poulos, 1949).

No residue limitation has been placed on elemental sulfur or lime sulfur in recognition of their low order of mammalian toxicity. In view of this fact and the possible advantages of a dry treatment, sulfur dust might be useful under special circumstances. However, these inorganic sulfur compounds are much less effective in controlling peach decay than some newer organic fungicides; i.e., sodium o-phenylphenate and 2,6-dichloro-4-nitroaniline (Section VI,J), and therefore should receive relatively little attention in future research.

2. Sulfur Dioxide and Bisulfites

The value of sulfur dioxide in the preservation of foodstuffs has been recognized since antiquity and its many applications in this field have been comprehensively reviewed by Joslyn and Braverman (1954). The fumigation of fresh grapes after harvest with sulfur dioxide for control of Botrytis rot has been a standard commercial practice in California since 1928 (Jacob, 1929; Winkler and Jacob, 1925).

Sulfur dioxide is a mobile liquid when confined in a cylinder and has a vapor pressure of 3.3 atmospheres at 20°C; it boils at -10°C at atmospheric pressure and its vapor density is 2.3 times that of air; SO₂ is soluble in water to the extent of 10.5% (by weight) at 20°C. Aqueous solutions of sulfur dioxide contain SO₂, sulfurous acid, bisulfite ions and sulfite ions, in accordance with the equilibrium,

\[
\begin{align*}
\text{SO}_2 & \rightleftharpoons \text{H}_2\text{SO}_3 \rightleftharpoons \text{HSO}_3^- \rightleftharpoons \text{SO}_4^{2-} \\
& \text{pK}_1 = 1.5 \quad \text{pK}_2 = 7.0
\end{align*}
\]

(1)

According to Remy (1956), only a few per cent of the dissolved SO₂ in aqueous solution exists as sulfurous acid. The concentration of undisassociated and ionic species may be calculated at any given pH (see Section VI,E) and these values have been graphically presented by Joslyn and Braverman (1954). The antimicrobial properties of solutions of sulfur dioxide and bisulfites have been attributed to the concentration of sulfurous acid (SO₂ + H₂SO₄) per se with little contribution from the ionic species present. Cruess et al. (1931) showed that at pH 3.5, two to four times more SO₂ was required to inhibit growth of fungi than at pH 2.5, while at pH 7 more than 25 times more SO₂ was required than at pH 2.5.

Couey and Ueta (1961; Couey, 1965) have shown that hydrated or wet spores of Botrytis and Alternaria are much more sensitive to SO₂
gas than are dry spores and concluded that the lethal effect of SO₂ gas must be due to the sulfurous acid formed in moist spores.

The fumigation of grapes with SO₂ during low-temperature storage makes it possible to market this fruit the year around. The widespread use of SO₂ in California is due to two circumstances. Certain grape varieties grown in California (e.g., Emperor and Tokay) may be stored for a number of months at 32°C without serious loss in quality. Even at this low temperature, however, *Botrytis cinerea* may slowly develop causing serious decay losses by forming “nests” of decayed berries. Secondly, the vinifera grape (*Vitis vinifera*) which is the backbone of the California grape industry is remarkably tolerant to SO₂ gas. Most other fruits and vegetables, including the American (eastern) varieties of grapes cannot be fumigated with SO₂ because of their susceptibility to injury at concentrations of SO₂ that would bring about a significant measure of decay control (J. M. Harvey and Pentzer, 1953).

Two general methods of applying SO₂ to grapes are currently in commercial usage (Ryall and Harvey, 1959). Virtually all California grapes are fumigated by direct volatilization of liquid SO₂ into the atmosphere of the storage room or into a loaded railroad car. The SO₂ may be delivered to the chamber either as a gas or as a spray of liquid SO₂ (Gentry and Nelson, 1963). The second method which is less generally used involves the generation of SO₂ by the hydrolysis of sodium bisulfite placed inside the container of grapes at the time they are packed.

The usual program for direct fumigation of grapes is an initial application of SO₂ at a concentration of 1% (by volume) for 20 minutes as soon after harvest as possible. If the grapes are held in storage the treatment is repeated every 7-10 days with 0.25% concentration for 30-60 minutes (J. M. Harvey and Pentzer, 1960; Nelson and Baker, 1963). Since postharvest decay of grapes is principally a result of incipient field infections which are not detectable at harvest and which cannot be eradicated by SO₂, the purpose of the periodic fumigation treatments is to prevent the spread of *Botrytis* during storage (J. M. Harvey, 1955; Nelson, 1958). Lesser benefits of SO₂ fumigation are the inactivation of spores contaminating the berries and packages (Couey and Uota, 1961) and the prevention of decay from inoculations occurring at the time of harvest.

Most of the grapes shipped from California receive a final SO₂ fumigation after loading in the railroad car. A cylinder containing the desired weight of liquid SO₂ (usually 3–5 lb) is immersed in hot water and the vaporized SO₂ is lead through a tube into the loaded railroad cars. Recently it has been suggested that this fumigation could be more
efficiently carried out by atomizing liquid SO₂ into the atmosphere of the railroad car and by distributing the gas with a portable fan (Gentry and Nelson, 1963; Uota and Harvey, 1964).

Nelson and Richardson (1961) regard an effective SO₂ fumigation program as a compromise between decay control and fruit injury, and have stressed the importance of uniformly distributing the minimum effective concentration of SO₂ within the fumigation chamber. Injury is manifested by bleached areas in the skin (around the cap stem in the case of the Emperor variety) and by the exudation from the berries which gives the grapes a glazed appearance and makes them sticky to the touch (Nelson and Tomlinson, 1958). Injury is accumulative and therefore the applied dosage is most critical for grapes destined for long term storage. Nelson and Baker (1963) found that relatively low concentrations (0.05-0.1%) of SO₂ applied at 7-day intervals gave better control of Botrytis with less fruit injury, than higher concentrations applied at less frequent intervals. Depending upon the variety, grapes begin to show injury symptoms when the berries have absorbed 20-55 ppm SO₂ (Pentzer et al., 1932; Reyneke and Piaget, 1952).

Considerable effort has been directed to the development of a formula for calculating the quantity of SO₂ required for an efficient fumigation under a given set of conditions, and to more effective methods for uniformly distributing the desired concentration throughout the load of grapes. Factors which must be considered in calculating the quantity of SO₂ required are relative humidity, free space in the storage room, units of packaged grapes in the room, and the absorptive capacity of the fruit. Relative humidity, through its effect on hydration of the storage boxes, greatly influences the quantity of SO₂ required to maintain a given concentration in the atmosphere (Cant and Nelson, 1957; Nelson and Baker, 1963). Air movement and ease of penetration of the packages is essential for efficient fumigation with SO₂.

The fumigation of grapes with SO₂ evolved by the hydrolysis of sodium bisulfite in the shipping container is a commercial practice in California and South Africa. A small quantity (ca. 5 gm) of sodium bisulfite or sodium metabisulfite is sifted into the pad upon which the grapes are packed, or more commonly today, the bisulfite is sifted on top of a sawdust-filled chest which is then vibrated in an effort to better distribute the bisulfite throughout the grapes (Pentzer, 1939). In principle, the bisulfite is slowly hydrolyzed over a period of weeks and the evolved SO₂ checks the spread of Botrytis on the grapes. The difficulties encountered in such a system with regard to a uniform rate of evolution and distribution of the evolved fumigant throughout the
package have been discussed in Section V,A,1,a. The effect of acidity on the rate of evolution of $\text{SO}_2$ from bisulfite solutions have been considered (Reyneke and Piaget, 1952) and unique formulations which partially control the evolution of $\text{SO}_2$ have been described (Van der Plank, 1939; Van der Plank and Van Wyk, 1940). The effectiveness of such formulations depends upon a constant level of moisture in the atmosphere or in the packaging materials, a condition which is seldom realized in commercial practice. Furthermore, the distribution of evolved $\text{SO}_2$ throughout the package is strongly influenced by the moisture content of the packaging materials (Nelson et al., 1963).

In summary, the advantages of $\text{SO}_2$ for treatment of grapes are that it is effective if properly applied, it retards browning of the grape stems, does not leave visible or toxic residues, and the treatment is relatively inexpensive to apply (Nelson et al., 1963). The disadvantages of the $\text{SO}_2$ treatment are that it causes bleaching of red grapes, repeated application accentuates problems of wetness and stickiness of the berries, effective application demands that the grape containers be well ventilated thereby making the berries vulnerable to shrinkage from water loss, the treatment must be applied at frequent intervals on storage grapes, penetration of the gas into sawdust packs is poor, the gas dosage required is affected markedly by moisture conditions, and $\text{SO}_2$ is highly corrosive to metal surfaces under conditions of high humidity. Dibromotetrachloroethane has been intensively investigated as a possible substitute for $\text{SO}_2$ since it is highly effective for control of Botrytis but does not possess many of the disadvantages of the $\text{SO}_2$ treatment (Section VI,D,1).

It has recently been reported that $\text{SO}_2$ fumigation at 0.13–0.25% for 20 minutes was effective in controlling Botrytis, Cladosporium, and Alternaria on red raspberries without causing injury or a serious delay in ripening. Injurious effects were noted at 0.5% however (Cappellini et al., 1961; Cappellini and Eyal, 1964).

3. Organic Sulfur Compounds

A mixture of sodium dimethylthiocarbamate and sodium 2-mercaptoethanol in a synthetic polyethylene polysulfide latex has been used as a fungicide paste applied to the cut main stem of bananas to prevent entry of decay fungi at this site (Wardlaw, 1961; Meredith, 1961a). Within the last few years a large percentage of bananas from Central America have been shipped as individual hands and it has become the practice to dip the cut hand stem (cushion) in a 0.46% suspension of maneb (manganese ethylenebisdithiocarbamate) or 0.16%
thiram (tetramethylthiuramdisulfide). Ziram (zinc dimethylthiocarbamate) may be used as an 8% paste for brush application to cut stems or crowns. In the United States the residue tolerance on bananas for maneb is 15 ppm on the peel and 2 ppm in the pulp, and for thiram, 7 ppm on the peel and 1 ppm in the pulp (U.S. Dept. Agr., Pesticides Regulation Division, 1964).

Zineb and ziram are used commercially for reduction of surface mold (Cladosporium and Alternaria) on cantaloupes. The residue tolerance for both of these fungicides is 7 ppm expressed on the weight of the whole melon. Thiram applied as field sprays or as a postharvest dip has given good control of postharvest rot of strawberry in England (M. H. Moore and Tew, 1955; Edney, 1964).

Thiourea and thioucarbamide as 5% aqueous solutions have provided highly effective control of Penicillium decay as well as Diplodia and Phomopsis stem end rots on oranges (Childs and Segler, 1946; Hopkins and Loucks, 1946). Thiourea possesses some local systemic activity and therefore is superior to borax in delayed applications for control of stem end rots. Owing to its mammalian toxicity, thiourea has never been adopted commercially in the United States although it was used to a limited extent for a few years in certain other citrus producing areas. Certain alkyl thionocarbamates \( [R-S-\text{C(O)NH}_2] \) have been shown to be effective for control of citrus fruit decays. Berry (1958b) reported that ethylthionocarbamate was more effective for control of fruit decay than sodium o-phenylphenate, despite the higher in vitro activity of the phenol. This finding implies that ethylthionocarbamate, like thiourea, has the property of efficiently penetrating the tissue of the fruit.

Pyridine \( N \)-oxide-2-thione, a compound possessing a high degree of antifungal activity in vitro, has been reported to be ineffective for control of Rhizopus on strawberries and M. fructicola and Rhizopus on peaches (DiMarco and Davis, 1957a,b). The author has also found this compound to be ineffective against Penicillium on citrus fruits. However, it has been reported that brown rot of peaches was reduced by dipping the fruit in a 1% suspension of the zinc salt of pyridine-\( N \)-oxide-2-thione (Szkolnik and Hamilton, 1957).

D. Hydrocarbons and Derivatives

1. Biphenyl

The use of biphenyl to control citrus fruit decay during shipment has been a major factor in the development of world trade in citrus fruits. With the possible exception of sulfur dioxide on grapes, biphenyl has
be used as a postharvest fungicide with success unmatched by any other chemical treatment.

Biphenyl (diphenyl) is a white crystalline solid which melts at 71°C and has a vapor pressure of approximately 0.01 mm Hg at 25°C (Bradley and Cleasby, 1953; Farkas and Aman, 1940). The vapor pressure curve from 0–40°C is graphically presented in the recent review of Rajzman (1965). Biphenyl is very soluble in most organic solvents but a saturated water solution at 25°C contains only 10 ppm biphenyl (Andrews and Keeler, 1949).

Although biphenyl cannot be considered a broad-spectrum fungicide, its vapors are strongly inhibitory to mycelial growth of P. digitatum, P. italicum, Diplodia natalensis, Phomopsis citri, B. cinerea, Aspergillus, Moniliia, Rhizopus, and other plant pathogenic fungi (Ramsey et al., 1944; Heiberg and Ramsey, 1946; Eckert and Kolbezen, 1963a). Bacteria, yeast, Phycomycetes, and resistant strains of Penicillium and Diplodia are not inhibited. An extensive list of sensitive and resistant species are given by Heiberg and Ramsey (1946) who suggested that biphenyl-enriched media may be useful for selective isolation of microorganisms. The typical response of sensitive fungi to biphenyl is a stunting, thickening, and profuse branching of developing hyphae. Spores of different fungal species vary in their ability to put forth germ tubes in the presence of biphenyl vapors, but germ tubes of all sensitive fungi are greatly inhibited, malformed, and sometimes burst at the tips. Conidia of P. digitatum frequently continue to swell beyond the point at which germ tubes normally emerge and sometimes burst before appearance of the germ tubes. Germ tubes which emerge after some delay, are profusely branched. These morphological abnormalities induced by biphenyl are illustrated in the publications of Ramsey et al. (1944) and Duran (1962).

The inhibition of fungi by biphenyl can be demonstrated either by incorporating this compound in the substrate or by exposing the culture to its vapors. The fact that vapor action is not essential can be demonstrated by inhibition in liquid culture (Eckert and Kolbezen, 1963a). The action of biphenyl is clearly fungistatic; saturated solutions containing excess solid biphenyl are not lethal to most fungi even after several days' exposure. With the exception of cells which lyse, growth of inhibited hyphae or spores is resumed in a normal manner within a few hours after the culture is removed from the presence of biphenyl (Farkas and Aman, 1940; Ramsey et al., 1944). By using butylphthalate as a solvent to reduce the vapor pressure of biphenyl, Farkas and Aman (1940) demonstrated that at 25°C a sustained vapor concen-
tion of 14 µg biphenyl per liter of air reduced the growth of *P. digitatum* on agar medium 50%, 30 µg/liter reduced growth 75%, and 80 µg/liter (saturated vapor concentration) gave complete inhibition. Upon removal of the source of biphenyl vapors, the cultures resumed their normal rate of growth immediately, indicating that biphenyl absorbed by the fungal cells and by the substrate is in very mobile equilibrium with that in the vapor phase. This fact is highly significant in the application of biphenyl as a fruit treatment.

Tomkins (1936) in England first reported that biphenyl-impregnated fruit wrappers were effective in reducing *Penicillium* decay of oranges and in preventing sporulation of this fungus on the surface of decayed fruit. He also recognized that a faint odor of biphenyl was detectable on treated fruit, but that the odor was lost after the fruit were exposed to fresh air for several days. Farkas (1938, 1939) conducted extensive commercial shipping tests from Israel to England and showed that untreated oranges developed 6 or 7 times more *Penicillium* decay than fruit which were wrapped with biphenyl-impregnated tissue paper. The efficacy of biphenyl for control of *Penicillium* has been confirmed by more recent large-scale commercial test shipments (Roistacher et al., 1960; Rygg et al., 1961) as well as by 20 years of commercial experience. Biphenyl also reduces decay of citrus fruits caused by the stem end rot fungi, *D. natalensis* and *P. citri* (Godfrey and Ryall, 1948; Leggo et al., 1964; Littauer, 1956; Winston, 1948; Hopkins and McCormick, 1959b). Reports that biphenyl is not effective against stem end rot (Loucks and Hopkins, 1946) may indicate that the extent of infection is critical. Biphenyl has been tested for control of *Botrytis* on grapes (Tomkins, 1936), *Rhizopus* on peaches (Haller, 1952), and *Fusarium* on potatoes (McKee and Boyd, 1962) with favorable preliminary results, but in each case the odor or flavor of the commodity was adversely affected. Biphenyl is injurious to apples and bananas (Tomkins, 1936).

Commercial formulations of biphenyl consist of tissue wrappers or paper sheets impregnated with a heated solution of biphenyl in a mixture of paraffin and mineral oil. About 1% lemon oil may be added to the formulation to mask the odor of biphenyl (Christ, 1964a; Mispley and MacRill, 1956). When the mixture cools on the paper, the biphenyl partially crystallizes out, resulting in an intimate mixture of microcrystals imbedded in an oil matrix. Treated tissue wraps are usually 10 inches × 10 inches and contain 40–50 mg biphenyl each. Treated paper sheets are approximately 10 inches × 16 inches and contain approximately 2.35 gm biphenyl. One sheet is usually placed at the top and another at the bottom of an 11 × 11 × 17-inches fiberboard fruit carton.
Prior to 1952 it was standard practice in all major citrus production areas to wrap individual fruit in biphenyl-impregnated wrappers. Although this practice is still common in South Africa, Israel, and Brazil, it was discontinued in the United States around 1952 when the standard wood box was replaced by a one-half box fiberboard container. The usual procedure in the United States at the present time is to place two biphenyl-impregnated sheets in each carton of citrus fruits. The individual fruit are not wrapped.

As originally noted by Tomkins (1936) the biphenyl treatment of citrus fruits performs two important functions—it reduces the number of decayed fruit and it also prevents the sporulation of Penicillium on the surface of decaying fruit. The latter function is absolutely essential to the successful shipment of unwrapped fruit (Eckert, 1959a). The effectiveness of the biphenyl treatment is influenced by the type of formulation, ventilation of the shipping container, variety of fruit, and sensitivity of the pathogen to biphenyl. The tissue-wrap formulation is considerably more effective in prevention of decay, per se, than is the sheet formulation (Christ, 1964a). This would be anticipated in view of the comparatively large surface area of the wrap treatment and the closeness of the solid biphenyl to the individual fruits. Both factors lead to a rapid buildup of biphenyl vapors in the microenvironment surrounding each fruit. On the other hand, biphenyl is lost more rapidly from the tissue wrap than from the pad and in long periods of shipment, the biphenyl in the tissue may be largely depleted before the end of the shipment. The wrap formulation was devised for use in slat wood boxes which have a high degree of ventilation. Under these conditions it is essential that the biphenyl formulation be able to rapidly supply biphenyl vapors to the atmosphere surrounding the fruit to compensate for losses due to leakage from the container. The individual wrappers are somewhat effective as barriers to the spread of decay so that it is not absolutely essential that all tissue wraps continue to evolve biphenyl vapors for the entire marketing period. By contrast, the total surface area of the sheet formulation is much less than that of the wrap formulation, consequently the rate of biphenyl evolution is lower and the distribution of the vapors is less efficient. Thus the overall effectiveness of the sheet formulation depends, to a large extent, upon the ability of the walls of the container to prevent a mass flow of air through the container which would purge the biphenyl vapors from the atmosphere surrounding the fruit. It is essential that the pad formulation maintain an inhibitory concentration of biphenyl in the carton atmosphere, since, in the absence of individual fruit wraps, sporulation of Penicillium on
decayed fruits will cause serious spore soilage of the remaining fruit in the container. The adverse result of excessive ventilation on the effectiveness of sheet formulation have been noted (Eckert and Kolbecen, 1963a; Rygg et al., 1961, 1962, 1964), but other factors such as rate of cooling of the fruit and control of biphenyl-resistant fungi may be important considerations for maintaining a moderate degree of ventilation (Harding and Savage, 1962; Rygg et al., 1961).

It has been a common observation that the biphenyl treatment is more effective in the control of *Penicillium* on lemons than on oranges, even when fruit of the same size are compared. This phenomenon might be explained by the fact that oranges absorb more biphenyl vapor than do lemons (Rygg et al., 1964; Hayward and Edward, 1965, 1964; Rajzman, 1965), and might, therefore, reduce the vapor concentration around each fruit to a lower level than for lemons. Rygg et al. (1964) analyzed the vapor concentration of biphenyl in fiberboard containers of oranges and lemons and found the concentration to be slightly higher in the atmosphere surrounding the lemon fruit.

It has been known for years that species of fungi (e.g., *Penicillium* and *Diploodia*) that are usually sensitive to biphenyl may, by constant exposure, develop saltants that possess stable resistance to this compound (Farkas and Aman, 1949; Littauer and Gutter, 1953; Harding, 1959a). More recently it has become apparent that biphenyl-resistant strains of *Penicillium* develop in packing houses, often in alarming proportions (Duran, 1962; Duran and Norman, 1964; Harding, 1964) and considerably reduce the effectiveness of the biphenyl treatment in overseas fruit shipments (Rygg et al., 1961). It has been adequately demonstrated that the practice of treating lemons with sodium o-phenylphenate before storage may cause an increase in the population of spores of biphenyl-resistant strains of *P. digitatum* in packing houses. Owing to the structural similarity of o-phenylphenol and biphenyl, strains of *Penicillium* that possess resistance to the former compound are also resistant to the latter. Suppression of sensitive strains by residues of sodium o-phenylphenate on lemons favors the proliferation of resistant strains (Harding, 1962, 1964). As a result of these findings, sodium o-phenylphenate is no longer used to any extent as a prestorage treatment for lemons in California. The use of biphenyl in lemon storage rooms to retard decay has been attempted on several occasions in California with the inevitable buildup of biphenyl-resistant strains of *Penicillium* which later caused serious decay losses during shipment. It is interesting to note that a similar treatment is currently being evaluated on Florida lemons (Oberbacher and Grierson, 1961).
Although biphenyl is unquestionably a commercial success for control of postharvest decay of citrus fruits, it has certain disadvantages which were recognized soon after its adoption for commercial use. In the first publication on the effectiveness of biphenyl, Tomkins (1936) concluded "thus it is possible that the advantage to be gained from reduced rott ing by the use of wraps treated with diphenyl might outweigh the dis advantages of the slight smell acquired by the orange." This conclusion has been borne out by over 20 years of commercial experience with biphenyl; nonetheless, the temporary odor of treated citrus fruits has, on occasion, caused consumer resistance to biphenyl-treated fruit. A number of odor and taste panel tests have confirmed that under usual handling conditions the odor of biphenyl may be detected for only a few days after the fruit are removed from the presence of biphenyl (Eckert and Kolbezen, 1963a; Rajzman, 1965). Biphenyl also may cause a temporary tackiness of fruit with certain "wax" coatings but this effect is lost after the fruit have been aired out for a few hours. Another adverse effect of biphenyl on lemons is that it accelerates the physiological decline of the button (calyx + receptacle), predisposing the fruit to Alternaria rot (E. M. Harvey and Atrops, 1953). Biphenyl also causes a slight depression in the respiration rate of citrus fruits, but has no effect on their gross chemical composition (Eaks, 1955).

Although biphenyl provides adequate protection against decay caused by Penicillium, Diplodia, and Phomopsis, which are the major decay problems in citrus fruit shipments, it does not control fruit diseases caused by Geotrichum candidum, A. citri, T. viride, or C. gloeosporioides (Eckert, 1959b; Eckert and Kolbezen, 1963a; Harding and Savage, 1962; E. M. Harvey and Atrops, 1953; Christ, 1964a), nor does it reduce decay by resistant strains of Penicillium (Eckert and Kolbezen, 1963a; Harding, 1962; Duran, 1962). Contamination of packing houses with spores of biphenyl-resistant strains of Penicillium to the extent of only 0.5% of the total spore population would greatly decrease the efficiency of the biphenyl treatment (Harding, 1964). A serious problem of resistant strains of P. digitatum arose in California lemon packing houses during 1958 to 1960 but has since been brought under control by a rigid program of sanitation and elimination of the sodium o-phenylphenate wash treatment before storage (Harding, 1964; Fig. 1).

Rajzman (1965) has published an extensive review on residues of biphenyl in treated fruit. Biphenyl is absorbed by the peel of the fruit and the residues are located mainly in the oil glands. The peel may contain a few to several hundred parts per million of biphenyl whereas the pulp contains residues under 1 ppm. In order of importance, the fac-
tors influencing the level of biphenyl residues are variety and maturity and size of fruit, storage temperature, time of exposure, quantity of biphenyl in the fruit container, and changes in the surface of the fruit brought about by washing or waxing (Rajzman, 1965; Hayward and Edwards, 1964, 1965). Biphenyl possesses a relatively low order of mammalian toxicity and the quantities consumed as residues in citrus fruits are considered to be innocuous. The permissible residue of biphenyl in citrus fruits in the United States has been set at 110 ppm on a whole fruit basis (U.S. Dept. Agr., Pesticide Regulation Division, 1964). Tolerances for other countries have been tabulated by Rajzman (1965).

2. Halogenated Hydrocarbons

Chloropicrin and methyl bromide are generally injurious to living plant tissues at concentrations that prevent decay. For this reason these materials have not found application for control of postharvest diseases, although methyl bromide is widely used to eradicate insect pests on fruits after harvest (Lindgren and Vincent, 1962). Even dormant gladiolus corms lack the necessary resistance to methyl bromide to permit its use for control of Sclerotinia rolfsii (Carpenter and Gammon, 1955), although this fumigant may have some application for control of fungi on stored grains (Yanai et al., 1964).

A number of reports in the literature indicate that polyhalogenated ethylene and ethanes can control decays of stone fruits, strawberries, citrus, and grapes. Vandermark and Sharvelle (1952) reported that vapors of several liquid chlorinated ethanes and ethylenes reduced decay of peaches, but that all except trichloroethylene had a tendency to injure fruit at concentrations required for decay control. W. L. Smith et al. (1966) corroborated the effectiveness of trichloroethylene and tetrachloroethylene on peaches reporting that both fumigants (at 1 : 20,000) were highly effective for control of Rhizopus, but that best control of M. fructicola was obtained by the combination of sulfur dust followed by fumigation with tetrachloroethylene. Berry (1958a) found that a proprietary mixture of sym-dichloroethane and trichloroethane inhibited spore germination of D. natalensis, P. citri, and P. digitatum and delayed, but did not prevent, decay of oranges. This mixture of dichloroethane and trichloroethane has been reported to substantially reduce surface spotting caused by Cladosporium and Alternaria on fresh figs (J. M. Harvey, 1956b). Patents have been issued claiming the control of mold growth on strawberries, peaches, and apples by various chlorinated aliphatic hydrocarbons (Keller, 1957, 1958). Pentabromo-
ethane in alcohol solution is said to be effective against *Penicillium* decay of oranges (Winston et al., 1953), but vapors of hexachloroethane are not effective against this disease although this latter compound is both volatile and fungistatic *in vitro* (Tomkins, 1938).

Dibromotetrachloroethane, a fungicide first proposed for control of dry rot of potatoes during storage (C. E. D. Smith and Wynne, 1958), has recently shown promise for control of postharvest decays of citrus fruits and grapes. Dibromotetrachloroethane is a crystalline solid which decomposes before melting at 113°C. Its vapor pressure is estimated at approximately 0.1 mm Hg at 25°C and its water solubility at 0.2 ppm at the same temperature (Eckert and Kolbezen, 1963a). Dibromotetrachloroethane has been shown to be a broad-spectrum vapor-phase fungicide for control of citrus fruit decay (Eckert et al., 1962; Eckert and Kolbezen, 1963a; Chriet, 1964b). It has several advantages over biphenyl for this application; i.e., it possesses more intense fungistatic activity, has a broader antifungal spectrum, and its odor disappears from treated fruit more rapidly than that of biphenyl. Dibromotetrachloroethane has also shown promise for control of *Botrytis* on stored grapes (Chiarappa et al., 1962; Nelson et al., 1963). For stored grapes, this fungicide has the following advantages over SO₂ or sodium bisulfite: its effectiveness is independent of the moisture content of the packaging materials; it offers an opportunity to completely close the storage containers thereby preventing shrinkage losses; and it has no tendency to injure the berries (Nelson et al., 1963). Dibromotetrachloroethane has two undesirable features that make it difficult to formulate, particularly for application to citrus fruits. The vapor pressure of this compound is about ten times higher than that of biphenyl necessitating some auxiliary means to control its rate of volatilization in the fruit container. Secondly, vapors of dibromotetrachloroethane are lachrimatory making it necessary to prevent leakage of its vapors from the fruit container. Investigations on the application of dibromotetrachloroethane are still in progress and at the present time, this compound has no legal status for use as a postharvest fungicide.

Tetraiodoethylene is used commercially as a 0.3-0.8% suspension in water or as a 0.2-0.3% suspension in a wax emulsion to control *Alternaria* and *Cladosporium* on melons. A tolerance of 15 ppm has been established in the U.S.A. for residues resulting from this treatment (U.S. Dept. Agr., Pesticides Regulation Division, 1964). The writer is not aware of any published reports on the effectiveness of this treatment.
3. Chloronitrobenzenes

Pentachloronitrobenzene and 2,3,5,6-tetrachloronitrobenzene ("tecnazene") are low vapor pressure solids which are reported to control Fusarium and Phoma on stored potatoes (Harrison and Downie, 1960; McKee and Boyd, 1962) and Botrytis on gladiolus corms (Hawker, 1946). Tetrachloronitrobenzene is also used to inhibit sprouting of potatoes in storage.

E. Phenols

1. o-Phenylphenol

Tomkins (1937b) reported that decay of several varieties of fruits could be reduced by wrapping them in tissue paper impregnated with o-phenylphenol, but that this treatment caused severe scalding of the fruit unless mineral oil or a high molecular-weight base, such as benzidine or hexamethylenetetramine (hexamine), was added to the formulation to reduce the volatility of the phenol (Tomkins, 1937b, 1939). The effectiveness of wraps impregnated with o-phenylphenol was confirmed by South African workers (Van der Plank et al., 1940) who further found that vegetable oils (glycerides) were more effective than mineral oil in preventing o-phenylphenol injury to oranges and lemons. Sharma (1936) first recognized that the dissociated form of o-phenylphenol was practically nonphytotoxic and described a method for treating fruit with a solution of sodium o-phenylphenate containing excess sodium hydroxide to suppress hydrolysis of the o-phenylphenate ion. The antifungal and phytotoxic properties of dissociated and undisassociated forms of phenols have been considered in general by several investigators, and with particular reference to o-phenylphenol by Van der Plank and Rattray (1940) and by Long and Roberts (1958). The undisassociated phenol is much more fungicidal and phytotoxic than the phenate ion.

o-Phenylphenol is a very weak acid which is soluble in water to the extent of about 800 ppm at 25°C (Van der Plank and Rattray, 1940; Long and Roberts, 1958). The pKd of o-phenylphenol has been reported as 9.7 (Golumbic and Weller, 1950) and 9.95 at 25°C (Kieffer and Rumpf, 1954). Sodium o-phenylphenate is very soluble in water; a 0.5% solution of the tetrahydrate salt having a pH of about 10.3. Within a range of 2 pH units on either side of the pKd value, a slight change in the pH of the solution markedly affects the concentration of phenol and phenate present, and therefore has a pronounced effect upon the fungi-
cidal and phytotoxic properties of that solution. The concentration of the ionic and undissociated species in a given solution may readily be calculated from the expression,

\[ \text{pH} = pK_a - \log \frac{[\text{phenate}]}{[\text{phenol}]} = \log \frac{c-x}{x} \] (2)

where \( x \) and \( c - x \) are the molar concentrations of an \( o \)-phenylphenol and \( o \)-phenylphenate, respectively, and \( c \) is their sum. The calculated concentration of phenol and phenate in a 0.5% solution of sodium \( o \)-phenylphenate tetrahydrate are given in Table I.

It is obvious from the above equilibrium expression that the concentration of free phenol in any solution is in direct proportion to the concentration of the salt. Furthermore, Table I shows that a shift of pH from 11.0 to 12.0 decreases the concentration of free phenol by a factor of 9.4, whereas the concentration of phenate ion increases only slightly.

At a fixed concentration of sodium \( o \)-phenylphenate, a decrease from pH 12.0 to 11.0 causes a great increase in the fungicidal effectiveness of the solution against spores of \( P. \) digitatum in vitro (Eckert, 1962) and also increases, to a modest degree, the efficacy of the solution in preventing Penicillium decay of citrus fruit (Long and Roberts, 1958; Van der Plank and Rattray, 1940). However, such a decrease in pH of solutions of sodium \( o \)-phenylphenate always is accompanied by an increase in the phytotoxicity of the solution to sensitive varieties of citrus fruits. This injury is due almost entirely to the undissociated \( o \)-phenylphenol present which would be expected to penetrate the waxy cuticle of the fruit with greater ease than the charged phenate ion. This theory has

<table>
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<th>pH</th>
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<tr>
<td>12.0</td>
<td>3171</td>
<td>28</td>
</tr>
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</table>
been documented by observations that neutral solutions containing 400 ppm o-phenylphenol give injury symptoms identical to those obtained by treating oranges in solutions of sodium o-phenylphenate at pH values which result in 400 ppm of free phenol (Long and Roberts, 1958; Van der Plank and Rattray, 1940). Furthermore, residues of o-phenylphenol in the peel of citrus fruits increase markedly as the pH of the solution decreases, clearly demonstrating that the lipophylic o-phenylphenol passes into the fruit rind with relative ease, whereas the ionized molecule is excluded by the intact cuticle (Hayward and Grierson, 1960; Long and Roberts, 1958).

Since o-phenylphenol is considerably more fungicidal than sodium o-phenylphenate and solutions of the latter increase in effectiveness against *Penicillium* on citrus fruits with a decrease in pH, it might be concluded that the free o-phenylphenol was the effective moiety and that the o-phenylphenate ion functioned as a concentration buffer for the phenol. This does not seem to be the case, however, since the author has observed that solutions containing 300 ppm or less of o-phenylphenol at pH 5 are quite ineffective against *Penicillium* on oranges, whereas solutions of sodium o-phenylphenate at pH 11.8 containing 45 ppm o-phenylphenol are extremely effective in preventing decay even when the fruit are subsequently rinsed. Furthermore, the latter solution of sodium o-phenylphenate has little effect upon the viability of ungerminated *Penicillium* spores exposed to the solution and then rinsed, under the same conditions as the fruit. Apparently, the o-phenylphenate ion in the solution is of major importance in the control of fruit decay. A plausible mechanism is that the o-phenylphenate ion is precipitated (as o-phenylphenol) in the acidic microenvironment of injuries in the fruit rind. The accumulated o-phenylphenol then would inhibit the development of fungi attempting to invade fruit at these sites. Analysis of the rind of treated fruit has shown that the magnitude of the o-phenylphenol residue is proportional to the number of injuries on the surface of the fruit.

Attempting to improve the effectiveness of the treatment or economize on the use of sodium o-phenylphenate by decreasing the pH of the solution is a poor practice in the commercial treatment of fruit. Better methods for improving the treatment are to increase the temperature of the solution, the exposure time, or the concentration of sodium o-phenylphenate.

The importance of controlling the pH of the sodium o-phenylphenate treatment to prevent injury to the fruit cannot be overemphasized. It has been repeatedly pointed out that citrus fruits will be injured by a
9. APPLICATION AND USE OF POSTHARVEST FUNGICIDES

Solution containing 400 ppm free phenol even at ambient temperatures (Long and Roberts, 1958; Sharma, 1936; Van der Plank and Rattray, 1940), and very sensitive fruit such as the navel orange may begin to show injury at 200 ppm free phenol. Furthermore, the injurious effects of free o-phenylphenol are accentuated by increasing the temperature or exposure time. Since at any fixed pH level, the concentration of free phenol is directly proportional to the concentration of o-phenylphenate, it is apparent that a higher pH level will be required to prevent injury from treatment with 2% sodium o-phenylphenate as compared to 0.5% sodium o-phenylphenate. Thus a 2% solution should be maintained at a pH of 11.7 or higher (Long and Roberts, 1958) whereas a pH of 11.5 is satisfactory for a 0.5% solution. Any substances of greater acid strength than o-phenylphenol, such as CO₂ or even soil, will liberate free o-phenylphenol; thus it is necessary to constantly readjust the pH of the solution to prevent injury to the fruit.

Although rigid control of alkalinity is widely practiced as a means to prevent phytotoxicity of sodium o-phenylphenate, hexamethylenetetramine (hexamine) effectively prevents injury in those situations where the pH cannot be adequately controlled (Hopkins and Loucks, 1950). Hexamine precipitates free o-phenylphenol as the insoluble hexamine phenate (3:1 complex) before the free phenol reaches a phytotoxic concentration, and also exerts a buffering effect on the solution at about pH 11.8 (Long and Roberts, 1958).

During the last decade, sodium o-phenylphenate had come into widespread commercial use as a treatment for control of decay of citrus fruits, pome fruits, stone fruits, sweet potatoes, and other perishables. Citrus fruits are submerged in the solution of sodium o-phenylphenate or the solution is flooded or foamed onto the fruit moving on a conveyor. Sodium o-phenylphenate is also incorporated in water emulsion waxes and o-phenylphenol in organic solvent "waxes" (Newhall and Grierson, 1955; Hopkins and McCornack, 1959a,b, 1964). In Florida, it is recommended that solutions for dipping or flooding onto oranges contain 2% sodium o-phenylphenate tetrahydrate, 1% hexamine, and 0.2% sodium hydroxide. The pH is maintained between 11.5 and 12.2 and the temperature of the solution held at 90°F (Hopkins and McCornack, 1959b, 1964). This treatment in Florida apparently provides control of both Penicillium and the stem end rot fungi, Diplodia and Phomopsis (McCornack and Hopkins, 1965; Hatton and Winston, 1958), although it is reported to be ineffective for control of Phomopsis in Australia (Leggo et al., 1964). In California, the solution used for bath treatment of oranges usually contains 0.5% sodium o-phenylphenate tetrahydrate...
with the pH maintained in the range 11.5-11.8, and at temperatures of 90-115°F. Hexamine is not used in the California formulation. Formulations used for foam application contain 2% sodium o-phenylphenate tetrahydrate at pH 11.3-11.8. Control of pH is not a problem in foam applications since the solution is not recirculated. Sodium o-phenylphenate is not commonly used as a prestorage treatment for lemons in California because it favors the proliferation of strains of *P. digitatum* that are resistant to biphenyl (Section VI,D,1). The foam application method has gained widespread popularity as a poststorage treatment for lemons (Harding and Savage, 1965). With formulations of sodium o-phenylphenate that do not contain hexamine, it is always necessary to rinse the fruit after treatment lest absorbed CO₂ liberate injurious quantities of o-phenylphenol on the rind of the fruit. Even with hexamine added to increase the safety of the treatment, rinsing is recommended to avoid leaving a dulling film of hexamine phenate on the surface of the fruit (Hopkins and Loucks, 1953).

Although solutions of sodium o-phenylphenate are used to a considerable extent for commercial control of *P. expansum* and *B. cinerea* on apples and pears in Washington and Oregon, few data have been published on the efficacy of the treatment. The standard commercial treatment consists of immersing or flooding the fruit with a 0.6% solution of sodium o-phenylphenate tetrahydrate at pH 11-12 and a temperature of 40-70°F. The treatment is most effective on pome fruits which are slightly immature. Decay control is relatively poor on fruit which have been stored for some time. According to Pierson (1960), sodium o-phenylphenate is more effective on pears if the fruit are not rinsed after treatment, but that this practice leads to injury. One of the major assets of the sodium o-phenylphenate treatment for pome fruits is that it inactivates spores of pathogenic fungi present in the washing water which might otherwise be a source of inoculum for fruit during the cleaning operation.

The practice of hydrocooling peaches has attracted much attention over the past 10 years, but it appears that this treatment may increase decay unless a fungicide is added to the cooling water (DiMarco and Davis, 1957a; Van Blaricom, 1959). Sodium o-phenylphenate has shown considerable promise for this application (DiMarco and Davis, 1957a; W. L. Smith et al., 1956, 1957; McClure, 1968; W. L. Smith and Redit, 1962; Sommer and Mitchell, 1959; Van Blaricom, 1959). The peaches are flooded or submerged for 15 to 20 minutes in 33-34°F water containing 0.1% sodium o-phenylphenate at pH 11.5, and not rinsed there-
after. The concentration of sodium o-phenylphenate is critical; at pH 11.5, a concentration of 0.25% may cause fruit injury and below 0.1% the treatment is ineffective (W. L. Smith and Reidt, 1962; Van Blaricom, 1969). The treatment is quite effective for control of *M. fructicola*, but much less active against *Rhizopus*. Since ice is commonly used as the refrigerant for hydrocooling, difficulty is experienced in controlling the concentration and the pH of the treating solution. It has been suggested that this situation could be corrected by incorporating sodium o-phenylphenate in the ice (W. L. Smith and Reidt, 1962). The treatment of peaches and nectarines with 0.6 and 1.0% sodium o-phenylphenate, respectively, has also been registered for use in the United States, provided the fruits are lightly rinsed after treatment (U.S. Dept. Agr., Pesticides Regulation Division, 1964).

Sodium o-phenylphenate has been reported to be effective in preventing black rot (*Endoconidiophora*) and soft rot (*Rhizopus*) of sweet potatoes (Kushman, 1961; Martin, 1964). A concentration of 0.8 to 1.0% sodium o-phenylphenate tetrahydrate is recommended for cured, and 0.4 to 0.5% for uncured sweet potatoes (Kushman et al., 1965). Treatment of Irish potatoes with 0.1% sodium o-phenylphenate does not control bacterial soft rot, but injures the periderm (Cates and Van Blaricom, 1961).

Sodium o-phenylphenate has been reported to be effective also for control of pathogenic fungi on figs (J. M. Harvey, 1966b), melons (Lipton and Stewart, 1961), and mangoes (Mathur and Subramanyam, 1956) but not on strawberries (DiMarco and Davis, 1957b).

It should be noted that the first method of application described for o-phenylphenol, i.e., impregnated into fruit wrappers, was never developed into a commercial treatment. Biphenyl is much more satisfactory for this application by reason of its greater volatility and negligible phytotoxicity. Although some progress was made in reducing the phytotoxicity of o-phenylphenol with hexamine and vegetable oils (Tomkins, 1939; Van der Plank et al., 1940), the treatment is still hazardous for use on tender varieties of citrus fruits (Tomkins, 1963). Due to a certain amount of consumer resistance to biphenyl-treated citrus fruits in recent years, the interest in o-phenylphenol-impregnated wrappers has been revived. It has been reported that 12–24 mg of sodium o-phenylphenate and hexamine impregnated into 625-sq cm paper wrappers controlled *Penicillium* on oranges, without causing injury or imparting an odor to the fruit (Lattar and Gutter, 1962). However, these wrappers were less effective than biphenyl-impregnated wrappers in controlling...
stem end rots. It is also reported that the acetate and isobutyrate esters of o-phenylphenol are effective in fruit wrappers for preventing decay of oranges and tomatoes without causing injury (Tomkins, 1963).

Residue tolerances have been granted in the United States for post-harvest application of o-phenylphenol and its sodium salt to a number of fresh fruits and vegetables (U.S. Dept. Agr., Pesticides Regulation Division, 1964). Tolerances for some of the most extensively treated commodities are: apples and pears, 25 ppm; citrus fruits, 10 ppm; peaches and plums, 20 ppm; sweet potatoes, 15 ppm.

Prior to the introduction of sodium o-phenylphenate, sodium o-phenylchlorophenate was used as a commercial treatment for control of decay in apples and pears in Washington and Oregon from 1947 to the early 1950's. This treatment was highly effective (English, 1948; Kienholz et al., 1949) but it caused severe dermatitis to fruit handlers and therefore was supplanted in the late 1950's by sodium o-phenylphenate.

Treatment of flower bulbs after digging with sodium 2,4,5-trichlorophenate is reported to give good control of Fusarium basal rot (Aycock, 1959; Magie, 1954, 1964a).

2. Hydroxybenzoic Acid Derivatives

Salicylanilide (the anilide of o-hydroxybenzoic acid) was originally developed as a textile preservative, but has some applications as a postharvest fungicide for bananas and oranges. Salicylanilide is a weak acid (pKₐ 7.45) which is practically insoluble in water (Baichwal et al., 1960). However, the sodium and ammonium salts are very water soluble. Bates (1933) reported that oranges dipped in an aqueous solution of 1% sodium salicylanilide developed less Penicillium decay than fruit treated with 3% borax. The efficiency of this treatment was confirmed in commercial shipments by Nattrass (1936). It has been found in Australia that sodium salicylanilide is more effective than sodium o-phenylphenate for control of Phomopsis stem end rot (Hall and Long, 1950; Leggo et al., 1964). Certain hydroxyamine salts of salicylanilide are said to be superior to the sodium or ammonium salts for control of Diplodia and Phomopsis stem end rots (Sidle and Gerwe, 1951). Salicylanilide has been used as a commercial treatment for oranges in Brazil.

Sodium salicylanilide is highly effective for control of Nigrospora sphaerica on bananas and has been used on a commercial scale for this purpose in Australia. (Simmonds, 1949; Wardlaw, 1961). This compound has also been tested extensively for control of Gloeosporium on bananas in Jamaica. Although small-scale tests were encouraging, the
treatment did not perform well in large-scale shipping trials, presumably due to an excessive delay between natural inoculation and application of the treatment (Meredith, 1960c, 1961a,b).

The esters of p-hydroxybenzoic acids have been used extensively as preservatives but have not been tested widely for control of postharvest diseases. These compounds are weakly acidic phenols and form alkali metal salts analogous to sodium o-phenylphenate. Kushman (1961) reported that the sodium and potassium salts of propyl-p-hydroxybenzoic acid were effective in reducing both soft rot (Rhizopus) and black rot of sweet potatoes.

F. Ammonia and Amines

1. Ammonia

Botini (1927) first reported that ammonia gas, sublimed from ammonium carbonate, was effective in preventing decay of citrus fruits. Ammonium bicarbonate was subsequently tested in England, Israel, and South Africa (Temkins and Trout, 1931, 1932; Grasovsky and Shiff, 1934; Bates, 1933). This treatment reduced both Penicillium and Diplodia decays of oranges, but also caused some injury to rind of the fruit. The treatment was therefore considered too hazardous to be generally useful for commercial shipments.

In the early 1950's, a comprehensive study was undertaken at the California Citrus Experiment Station to evaluate the feasibility of using ammonia as a substitute for biphenyl in citrus shipping cartons. These investigations were justified by the observation that constant low concentrations of ammonia gas (ca. 50-200 ppm vol./vol.) effectively controlled Penicillium decay and yet were not injurious to citrus fruit exposed for a period of many hours (Eckert et al., 1963; Rostaucher et al., 1955, 1957). The problem then was to devise a stable formulation that would generate ammonia gas at a controlled low rate when placed in a carton of citrus fruit. These investigations were further encouraged by the fact that low concentrations of ammonia gas did not leave a detectable residue on the fruit, had no measurable effect on fruit quality, and did not appear to present any major toxicological problems (ammonia was subsequently declared to be exempt from the requirement for a tolerance, when applied to citrus fruit after harvest).

Two basic ammonia-generating formulations were developed: one based on the hydrolysis of ammonium salts of weak acids and the other a mixture of a stable ammonium salt with an alkaline carbonate or
oxide. The formulations were prepared either as compressed tablets or paper sheets impregnated with the reactants (Gunther et al., 1956, 1959a; Eckert et al., 1963; Roistacher et al., 1958). Under constant environmental conditions it was found possible to control the release of ammonia from the formulation, either by choice of ingredients or by the physical nature of the formulation. Promising formulations were tested extensively with fruit, both in controlled environments and in commercial shipments with the following conclusions regarding the utility of ammonia to prevent decay in commercial shipments of citrus fruits.

1. The rate of ammonia generation by moisture activation is very difficult, if not impossible, to control under the variable humidity conditions which exist within fruit containers during commercial shipments (Harding, 1959b; Kolbezen and Eckert, 1963).

2. Although it was demonstrated that an atmosphere of 100–400 ppm NH₃ would prevent sporulation of Penicillium on lemons yet not injure the fruit, none of the formulations devised were capable of providing such an atmosphere for more than a few days (Eckert et al., 1963). Therefore, soilage of fruit by spores was a serious shortcoming of the ammonia treatment in commercial shipping trials (Roistacher et al., 1960).

3. The sorptive capacity of fruit and cellulosic substances for NH₃ is considerable (Gunther et al., 1959b; Roistacher et al., 1957); thus, only formulations with relatively large surface areas are capable of adequately distributing the evolved NH₃ throughout the atmosphere inside the container (Eckert et al., 1963).

4. Sensitive lots of citrus fruits show darkening of skin defects and injured buttons as a result of being treated with ammonia gas (Hopkins and McCormack, 1957; Roistacher et al., 1960; Eckert et al., 1963; Eaks, 1959).

In view of these difficulties, it is now considered unlikely that existing ammonia formulations would ever be acceptable, by present standards, for fumigation of citrus fruits during shipment. Ammonia-generating formulations have also been evaluated for control of post-harvest diseases of cranberries and other small fruits without promising results (Harding, 1957; Vaughan, 1962). As an extension of their investigations on in-package ammonia generators, Roistacher et al. (1955, 1957) evaluated the effectiveness of ammonia gas delivered directly into the atmosphere of de-greening rooms for control of Penicillium decay of citrus fruits. Such fumigations reduced decay without injury to the fruit when the applied dosage was within the range of
500-3000 ppm-hours (parts per million by volume ammonia × hours exposure). An effective and convenient fumigation schedule consisted of 100 ppm NH₃ for 9 to 10 hours. Despite the effectiveness of ammonia fumigation, the treatment has never achieved widespread popularity in California, probably because of slight inconvenience involved and the shortage of adequately trained people in most packing houses. Leggo and Seberry (1964) have described a simplified fumigation procedure whereby ammonia gas is volatilized into the atmosphere by heating a solution of ammonium hydroxide in the fumigation chamber. Apparently this method is acceptable to Australian packing house operators. The possibility of periodic ammonia fumigation during long-term storage of lemons has been considered (Roistacher et al., 1955), but this practice may lead to serious darkening of the fruit buttons (Eaks, 1959; Eckert et al., 1965). Ammonia fumigation has been suggested as a means of retarding Rhizopus rot in peaches (Eaks et al., 1958), although this fruit is much more sensitive to ammonia than are citrus fruits.

2. Amines

A number of aliphatic amines have been evaluated as fungicides for control of Penicillium decay of citrus fruits. When injected into the fruit containers, highly volatile aliphatic amines are fairly effective in reducing decay of inoculated fruit (Eckert and Kolbezen, 1963b). However, some fruit injury is usually associated with dosages that control disease. Formulations of amine salts in a manner analogous to the in-package ammonia generators described in the previous section, produced a treatment with the same basic weaknesses as the latter.

2-Aminobutane is unique among the aliphatic amines in that neutral solutions of its salts are fungistatic to microorganisms responsible for certain postharvest diseases. Recent tests have shown that solutions of 2-aminobutane salts are effective for control of Penicillium spp. on citrus fruits and apples, M. fructicola on peaches, and Botrytis on cut flowers (Eckert and Kolbezen, 1962a, 1964; Smoot and Melvin, 1964; McCormack and Hopkins, 1965; MacLean and Dewey, 1964; Magie, 1964b; Jarrett and Gathercole, 1964). Furthermore, fumigation with 100 ppm (by volume) gaseous 2-aminobutane for 4 hours is extremely effective for control of Penicillium spp. on citrus fruits and apples (Eckert and Kolbezen, 1966).

The ability of gaseous ammonia and volatile aliphatic amines to reduce decay appears to be due to their basicity and the fact that they are absorbed by injuries on the surface of the fruit where they increase
the pH beyond the range suitable for development of pathogenic fungi (Eckert and Kolbezen, 1963b). This is primarily a fungistatic effect, for as soon as the volatile amine is dissipated or neutralized, growth of the pathogenic fungus resumes (McCallan and Weedon, 1940; Eckert and Kolbezen, 1963b). High concentrations of amine vapors may be lethal to a certain percentage of the exposed fungal spores, but it has been the author's experience that dry *Penicillium* spores are little affected by concentrations of ammonia or amines that provide very effective control of decay in inoculated fruit.

In contrast to ammonia and all other aliphatic amines tested by the author, neutral salts of 2-aminobutane possess fungistatic properties (Fig. 3). Thus 2-aminobutane, applied either as the free base or as a solution of a neutral salt, is a very effective treatment for prevention of fruit decay. 2-Aminobutane is somewhat more effective when applied as the free base (e.g., by fumigation), since the growth of pathogenic fungi is inhibited first by the high pH created at the site of attack on the fruit, and later by residues of fungistatic 2-aminobutane salts that persist on the surface of the fruit after neutralization of the amine by acids of the fruit.

Aqueous solutions of pyrrolidine and 2-aminopyridine (free bases) have been reported as effective for control of citrus fruit decays (Winston and Meckstoth, 1933; Lauriol, 1954). The former was more effective in controlling *Penicillium* than the latter. 2-Aminobutane is more active against *Penicillium* than pyrrolidine (Eckert and Kolbezen, 1964).

Florestano and Bahler (1957) reported that 1-piperidino-2-phenyl-3-butanone hydrochloride was as effective as sodium borate in preventing citrus fruit decay; however, this compound apparently has not been evaluated beyond the laboratory.

It is very interesting to note that quaternary amine salts which have well-characterized antifungal properties are not effective for control of fruit decay and may injure citrus fruits (Haller, 1952; Lauriol, 1954).

G. Organic Acids

The long and successful use of organic acids as microbial inhibitors in the processed food industry has inevitably led to the evaluation of these compounds for control of postharvest diseases of various fruits and vegetables. Sorbic acid (2,4-hexadienoic acid) and dehydroacetic acid (3-acetyl-6-methyl-2,4-pyridinedione) have received most attention
9. APPLICATION AND USE OF POSTHARVEST FUNGICIDES

in this field because of their greater antifungal activity (Bandelin, 1958), although possible applications for benzoic acid (Branley and Mason, 1939; Lewis and Payne, 1958) and propionic acid (Schroeder, 1964; Wolford and Anderson, 1945) have also been described. Organic acids, like phenols, exhibit greatest antifungal activity in their undissociated forms. The carboxylic acids benzoic, propionic, and sorbic have pKₐ values in the range 4–5; therefore, their activity is greatest below pH 4, and is greatly diminished at pH values of 6 or higher (Bandelin, 1958; Bell et al., 1969; Beneke and Fabian, 1953; Cruess et al., 1931). In contrast, the antifungal activity of dehydroacetic acid persists at pH 7, but is weak at pH 9 (Bandelin, 1958). It would seem advisable to adjust the acidity of solutions of organic acid fungicides to as low a value as possible to suppress dissociation, but at the same time maintaining a reasonable concentration of sparingly soluble acids, e.g., sorbic and benzoic acids, in solution. Lewis and Payne (1958) have described a method for treating citrus fruits, in which benzoic acid is solubilized with a surfactant in order to hold this fungicide in solution in its undissociated form. It should be kept in mind, however, that some commodities may not tolerate undissociated acids as well as their ionic form (Section VI,E). Another factor that might influence the effectiveness of carboxylic acids is that the final form of the residual acid will be determined by the pH of the host tissue. It has been pointed out in this regard that the pulp of most fruits is sufficiently acid to hydrolyze salts of sorbic acid (Beneke and Fabian, 1955). The pH of superficial tissues is the more pertinent consideration in the treatment of fresh fruits and vegetables, however.

Sorbic acid (0.1%) has been reported to reduce decay of fresh figs caused by Alternaria and Cladosporium (J. M. Harvey, 1956b), but 0.5% sorbic acid was found to be relatively ineffective for control of decays of peaches and strawberries (DiMarco and Davis, 1957a,b; Van Blaricom, 1959). In these screening trials with strawberries, dipping the berries in 10% sodium propionate has virtually no effect upon subsequent decay (DiMarco and Davis, 1957b).

A number of reports have established that dehydroacetic acid is beneficial in retarding the development of decay organisms on fresh strawberries (R. C. Moore and Oberle, 1961; Thompson, 1958; Tuli et al., 1962; Young and Beneke, 1962). Dehydroacetic acid is usually applied as a 0.1–0.5% solution of the sodium salt. A residue tolerance of 65 ppm (as dehydroacetic acid) has been established for treated strawberries in the United States (U.S. Dept. Agr., Pesticides Regulation Division, 1964). Despite the effectiveness of dehydroacetic acid, shippers are re-
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Hesitant to apply aqueous treatments to strawberries after harvest because of attendant delays in handling and justifiable apprehension regarding the influence of water, per se, on the market life of the berries.

Postharvest dip treatments of 0.5-1% sodium dehydroacetic acid have been reported to retard *M. fructicola* and *Rhizopus* on peaches (Luvisi and Sommer, 1960; Shurtleff and Shutak, 1952; W. L. Smith and Redit, 1962), while in other trials the results have been less favorable (Van Blaricom, 1959). Preharvest sprays of dehydroacetic acid have also reduced storage decays of peaches (Hawthorne and Horne, 1957; Shurtleff and Shutak, 1952). Delayed ripening and injury to the skin of peaches have been observed to result from treatment with dehydroacetic acid (Luvisi and Sommer, 1960; Van Blaricom, 1959). Dehydroacetic acid has not provided beneficial results in trials on tomatoes or cherries (Ayres *et al.*, 1964; Salunkhe *et al.*, 1962).

H. Aldehydes and Epoxides

Formaldehyde is used extensively as an inexpensive and effective fumigant to decontaminate picking boxes, packing facilities, and storage rooms. Formalin (37% aqueous formaldehyde) is usually applied as an aerosol or added to air washers used to humidify storage rooms. The direct application of aldehydes to living fruits generally has not been successful because dosages required for decay control most often cause injury to the host.

R. E. Smith (1907) suggested that *Phytophthora* brown rot of lemons might be controlled by adding a small quantity of formaldehyde to the water used to wash the fruit in order to kill zoospores of the fungus pathogen. This treatment has not been seriously considered for commercial use because of the superiority of the hot-water treatment for control of this disease. Du Plessis (1938) evaluated formaldehyde and paraformaldehyde as fumigants for grapes and although the treatments reduced *Botrytis* rot some injury resulted from most treatments. Trioxane, paraformaldehyde, and 1-hydroxymethyl-5,5-dimethylhydantoin, all of which slowly liberate formaldehyde, have been evaluated in the author's laboratory for control of *Penicillium* decay of citrus fruits without any measure of success. Acetaldehyde was one of the first volatile fungicides reported to be effective for control of decay of fresh fruits (Tomkins and Trout, 1932) but apparently this treatment did not stand up under more intensive evaluation.

Epoxides, like aldehydes, are too injurious for direct application to
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living fruits. However, these reactive compounds have been successfully applied as fumigants for control of yeasts and saprophytic fungi on partially dehydrated fruits such as dates, figs, and prunes. Ethylene oxide, mixed with a chlorinated hydrocarbon or with isopropyl formate to reduce its volatility, or pure propylene oxide, is injected directly into the sealed package of dehydrated fruit (J. M. Harvey and Pentzer, 1953; Mrak and Stadtmann, 1946; Whelton et al., 1946).

I. Positive Halogen Compounds and other Oxidizing Substances

1. Hypochlorous Acid

The chemical reactivity of the elemental halogens decreases in the order chlorine, bromine, iodine. Gaseous elemental chlorine is extremely fungicidal but is injurious to fruit at dosages which prevent decay (Klotz, 1936; McCallan and Weedon, 1940), whereas elemental iodine is more selective in its action. Elemental chlorine hydrates in water solution to form hypochlorous acid \( pK_a = 7.46 \), which on dissociation yields hypochlorite ions. At pH values greater than 5 the concentration of elemental chlorine in solution is negligible and the relative concentrations of hypochlorous acid and hypochlorite ions are governed by the pH of the solution (Table II).

It is a common analytical convention to express the strength of hypochlorite solutions in terms of parts per million or per cent "available chlorine," viz., the oxidizing power of the solution determined iodometrically. Since one gram atom of positive chlorine liberates one mole of \( I_2 \), a solution which is said to contain 100 ppm "available chlorine" actually contains only 50 ppm of \( Cl^- \). This convention is desirable since it provides an exact measure of the potential chemical activity of the

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hypochlorite solution. Unfortunately, many plant pathologists have expressed the concentration of hypochlorite solutions in terms of parts per million of chlorine, which presumably indicates a calculated concentration prepared from calcium hypochlorite or by dilution of a concentrated solution of sodium hypochlorite. The latter procedure yields solutions which may vary greatly in actual oxidizing power and this could be one factor responsible for the highly variable reports regarding the effectiveness of hypochlorite solutions for control of postharvest diseases. Furthermore, the pH of the solution is rarely specified in published reports, further complicating the interpretation of conflicting statements in the literature.

Solutions of hypochlorous acid and its salts have been widely employed to reduce microbiological contamination of water used to wash or hydrocool fruits and vegetables, and to bleach superficial mold and scars on the surface of fruit. It has been adequately demonstrated that both the antimicrobial effectiveness and the bleaching action of chlorine solutions is a function of the concentration of undissociated hypochlorous acid in the solution (Allen, 1950; Ridge and Little, 1942; Van der Plank, 1945). On the other hand, solutions which contain a major portion of the total available chlorine in the form of hypochlorite ions are the most stable. In formulating a hypochlorite solution for the treatment of fresh fruits and vegetables, it is of utmost importance to strike a practical balance between reactivity and stability of the solution. Furthermore, it is possible that high concentrations of hypochlorous acid might injure sensitive commodities whereas the less reactive hypochlorite ion ensures greater safety for the treatment.

As pointed out by Van der Plank (1945) the principle factors affecting stability of hypochlorite solutions are acidity, concentration, and temperature. The most stable solutions are those containing equivalent quantities of hypochlorous acid and sodium hydroxide (pH 10.26 for a solution containing 0.6% available chlorine) and the most unstable are those below pH 8 in which most of the chlorine is in the form of hypochlorous acid. The pH selected for a particular application depends principally upon the concentration and temperature required. For example, Van der Plank found that solutions containing 1000 ppm available chlorine at pH 7.6 showed no loss of active chlorine on standing for 7 days at 20°C in the dark. Such low concentrations are commonly employed in the water of hydrocoolers. Solutions containing 0.5% available chlorine, which are recommended for control of decay, suffer appreciable losses of active chlorine below pH 8 under the same conditions. Van der Plank (1945) recommended that solutions containing 0.5-0.7%
active chlorine be adjusted with sodium carbonate to a minimum pH of 8.5 to ensure stability and if the solutions are to be heated to 48°C, the pH should be maintained at no less than 9.2. It has been recommended that hydrocooler water containing 100 ppm chlorine should be maintained in the range of pH 8.0–8.3, which is said to be the best compromise between stability and effectiveness for a practical treatment period (Link and Pancost, 1949). Methods for adjusting and buffering the pH of hypochlorite solutions have been comprehensively treated in the publications of Van der Plank (1945) and Ridge and Little (1942).

Although solutions of hypochlorous acid exhibit rapid fungicidal action against suspensions of fungus spores in vitro (Baker and Heald, 1932; Hwang and Klotz, 1938; Van der Plank, 1945), they are relatively ineffective in preventing decay of inoculated citrus fruits (Bates, 1933; Fidler et al., 1949; Van der Plank, 1945). Hypochlorite solutions have been extensively evaluated for the control of M. fructicola and Rhizopus on peaches, with major emphasis on the chlorination of water in hydrocoolers (Cardinell and Barr, 1952; Daines, 1965; McClure, 1958; W. L. Smith et al., 1957; Van Blaricom, 1959). Although 100 to 200 ppm chlorine is the preferred concentration range for hydrocoolers, peaches apparently can tolerate at least 1000 ppm chlorine. At higher concentrations some surface injury to the fruit may occur as well as an actual increase in decay (Cardinell and Barr, 1952; Poulos, 1949). The hypochlorite treatment has been observed to reduce decay due to Monilinia in some instances, whereas in other tests by the same investigator, the treatment had no effect upon the incidence of the disease. The hypochlorite treatment has generally been found to be less effective for control of Rhizopus on peaches as well as on melons (Lipton and Stewart, 1961). The hypochlorite treatment also has been reported as ineffective for control of bacterial soft rot on spinach (Friedman, 1951). On the other hand, hypochlorite solutions have been beneficial against fungi which develop superficially on fruit, such as Alternaria and Cladosporium on melons (Lipton and Stewart, 1961) and sooty blotch (Stomatospeltis citri) on oranges (Van der Plank, 1945).

The use of hypochlorite in cleaning baths and hydrocoolers is unquestionably a beneficial practice since it indirectly controls decay by reducing the population of pathogenic microorganisms in the water. Indeed, operations such as washing potatoes and hydrocooling vegetables could not be successfully carried out were it not for chlorine to prevent the buildup of soft rot bacteria in the water. This applies also to apple washers and hydrocoolers for peaches, but in these cases sodium
o-phenylphenate appears preferable to hypochlorite since the phenol not only maintains the water in a sanitary condition but also reduces decay of inoculated fruit.

The relative ineffectiveness of hypochlorous acid in preventing decay of inoculated fruit, despite its rapid fungicidal action against the causal microorganisms in vitro, has been attributed to its inability to penetrate into wounded host tissues (Van der Plank, 1945). Hypochlorous acid is a highly reactive and nonspecific oxidizing agent and undoubtedly is reduced by organic substances of the fruit peel when it attempts to diffuse into wounded tissue. The decay-preventing properties of hypochlorite solutions are potentiated by the presence of 1-2% boric acid in the solution. These low concentrations of boric acid are inactive alone (Van der Plank, 1945).

2. Chloramines

Inorganic and organic compounds containing active chlorine bonded to a nitrogen atom have attracted interest as decay control agents because they generally are more stable than hypochlorous acid in neutral and slightly acidic solutions. These compounds are apparently able to penetrate in an active state into injured host tissue where they react with the pathogen directly or slowly hydrolyze giving HOCI.

Nitrogen trichloride has been extensively used as a fumigant to control decay of citrus fruits (Klotz, 1936; Littauer, 1947, 1956; Ryall and Godfrey, 1948), melons (Barger et al., 1948) and certain vegetables (Pryor, 1950). Nitrogen trichloride is a dense yellow liquid which boils around 71°C and is explosively unstable when concentrated. NCl₃ is freely soluble in organic solvents but practically insoluble in water. For fumigation of perishable commodities, NCl₃ is generated in the packing house by chlorination of aqueous ammonium chloride, and then immediately volatilized and diluted with a stream of air which carries the dilute NCl₃ to the fumigation chamber. Lemons in storage may be fumigated 3 times weekly with vapor concentrations of 0.5-0.75 mg NCl₃/ cu ft. The treatment is not only effective in reducing the number of decayed fruit, but also accelerates the desiccation of rotten fruit and inhibits the sporulation of Penicillium on their surfaces. Oranges may be fumigated for 3 to 4 hours at dosages of 1.5-3.0 mg NCl₃/cu ft. This treatment may be repeated if the oranges are held for several days before packing. The review of Pryor (1950) should be consulted for specific details regarding the fumigation of vegetables with NCl₃. Dichloroisocyanuric acid decomposes in the presence of water to give NCl₃ and papers impregnated with the former compound or one of its salts are effective in reducing decay of packaged fruits (Eckert et al.,
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Papers impregnated with N-bromo (or chloro) hydantoins are also effective (Eckert and Kolbezen, 1962b). The efficiency of NCl₃ in preventing decay of inoculated fruits may be explained by its low water solubility and lower chemical reactivity (cf. hypochlorous acid), properties which could lead to selective action against the pathogen.

Certain volatile alkyl chloramines have been shown to control decay of oranges and grapes, but these compounds do not appear to offer any advantage over NCl₃ (Klotz, 1936; Van der Plank and Rattray, 1939). The major limitation on the use of nitrogen trichloride as postharvest treatment is that NCl₃ and its decomposition products are highly corrosive to metals and will weaken wood boxes which are exposed repeatedly to this fumigant.

Attempts have been made to increase the stability of neutral and slightly acid solutions of chlorine through the use of chloramines (Pryor, 1950). Since low concentrations of HOCl exist in equilibrium with chloramines in solution, the latter function as reservoirs of highly reactive chlorine at low pH values where the hypochlorite ion cannot exist. A satisfactory chloramine for this purpose should be reasonably stable (not subject to decomposition by chlorine), nonvolatile, and have a low combining weight with respect to chlorine. Solutions of melamine (2,4,6-triamino-1,3,5-triazine) have been chlorinated to give more stable solutions of active chlorine. N-Chloro-p-toluenesulfonylamide (chloramine T) has been used as a chlorine carrier in the commercial treatment of vegetables, but the relatively high equivalent weight of this compound would seem to make it a poor choice for this purpose.

3. Iodine and Bromine

Elemental iodine has been intensively evaluated as a volatile decay inhibitor for oranges, grapes, and tomatoes with considerable success (DuPlessis, 1938; Nattrass, 1939; Tomkins, 1934, 1935). Plums, apples, peaches, and grapefruit were injured by the treatment (Tomkins, 1934; Wardlaw, 1936). Although the vapor pressure of iodine is considerably greater than other fungicides which have been advocated for impregnation of fruit wrappers, Tomkins (1934) suggested that the volatility was reduced by the formation of a cellulose-iodine complex and could be further controlled by the ratio of KI/I₂ in the formulation. The iodine treatment was eventually abandoned because of excessive cost and staining of the packaging materials (Tomkins, 1964; Farkas, 1938). A novel treatment with iodine consists of coating fruit with KI and then periodically fumigating with ozone to release elemental iodine (Marks and Strandskov, 1955).
Although bromine and iodine possess excellent bactericidal and fungicidal properties (Marks and Strandskov, 1950; McCallan and Wellman, 1942), and tend toward greater selectivity in their action, these halogens have not been extensively tested as solution treatments for control of postharvest diseases. Their slower rate of action would make them less suitable than chlorine for treatments in which the exposure time was limited. Bromine and iodine have quite low hydrolysis constants, $4.2 \times 10^{-9}$ and $4.6 \times 10^{-13}$, respectively (Holst, 1954) and thus these halogens exist in elemental form in neutral solutions rather than as the hypohalous acid as in the case of chlorine. The hypohalous acids of bromine and iodine are too unstable for treatment of fruit at practical levels of alkalinity. Some interest has been shown in the application of organic complexes of iodine for treatment of fruit (e.g., Van Blaricom, 1959), but these compounds do not appear to show much promise for control of postharvest diseases.

4. Peracetic Acid

Peracetic acid, a powerful oxidizing agent that slowly decomposes in aqueous solutions to give $\text{H}_2\text{O}_2$, has shown some promise for control of pathogenic fungi on sweet potatoes (Kushman, 1961) and figs (J. M. Harvey, 1956b). A reduction in decay of strawberries was obtained by a 0.5% peracetic acid dip (Lowings, 1956) but not with a 0.1% treatment (DiMarco and Davis, 1957b).

5. Ozone

Ozone has been advocated for many years as a fumigant for control of postharvest decay. Tests have shown that concentrations of ozone tolerated by treated commodities will not control decay of inoculated fruit (Barger et al., 1948; Hopkins and Loucks, 1949; Klotz, 1936), but will prevent the growth of surface molds, reduce the viable spore count in the atmosphere, and destroy offensive odors in the storage room (Schomer and McColloch, 1948). More recently, fumigation with ozone has been reported to control Botrytis on gladiolus flowers during shipment (Magie, 1961).

J. Captan, 2,6-Dichloro-4-Nitroaniline, and Miscellaneous Organic Fungicides

1. Captan

Captan (N-trichloromethylmercapto-4-cyclohexene-1,2-dicarboximide) has been evaluated both as a preharvest and as a postharvest treat-
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9.

ment for control of decay during storage and marketing. Some applications of captan before harvest have been considered in Section II.A. Postharvest treatments have been evaluated for control of decays of strawberries (Becker et al., 1955a; DiMarco and Davis, 1957b; Salunkhe et al., 1962, Thompson, 1958), peaches (DiMarco and Davis, 1957a; Van Blaricom, 1959), cherries (Pierson, 1958), pears (Pierson, 1960), figs (Harvey, 1956b), and potatoes (Cates and Van Blaricom, 1961). In the tests reported, captan was applied at concentrations ranging from 0.1 to 1% active material as an aqueous dispersion of wettable powder formulations. In most instances the performance of the captan treatment was rated as good to fair depending upon the concentration used and the pathogens involved. In the case of peaches, captan has provided satisfactory control of *M. fructicola*, whereas its effectiveness against *Rhizopus* has been poor (Ogawa et al., 1963c; Van Blaricom, 1959). Difolfan [N-(1,1,2,2-tetrachloroethyl)sulfenyI)-cis-Δ-4-cyclohexene-1,2-decarboximide], an analog of captan, also is more effective against *Monilinia* (Ogawa et al., 1964). Captan has been registered in the United States as a postharvest dip (0.12% captan) for a number of fruits and vegetables. The residue tolerance in the United States for this application is 100 ppm (U.S. Dept. Agr., Pesticides Regulation Division, 1964).

The availability and favorable residue status of captan have probably been responsible for the extent to which the compound has been evaluated as a postharvest treatment. Actually captan does not appear to be well suited for this purpose since it must be applied as a wettable powder (Section V,B) and may leave visible residues on peaches and strawberries when applied at effective concentrations (DiMarco and Davis, 1957a; Van Blaricom, 1959).

(2,4,5-Trichlorophenoxythio)-trichloromethane, a compound structurally similar to captan, has been reported to be effective as a volatile fungicide for control of *Gloeosporium* on apples and *Penicillium* on oranges (C. H. Fawcett et al., 1958; Spencer and Wilkinson, 1960).

2. 2,6-Dichloro-4-Nitroaniline

Clark et al. (1960) in England first described the antifungal properties of 2,6-dichloro-4-nitroaniline (DCNA). These investigators characterized DCNA as being practically insoluble in water and nonvolatile. They reported that it was extremely inhibitory to the mycelial growth of *Botrytis*, but did not prevent germination of spores of this fungus.

Preliminary tests in the United States demonstrated that *Rhizopus* on sweet cherries (Ogawa et al., 1961) and on peaches (Cappellini and
Stretch, 1962; Dewey and MacClean, 1962) could be controlled by dipping the fruit in aqueous suspensions of 1000-2000 ppm DCNA. If applied within 12 hours after inoculation, this treatment not only reduced the number of decayed fruit but also suppressed the development of *Rhizopus* on the surface of diseased fruits, thereby preventing secondary infection. DCNA also has provided good control of *Rhizopus* on peaches when applied in a hydrocooler (Daines, 1965). The effectiveness of DCNA against *Rhizopus* decays is highly significant since none of the earlier treatments, except nystatin (Section VI.K), had provided exceptional control of this disease.

DCNA is much less effective in preventing decay by *M. fructicola* (Cappellini and Stretch, 1962; Dewey and MacClean, 1962; Ogawa et al., 1964) and *P. expansum* (Ogawa et al., 1963b) than that caused by *R. stolonifer*. Ogawa et al. (1965a,c) further reported that DCNA does not control decay of peaches caused by *Rhizopus arrhizus* nor by a resistant variant of *Gilbertella persicaria*.

Despite the low volatility of DCNA, its vapors strongly inhibit aerial growth of *Rhizopus* on decaying fruit (Ogawa et al., 1963b) and fruit wraps impregnated with 2000 ppm DCNA have greatly reduced the contact spread of *Rhizopus* in packed boxes of peaches (Luepschen, 1964).

Postharvest decay of peaches by *Rhizopus* was controlled in California by several field sprays of DCNA and the effectiveness of different spray schedules was correlated with the magnitude of residues of DCNA remaining on the fruit at time of harvest (Ogawa et al., 1964). A residue of 10 ppm DCNA gave effective control of *Rhizopus*, but 19 to 30 ppm were required to control *M. fructicola*. Since a residue of 10 ppm “Difoltan” effectively reduced *Monilinia* rot, it was suggested that field sprays with a combination of the two fungicides might give better protection against postharvest decay than either fungicide alone. Field sprays of DCNA have not provided effective control of *Rhizopus* under New Jersey conditions (Daines, 1965). DCNA has also shown promise for control of *Rhizopus* on sweet potatoes (Martin, 1964; Kushman et al., 1965).

Residue tolerances established in the United States for DCNA are 20 ppm for peaches, nectarines, and cherries; 15 ppm for strawberries; and 10 ppm for sweet potatoes (Larrick, 1965).

Smoot et al. (1960) have reported that certain esters of carbanilic acid are more effective than sodium o-phenylphenate in preventing decay of oranges. The low water solubility of these compounds, necessi-
tating their application in alcohol solutions, appears to limit the usefulness of these compounds as postharvest treatments.

**K. Antibiotics**

Research on the application of antibiotics to the control of postharvest diseases received considerable attention from 1950 to 1960. The activity in this field has assumed a more moderate pace in recent years as the potential and limitations of antibiotics have been more fully recognized.

**1. Antibacterial Antibiotics**

Most research on antibacterial antibiotics has been directed to the problem of bacterial soft rot on leafy vegetables, where an increase in market life of only a few days would be of great economic significance. Treatment of leafy vegetables with solutions of active chlorine compounds reduces viable inoculum on the leaves and in the wash water but does not prevent decay after inoculation has taken place (Friedman, 1951). There is a real need for a treatment to retard spoilage of these commodities, and attention focused on antibiotics because of their effectiveness against soft rot bacteria and the low concentrations required did not leave visible residues on the treated crop.

The first tests demonstrated that bacterial soft rot of packaged spinach could be delayed 2 to 3 days at 70°F by dipping in 1000 ppm streptomycin (W. L. Smith, 1955) and that even lower concentration retarded the development of this disease on lettuce (Cox, 1955). Subsequent tests have shown that oxytetracycline and chlortetracycline are more effective than streptomycin in retarding bacterial soft rot on several leafy vegetables and that solutions containing 25-50 ppm of the tetracyclines effectively delay the onset of decay (Becker et al., 1958b; Carroll et al., 1957; Francis, 1960; Koeh and Carroll, 1957). Dip treatments of streptomycin, oxytetracycline, and chlortetracycline have provided control of soft rot on potatoes in laboratory tests, but not in field trials (Bonde, 1953; Cates and Van Blaricom, 1961). Concentrations greater than 250 ppm chlortetracycline were phytotoxic to new potatoes and caused an increase in decay.

Although the necessity and effectiveness of antibiotics for retarding soft rot decay of leafy vegetables is well established, the possible adverse effects of continuous low levels of medicinal antibiotics in the human diet has raised serious questions regarding the widespread use
of antibiotics in unprocessed foods. Several aspects of this problem have been considered in detail by Goldberg (1964). Residues of oxytetracycline in plant tissues are inactivated by heat whereas streptomycin is only partially destroyed under the same conditions (Becker et al., 1958b; Goodman et al., 1958). The persistence of streptomycin in treated commodities would appear to remove this antibiotic from further consideration as a postharvest treatment until such time as the public health question has been completely resolved. Oxytetracycline is permitted for use on fish because it is heat labile, but this property is not pertinent to its proposed uses on fresh vegetables.

2. Antifungal Antibiotics

The related antifungal polyene antibiotics, nystatin and pimaricin, have shown promise for control of Monilinia and Rhizopus on peaches (DiMarco and Davis, 1957a; Van Blaricom, 1959), strawberry rots (DiMarco and Davis, 1957b; Salunkhe et al., 1962; Ayres and Denisen, 1958), Glaspergium on bananas (Meredith, 1960a,b,c), Botrytis on stored rose stocks (Stessel, 1958), and Botrytis on orchid flowers (Frank et al., 1959). A concentration of 100 ppm has been used in most tests. Probably the most outstanding attribute of nystatin is its effectiveness against Rhizopus rots, which, prior to the introduction of DCNA (Section VI,2), were not adequately controlled by any postharvest fungicide (DiMarco and Davis, 1957a,b; Van Blaricom, 1959). Both nystatin and pimaricin are strongly inhibitory to most phytopathogenic fungi with the exception of those in the class Oomycetes (Eckert and Tsao, 1962; Frank et al., 1959). The water soluble pimaricin would appear to be more adaptable as a postharvest treatment, but nystatin which is water insoluble has been more readily available in the United States. Both pimaricin and nystatin suffer the shortcoming that their aqueous solutions rapidly lose activity in the presence of light. Since these antibiotics have clinical applications, their use as postharvest fungicides would be objectionable if significant residues persisted on treated foodstuffs. It is reported that nystatin is inactivated on the peel of the banana within 5 to 10 days after application (Meredith, 1961a). Residues of nystatin are permitted on the peel but not in the flesh of bananas in Great Britain (Goldberg, 1964). Actidione has been evaluated to a limited extent for control of strawberry decay, with variable results (Becker et al., 1958a; Thompson, 1958; Young and Beneke, 1952). Actidione is phytotoxic to banana fruits at concentrations required to control decay (Meredith, 1960a).
9. APPLICATION AND USE OF POSTHARVEST FUNGICIDES

L. 2,4-Dichlorophenoxyacetic Acid and Carbon Dioxide

1. 2,4-Dichlorophenoxyacetic Acid (2,4-D)

Stewart (1949) observed that field sprays of 2,4-D increased the storage life of citrus fruits by delaying senescence of the fruit buttons (receptacle and calyx). Spores of A. citri are present under the buttons of most lemon fruit at the time of harvest and senescence of this fruit part precedes the onset of Alternaria stem end rot (Bartholomew, 1926). Commercial tests demonstrated that 2,4-D and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) applied to lemons before storage at concentrations of 100–1000 ppm in a water-emulsion wax consistently reduced button deterioration, rate of coloration, and water loss by the fruit during storage (Stewart et al., 1952).

In vitro studies have demonstrated that both 2,4-D and 2,4,5-T are only weakly fungistatic and therefore the effectiveness of these compounds in preventing Alternaria rot is a result of their physiological action on the lemon fruit (Erickson et al., 1958). It has been demonstrated that the albedo of the rind of immature lemons possesses considerable resistance to the growth of Alternaria, but that this resistance is lost during storage. Prestorage treatment of the lemons with 2,4-D delays the loss of resistance by the fruit rind (DeWolfe et al., 1959).

The current practice is to treat lemons before storage with a water-emulsion wax containing 500 ppm 2,4-D in the form of the isopropyl ester. The isopropyl ester is preferred over salt formulations because the esters are slightly volatile leading to better distribution and low residues from the treatment. The residue tolerance in the United States for postharvest application of 2,4-D (isopropyl ester) on lemons is 5 ppm (J. L. Harvey, 1964). 2,4-D and 2,4,5-T are also reported to be effective for control of Diplodia and Phomopsis stem end rots of oranges (Loest et al., 1954; Leggo et al., 1964), Penicillium and Alternaria on mandarins (Loeb et al., 1963), and in extending the storage life of limes (Gates, 1949; Hatton, 1959).

2. Carbon Dioxide

One of the principle shortcomings of refrigeration for control of postharvest diseases is that it is often difficult to rapidly cool the commodity to a temperature that prevents growth of pathogenic microorganisms (Section III,B,4). Brooks and his associates (1932, 1936) made an extensive study of the value of gaseous CO₂ for retarding
metabolic activity and decay of fruits and vegetables while being cooled to the optimum storage temperature. These investigators found that grapes, peas, and sweet corn could tolerate 40% CO₂ for several days without showing any adverse effect from the treatment. Plums, cherries, blackberries, pears, apples, and oranges were somewhat less tolerant, but could be treated with 30% CO₂ for 2 days without adverse effect upon fruit quality. Peaches, apricots, strawberries, and red raspberries were most liable to injury, but were safely treated with 30% CO₂ for 24 hours. Fruits and vegetables were more susceptible to CO₂ injury at higher temperatures. These investigators (Brooks et al., 1936) concluded that a 1 to 2-day treatment with CO₂ at temperatures usually prevailing in refrigerator cars after loading had the same effect in preserving fruit quality as immediate storage at 32°F. The CO₂ treatment retarded the development of R. nigricans, M. fructicola, and P. expansum on peaches and apricots; Cladosporium, Rhizopus, and Monilinia on sweet cherries; and Penicillium on oranges. After removal of inoculated peaches from the CO₂-enriched atmosphere, the decay lesions were smaller than those which had not been treated with CO₂, but subsequent rate of lesion enlargement was usually the same as in the control fruit.

The effectiveness of the CO₂ treatment has been confirmed for the control of decay of strawberries and raspberries (Winter et al., 1937, 1939) as well as for cherries (English and Gerhardt, 1942; Gerhardt and Ryall, 1939). English and Gerhardt (1942) have stressed that the CO₂ treatment on cherries is more effective at low transit temperatures. The commercial practice is to place 800 to 1000 lb of solid carbon dioxide in refrigerator cars containing sweet cherries and strawberries.

High concentrations of gaseous CO₂ apparently retard fruit decay by a direct inhibitory effect on the pathogenic fungus, although an indirect effect on resistance of the host has not been ruled out. A temporary inhibition of the pathogen, coupled with a slight desiccation of the fruit during cooling may explain the observation that the number of decayed fruit is reduced by the CO₂ treatment (Brooks et al., 1932; English and Gerhardt, 1942). However, the more recent report by Montgomery (1958) that the incidence of Gloeosporium alba on apples is reduced by storage in an atmosphere of 3% O₂–5% CO₂ implies that such conditions increase the resistance of the host to spread of the pathogen. The level of metabolic CO₂ that can be tolerated by peaches and nectarines in cartons with polyethylene liners is not sufficiently high to significantly influence the rate of decay (Lavisi and Sommer, 1960). In contrast, sweet cherries can tolerate a higher concentration
of CO$_2$ and show less decay and a better appearance if stored under 6 to 9% CO$_2$. This is approximately the composition of the atmosphere which results when sweet cherries are sealed in 1.5-mil polyethylene and held at 31°F (Schomer and Olsen, 1964).

VII. CURRENT AND FUTURE TRENDS IN THE DEVELOPMENT OF POSTHARVEST FUNGICIDES

Few new postharvest fungicides have been developed into commercial treatments since 1940. Slow progress in this field may be attributed to the relatively small number of investigators devoting their full attention to the control of postharvest diseases and to the inability of most fungicide screening programs in industry to reveal compounds that might be useful as postharvest fungicides. Traditionally, the major efforts of the chemical industry have been focused on the development of broad-spectrum protectant fungicides for growing crops and for use as industrial preservatives. Fungicides with properties required for these applications are only rarely useful as postharvest treatments.

The number of investigators engaged in postharvest research has increased in recent years as a result of new problems created by technological advances in handling and marketing perishable commodities, as well as by the demands of the consumer for higher quality products. Certain segments of the chemical industry have noted the increasing demand and potential for postharvest fungicides, as evidenced by their active screening programs to find compounds with properties suited for these applications.

No satisfactory chemical treatment has yet been developed for control of bacterial soft rot of leafy vegetables. The initial success of medicinal antibiotics in suppressing this disease clearly indicated the benefits that could be anticipated if potent antibacterial compounds with no clinical applications could be developed for the sole purpose of treating fresh vegetables.

There is also need for volatile fungicides for fumigation of fruits during ripening or de-greening, and while in storage or transit. Moreover, fruits such as strawberries that are exceptionally prone to decay could be more effectively treated by fumigation than by aqueous formulations of fungicides. Sulfur dioxide and nitrogen trichloride are the only fumigation treatments that have been used extensively and even these are not applicable or acceptable treatments for most commodities. The ideal fumigant should be easy to apply and monitor without specialized equipment, should be effective at vapor concentrations of only a frac-
tion of injury threshold for the treated commodity, and should be chemically unreactive in contrast to sulfur dioxide and nitrogen trioxide.

The trend toward marketing fresh commodities in containers that impede mass transfer of air, e.g., fiberboard cartons and plastic films, should increase the interest in developing vapor-phase fungicides for application to individual containers. This type of treatment, exemplified by biphenyl and dihydrogenotetrachloroethane, presents a means for suppressing the growth of pathogenic fungi for the entire marketing period as contrasted with the short-term effectiveness of the usual postharvest treatments.

Although several effective fungicides are available for application in water, there is room for considerable improvement in this type of treatment. For example, 2-aminobutane has the advantage over sodium o-phenylphenate that it can be used in a neutral solution whereas the latter fungicide must be applied at pH 11.5 or higher to prevent injury to fruits. The necessity for applying the water-insoluble fungicide DCNA as an aqueous dispersion may limit the usefulness of this compound in certain types of mechanical washers. A water-soluble fungicide which was equally effective against Rhizopus would represent a distinct improvement over the existing treatment.

It is highly desirable that postharvest fungicides accumulate selectivity and persist in an active form at potential sites of attack by the pathogen. Attempting to protect a commodity with a uniform coating of a fungicidal substance rarely produces the desired effect without resorting to impractically high levels of application. Many of the successful postharvest fungicides possess the ability to accumulate at specific sites on the host at concentrations many times higher than was present in the treating solution. It is also essential for effectiveness that a fungicide penetrate host tissue to at least the depth of the pathogen. Many compounds are extremely active against a pathogen in vitro, but are completely ineffective in preventing decay of inoculated fruit. Conversely, some compounds such as thiourea and 2-aminobutane exhibit only a modest degree of fungistatic activity in vitro, but are quite effective in controlling fruit decays. These possibilities should be borne in mind when screening compounds as potential postharvest fungicides.

Ultimately it should be possible to uncover compounds that uniformly permeate the outer tissues of the host, creating an environment unsuitable for the development of pathogenic microorganisms. Although no true systemic treatments have been described for postharvest appli-
cations, DCNA apparently can penetrate peach tissue to a limited extent and therein retard the development of *Rhizopus* in infected fruit (Ogawa et al., 1963c). With some effort it should be possible to develop local systemic fungicides that would prevent growth and sporulation of *P. digitatum* on the surface of decayed citrus fruits, thereby solving one of the most serious disease problems in the marketing of citrus fruit. Little effort has been made to find compounds that increase the resistance of fruit to fungal invasion, although the success of 2,4-D and related compounds in the control of stem end rots of citrus fruits has established a clear precedent for such treatments.

The state of the art of treating perishable commodities with fungicides after harvest is yet in its infancy. Postharvest treatments of far greater sophistication must be developed within the next decade to keep abreast of eminent revolutionary developments in mechanical harvesting, controlled ripening, and consumer packaging. This challenge will have to be met by conscientious screening programs aimed at uncovering fungicides suitable for postharvest use and by the development of formulations tailored to suit the requirements of each individual application. Little progress can be expected by perpetuating the prevalent practice of attempting to adapt field fungicides for postharvest use.

ACKNOWLEDGMENT

The author wishes to express his appreciation to Dr. M. J. Kolbren and Mr. H. F. Fitzpatrick for many valuable suggestions that have been incorporated in this chapter.

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CHAPTER 10

Application and Use of Fungicides as Industrial Preservatives

SEYMOUR S. BLOCK

I. Introduction

II. Textiles
   A. Copper Fungicides
   B. Metals other Than Copper
   C. Organic Protectants
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   E. Formulation of Textile Fungicides
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VI. Petroleum Products

VII. Leather

VIII. Drug and Cosmetic Preservatives

References

I. Introduction

"... to dust returnest," Longfellow wrote, "was not spoken of the soul." But it does depict the fate of soulless organic and inorganic matter whether in the natural state or shaped by man's manufacturing processes. Decay and rebirth may be nature's scheme but they do not always satisfy man's desire, even in this age of plenty and planned obsolescence. Fungi are in the forefront in this war of degradation. They show no greater respect for the cotton in a canvas awning sheltering the V.I.P.'s at the Waldorf Astoria than for cotton in the boll in a field in rural Georgia. They are at work in missile components, plastic coatings, television sets, pharmaceutical products, and paper

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mills. Industry has roused itself in defense against these relentless invaders and has amassed an arsenal of industrial fungicides for this worthy effort. Wessel and Bejuki (1959) have prepared a list of industrial fungicides (including wood preservatives) mentioning 403 products representing 140 different chemicals or combinations being marketed by 171 different companies. The nature of the defense and the type of weapons best suited for the purpose vary from product to product and industry to industry. An examination of the industrial fungicides that have been devised and evaluated for different industrial products should therefore be instructive.

II. Textiles

Next to wood products, cotton textiles consume a greater quantity of industrial fungicides than any other product. Tents, tarpaulins, awnings, sails, tobacco shade cloth, tennis nets, and fishing lines are continuously exposed to the weather whereas industrial filter cloths, shoe linings, diapers, and umbrellas are also subject to biological deterioration. Howard and McCord (1960), employing data of the National Cotton Council of America (1959), reported that a potential market of 154,650,000 lb of cotton exists in the first category and 270,370,000 lb for the second category, that is subject to microbiological attack but not in outdoor exposure. Estimating that the cotton receives 1% of its weight as fungicide for preservation, approximately 5 million lb of fungicides per year in the United States represents the market potential for fungicides for cotton goods. Cotton products such as awnings are cheaper than plastic and aluminum awnings but the latter became competitive because they have a longer service life. Preservative treatment at low cost to extend the normal service life, therefore, holds the key to the future use of cotton products.

Cotton and other cellulosic fibers are subject not only to biological degradation but also to actinic degradation. Deterioration due to sunlight may be greater than that produced by fungi, particularly in dry climates and unshaded exposure. While tensile strength measurements will not indicate the cause of deterioration, these agents can be differentiated by the determination of the cuprammonium fluidity of the cellulose solution. Microorganisms attack the cellulose by hydrolyzing off cellobiose units and consuming them, which does not affect the chain length of the remaining cellulose greatly, whereas the ultraviolet rays of sunlight oxidize and break the cellulose chain into smaller fragments, thus increasing the fluidity of the cellulose solution. In textiles for out-
door use, protection must be afforded against both types of damage. Protection may be provided simply by coating and sealing off the cellulose, or by chemically modifying the cellulose, or by the use of chemical inhibitors such as fungicides.

Rubber and plastic coatings, serving as physical barriers between the textile and microorganisms or weather, may prevent deterioration as long as they remain continuous and intact. They must be fairly heavy, however, and thereby change the "hand" of the product and increase its cost. Once the protective coating cracks or erodes, the fabric may become wet and degrade faster than if it could breathe and dry out. For this reason the rubber-coated cloth in inflatable life rafts and the vinyl-coated fabric in children's swimming pools are treated with fungicides.

The choice of a fungicide for the rot-resistant treatment of elastomer-coated cotton fabric must be made only after insuring compatibility of the fabric fungicide and coating under the conditions of processing (heat curing), storage, and use. Ashcroft (1965) reported tests with natural rubber, neoprene (GR-M), butyl rubber (GR-I), and Buna-S rubber (GR-S). Under simulated high-temperature storage conditions of 165°F for 7 days, no damaging effect was found with copper naphthenate, zinc naphthenate, Copper 8-quinolinolate, pentachlorophenol, and a resin amine, but 5-chloro-2-mercaptobenzothiazole, sodium phenylphenate, salicylanilide, phenylmercuric acetate, and 2,2'-methylenebis(4-chlorophenol) (dichlorophene) showed a tendency to accelerate the degradation of the cotton. None of the rot-resistant treatments caused significant weakening of the fabric when exposed to dry heat of 325°F for 1 hour for vulcanization of the rubber. The heating was noted to have an effect on prevention of rot as established by soil burial tests. About half of the fungicides decreased the protection while the other half increased it. For lightweight, vinyl-coated fabrics used in shoe liners, wall coverings, sporting goods, and upholstery, Kien and Stewart (1967) mention the use of dichlorophene, chlorinated salicylanilides, chlorinated phenols, phenylmercuric acetate, quaternary ammonium compounds, and tetrachlorophenol amine salt.

While other methods of treatment will resist decay of cotton cloth brought about by cellulose-decomposing fungi, only fungicides inhibit surface-growing mildew and algae that stain and dirty the material. Pullularia pullulans is commonly found growing on the underside of a fabric that may reach 180°F due to heating in sunlight. (Kien and Stewart, 1967). The fungicides most effective in combating decay and mildew of cotton cloth were obtained after extensive research and
testing during and following World War II. The most important of these are copper fungicides, with copper naphthenate having been used to treat a greater quantity of cellulosic textiles than any other chemical or mixture.

**A. Copper Fungicides**

Copper naphthenate is produced from the reaction of an inorganic copper salt with naphthenic acids obtained from the distillation of petroleum crude oils. The product is a blue-green resinous soap with a petroleum odor, which dissolves readily in petroleum solvents. It is a cheap compound that may be applied easily from cheap, volatile solvents simply by passing the cloth through a bath of the solution and allowing the solvent to evaporate. It adheres to the fibers without dusting off and is quite resistant to leaching by water. Copper naphthenate is not a pure compound but is a mixture of salts of the naphthenic acids as they are found in the petroleum distillate. Since solid copper naphthenate contains only 10% copper metal, and 0.4–0.8% as copper is required for effective protection, it is evident that the cloth must carry from 4 to 8% copper naphthenate. This large amount of compound stiffens the fabric and gives it color and odor. Furthermore, it is usually employed in combination with other treatments, such as fireproofing, which adds to the weight and stiffness. Nevertheless, its ready availability, cheapness, and dependability made it the fungicide of almost exclusive use for tents, gun covers, tarpaulins, etc., during World War II. The U.S. Army Quartermaster procured 1.5 billion yards of cotton duck during the war and most of it was protected with copper naphthenate.

Laboratory tests demonstrated copper naphthenate to be superior to copper soaps such as copper oleate, copper tallate, and copper resinate. Marsh et al. (1944) showed the greater antifungal potency to be due to the activity contributed not only by the copper ion but by the naphthenic acid. They demonstrated that in copper oleate-treated fabric, copper-resistant fungi were able to utilize the oleic acid and dissolve and leach the copper, thus permitting copper-sensitive cellulolytic fungi to attack the cellulose. With copper naphthenate, the naphthenic acid being antifungal, the copper-resistant fungi could not proliferate and leach the copper. It should be recognized that the copper ion is extremely toxic to microorganisms that attack cellulose. Reese (1947) reported that as little as 1 ppm copper ion is toxic to cellulose-decomposing bacteria. While naphthenic acids are only mildly
10. FUNGICIDES AS INDUSTRIAL PRESERVATIVES

Antifungal, their presence in copper naphthenate at nine times the concentration of the copper, that is, about 5% of the weight of the cloth, makes up for any lack of dramatic potency. Copper naphthenate has a low order of toxicity to humans and does not cause irritation in contact with the skin in the concentrations regularly used.

Copper hydroxynaphthenate has been employed as a substitute for copper naphthenate. Employed during the war, it extended the supply of naphthenic acid and produced a finish with less color and odor than copper naphthenate, but gave less protection for an equal concentration of copper (Abrams, 1948). It consists of a mixture of copper naphthenate with excess alkali. Since it is not soluble in hydrocarbon solvents, it is not applied as effectively as copper naphthenate (Shanor, 1945).

Copper 8-quinolinolate, the copper chelate of 8-hydroxyquinoline (8-quinolinol), is the textile fungicide of choice when cost and color are not restrictive. It combines remarkable antimicrobial activity with very low toxicity to humans, and is free from odor. It is very insoluble in water and common organic solvents (Benignus, 1948). It has a characteristic yellow-green color. Because of its insolubility in solvents it was first applied as a dispersion or by the two-bath process. In the latter process, the fungicide is precipitated in situ by first immersing the cloth in an aqueous solution of the sodium salt of 8-quinolinol and then in a bath of copper acetate.

A process was later developed for complexing "copper 8" which made it soluble in cheap organic solvents thereby greatly extending its usefulness, although further increasing its cost (Block, 1952). Several solubilized formulations are now commercially available. Despite its high unit cost, however, "copper 8" has found wide acceptance, since it is effective at a fraction of the concentration of copper naphthenate. It is commonly used at 0.1-0.2% copper, which is equivalent to 0.5-1% of the compound on the cloth. The cost differential over copper naphthenate may be about 2.5 times for straight "copper 8" and eight times for the solubilized products. These figures are based upon a cost of 30¢ a pound for copper naphthenate, $3 a pound for "copper 8," and $10 a pound for "copper 8" in a soluble form. "Copper 8" has another advantage over other copper fungicides, namely, that it does not accelerate cellulose decomposition in the presence of sunlight. It is active against a wide spectrum of microorganisms including the copper-tolerant fungi. There is a considerable literature on the mechanism of action of this and other chelate fungicides (Chapter 9, Volume II).

Copper formate is a second-generation textile fungicide, having made
its appearance in the 1950's rather than the 1940's (Abrams and Bottoms, 1956). It is an important treatment nonetheless, particularly in view of its low cost. At $1.72 per pound of copper as copper formate it compares favorably, costwise, with copper naphthenate at $3 per pound of copper, both being applied at a concentration range of 0.4-0.8%.

Whereas it was found to provide considerable protection against microbiological deterioration (Klens and Stewart, 1957), it does not possess the convenience and simplicity of application of copper naphthenate. It is applied by passing the fabric through a bath of cupric formate and then heating the fabric to reduce the copper and precipitate cuprous oxide in the fibers. Although reference has been made to cross-linking the cellulose by copper formate, to explain its protective properties (Abrams and Bottoms, 1956), the work of Rose et al. (1959) has clearly shown that this is not the case but that copper formate is merely converted to insoluble copper oxides and free copper. This is borne out by the loss of copper in weathering, by leaching with ammonium hydroxide, by the solubility of the treated cotton in cuprammonium solution, and by the demonstration that cotton heated in Fehling's solution produces a coating of cuprous oxide that provides equivalent rotproofing.

Copper soaps of fatty and oleoresinous acids such as copper oleate, copper stearate, copper "tallate," and copper resinate, while inferior to copper naphthenate in performance (Goodavage, 1943), still have given useful protection to cotton against microbiological breakdown. They dissolve readily in mineral spirits and are resistant to leaching by water. A more recent preparation that is worthy of notice is a resin amine copper soap made soluble by complexing with 8-quinolino1 and 2-ethylhexanoic acid. This dehydroabietylamine-copper complex was found to rank between copper naphthenate and "copper 8" as a protectant against microbiological attack (Rose and Bayley, 1955). It may be noted also that in its ingredients and cost it holds a similar position.

When a flammable solvent is not desired, copper-ammonium complexes such as cuprammonium fluoride, cuprammonium hydroxide, or cuprammonium carbonate may be applied. The copper salts are dissolved in aqueous ammonia to give complexes stable in alkaline solution. After passing the cloth through the solution, the ammonia is driven off by heating the fabric to 80°C, thereby precipitating the insoluble basic salt in the fabric. A range of 1-1.5% copper is employed. The treatments lost favor because of difficulty in application control and their lack of protection against surface-growing molds (Goodavage, 1943).
A number of other copper protectants for cotton and other cellulosic textiles have shown promise but have received less commercial exploitation. Copper chromate was found to give excellent protection (Armstrong, 1941; Block, 1949), and deserves further attention. In tests by Rose and Bayley (1954), oxides of chromium and iron with copper carbonate or copper naphthenate, "copper 8," or copper dehydroabietylamine revealed that the presence of chromium and copper together in inorganic form confers marked protection against actinic breakdown of cellulose. It also prevents the severe loss of copper, which in the absence of chromium, occurs during weathering. To a less degree these were also noted with chromium-treated fabrics after-treated with the copper organics. They attribute the retarded loss of copper in the presence of chromic oxide to a complex formation between copper and chromium ions.

Copper cupferron, the chelate of copper and N-nitroso-N-phenylhydroxylamine, has been prepared in a solubilized form which may be applied from volatile solvent in a single bath treatment (Klens and Stewart, 1957). This chelate has the characteristic high antifungal activity of "copper 8," and in abbreviated weathering tests showed the same protection against deterioration when both were evaluated at 0.05-0.2% copper. When neutrality in color is desired, copper cupferron has the advantage of being an off-white to grayish-green color, depending on the concentration used.

An interesting new development is copper borate solubilized for aqueous application in a complex with zirconium (Conner et al., 1964). Solutions of zirconyl acetate and zirconyl ammonium carbonate dissolve insoluble copper borate producing stable, soluble complexes. The cotton fabric is padded through either of these solutions and then dried at 145°C to produce insoluble basic copper bis(borato zirconyl acetate) or copper bis(borato zirconic acid) on the fabric (see Fig. 1). Ten-ounce cotton duck treated with 0.4% copper as copper bis(borato zirconic acid) by the above process retained 100% of its strength after 5 weeks of soil burial. In 6 months of outdoor exposure at New Orleans, samples of fabric treated to give 0.2 and 0.4% copper add-ons with 2.5% ZrO₂ showed no change in appearance and retained 60 to 70% of its strength. The control during this period retained only 25% of its original tensile strength and showed heavy growth of fungi and algae. Copper borate dissolved in zirconyl acetate to contain 0.07% copper and 2.4% ZrO₂ retained 72% of its strength without visible evidence of mildew or algae after 14 months' outdoor exposure. The experiments suggest that the known strong affinity of zirconium compounds for...
Basic copper bis (borato zirconyl acetate)
Mol. wt. 513.62
% Cu = 12.37 by theory  % Cu = 12.26 by x-ray analysis
% Zr = 35.51 by theory  % Zr = 36.00 by wet analysis

Fig. 1. Complex of copper borate and zirconyl acetate produced on cloth.

Cellulose will explain the resistance to weathering in these treatments. In cost, these treatments fall between copper naphthenate and copper 8-quinolinolate.

B. Metals Other Than Copper

Copper has certain disadvantages among which are that it may tender cellulose upon exposure to ultraviolet light, it deteriorates rubber and cannot be used on rubber-treated fabrics, and it has color. Zinc naphthenate has served in many applications where one or more of the factors above have ruled out the use of copper naphthenate. It has only about one-half the protective power of copper naphthenate (Cavill et al., 1949) thereby requiring twice the concentration, that is, about 10-15% of the weight of the cloth. Zinc dimethyldithiocarbamate is a good fungicide which has had some use on textiles but was found to be unstable in the presence of acids and is dissolved by alkalies (Shanor,
The lack of solubility in organic solvents resulted in its application by dispersion in water, which is not a very satisfactory method for impregnating the fibers, or by a two-bath process. The mercurials, inorganic and organic, have not proven to be very popular in rot-proofing textiles. They do not stand up well in sunlight and in the presence of reducing agents. Furthermore, they may produce irritation when in contact with the skin. In soil burial tests, mercuric naphthenate or phenylmercuric naphthenate added to copper naphthenate reduced the loss of copper and the breakdown of the fabric (Bayley and Weatherburn, 1947). This result might be explained by the inhibition of copper-tolerant fungi by the mercury.

Cadmium selenide is not a fungicide. In a laboratory test it gives no protection against cellulose breakdown or surface mildew. Yet in field exposure it was found to protect cotton fabric against both these hazards. An army duck with a selenium content of 0.13%, along with a coating of sun-screening pigments totaling 2.25% of the weight of the cloth retained 60% of its strength and was free from visible attack after 2 years' weathering (Brysson et al., 1963). Interestingly enough, cadmium sulfide does not show a similar effect. In the tests the fabric was treated with an aqueous emulsion of alkyd resin binder with the compound and then allowed to air dry. The authors propose a mechanism based upon a sunlight-catalyzed oxidation of inactive selenide to antimicrobially active selenite ion. When sun-screening pigments were used with the cadmium selenide, the rate of loss of selenium from the fabric was cut in half over that without them and the protective life of the fabric was thereby extended.

C. Organic Protectants

The most important of the nonmetallic fungicides for fabrics is 2,2'-methylenebis(4-chlorophenol) known as "dichlorophene" or "Compound G-4." A relative of hexachlorophene, it was found in the search for a soap antiseptic, but its properties suited it much better as a fabric preservative. A methylene-bridged chlorophenol, it is strongly antifungal to rot and surface organisms and resistant to leaching (Marsh and Butler, 1946). Its toxicity to man is low and it is nonirritating to the skin. A further desirable characteristic is its white color. When employed with a water repellent it was shown to stand up well in soil burial and above-ground weathering (Barghoorn, 1945). Application is made in a one-bath treatment by dissolving the compound in alcohol.
and diluting with mineral spirits. Aqueous application can be made with ammonia and heating, or with the two-bath process employing the sodium salt and a weak acid.

Pentachlorophenol, because of its powerful fungicidal activity, low cost, and lack of color has found use as a textile preservative. It has, however, several disqualifying characteristics, among which is the liberation of hydrochloric acid upon decomposition in sunlight, a reaction which accelerates deterioration (Bertolet, 1943). It is a primary skin irritant and performs poorly in the soil burial test. Indications are, however, that in a properly formulated finish, with a good water repellent to prevent leaching, and pigments and binder to screen sunlight, that pentachlorophenol will perform well at low cost. With a heavy finish containing 0.95% pentachlorophenol, fabric retained its full strength after 6 weeks' burial in soil (Bertolet, 1944). Lauryl pentachlorophenol has been found to meet some of the objections to pentachlorophenol based on its skin-irritating properties and has been shown to give satisfactory decay resistance in soil-burial tests at 0.5–2.0% when used with a water repellent (Hueck and La Brijn, 1960).

Salicylanilide or "Shirlan" is one of the older textile fungicides, and was developed especially for use on textiles after considerable research (Fargher et al., 1930). Unlike pentachlorophenol it does not generate hydrochloric acid upon weathering but is susceptible to loss by leaching. Considerable improvement in its performance has been obtained with the common wax–aluminum acetate water repellent (Furry et al., 1941), but its principal application has been for mildew prevention on fabrics being shipped and stored, the purpose for which it was originally developed.

While many sulfur compounds have antifungal activity, the only compound other than zinc dimethylthiocarbamate to be accepted commercially as a textile preservative is 2-mercaptobenzothiazole. It has been marketed as a synergistic mixture of the zinc salts of mercaptobenzothiazole and dimethylthiocarbamate (Somerville, 1957). Darby and Kempston (1962) confirmed the high activity of the individual ingredients and the synergism of the proprietary mixture but were unable to reproduce the synergistic activity to their satisfaction with their own mixtures.

Several amines and amine salts, especially the quaternaries, have aroused interest and found some use as fabric fungicides. Among these are dodecylamine, dehydroabietylamine, dodecylamine salicylate, dodecylmethylenenyl ammonium cyclcopentane carboxylate salt, trimethyl cetyl ammonium pentachlorophenate, and quaternary ammonium naph-
10. FUNGICIDES AS INDUSTRIAL PRESERVATIVES

The last two compounds have been employed in treating lightweight products such as thread and bed sheets (Anonymous, 1946; Kliens and Stewart, 1957).

Other fungicides, metallic and nonmetallic, that have shown merit in tests on cotton materials are copper pentachlorophenate, copper 3-phenylsalicylate, copper 2-nitroso-1-naphthol, copper dimethylglyoxime, zinc 8-quinolinol, phenylmercuric acetate, tetrabromo-o-creosol, e-phenylphenol, p-chloro-m-xylene, o-chlorobenzal rhodanine, and 4,5-dichlorobenzoxazolinone. In the search for materials suitable for use in the war in the South Pacific a great many candidate compounds were considered and tested (Siu, 1951).

D. Cellulose Modifiers

Cellulose can be modified by chemical reaction so that it is no longer susceptible to microbiological attack. These treatments are not fungicidal, but they are preservative and as such they are competitive with fungicidal treatments. Cellulose-modifying processes include acetylation (Goldthwait et al., 1951), cyanoethylation (Grant et al., 1955), and methylolmelamine-resin treatment (Berard et al., 1961). Cotton fabric so treated may resist decomposition in soil burial practically indefinitely and has been shown to be resistant to microbiological breakdown for over 2 years. It is not, however, impervious to degradation resulting from actinic radiation. The disadvantages that may offset the obvious superiority in rot protection are high initial losses in tensile strength, high add-on weight, relative complexity of treatment, and increase in cost. These treatments do not prevent the growth of algae and surface molds that adhere to the fabric and grow on nutrients other than cellulose. Thus, fungicides are necessary in conjunction with cellulose modifiers to keep the fabric looking clean. An interesting exception has been reported in the case of certain phosphorus-containing flame retardants which are cellulose reactive. They were found to be strong inhibitors of both mildew and decay organisms (Bullock et al., 1964).

E. Formulation of Textile Fungicides

Since a multiplicity of forces and environmental factors in weathering are instrumental in the breakdown of preservative-treated fabrics, formulation is very important for achieving maximum performance from any fungicide. Copper naphthenate and other fungicides are found to
resist leaching and prevent rotting longer when used with water repellents based on wax or resins. Many copper fungicides and halogenated phenols when subjected to sunlight and moisture accelerate the tendering of cellulose. Pigments in the coating formulation that shield the fungicide from the sun give the fabric extra service life (Barthoorn, 1945). Bayley et al. (1965) have demonstrated that as pigments on cotton subject to actinic degradation, copper and barium chromates show enhanced cellulose breakdown at lower level of treatments, whereas lead and zinc chromates resemble copper 8-quinolinate in being protective of the cellulose over the entire range of treatments.

During World War II, a popular treatment for army tents purchased by the military contained chlorinated paraffin, antimony oxide, yellow iron oxide, lampblack calcium carbonate, and copper naphthenate. This gave an olive drab color and came under the army specification for fire-, water-, and weather-resistant cotton duck. In weathering tests with the fungicide alone, it was found that although it retarded mildew it caused the fabric to break down faster than the untreated fabric. Chlorinated paraffin, necessary for chlorine generation in producing fire resistance, was found to yield hydrochloric acid when exposed to light and heat and also degraded the fabric faster than the untreated control. Yet, the whole formulation was shown to retain almost all its strength after 1 year’s weathering at Miami, Florida (Heffner, 1954).

The beneficial result of the formulation is attributed to the sun-screening properties of the pigments. The acid-neutralizing presence of the calcium carbonate no doubt aided in controlling the damaging action of free hydrochloric acid.

F. Evaluation of Textile Fungicides

Evaluation of the relative merit of textiles fungicides based on even an extensive literature may be hazardous. Uncertainties arise from differences in the pretreatment of the fabric, the weight of the cloth, the formulation and concentration of the fungicide, and the method and environment of the exposure. Outdoor weathering tests usually require at least 2 years for conclusive results, thus stimulating interest in accelerated laboratory tests. The most popular and dependable of these is the soil burial test. It predicts rather faithfully what may be expected in prolonged exposure where microbiological agents are the principal deteriorating factor. Soil burial for 1 week and 2 weeks give similar results to outdoor shade exposures for 1 and 2 years in Florida (Block, 1949).

Employing the soil-burial test, Darby and Kempton (1962) com-
pared a selected variety of textile fungicides, determining the ED₅₀ in breaking strength after 10 days' burial. In this way the time factor was a constant and greater uniformity between replicates was achieved. The results in Table I show the fungicides in general use to have an ED₅₀ of 0.5% or less based on the weight of the cloth.

Based upon extensive field testing by technical missions of the United States Office of Scientific Research and Development and the U.S. Army Quartermaster, Barghoorn (1945, 1946) concluded that copper 8-quinolinolate was the most suitable for light fabrics, and copper naphthenate with sun-screening pigments and "copper S" were best for heavy tent fabric. In exposure tests of manila ropes, Miller and Bell (1959) found "copper S" to be much superior to copper naphthenate. Jute fabrics gave the longest protection with copper pentachlorophenate when exposed to tropical conditions (Bomar, 1960).

III. PAPER AND PULP

By its very nature the paper-making industry is subject to problems arising from microorganisms. It employs as its basic ingredient vegetable fibers in a wet or moist condition. These must be stored and

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylmercuric acetate</td>
<td>0.01</td>
</tr>
<tr>
<td>Zinc salt of dimethylthiocarbamate and 2-mercaptobenzothiazole</td>
<td>0.025</td>
</tr>
<tr>
<td>Zinc dimethylthiocarbamate</td>
<td>0.035</td>
</tr>
<tr>
<td>4,5-Dichlorobenzoxazolinone</td>
<td>0.05</td>
</tr>
<tr>
<td>Copper 8-quinolinolate</td>
<td>0.06</td>
</tr>
<tr>
<td>2-Mercaptobenzothiazole</td>
<td>0.1</td>
</tr>
<tr>
<td>Copper naphthenate</td>
<td>0.25</td>
</tr>
<tr>
<td>Copper 8-nitroso-4-phenylhydroxylamine</td>
<td>0.35</td>
</tr>
<tr>
<td>2,2'-Methylenebis(4-chlorophenol)</td>
<td>0.6</td>
</tr>
<tr>
<td>Salicylamide</td>
<td>0.5</td>
</tr>
<tr>
<td>Trimethylsilyl ammonium-pentachlorophenate</td>
<td>0.6</td>
</tr>
<tr>
<td>Dehydroabietylamine</td>
<td>0.6</td>
</tr>
<tr>
<td>Dodecyldimethylbenzylammonium cyclo-pentane carbonate salt</td>
<td>0.7</td>
</tr>
<tr>
<td>Copper oleate</td>
<td>1.5</td>
</tr>
<tr>
<td>8-Hydroxyquinoline</td>
<td>4.5</td>
</tr>
</tbody>
</table>
processed, giving rise to possible deterioration of the fibers and undesirable microbial growths in the water systems, which are called slimes because of their consistency. Slimes clog screens and pipes and accumulate in the paper machines where they may break loose and get into and weaken the roll of paper. A shutdown to start a new roll, or to clean out the slime from the system can be costly. A study of a paper mill producing 240,000 tons annually on a 310-day operating year showed possible savings of $360,000 annually by a slime-control program (Stitt, 1948). High-yield pulping, reuse of the water in the paper machines, and addition of fillers, glues, and sizes to the paper are all practices which favor the growth of microorganisms (Drescher, 1963). Use of microbiocides aid in sanitation, thus reducing the requirement for shutdowns for cleanup purposes, and also help to produce a better product.

The problem of slime growth in the paper mill is not an easy one to eliminate. Many different microorganisms are found in the system, i.e., bacteria, fungi, and algae. The slimes themselves are composed of mixtures of organisms. The flora varies from one part to another and even from one paper machine to another. These differences result from variation in the type of nutrients available, pH, temperature, oxygenation, and operator control (Wolfson, 1963). Thus the selection of a chemical inhibitor is not a simple one. A number of the factors to be considered in the selection of a slimicide for any particular system are discussed by Shema (1963). A discussion of the biological nature of slimes and a plea for further research on their prevention has been made by Deleporte and Vlassoff (1960). In laboratory tests with chlorine dioxide as a microbiocide the results obtained in reducing bacterial count varied considerably depending upon the system and the composition of the water (Alper, 1960).

Different methods of testing biostatic agents for slime control (the terms slimicide, microbiocide, biostatic agent, etc., are being used synonymously) include (1) the agar plate test with representative bacteria and fungi (i.e., Aerobacter aerogenes, Bacillus mycoides, Aspergillus niger, and Penicillium expansum); (2) the millstock or white-water method using a commercial paper pulp and an inoculum taken from an operating paper mill; and (3) the spore pulp method which differs from the millstock method in that the substrate is made up of nine different kinds of paper pulp prepared in the laboratory. A comparison of these methods was made with a group of representative chemicals, most of them being proprietary products (Conkey and Carlson, 1962). These authors found greatest reproducibility of results,
10. FUNGICIDES AS INDUSTRIAL PRESERVATIVES

99%, with the agar plate method, and about 90% for the other two methods. The only agreement among all methods was with phenylmercuric acetate, which rated first by each test. The authors conclude that the choice is between a general, reproducible test and a nonreproducible, specific-use environment test. The agar plate test is recommended for screening new materials and the other methods provide reasonably reliable prediction of performance in the mill.

Since 1941, the Biological Control Committee of the American Paper and Pulp Association has sponsored testing of all biostatic agents offered to this industry. All of the compounds are tested by the agar plate test method employing the organisms already noted. Phenylmercuric acetate is employed as a standard chemical giving usual inhibition levels as follows: *A. aerogenes*, 2.0 ppm; *B. mycoides*, 0.2 ppm; *P. expansum*, 6.0 ppm; and *A. niger*, 5.0 ppm. It should be noted that the bacterial plates are inoculated with a bacterial suspension by streaking the surface of the agar, whereas the fungus plates are inoculated with disks of a mycelial mat. Further, different media at different pH are employed for the bacteria and fungi. Nevertheless the comparative results between different potential slimicides is of great value. The list of materials and the results of the tests are published every 5 years, the latest having been 1963 (Conkey and Carlson, 1963).

In this cumulative list are several hundred proprietary products, their trade names, active constituents, and test data. Among the more active chemicals against all four organisms were: a number of different organic mercurials, dimethyldithiocarbamate-dimorpholine salt, p-chloro-m-cresol, salicylanilide, chloroisonaphthene, 2-chlorothianaphthene-1-dioxide, copper pentachlorophenate, numerous halogenated phenols and their sodium salts, bis(tributyl tin) oxide, 3-5-dimethyltetrahydro-1,3,5,2-H-thiaazain-2-thione, 8-hydroxyquinolium methanesulfonate, 2-thiocyanopyridine oxide, tetrachloromethylenecyclopentadiene-1,3, sodium 2-pyridinethione, 2-nitro-1-phenylpropene, and 2-bromothianaphthene-1-dioxide.

It should be clear from the comparative results in the agar plate and millstock tests that the better chemicals in the former test may not be the better materials in the paper mill and that some of the many compounds that do not perform as well in the agar test may do an excellent job under actual conditions of use. Chlorine deserves special attention as a disinfectant for water systems. It is of great use and value in the paper mill's raw-water supply; some mills use it as an adjunct to other slime treatments. In mills where there is a continuous outflow of clarified water from the paper machine systems it
carries away water-soluble slime-control chemicals and prevents a buildup of minimum levels to kill microorganisms. Chlorine, because of its low cost and the low concentration needed to prevent slime formation, may therefore be added continuously.

When there is not a continuous outflow of water, a combined form of chlorine, chloramine, the product of chlorine and ammonia or amines, is preferred because it is residual, less corrosive, and does not degrade the paper fibers. A chloramine residual of 0.2–0.3 ppm for acid systems and 0.4–0.6 ppm for alkaline systems is said to produce excellent microbiological control for 1 to 2 cents per ton of paper produced (Schiratzinger, 1963). For high-concentration alkaline stock preparation systems, calcium hypochlorite can be employed; for acid stock preparation systems, sodium chlorite may be employed. Chlorine dioxide, which is slowly released by sodium chlorite, has the same antimicrobial effect as chlorine (Schiratzinger, 1963). A stabilized solution of chlorine dioxide (4–5% ClO₂) has been produced and is said to have been used very successfully in paper mills for slime control (Alper, 1960). The merits claimed for it are that it does not attack the cellulose fibers; it is not absorbed by the fibers and removed from the water system as in the case of certain organic slimicides; it leaves no toxic residues or objectionable odors on the paper; and it does not contribute to stream pollution. Like chlorine, it is active against a broad spectrum of microorganisms, but can only be used on white papers because of its bleaching properties.

Apart from the need for sanitation in the aqueous systems in paper making, fibers in the form of pulp are subject to fungal attack, causing staining and decay. The pulp must be stored with at least 50% moisture or the fibers lose strength. The fungi attacking pulp are reviewed in a paper by Russell (1961) who stated that, at the time of writing, only two preservatives were used commercially for the treatment of pulp. One was phenylmercuric acetate and the other was a mixture of phenylmercuric acetate and 8-quinolinc in equal amounts. Since phenylmercuric acetate has the disadvantage of being inactivated by the mercury-resistant fungus, *Penicillium roquefortii*, the mixture was said to have been preferred, and was used at a rate of 50–100 ppm of the dry weight of the wood pulp to give protection for long periods of storage (Russell, 1961). For paper pulp made from straw the best results for inhibiting decay were obtained with sodium pentachlorophenate, at 1000 gm per ton or mixed with phenylmercuric acetate at 40 gm per ton of dry pulp (Popescu and Esanu, 1962).

For the manufacture of paperboard resistant to microbial degrad-
tion, sodium pentachlorophenate was found to be most suitable (A. D. Shapiro and Volina, 1961). It is used in boxboard such as is employed for packaging electrical equipment for shipment to the tropics and in bituminized board for use in automobile manufacture. The minimum effective concentration was found to be 0.3–0.6% of the weight of the board or 4–5% of the adhesive weight. Satisfactory results were also obtained with 8-quinolinol and with salicylanilide but these were more costly. For paper claimed to be mold-resistant (for wrapping foods, etc.) a treatment with 0.1–5% (by weight of the paper) of the reaction product of equimolar amounts of diphenyl, o-phenylphenol, and a resin amine in a microcrystalline wax binder is described (Mosinee Paper Mills Co., 1961). Another formulation for imparting fungus resistance to paper employs an oil-in-water emulsion of solubilized copper 8-quinolinolate or zinc complex preparations in amounts to supply 0.02–0.2% copper or 0.04–0.4% zinc by weight of the paper (Mosinee Paper Mills, 1960). An antirust and fungitoxic paper for wrapping metals and nonmetals during storage contains an amine nitrite as a vapor-phase corrosion inhibitor and an amine phenate, cyclohexyl ammonium nitrophenate, as a fungicide that does not adversely affect the rate of vaporization of the nitrite (Wachter and Wieland, 1960).

IV. RUBBER, PLASTICS, AND PAINT

Rubber, plastics, and paint have something in common, namely, they are customarily products of organic polymers. They may contain fillers or pigments of an organic or inorganic nature, plasticizers for flexibility, and other ingredients. Their composition, treatment, and use, however, may be quite different.

Rubber was the first commercial elastomer and plastic. An elastomer is a material which has elastic properties and a plastic is a material which has the capacity for being molded to a desirable shape. Natural rubber is produced by many plants and undergoes biological decomposition in nature. The latex is much more susceptible to microbial attack than the vulcanized rubber. Hevea latex can be preserved with 0.001% of an emulsion or soluble salt of 2,2'-thiobis(4,6-dichlorophenol). The compound is applied as a spray at the tapping cup, or as an anticoagulant in the cup. With a latex concentrate containing 0.2% ammonia and 0.3% of the germicide, the bacterial count was fewer than 10 per milliliter after 6 months' storage. Without the germicide the count was $1.2 \times 10^5$ in 3 days and the latex was completely coagulated in 6 months (Graham and Taysum, 1962).
Whereas bacteria and fungi may grow on vulcanized rubber, it is actively destroyed by species of proactinomyentes and actinomyces, which may consume 20-40% of the rubber on which they are cultured. Bacteria act much more slowly in decomposing the rubber, whereas fungi do not consume the rubber hydrocarbon but grow on other organic materials that are on or in the rubber (Nette et al., 1959). Chlorinated rubber and silicone rubber are resistant to attack but there is considerable disagreement concerning the susceptibility of other synthetic rubbers (Lightbody et al., 1954). Microorganisms do not usually destroy the rubber but they may promote its susceptibility to chemical breakdown by oxidation and they can cause it to fail in its function, as in the case of buried electrical cable, which must be intact to serve as an insulator.

The growth of Penicillium on smoked crepe or sheet rubber has been prevented with p-nitrophenol (Morris, 1927). Copper compounds cannot be tolerated in rubber since they act as oxidation catalysts, but zinc compounds have been used. These include the rubber vulcanization accelerators zinc mercaptobenzothiazole and zinc dimethylthiuramdisulfide. The patent literature also mentions zinc salicylate and zinc benzoate (McDermott and Funk, 1930). In agar plate tests of over 100 compounds, mostly phenolics, against the bacteria, fungi, and actinomyces commonly found degrading rubber, alkyl phenols such as thymol, o-phenylenediamine, and nitrophenols, were characterized by a broad spectrum and strong antibacterial and antifungal activity. Particularly active compounds were 4-chlorothymol, pentachlorophenol, 1-nitroso-2-naphthol, and 8-quinolinol (Kost et al., 1959). It should be recognized that an agar plate test in which the compound is not incorporated in the rubber and does not undergo the heat and chemical reaction of vulcanization, cannot necessarily be expected to indicate the performance of the compound were it so incorporated. Experience with polymers has shown that a very potent fungicide and bactericide can be rendered completely inactive by such treatment. In tests in which 18 azomethane derivatives of thiophene, as well as zinc, nickel, and antimony salts of salicylanilide were incorporated in rubber (natural and butadiene mixture, polychloroprene, and butadiene) the samples were tested for thermal stability at 65°C prior to subjecting them to microorganisms on nutrient agar. The salicylanilide salts and most of the thiophene derivatives were resistant to the heat and protected the rubbers from biological attack (Dubrovin, 1959).

The use of antimicrobial agents for incorporation in bathing mats, rubber footwear, rubber mattresses and aprons, sterile medical supplies,
10. FUNGICIDES AS INDUSTRIAL PRESERVATIVES

etc., has been discussed by Hofman (1962). This author recommends a trade-name product "Antimykotikum A" as combining good rubber-technological characteristics with effective antimicrobial action. For rubberized fabrics, shoe linings, etc., he proposed additional impregnation with 2,2-methylenebis(4-chlorophenol), quaternary ammonium compounds, zinc dithiocarbamate, or thiram to increase the antimicrobial effect.

In a discussion of the microbiological susceptibility and deterioration of synthetic plastics, care should be made to distinguish between the polymer itself and the finished product incorporating plasticizers, fillers, and other modifiers. Lightbody et al. (1954) summarized the tests of different workers on different plastics where the data was obtained on only the pure plastic material. These results, presented in Table II, represent the resistance of the more important plastics to microorganisms as determined by a variety of tests, including humidity chamber, soil burial, and pure culture tests. Changes in electrical properties were also recorded.

Regardless of the susceptibility of the pure polymer, work of the Bureau of Ordnance of the U.S. Navy on a large number of plastic formulations with different plastics having fillers such as wood flour, nylon fabric, glass cloth, and paper, etc., makes it clear that the plastic notwithstanding, the individual product in each case must be evaluated if fungus resistance is to be assured (Alaka Research Laboratories, 1952). Other than the filler, much of the problem with microbial attack of plastics can be blamed on the plasticizer. Without the plasticizer most plastics would be too brittle to use. Yet, many plasticizers provide food for fungi and bacteria. A compilation of considerable government work on the fungal resistance of plasticizers gives the results of tests with about 150 plasticizers as determined in pure culture tests (Brown, 1945). Acetates, laurates, oleates, and other compounds containing fatty acid residues are especially susceptible. This includes vegetable oils with the exception of tung oil. Esters of straight-chain dicarboxylic acids are more resistant than those of monocarboxylic acids. In fact, a patent claims glycol esters of C_6—C_8 straight-chain dicarboxylic acids for making polyvinyl chloride plastic fungus resistant. It is recommended as 10% of the weight of the composition or, when used as the only plasticizer, up to 50% is preferred (Bockstahler and Coe, 1960). As a group, the phthalates are resistant, also the phosphates, although exceptions were found in both groups. Glycerol and sorbitol derivatives were found to support luxuriant growth. Glycerol and glycolic acid derivatives containing aliphatic chains no longer than 10 carbon atoms...
<table>
<thead>
<tr>
<th>Material</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acrylon</td>
<td>Good</td>
</tr>
<tr>
<td>Polymethylmethacrylate</td>
<td>Good</td>
</tr>
<tr>
<td>Polyacrylonitrile (&quot;Orel&quot;)</td>
<td>Good</td>
</tr>
<tr>
<td>Acrylonitrile-vinyl chloride copolymer</td>
<td>Good</td>
</tr>
<tr>
<td>(&quot;Drycal&quot;)</td>
<td></td>
</tr>
<tr>
<td>Cellulose derivatives</td>
<td></td>
</tr>
<tr>
<td>Cellulose acetate</td>
<td>Good, poor</td>
</tr>
<tr>
<td>Cellulose acetate butyrate</td>
<td>Good</td>
</tr>
<tr>
<td>Cellulose acetate propionate</td>
<td>Good</td>
</tr>
<tr>
<td>Cellulose itaconate</td>
<td>Poor</td>
</tr>
<tr>
<td>Ethyl cellulose</td>
<td>Good</td>
</tr>
<tr>
<td>Rayons</td>
<td></td>
</tr>
<tr>
<td>Acetate rayon (&quot;Etron&quot;)</td>
<td>Good</td>
</tr>
<tr>
<td>Saponified acetate rayon</td>
<td>Slightly more resistant than cotton</td>
</tr>
<tr>
<td>Cellulose derivatives</td>
<td></td>
</tr>
<tr>
<td>Cellulose acetate butyrate</td>
<td>Good</td>
</tr>
<tr>
<td>Cellulose acetate propionate</td>
<td>Good</td>
</tr>
<tr>
<td>Cellulose itaconate</td>
<td>Poor</td>
</tr>
<tr>
<td>Ethyl cellulose</td>
<td>Good</td>
</tr>
<tr>
<td>COPRAMMONIUM rayon</td>
<td></td>
</tr>
<tr>
<td>Viscose rayon</td>
<td>Poor</td>
</tr>
<tr>
<td>Phenol formaldehydes</td>
<td></td>
</tr>
<tr>
<td>Phenol formaldehyde</td>
<td>Good</td>
</tr>
<tr>
<td>Phenol aldehyde formaldehyde</td>
<td>Poor</td>
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<tr>
<td>Resorcinol formaldehyde</td>
<td>Good</td>
</tr>
<tr>
<td>Melamine formaldehydes</td>
<td>Good, poor</td>
</tr>
<tr>
<td>Melamine formaldehyde</td>
<td></td>
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<tr>
<td>Urea formaldehyde</td>
<td></td>
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<tr>
<td>Protein formaldehydes</td>
<td></td>
</tr>
<tr>
<td>Zein formaldehyde (&quot;Vicara&quot;)</td>
<td>Good</td>
</tr>
<tr>
<td>Casein formaldehyde</td>
<td>Poor</td>
</tr>
<tr>
<td>Polyamides</td>
<td></td>
</tr>
<tr>
<td>Nylon</td>
<td>Good</td>
</tr>
<tr>
<td>Polystyrene</td>
<td>Good</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>Questionable, good</td>
</tr>
<tr>
<td>Polyethylene</td>
<td></td>
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<tr>
<td>Polytetrafluoroethylene (&quot;Teflon&quot;)</td>
<td>Good</td>
</tr>
<tr>
<td>Polyethylene</td>
<td>Questionable, good</td>
</tr>
<tr>
<td>Polysobutylene</td>
<td>Good</td>
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<tr>
<td>Styrenes</td>
<td></td>
</tr>
<tr>
<td>Polystyrene</td>
<td>Good</td>
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<tr>
<td>Polyvinyl chloride styrene</td>
<td></td>
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</tbody>
</table>
were resistant. Among the plasticizers found resistant were chlorinated hydrocarbons, diphenyl sulfone, and benzophenone (Lightbody et al., 1954).

To prevent fungus growth on plastics, selecting a composition that does not provide nutrients in itself helps, but is not always sufficient when superficial dust, dirt, or grease on the surface of the plastic supplies enough nutrient for the fungus to extend luxuriant superficial growth. This growth will not destroy the plastic but it could destroy its function when it performs as insulation in an electronic system. The work of Luce and Mathes (1948) demonstrated that subvisual growth of fungi has a pronounced effect in lowering the electrical resistance of plastics by providing a moisture bridge across the insulation. In plastic sheeting, such as used in raincoats and shower curtains, fungus growth is not only unsightly but when it feeds upon the plasticizer it embrittles the film and causes it to crack.

Fungicides and germicides that have found greatest use in plastics are the phenylmercurials, copper 8-quinolinolate, zinc 8-quinolinolate, zinc dimethyl dithiocarbamate, and mercaptobenzothiazole. In polyvinyl plastic raincoats for the U.S. Army, 1% of solubilized copper 8-quinolinolate based upon the weight of the plasticizer was used to arrest fungus growth and 0.1% of phenylmercuric acetate was employed as an antibacterial agent (Daoust et al., 1951). In polyethylene, polystyrene, acrylonitrile, and cellulose acetate butyrate plastics, bacteriostatic agents such as 2,2’thiobis(4,6-dichlorophenol) or 2,2’-methylenebis(3,4,6-trichlorophenol) are claimed to produce a bacteriostatic plastic that may be safely used in contact with the skin (Dubin and White, 1959). Cumene hydroperoxide and other aromatic hydroperoxides and their sodium salts at a concentration of 0.5-5.0% are said to render thermo-
plastic and thermo-setting resins resistant to bacteria and fungi (McCall and Seiler, 1961).

Paints, varnishes, and lacquers are usually polymers with other ingredients, which are applied as a film to a surface on the site. Paint films are seldom broken down by microorganisms but since they are used principally for decoration the aesthetic value of a white paint finish splotched and blackened by mildew may be said to have deteriorated. Fungi growing on the surface of a paint film causes dirt to adhere to it and the fungus then obtains nutrients from the dirt and proliferates, thus compounding the problem. It was once thought that mildewing of paint surfaces was only a problem in the warm humid areas of the South but examinations of paint films from various parts of the country and Canada showed that this is not so, and what was often taken for dirt on paint in the northern areas was actually mold. Numerous species of fungi are found on paint films but the most prevalent one by far is *P. pullulans* (Goll and Coffey, 1948). On paint surfaces subject to high humidity indoors, as in textile mills, locker rooms, and breweries, one finds a preponderance of species of *Aspergillus* and *Penicillium*. Bacteria are found in cases of spoiled water-emulsion paints, where they may attack the emulsifying agent and the vehicle, resulting in gases and fermentation and putrefaction products. *Pseudomonas aeruginosa* and other species of this genus are commonly found in cases of such spoilage (Ross, 1963). Bacteria have also been found in appreciable numbers on deteriorating paint films at the interface between the wood and the paint. *Flavobacterium marinum* is the most common, and it is suggested that this growth is a cause of deterioration of paint films by peeling (Ross, 1963).

The composition of the paint has a significant effect on the resistance of the coating to microorganisms. The natural vegetable oils, fatty acids, glycerol, casein, and glue are all food for bacteria and fungi, whereas modified oils and synthetic resins are less susceptible or inert to attack. Tung oil is more resistant to mildew than linseed oil in paint, and this may be due more to the physical characteristics of the coating than any difference in susceptibility of the oils. The tung oil coating is considered to be more water-resistant than linseed oil films. Ross (1958) has shown that the fungi and bacteria most commonly found on paint are able to utilize the polymerized oil films as a source of carbon in their growth and metabolism.

Formulation of a paint can affect its mildew resistance. Commercial paints today purposely use chalking titanium dioxide pigment to promote cleaning of the surface. This also removes or hides mildew. The
use of a large ratio of pigment-to-oil makes the paint less susceptible to mold, but both this practice and chalking have their limitations as they lead to other types of paint failure. As a paint pigment, zinc oxide was employed long before it was realized that it helped to control mildew. It produces a hard paint film that not only promotes fungus resistance but is also toxic to fungi (Salvin, 1944). Paints with 50% zinc oxide were found to be mildew-free after 5 years' exposure (Vannoy, 1948). Barium mctaborate, a white pigment with limited hiding power but with mold- and bacteria-inhibiting characteristics, has been recently introduced. Four years' exposure tests with this product were made (Ross, 1963) comparing a preserved linseed oil paint over a similarly preserved and unpreserved undercoat. After the third year, the finish with the unpreserved undercoat began to crack and peel and had a higher bacterial count than the finish in which both coats contained the preservative pigment.

Whereas preservative pigments must be employed in paints in large quantities, fungicides in much smaller quantities protect paint films. Commercial practice, based upon a considerable literature of testing experience favors organic mercurials such as phenylmercuric propionate, di-(phenylmercury) deodcenyl succinate, and phenylmercuric oleate; chlorinated phenols such as tetrachlorophenol and pentachlorophenol; and copper 8-quinolinate as a powder or a solubilized formulation. The organic mercurials are used in concentrations of 0.05-0.5% (based on Hg content) of the dry weight of the paint. The chlorophenols are employed in 3–5% concentrations and copper 8-quinolinate in 0.5%. The organic mercury's are preferred because of their effectiveness in low concentration, thereby keeping the cost low. "Copper 8" is very effective but has a yellow-green color. Tetrachlorophenol at 4% has proved very effective for white paints as evidenced from use in tropical climates by the U.S. Army Corps of Engineers (Shapiro, 1958) and by the U.S. Navy Bureau of Yards and Docks. In furniture lacquers, 3% pentachlorophenol kept the finish in perfect condition in Ghana and Aruba for over a year whereas an untreated lacquer lost its gloss in 2 to 3 months (Zinay and Medireka, 1961). "Copper 8" at 0.5–1.0% does an excellent job and is employed in food plants where its low toxicity is an asset and in outdoor applications that will tolerate its yellow-green color. In varnish for treating electrical equipment, salicylanilide has proved most effective in combating invasion of mildew (Leonard and Pitman, 1951) but it must be employed at a concentration of at least 8%. The organic tin compounds were hopeful candidates for use in outdoor paints and lacquers but as yet have not
not expectations. Combinations of fungicides have sometimes been found to be more effective than each separately; for example, 0.08% of a mixture of 40% phenylmercuric quinolinolate and 60% copper 8-quinolinolate showed the same activity as 0.2% of phenylmercuric quinolinolate alone (Hirschfeld, 1959). Ramp and Grier (1961) reported excellent practical results with a combination of phenylmercuric propionate and 2,4,6-o-chloroanilino-s-triazine.

V. ELECTRICAL AND ELECTRONIC EQUIPMENT

Military forces fighting in the jungle territories of the South Pacific during World War II found that electrical and electronic equipment rapidly became mildew infested and inoperative. The question that arose was whether the failure was due to the fungus or merely to moisture, which incidently fostered fungus growth. Leutritz and Herrmann (1946) exposed plastic insulation materials to 97% relative humidity and fungi and in less than 2 hours most of the materials dropped from 2,500 to 1,000,000 MΩ initial resistance to 1000 MΩ. It is obvious that the fungus could not have grown in this time. After 2 months, when fungi had grown on all the specimens, the humidity was dropped and rapid recovery was effected, despite the presence of the fungus. The investigators concluded that the common commercial types of insulating material made from organic and inorganic-filled plastics are so rapidly affected by water absorption that fungus effects are not responsible for electrical leakage of insulation. On the other hand, Luce and Mathes (1948), on the basis of their experiments, incriminated fungi as causative agents. By employing fumigating vapors to control fungus growth they were able to separate the effect of moisture from the effect of fungi. They noted that with braided wire insulation possessing poor surface resistivity under high humidity, resistance fell rapidly in the presence of the fumigant. Fungus was obviously not implicated. However, with unbraided wire with superior electrical characteristics, it maintained its electrical resistance for 60 days in an atmosphere of 100% relative humidity and methyl bromide. Without the methyl bromide and with fungus inoculation the electrical resistance of this wire dropped from 1 million MΩ to 5 MΩ. In this case fungus was certainly responsible.

Blake et al. (1952) devised a different method for testing fungal susceptibility of wire insulation. They removed the wire from the insulation and in its place forced sterile nutrient agar, sealed the ends,
10. FUNGICIDES AS INDUSTRIAL PRESERVATIVES

and buried it in soil for extended periods. The agar was then examined for organisms, since any present would have had to gain access through the insulation. It was thus discovered that fungi were able to invade certain types of rubber insulation used for wire.

The problem of failure of electrical and electronic equipment can be very expensive in money or lives as, for example, when the failure is in a cable buried underground or in a portable transmitter used by soldiers in jungle warfare. To protect equipment from moisture and fungus, military electrical equipment was "tropicalized." This consisted of coating with wax, varnish, or lacquer containing fungicides, the components of the system and often all the equipment that would not be harmed by such coating. This protected the equipment from moisture and corrosion as well as fungi. The fungicides are essential, for although fungi grow better on components that contain cotton and paper and are especially vulnerable to attack, they find sufficient nutrient in dust and grease that is on the plastic parts and even the metal chassis to be able to grow on these surfaces. The fungus filaments contain moisture and salts and serve as bridges for electrical leakage. As well as surface coating, parts may be pressure impregnated with plastics, or better yet, whole systems may be "potted" in plastic, when this is feasible. To instruct industry in this subject, the Signal Corps of the U.S. Army published in the forties a graphic booklet for manufacturers of Signal Corps equipment entitled, "This Is Serious—Tropicalization—Why, How, What?"

Many different fungicides were investigated for use in coatings for tropicalization (Horner and Comlen, 1945), among which were the organic mercurials, the chlorinated phenols, and salicylanilide. Phenylmercurials in waxes were troubled by sublimation of the fungicide at high temperatures (Horner et al., 1945). Chlorinated phenols are also somewhat volatile and present a potential danger of corrosion due to release of chlorine on decomposition. Preferred above other fungicides was salicylanilide, as indicated by Military Specification MIL-V-173A (1952) calling for 7–8% salicylanilide as the only fungicide named. The work of Leonard and Bultman (1955) is most intriguing. They compared fungicidal varnish films exposed in a Panama jungle for their ability to suppress fungus growth. Compared over a period of 33 months at 8, 4, and 2% concentration in the varnish were pentachlorophenol, salicylanilide, phenylmercuric phthlate, uranyl nitrate, copper 8-quinolinolate, and p-toluenesulfonamide. While the last chemical showed no antifungal activity whatever in laboratory tests, it proved to be much superior to all the other chemicals.
VI. PETROLEUM PRODUCTS

It is probably true that all natural organic materials are susceptible to degradation by microorganisms. Petroleum products have been found to be no exception to this general premise. Petroleum oil poured on the ground is consumed by soil microorganisms. Methods of oil prospecting have been based upon microbial analysis of soils (Ritzman, 1961). A recent patent (Ritzman, 1962) describes a method for refining petroleum by using bacteria to remove the unwanted sulfur-, nitrogen-, and oxygen-containing contaminants that produce sludge. Dramatic interest in the action of microorganisms on petroleum resulted a few years ago from a series of disastrous crashes of jet aircraft. Microbial slimes were found fouling fuel filter systems and water separators. Aircraft were found with large amounts of sludge and bacterial growth on the wing tank bottoms and sides. When these deposits were removed, widespread corrosion was found in the areas covered by the sludge (Ward, 1963). Churchill (1963) lists both bacteria and fungi found in the green or brown slimes or bacterial mats. The predominating fungi were Hormodendrum hordei or Cladosporium, and the predominating bacteria were P. aeruginosa, A. aerogenes, and a Bacillus. While no air crash could be said to have been caused by microbial activities, Churchill does note there was filter plugging and fuel gauge malfunction. Also, in the wing tanks there was degradation of the top-coating material and pitting corrosion of the aluminum wing tanks, thus weakening the structure.

Microbially induced problems are not limited to aircraft fuels but are also found in turbine fuels, where microorganisms live and proliferate at the interface of the oil and the aqueous phase under the oil in the tank. The problem with aircraft became acute with the large-scale use of jets and the kerosene-type jet fuel. These fuels contain a large percentage of straight-chain paraffins and it has been shown that microorganisms taken from contaminated fuels utilize long, straight-chain hydrocarbons such as dodecane and cetane much more rapidly than the saturated naphthenes which are also found in these fuels (Smith et al., 1962). There is little problem with the aromatic hydrocarbons that are found in high octane gasolines. Furthermore, the anti-knock additives of the latter are toxic to microorganisms. The rate of consumption of oil by microorganisms may amount to 100 gal per year when the fuel is in 55,000-gal tanks and emulsification increases the area and rate 10-fold. Gasoline, kerosene, lubricating oil, and paraffin wax are oxidized in ascending order of ease. The problem
10. FUNGICIDES AS INDUSTRIAL PRESERVATIVES

is obviously not how much fuel is lost but the presence of the bacteria and their products. Bacteria may destroy rust inhibitors and deteriorate transformer oils and lubricants and diesel fuel in laid-up engines.

It is agreed that elimination of water from petroleum products and water bottoms from storage tanks would eliminate microbial deterioration but this has not been considered practical. Good housekeeping measures to filter out the sludge and dirt, remove as much entrained water as possible, and eliminate surfactants that allow the oil to absorb more water have been suggested and put into practice. Antimicrobial coatings for lining storage tanks have been of interest. Paracyn-lined jet-fuel storage tanks are claimed to have been found free of bacteria in 14 years' experience with 60,000 tanks. Laboratory tests indicate that the lining is bacteriostatic (McGregor, 1963). In another report (Griffith, 1961) a type-53 concrete fuel tank lining, a blend of saran and polysulfide latexes, with 1% of finely divided copper 8-quinolineolate, p-toluene sulfonamide, or tri nitro benzene was recommended. No greater amount of fungicide was suggested pending determination of the effect of the fungicides on the fuel. A study of the fungicidal and bactericidal benefits of metallic silver in the lining was reported under study.

Some authorities have little hope for the effective use of antibacterial and antifungal additives to control this biological deterioration of fuel and its associated problems. They argue that (1) inhibitors treat the effect and not the cause of the trouble, (2) economical chemicals for this purpose will not be found, (3) these chemicals may be harmful to the fuel and engine parts, and (4) resistant microbial strains will develop (Stormont, 1961). The usefulness of borates in water bottoms of fuel storage tanks for control of microbial growth at the interface has been known for some time. New interest in boron compounds has arisen from the bactericidal effect of boron motor fuel additives in gasoline. Several years' experience with these fuels in tankage free of slime and other microbiological products are reported (Stormont, 1961). Examples of organic boron additives for inhibiting bacteria in petroleum fuels in storage are potassium ethylene glycol borate; sodium 1,2-propanediol borate; potassium 1,4-butanediol borate; and sodium triethylene glycol borate (Willcockson, 1962). These are hydrocarbon soluble and can be used with or without a water bottom in the storage tank.

The U.S. Air Force found potassium dichromate to be effective in water bottoms at a 2% concentration in a service test at a southern
It is also evaluating potassium dichromate in a biocidal wash-rinse procedure for cleaning contaminated aircraft wing tanks (Churchill, 1963). Microbial gel deposition in petroleum distillate fuels in contact with an aqueous phase are said to be controlled by addition of oil-soluble materials such as a 40% solution of formaldehyde in n-propyl alcohol, o-phenylphenol in n-propyl alcohol, or by water-soluble and oil-insoluble compounds such as certain quaternary ammonium chlorides. The water-soluble compounds are dissolved in the water bottom or are added to the oil with a cosolvent. A concentration range of 0.01-1% is effective, although 0.1% usually suffices (Summers, 1961).

Twenty substances were evaluated in JP-5 jet fuel using 17 organisms obtained from JP-4 fuel storage tanks. Four n-alkyl quaternary ammonium chlorides and an n-alkyl isoquinolium chloride prevented bacterial growth when incorporated in the solution at not less than 50-100 ppm. Only the isoquinolium salt caused no turbidity to either the fuel or the water phase. Silver metal was toxic to bacteria at very low concentrations but was not fungicidal at these concentrations. Tetraethyl lead and ethylene dibromide, the two principal ingredients of antiknock mixture, incorporated individually in JP-5 at 0.13 and 0.5 ml per gallon, respectively, were inhibitory to bacteria. In combination, however, they were less active (Klemme and Leonard, 1960).

Petroleum crude oil is obtained from wells that tap pools of oil deep in the ground. When these wells are pumped dry, it is still possible to obtain considerably more oil from isolated pockets by a process known as secondary recovery or water-flooding. Fresh or salt water is pumped into the well to displace the oil that is trapped in the interstices of the formation. Bacteria that are introduced with water may proliferate and interfere with this process by clogging the porous sands through which the water must pass. There is also another problem, that of microbially induced corrosion of the pipes and equipment used in the flooding process.

While many types of microorganisms play a role in this unhappy drama, the generally-recognized villain is the sulfate-reducing bacterium, Desulfovibrio desulfuricans. This organism is an anaerobic autotroph which is found in lakes, marshes, and rivers. It obtains energy for survival by reducing the inorganic sulfate present in water to hydrogen sulfide, which reacts with iron to produce black iron sulfide and the pitting-type corrosion in iron pipes. Corrosion may be produced in several ways. Hydrogen sulfide itself is corrosive, especially in the presence of oxygen. But more important is the electrochemical corrosion brought about by the bacteria. Iron and steel pipe in water
develops anodic and cathodic areas but an equilibrium is reached when no further corrosion occurs. Under the anaerobic conditions in which the sulfate reducer grows, the organisms utilize the hydrogen in their metabolism thereby acting as cathodic depolarizers and thus accelerating the electrochemical degradation. Iron sulfide which is produced is cathodic to metallic iron and this further aids in the corrosion (Wolfson, 1960). This corrosive damage is by no means limited to oil wells but is found in all types of wells, underground pipes, and tanks exposed to water. The sulfate-reducing bacteria do not restrict their activities to iron but attack underground cables and concrete pipe and tanks.

Other types of bacteria are also involved in corrosion. The sulfur-oxidizing bacterium, *Thiobacillus suboxydans*, oxidizes sulfur to produce sulfuric acid, the concentration of which has been known to reach 10%. Iron bacteria, which are found in water systems, remove iron from the water and deposit a sheath of iron hydroxide around themselves. They may clog filters, screens, and sand beds and they may provide anaerobic conditions that permit the growth of the corrosion-causing bacteria. Where the water used in flooding is stored in open tanks and ponds, algae are almost sure to grow and while they will cause plugging and fouling in themselves, they can also serve as a source of nutrient for some autotrophic bacteria.

Biocidal chemicals have two primary requirements. They must be effective in great dilution and they must be cheap, since a large amount of water is employed. Most of the chemicals that have been considered as slimicides in paper mills have received consideration in flood-water operations. Halogenated phenols have found use in this application. In a study of 63 phenolic compounds for their inhibition to *D. desulfuricans*, three at 25 ppm completely inhibited hydrogen sulfide production for 28 days. These compounds were 2-bromo-4-phenylphenol, 4-chloro-2-cyclohexylphenol, and 2-chloro-4-nitrophenol (Bennett et al., 1958). A number of germicidal quaternary ammonium salts and fatty primary amine salts have been effective in low concentration (Lagarde, 1961). The latter worker also found formaldehyde to be active at 10 ppm. Tri(hydroxymethyl) nitromethane slowly releases formaldehyde and is marketed for oil-well flooding systems (Commercial Solvents Corp., 1958). Sulfur bacteria were found to be more sensitive to substituted nitroparaffins than to mercurials or phenols. Of 200 tested the following six compounds completely inhibited *Desulfovibrio* for 28 days: 3-chloro-3-nitro-2-butanol, 2-chloro-2-nitro-1-butanol stearate, 2-chloro-2-nitrobutyl acetate, 4-chloro-4-nitro-3-hexanol, 1-chloro-1-
nitro- and 2-chloro-2-nitro-1-propanol (Bennett et al., 1960). Glutaraldehyde has been found to be effective in field and laboratory tests as low as 3-5 ppm (Union Carbide Chemicals Corporation, 1968). It is economical and completely water miscible. Triethyl tin chloride in 1-10 ppm is claimed for the protection of ferrous oil well casings in oil well brine from corrosion due to Desulfovibrio (Thompson, 1963).

A novel method for protecting pipelines, posts, and tanks buried or partially buried in the ground from bacterial corrosion involves the use of (1) a protective coating such as polyvinyl chloride or bitumen-impregnated wrapping containing a suitable germicide and (2) the treatment of a 1-6-inch contiguous layer of fill with a different germicide. A double defense is thereby provided against the organisms, by preventing acquired resistance to the germicide. Arsenicals, chlorophenols, and cresols are mentioned as germicides (Hitzman and Schneider, 1961).

Petroleum products known as cutting oils are subject to bacterial and fungal degradation and require preservation. Cutting oils are used in the metalworking industry as lubricants and coolants for machine cutting tools. As lubricants, they reduce friction and save power, and as coolants they reduce the heat generated by high-speed machines and prolong the life of the tool and prevent distortion of the metal that is worked. In 1957, the metalworking industry was estimated as operating 3.4 million production machines that require lubricants and coolants. One company alone, the Chicago Screw Company, is said to use 150,000 gal of cutting oil or 150 gal per machine per year (Anonymous, 1957). There are three types of cutting oils: the straight, the soluble, and the water-solution or synthetic type. The soluble, or emulsifiable oil, makes up about 35% of the market (as compared with 60% for the straight) and is the type most troubled by microorganisms. The soluble oil contains about 90% mineral oil, the rest of the composition being a coupling agent such as a glycol alcohol, an emulsifying agent, a wetting agent, an antifoam, a rust inhibitor, a water softener, and a germicide. The concentrate is diluted about 20- to 40-fold with water when used. The oil is contaminated with bacteria by the diluting water and in the general machine shop operation. Although the fresh oil may be nearly sterile, studies of the used emulsions from machine shops show them to contain from a few million to almost a billion bacteria per milliliter (Bennett, 1956). The bacteria may oxidize components of the formulation causing spoilage and producing bad odors, so that the material must be discarded prematurely. Even
the disposal of badly contaminated oil is a problem. But there is also
the problem of dermatitis to the machine operator and the further
health hazard of the possible dissemination of pathogenic microorga­
nisms which have been shown to survive in the emulsified oil (Bennett,
1956). A wide range of bacterial flora is represented in a contaminated
cutting-oil emulsion, but coliform bacteria, *Pseudomonads,* and *Desulf­
ovibrio* are of greatest interest from the standpoint of public health,
breakdown of the emulsion, and possible corrosion of the equipment.
The synthetic, water-based coolants, unlike the emulsifiable-oil type,
are more susceptible to molds and yeasts than bacteria. Water hardness
and level of the coolant appears to affect the floral type that will
predominate (Kitzke and McGrey, 1963).

Since it would be impractical to attempt to keep the coolant sterile,
microbial inhibitors are employed to control the spoilage. A great
many compounds have been screened (Bennett, 1956). They must be
soluble in oil and water, noncorrosive, and nonirritant to the operator.
The most active are the organic mercuries, but in practice there has
been a tendency to shy away from mercury because of fear of toxicity.
Interestingly enough, in 1956, a study showed that none of the com­
mercial cutting-oil inhibitors was satisfactory in controlling bacterial
growth (Wheeler and Bennett, 1956). Five compounds that these work­
ers found to be effective emulsion-oil inhibitors were: 2,4,5-trichloro­
phenol; 2,4,6-trichlorophenol; 2,2'-thiobis(3,4,6-trichlorophenol); glyc­
oxal; 1,3-dichloro-5,5-dimethyl hydantoin; and tris(hydroxymethyl) ni­
tromethane.

The high activity per unit cost of the last compound stimulated in­
terest in examining other substituted nitroparaffins. Several hundred
were tested for their activity against bacteria in a metal-cutting
fluid. Of these, 16 were selected for further study against pseudomonads
and sulfate-reducing bacteria in 6 different major cutting fluids of the
soluble oil type, diluted 1:25. All compounds, and formaldehyde, were
employed at 800 and 1000 ppm and the number of days during which
growth was completely inhibited was recorded. The compounds were
found to vary considerably in effectiveness with the different coolants.
Four compounds were found superior against both the aerobic and
anaerobic organisms in all the products. These were: (1) tris(hy­
droxymethyl) nitromethane; (2) 2-nitro-2-ethyl-1,3-propanediol dipropi­
onate; (3) 2-bromo-2-nitropropyl acetate; and (4) 2-nitro-2-ethyl-1,3-
propanediol. In most cases the coolants were completely protected for
a period of 50–100 days with these four compounds, even though they
had been reinoculated each week (Bennett and Futch, 1960). The com­
pounds were soluble in cutting fluids, were cheap, and relatively nontoxic. They were superior to 100 phenolics previously studied. Whereas tris (hydroxymethyl) nitromethane is believed to be antimicrobial, at least in part, due to its slow release of formaldehyde in an alkaline environment, the other nitroparaffins do not possess this property and must function by another mechanism. The release of formaldehyde introduces some difficulties in incorporating such a compound into the oil concentrate. The other nitroparaffins do not present such a problem.

In a comparison of nitroparaffins with trichlorophenol and sodium ethyl mercury thiosalicylate (Carlson and Bennett, 1960), the same cutting oil was employed and the oil-water dilution ratio was varied. It was found that this ratio has a profound effect on the activity of these inhibitors. Using *P. aeruginosa* and dilution ratios between 1:10 and 1:100, the organisms were usually most difficult to inhibit when the dilution was 1:40, being less difficult in the more dilute and more concentrated emulsions.

The mercury compound was generally the best inhibitor of the group. Of 16 nitroparaffins, numbers 2 and 4 of the list of four given above, and 2-nitro-2-methyl-1,3-propanediol were outstanding, giving inhibition at no greater than 50 ppm over the dilution range. Trichlorophenol gave inhibition with 50 ppm at a 1:20 but at a 1:30 dilution it took 1000 ppm of the compound to suppress the bacteria. The investigators offer no explanation for this dilution ratio phenomenon and why different inhibitors react differently toward the change in dilution. It may be related to the inversion of the emulsion from water-in-oil at the lower dilutions to oil-in-water at the higher dilutions. The compounds least affected by the changeover are probably more compatible toward both phases and may concentrate at the interface where the organism is likely to be found. The phenomenon is certainly an interesting one both from its practical and fundamental aspects.

VII. LEATHER

Leather is one of the materials most susceptible to mildew. A pair of shoes that have not been worn for some time covered with mildew is a common sight, especially to persons living in the warmer, more humid regions. Leather will support mold growth at 75% relative humidity, which is too dry for many other products (Block, 1953). But, while control of mildew on finished leather is a problem, control of bacteria is
a problem in the processes in the preparation of leather from skins. Leather is a protein, namely collagen, and any destruction of collagen by proteolytic bacteria in the manufacturing process will produce an inferior product.

The leather-making process is comprised of the following steps: curing, soaking, unhauling, bating, pickling, tanning, oiling, and finishing. After removal from the animal, the skin is dried in air or by dehydration with salt. This process helps preserve the skins until they can be processed further. Growth of chromogenic, halophilic bacteria stain the hide, producing a condition known as “red heat.” Paludrine was found to be very effective in controlling bacteria that produce these stains when used in 1–2% in the salt or 0.06% in the salt soak liquor to produce about 0.45% on the dry weight of the hide. Even in the absence of salt, paludrine was said to give better preservation in the same treatment time (6 hours) as sodium pentachlorophenate (Nandy and Sen, 1960). Sodium silicofluoride has also been mentioned in prevention of red stain (Kritziinger, 1954). “Salt stain,” also of bacterial origin, produces stains colored from brownish to blue-green but these can be prevented by a few percent of sodium carbonate added to the salt (Dahl, 1956).

When the hides are delivered to the tannery they are soaked to soften and clean them. There is greater danger of attack from putrefactive microorganisms in the presence of the blood and soluble nitrogenous products of the hide. The pH is maintained slightly on the alkaline side to reduce this danger and sodium hypochlorite is added as a disinfectant. The reduction in salt content after soaking and the conditions for enzyme action which also favor the growth of bacteria both contribute to make the hides susceptible to degradation in the enzyme unhauling and the bating steps. Investigations by Cordon et al. (1961) show that though the unhauling solutions are not sterilized, there is no damage to the hide, no putrid odor, and no change in the enzyme action if the bacterial population is kept under 100 million per milliliter. Without bactericides, the count reached 325 million per milliliter in 24 hours. All the bactericides tested kept it below 100 million for 2 days. The bactericides included 0.1 and 0.2% Storezol-S (a mixture of pine oil, cresol, α-phenylphenol, pentachlorophenol, and 2-naphthol), 0.02 and 0.04% sodium trichlorophenate, 0.05 and 0.1% of 2-naphthol, 0.1 and 0.15% BSM-11 (10% phenylmercuric acetate and 50% potassium trichlorophenate), and 0.015 and 0.025% phenylmercuric acetate. BSM at 0.1% held the population below 40 million per milliliter for 11 days.
In pickling, the hides are treated with sulfuric acid and salt and are no longer in danger of bacterial attack but they are susceptible to attack by molds. This may be prevented with organic acids, particularly acetic acid (Pleass, 1935). In vegetable tanning, bacteria, yeast, and molds are found and the greatest fear of economic loss due to microorganisms in this operation is the destruction of the tanning liquor, although there is also a possibility of destruction of the skin fibers. Preservatives commonly used in tanning liquors are cresols, naphthols, sodium silicofluoride, and p-nitrophenol (Dahl, 1956).

The finished leather, having been tanned, is relatively safe from destruction by microorganisms but, containing oils and greases added as leather lubricants in the finishing steps, it is very susceptible to molds. Not only is mildew unpleasant from an aesthetic standpoint, but in removing the oils and greases it can cause stiffness, cracking, and loss of tensile strength (Dahl, 1956). Many fungicides have been examined for preventing mold growth on leather (Musgrave, 1948; Horigan and Sago, 1948; Dahl and Kaplan, 1957). The one compound that has found acceptance, particularly by the military, as copper naphthenate has in the textile field, is p-nitrophenol. Used at about 0.3% of the weight of the leather, it has proven most generally satisfactory with regard to mildew control, toxicity, and cost. It is introduced into the leather by dissolving it in the greases in the finishing operations. While it is fairly water soluble, it is bound to the leather and not readily removed from the leather with water. Although it is yellow, the color is hidden by the color of the leather. If it is lost from the leather by excessive leaching, it may be reapplied by incorporation in leather dubbings.

Unfortunately, no fungicides have been found that will last for the service life of the leather articles. A few chemicals appear to be better for leather than p-nitrophenol with respect to antifungal activity, color, or resistance to leaching; although the supremacy of p-nitrophenol does not, as yet, appear to be threatened. A close relative, 2-chloro-4-nitrophenol is somewhat more fungicidal on leather than p-nitrophenol and not as water soluble (Dahl and Kaplan, 1958). Tetrachlorohydroquinone is colorless, nearly insoluble in water, less irritating to skin, and almost as effective as p-nitrophenol. Bis(4-nitrophenyl) carbonate and bis(2-chloro-4-nitrophenyl) carbonate are esters of the nitrophenols that evidence about the same toxicity as the free nitrophenols, probably due to hydrolysis (Dahl and Kaplan, 1958). Their advantages are lack of color and insolubility in water. Esters in addition to the carbonates were investigated (Dahl and Kaplan,
10. FUNGICIDES AS INDUSTRIAL PRESERVATIVES

1960) with similar results. The esters are inert reservoirs of the active phenols (without the objectionable properties of the latter) which release the phenol when the conditions for mold growth, moisture and warmth effect their hydrolysis. A promising nonphenolic fungicide for leather is 5,6-dichloro-2-benzoxazolinone. It protects very mildew-susceptible, vegetable-tanned, sole leather at 0.4% and other leather at lower concentrations. It is stable, colorless, nonvolatile, and relatively resistant to removal by leaching with water (Dahl and Kaplan, 1961).

VIII. DRUG AND COSMETIC PRESERVATIVES

The drug and cosmetic industry produces many different products, some of which must remain free of microorganisms upon storage and use, while others may be susceptible to microbiological spoilage in a variety of ways. Attack may be by bacteria, fungi, and yeasts. Products include ointments, lotions, syrups, extracts, jellies, powders, and others. They may be for topical or internal application. Toxicity, odor, color, and taste are all extremely important considerations.

Alcohols represent an important group of preservatives for this industry. These include ethyl alcohol, isopropryl alcohol, benzyl alcohol, phenethyl alcohol, glycerol, propylene glycol, and chlorobutanol as the more widely used members of this group. The lower alcohols are used primarily with materials that will tolerate relatively high concentrations of the alcohol. They may be used in lesser concentration to supplement the preservative action of syrups or of solutions that have high salt concentration. For acid solutions, Gabel (1921) recommended 15% ethanol whereas in neutral or alkaline solutions 17.5% was advised. Isopropanol is often used in place of ethanol. Glycerol helps in preservation, but when used as the only preservative at least 50% is required (DeNavarre, 1941). Propylene glycol has preservative properties similar to ethanol (Rae, 1938). Benzyl alcohol is active at 1-3% and is used in preparations intended for injection because it also has anesthetic properties (Nogueira, 1962). Phenethyl alcohol is bacteriostatic particularly toward gram-negative bacteria and is used to some extent in ophthalmic solutions. Chlorobutanol demonstrated its microbiocidal effectiveness in aqueous solutions of ophthalmic drugs contaminated with Pseudomonas aeruginosa (Lawrence, 1955) but, because of its instability upon autoclaving, it is not a safe antibacterial agent to add to parenteral preparations prior to autoclaving (Gladhart et al., 1935).

Essential oils contain individual compounds that have bacteriostatic and fungistatic properties. Cade (1957) published a review of the
subject and further investigations have been conducted by Maruzzella (1963). While some of the constituents such as thymol, benzaldehyde, and salicylaldehyde are very active antimicrobially, the essential oils are employed for flavoring or perfuming in such small quantities that they have little preservative value for the cosmetics or pharmaceuticals, which require other preservatives to make sure they resist spoilage.

Benzoic acid and sorbic acid used as food preservatives, are suitable for use in cosmetics and drugs. They suffer from the restriction that they are only effective in an acid medium (Rahn and Conn, 1944; Wickliffe and Entrokin, 1964). Although they are weak as preservatives, their low toxicity to man and consequent safety makes them appealing for use in preparations in which they might be effective. Both materials are reduced in activity by nonionic surfactants (DeNavarre and Bailey, 1956) but it has been shown with sorbic acid that the degree of inactivation varied with the nonionic surfactant employed (Blaug and Ahsan, 1961) and the unbound sorbic acid was antimicrobial. Dehydroacetic acid which has been used as a preservative in toothpaste and numerous cosmetic products is similarly inactivated by both nonionic surfactants (Wedderburn, 1958) and neutral to alkaline environments (Wickliffe and Entrokin, 1964).

The esters of p-hydroxybenzoic acid, or the “parabens” have been the most popular preservatives for the drug and cosmetic industry. Their literature and properties have been thoroughly surveyed (Neidig and Burrell, 1944; Sokol, 1952; Gottfried, 1962). The combination of high activity against bacteria and molds with desirable physical properties, low toxicity, and reasonable cost has led to their widespread use. The esters that are employed include the methyl, ethyl, propyl, butyl, and benzyl. The greatest antifungal activity was found in the hexyl and heptyl esters (Huppert, 1957) but the methyl and propyl esters are in the greatest use because of their higher water solubility. They are often used in combination, as for example, 0.18% methyl and 0.02% propyl. The parabens are quite stable and are active over a wide pH range, although they are more effective on the acid side. They are bound by anionic surfactants and other substances, the antimicrobial activity being determined by the unbound fraction (Pisano and Kostenbauder, 1959). The parabens are employed in hand lotions, facial masks, polio vaccine, ointments, ophthalmic preparations, and hair-waving fluids.

Phenols are also important preservatives. Phenol, itself, has a number of disagreeable characteristics which include skin irritation, high toxic-
ity, odor, volatility, and incompatibilities, but it has found recent interest as a virucide. o-Phenylphenol was reported to have a greater activity against microorganisms from deteriorating syrups than sorbic acid, benzoic acid, and the parabens according to Schimmel and Husa (1956). A purified grade recommended for use in cosmetics is said to be nonirritating, nonallergenic and active against a spectrum of bacteria and fungi. p-Chloro-m-cresol is quite active against microorganisms and has been employed in pharmaceutical solutions and in cosmetics (Wells and Lubowe, 1964). p-Chloro-m-xylenol is said to be active on the alkaline side but its activity is diminished in the presence of nonionic surfactants. It has been used widely in Europe.

Regardless of their potential hazards in toxicity and allergenic reaction, organic mercurials are so highly bacteriostatic and mycostatic as to be useful in subtoxic concentrations. For example, thimerosal (merthiolate) and the phenylmercuric salts are effective preservatives of parenteral and ophthalmic preparations in concentrations of 0.001–0.0005%. To provide water solubility thimerosal is used as the sodium salt, and the phenylmercuric as the nitrate or acetate. Phenylmercuric borate and phenylmercuric benzoate have also found application in cosmetics (DeNavarre, 1962). Organic mercurials are slow-acting bactericides and this has questioned their use in ophthalmic products where rapid bactericidal action is required (Riegelman et al., 1956). Mercurials are inactivated by sulfides and cannot be used in preparations containing them.

Quaternary ammonium salts are useful in ophthalmic and similar solutions, where their rapid bactericidal effects are required and where problems of incompatibility are minimized (Kohn, 1963). They are effective in very low dilutions, about 0.0001%, but they are incompatible with anionic surfactants such as soaps, nonionic surfactants, organic matter, and certain inorganic salts. Compounds of interest are cetyltrimethyl ammonium bromide, alkyldimethylbenzyl ammonium bromide, and diisobutylphenoxethyl dimethylbenzyl ammonium chloride.

Several newer antimicrobial preservatives for the drug and cosmetic trade deserve mention. Bronopol, or 2-bromo-2-nitropropane-1,3-diol, is claimed to demonstrate very high activity against P. aeruginosa and to be active in the presence of nonionics. Recommended as a preservative for shampoos, it is unstable in sunlight and alkaline solutions. Dioxin, or 2,4-dimethyl-6-acetoxy-1,3-dioxane is offered for use as a cosmetic preservative. It is said to be active over a wide pH range and maintains its activity in the presence of nonionics. Odor or discoloration problems are possible. Dowicide Q, a quaternary of hexa-
methylene tetramine and a halo hydrocarbon, liberates formaldehyde and is claimed to be active over a wide pH range and in the presence of nonionics (Scott and Wolf, 1962). The odor of formaldehyde could be objectionable in products that are used on the person. Chlorohexidine, or 1,1-hexamethylene bis[5-(p-chlorophenyl) biquanidine], has rapid sterilizing action when used at a concentration of 0.025% and is considered an excellent product for ophthalmic solutions (Anderson et al., 1964). Neomycin, one of the more stable antibiotics, is employed in ophthalmic solutions together with a quaternary ammonium compound (Dale et al., 1959). For aqueous hormone suspensions this was considered to approach the ideal from a self-sterilizing standpoint.

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10. FUNGICIDES AS INDUSTRIAL PRESERVATIVES


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1. INTRODUCTION

This chapter deals primarily with the use of chemicals to prevent the destruction of wood by fungi. Living organisms other than fungi are
known to destroy wood, and chemicals are used to combat them, but such treatments will receive only casual attention in this discussion. Of the various living organisms that attack wood, wood-destroying fungi are the most widespread and cause the greatest economic loss. Despite the extensive use of preservative treatments, it has been estimated that the loss of wood products through biological attack approaches $1.5 billion annually. Of this loss, about two-thirds is due to decay.

Under many conditions of use, wood is not subject to biological attack and untreated wood gives excellent service. Proper building practices assist in avoiding decay and termite damage, but under some conditions a hazard is unavoidable. Sometimes the use of naturally durable species is a satisfactory solution to the problem. Such species are of limited availability and do not always provide the degree of protection desired. Frequently the most practical solution lies in the use of preservative-treated wood.

As mentioned, living organisms other than fungi may attack wood products. Under some conditions, as in the storage of logs in ponds, bacteria may develop in wood. Although bacteria may increase wood's porosity and cause it to absorb excessive preservative in subsequent dip treatments, they generally do not present a serious practical problem.

Insects, especially termites, cause serious damage to wood. Experience has shown that treatments effective in preventing decay are also generally effective in controlling termite attack. For this reason, it is feasible to discuss the preservation of wood for land use from the standpoint of preventing decay.

The treatment of wood to be used in coastal waters falls into a special category of wood preservation, a discussion of which is outside the scope of this chapter. In saline or brackish waters, certain marine animals bore into wood and cause serious damage to harbor structures. They show marked resistance to many chemicals that are effective fungicides. Even those preservatives that do control their attack must be used in much heavier retentions than are customarily used where only protection against decay is needed.

Higher in the biological scale are organisms that occasionally damage wood. Rodents, for example, cause some damage to wood in use although less than to seedlings and young trees. In some parts of the United States, the damage to wood utility poles caused by woodpeckers is a problem of some magnitude.

The use of fungicides to protect living trees is confined mainly to ornamental trees or to trees that yield fruit, nuts, rubber, and other products with an intrinsic value much higher than wood. As a rule, a
tree that is cut for its wood has grown without the benefit of fertilizers or biocides. Compared with the use of fungicides to protect plant crops, their use in the growing of trees for wood is insignificant. In the protection of materials in use from biological deterioration, however, wood preservation is somewhat of a giant. On the basis of annual consumption, the use of coal-tar creosote for treating wood is the most important application of a fungicide to nonliving materials.

By usage, the term “wood preservation” has come to connote the treatment of wood with chemicals to prevent its destruction by living organisms. A related use of fungicides is their application to freshly cut lumber to prevent development of fungi that do not destroy wood but cause unsightly stains on the surface and in that way degrade the lumber. Such organisms are known as sap-stain organisms since their development takes place only in the sapwood of certain species, especially coniferous species such as pine. The economic loss due to such organisms would undoubtedly be very high were it not for the widespread practice of dipping lumber in antisap-stain chemical solutions. Chemicals of this kind are also applied to saw logs in storage and to freshly peeled poles before they are stacked for air seasoning prior to pressure treatment. Application of chemicals to wood for protection from living organisms is considerably broader than the subject discussed in this chapter.

II. Present Status of the Wood Preserving Industry

Historical accounts of the development of the wood preservation industry are given in other publications. The subject is briefly reviewed by Hunt and Garrott (1953).

The U.S. Forest Service annually publishes statistics on wood preservation (Merrick, 1963). Large-scale commercial treatments are covered quite thoroughly. Because of difficulty in covering small-scale non-pressure treatments, the industry is somewhat underestimated. Volumes of the principal wood products treated in 1963 are shown in Table 1. A total of 188,939,918 gal of liquid preservative, including 125,609,550 gal of creosote, was used as well as 30,329,298 lb of solid preservatives. This included over 5 million lb of chemicals used as fire retardants, which in some cases contained preservative salts. Solid pentachlorophenol accounted for 15,648,472 lb.

Most wood preservatives are used mainly in pressure treatments so that the statistics on pressure treatments give reliable figures for the use of those materials. Pentachlorophenol, however, is used to a con-
TABLE I
WOOD PRESERVATIVES USED IN 1963

<table>
<thead>
<tr>
<th>Preservative</th>
<th>Gallons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liquids</strong></td>
<td></td>
</tr>
<tr>
<td>Straight creosote</td>
<td>80,451,326</td>
</tr>
<tr>
<td>Creosote-coal tar solution</td>
<td>23,394,705</td>
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<tr>
<td>Creosote-petroleum solution</td>
<td>11,004,847</td>
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<tr>
<td>Creosote-pentachlorophenol solution</td>
<td>9,877,087</td>
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<tr>
<td>Other solutions</td>
<td>290,785</td>
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<tr>
<td><strong>Total creosote</strong></td>
<td>125,060,500</td>
</tr>
<tr>
<td>Coal-tar content of creosote-coal tar solution</td>
<td>11,732,439</td>
</tr>
<tr>
<td>Petroleum content of:</td>
<td></td>
</tr>
<tr>
<td>Creosote-petroleum solution</td>
<td>14,970,787</td>
</tr>
<tr>
<td>Petroleum-pentachlorophenol solution</td>
<td>36,564,241</td>
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<td>Other solutions</td>
<td>62,901</td>
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<td><strong>Total petroleum</strong></td>
<td>51,587,929</td>
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<table>
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<th>Preservative</th>
<th>Pounds</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solids</strong></td>
<td></td>
</tr>
<tr>
<td>Pentachlorophenol</td>
<td>15,648,472</td>
</tr>
<tr>
<td>Fluoride-chromate-arsenate-dinitrophophenol, Type A</td>
<td>3,442,077</td>
</tr>
<tr>
<td>Charoasted zinc chloride (including copperized CZC and CZC-FR)</td>
<td>1,808,988</td>
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<tr>
<td>Acid copper chromate</td>
<td>1,374,440</td>
</tr>
<tr>
<td>Fluoride-chromate-arsenate-dinitrophophenol, Type B</td>
<td>1,315,127</td>
</tr>
<tr>
<td>All other solid preservatives</td>
<td>818,328</td>
</tr>
</tbody>
</table>

*Fire-retardant formulation.

A considerable extent in nonpressure treatments of posts and lumber, and especially in dip treatments of millwork in solutions of pentachlorophenol and water-repellent materials dissolved in low-boiling petroleum oils. An unpublished estimate by an industry representative places the annual use of pentachlorophenol in nonpressure methods at 4 million lb.

Included among the miscellaneous materials receiving preservative treatment in 1963 were over 3 million sq ft of plywood. It is believed that most of this consisted of exterior grade plywood. For convenience in handling, it is common practice to treat the finished plywood rather than the individual veneers before gluing.
Preservatives are used to some extent in the manufacture of wood-base materials such as particle board, fiberboard, and hardboard. Statistics on such treatments are not available.

Particle board may be defined as a product made in panel form from dry wood particles that have been coated with a binder and are formed and bonded to shape by pressure and heat. In the past, it has been used mainly in interiors where preservative treatment was considered unnecessary. There is considerable interest in its use in sheathing whereby preservative treatment may be necessary in some cases.

The term “fiberboard” spans a wide range of products divided into several groups on the basis of density. Natural bonds are largely responsible for their strength although some binder may be used in their production. Although it is a relatively simple matter to add fungicides or insecticides during production, this is not commonly done because most products are used where decay or insect attack is not likely to occur. Some manufacturers of fiberboard add toxic chemicals for that part of their output that is intended for use in tropical areas where drywood termites are found. Research is in progress on the preservative treatment of hardboard for certain specialty uses where protection against fungi and insects may be needed.

There is little parallelism between statistics on the production and preservation of different wood products. This, of course, is largely due to differences in conditions of use which govern the need for preservative treatment. The disparity probably is accentuated because presently available methods of treatment are more satisfactory for some products than for others.

Cross ties and poles together comprise less than 3% of the wood cut annually in this country, but they account for over 60% of the treating business. These products have in common certain characteristics that are conducive to preservation: (1) they are nearly always used under conditions favorable to decay; (2) they are relatively expensive to replace; (3) they may be framed before treatment, thus avoiding removal of any part of the heavily treated outer zone which would reduce the effectiveness of the treatment; (4) they do not need to be clean, odorless, and paintable so that the effective, low-cost, oil preservatives may be used; and (5) they frequently are purchased in large quantities which minimizes unit production, sales, and distribution costs.

The importance of economics is illustrated by statistics on poles and posts, two products similar in technical aspects. On the basis of total volume cut, posts outrank poles by a substantial margin; but on the basis of volume treated, the ratio is more than 4:1 in favor of poles.
III. CHARACTERISTICS OF WOOD-ATTACKING FUNGI

Many saprophytic fungi grow in and damage the heartwood of trees, causing what is known as heartrot. Fungi that invade wood cut from a tree are also saprophytes; the wood cells apparently must be dead or dying before such fungi will enter them.

From a practical standpoint, it is convenient to classify wood-attacking fungi into four groups, according to the damage they cause. These groups are the molds, the sap-stain fungi, the decay fungi, and the soft rotters. Technical distinctions between them are not always clear.

If the fungus development is superficial, and can be removed by brushing or shallow planing, it is commonly termed "mold." Although molding per se has no significant effect on strength, the presence of heavy molding shows that moisture and temperature conditions have been favorable for the development of decay fungi also; hence incipient decay may be present but masked by mold. Molding may make wood more permeable to rainwater and thus promote decay of wood in storage. On the other hand, some molds that penetrate the interior may have a beneficial effect on the penetration of preservatives, as pointed out by Lindgren and Harvey (1952).

Sap-stain fungi penetrate the sapwood and, because of the dark color of the mycelium, impart to the wood a color ranging from gray to almost black—depending upon the fungus and wood species. Because the discoloration tends to penetrate to a considerable depth, it produces a permanent blemish that cannot be surfaced off. Sap-stain fungi, for the most part, depend on the nonstructural elements of wood, such as sugars and starches, for their food. Consequently, most strength properties are not seriously affected by sap-stain fungi, a small loss of toughness being the most noticeable change. Heavily stained wood tends to be more permeable to preservatives than sound wood.

Of the organisms that can invade wood, the decay-producing fungi are by far the most serious. They use the cell walls as food and gradually change the wood into a material that is practically devoid of its normal strength. The spread of decay may be accomplished by the dissemination of spores by air currents or it may occur merely by the spread of hyphae from a piece of decaying wood into an adjacent piece of sound wood. The rate of growth of fungi is dependent upon a number of factors, of which the most important are moisture, temperature, air supply, and the presence or absence of extraneous materials that have a toxic effect on the fungus.
For fungi to become established, wood must contain moisture in excess of what is called the fiber-saturation point, at which cell cavities are empty while the cell walls are completely saturated with water apparently held by loose chemical bonds. Wood that has been dried below a moisture content of approximately 30%, and then exposed to high relative humidity, cannot absorb the moisture needed for decay. It is only when such wood is wet with water that decay is possible. It appears that some free water in the cavities is necessary for growth of fungi. The average moisture content of wood at the fiber-saturation point is somewhat less than 30%. It tends to be higher in hardwoods than in conifers.

The fungus hyphae spread through the wood by penetrating the pits or puncturing the cell walls with bore holes. During the early stage of decay, called incipient decay, there is little loss of wood substance and often little loss in most strength properties except that there may be a large decrease in resistance to impact (toughness). As decay proceeds, the cell walls are decomposed by the enzymes of the fungus, and the breakdown of the wood is accompanied by formation of carbon dioxide and water. At the same time the structure of the wood gradually undergoes a profound change to the well-known condition of rot.

All wood-destroying fungi are aerobic and, while their requirements for oxygen are not great, they are vital as shown by the fact that when wood is kept waterlogged it does not decay. The area just below the groundline of fence posts and telephone poles generally provides a compromise between air and moisture supply that is most conducive to decay. In regions of low rainfall this band of maximum decay of round timbers is somewhat lower than in regions of higher rainfall.

Fungi are capable of growing over fairly wide temperature ranges which vary somewhat from species to species. At temperatures below this range they tend to remain dormant; temperatures considerably above this range cause death. The optimum temperature of most species of wood-destroying fungi lies between 75° and 90° F.

Wood-destroying fungi are killed by the temperatures prevailing in dry kilns or in treating cylinders in which wood may be steamed prior to impregnation with preservatives. The exposure time must be sufficient to heat the wood to the required temperature at the depth to which infection has penetrated.

Fungi that cause typical rot in wood are classified as Basidiomycetes. Some utilize cellulose to a much greater extent than lignin and produce so-called “brown rot”; others utilize both lignin and cellulose and produce “white rot.” Decay of coniferous wood species is most often, al-
though not always, due to brown-rot fungi, whereas decay of hardwoods is often caused by white-rot fungi. Fungi vary in their optimum growth conditions and apparently in their preference for wood species. Despite the large number of species of wood-destroying fungi, there is a tendency for the decay of some specific wood product to be due to a relatively small number of species of fungi.

Fungi vary in their susceptibility to different toxicants. A preservative that is highly toxic to one fungus may be relatively inert to others. This phenomenon has been recognized for a long time and has been studied by many investigators. Its importance in wood preservation is obvious. It will be referred to again in discussions of specific preservatives.

Until comparatively recent years, there was a tendency to overlook another form of wood degradation by fungi which had been observed in the middle of the nineteenth century but was mistakenly believed to be of little practical importance. This type of decay, called soft rot, is characterized by a gradual softening of the surface with a rather abrupt demarcation between the softened wood and sound wood. It is caused by fungi belonging to the Fungi Imperfecti and Ascomycetes groups which differ in many respects from the Basidiomycetes fungi. Whereas the latter have fairly exacting requirements of balance between moisture and air the soft-rot fungi tolerate a wider range. They frequently are found in wood that is too wet to contain sufficient air for Basidiomycetes fungi; at the same time, soft-rot fungi will tolerate periods of dryness and will resume growth when wood is wetted for durations too short for the common Basidiomycetes fungi to become established. Under microscopic examination wood attacked by soft-rot fungi is found to have hyphae growing longitudinally within the cell walls in contrast to the transverse growth characteristic of Basidiomycetes fungi. Bore holes through the cell walls are virtually absent in soft-rotted wood. The importance of soft rot in the decay of cooling towers was pointed out by Findlay and Savory (1953). Duncan (1960) has studied many organisms in wood removed from cooling towers, and has reviewed the literature on soft rot. She observed that, as a group, the soft-rot fungi have a higher tolerance of preservatives than do Basidiomycetes.

IV. THE PREVENTION OF DECAY WITHOUT THE USE OF FUNGICIDES

In the evolution of wood preservation, as in many other industrial processes, art preceded science. The gradual deterioration of wood exposed to damp conditions in contrast to its tendency to remain sound
when dry was probably among man's first observations. The ancient
theorists no doubt explained this phenomenon as due to a softening
effect of water. In line with this perception the earliest attempts to pre­
vent decay of wood consisted of applying oily or tarry materials. When
Noah besmeared the timbers of his ark with pitch, he, no doubt, was
following an old established custom. The aim to keep wood dry is still
recognized as good practice whenever feasible, and much can be done
to avoid decay in buildings by proper design and construction practices.

The role of fungi in the decay of wood was demonstrated by Hartig
(1874) in a comparatively recent year with respect to the countless
years during which wood has been used as a building material. After
Hartig's classical studies the emphasis on wood preservation experi­
ments shifted from a search for water-excluding materials to a search
for fungitoxic materials. This illustrates the effect that a discovery in
pure science may have on the direction of applied research. All preserv­
avitives in commercial use at present depend for their effect on their
toxicity to wood-destroying organisms. It is recognized, however, that
protection against decay is possible by other means, and a brief consid­
eration of several of these nontoxic methods may be in order.

Heavy treatments with nontoxic oils give disappointing results as a
rule when the wood used is in contact with ground. Better protection
is afforded by smaller amounts of toxic oils. Oils tend to be deposited
only in the gross cavities and the cell walls retain their ability to ab­
sorb moisture. When decay conditions are less favorable, as in the case
of window sash, nontoxic water-repellent waxes dissolved in volatile
 carriers are quite effective; nevertheless, toxics are practically always
added to such formulations.

According to Lindgren and Harvey (1952) some molds that do not
destroy the structural elements of wood appear to produce compounds
that have an antibiotic effect on wood-destroying fungi. This approach
has not been investigated very thoroughly.

In addition to cellulose, wood-destroying fungi require thiamine
which is usually present in wood. Thiamine is unstable under alkaline
conditions. Baechler (1959) has shown that if the pH of wood is raised
somewhat by basic materials, such as ammonia or triethylamine, and
the wood heated to temperatures used in kiln drying, decay is inhibited.
Panels treated in this manner, and exposed above ground in a warm
humid climate, have remained sound after 8 years; untreated controls
were destroyed by decay in 4 years.

Since thiamine is widely distributed in plant materials, there is much
reason to question the likelihood of such wood in contact with the soil
and vegetation remaining sound, even though a protective coating is applied. Nevertheless, the idea seems promising in view of the low cost of the treatment and the fact that much wood is used out of contact with the ground but under conditions favorable to decay. Baechler also pointed out the possibility of preventing decay by inactivating one or more of the six trace elements required by fungi. This idea has received only cursory attention.

A number of investigators (Goldstein, 1955; Goldstein, et al., 1961; Stamm, 1946; Stamm and Baechler, 1960) have shown that chemical reactions that modify the cellulose molecule render wood resistant to decay. Treated specimens have given good performance in field tests, but the cost of such treatments has limited their application to specialty products.

The natural durability of some species has been shown to depend, to a large extent at least, on the presence of extraneous materials that are toxic to fungi. When such wood is ground and extracted with hot water, the addition of the extract invariably causes an inhibition of the growth of wood-destroying fungi on nutrient agar. This was pointed out by Hawley et al. (1924) and has been confirmed by other investigators. In the case of some species, the inhibition caused by the extractive is less than would be expected from the known natural durability. The way in which the extractives are deposited during the growth of the tree may be a factor in their effect; for example, it is conceivable that they mechanically hinder the direct contact between hyphae and the cell wall—which contact seems to stimulate the secretion of cellulase.

V. REQUIREMENTS OF WOOD PRESERVATIVES AND GENERAL TYPES USED

The list of requirements that must be met by a wood preservative is similar in pattern to that which must be met by preservatives for other materials. Whether the objective is to protect wood, paper, textiles, cordage, paint, glue, or plastics against damage by fungi, the fungicide first of all must prevent the growth of the invading organisms. It must have satisfactory permanence. It must be relatively free from harmful effects on the material being protected, on finishes, and on processing equipment. It must be free from any health hazard that cannot be controlled satisfactorily. It must be available in adequate amounts, and at a cost that is economical in relation to the value of the material.
being protected and the increase in service life that can be attained. The relative importance of these individual requirements varies from one material to another as does the difficulty of meeting them.

In wood preservation, requirements on permanence are relatively high. In products such as telephone poles, an average service life of 35 years or more is generally desired. Since the intrinsic value of wood is relatively low, compared with other materials that may receive fungicidal treatment, the permissible cost of wood preservatives is also relatively low. As would be expected, wood preservatives tend to be materials of low cost and high permanence.

The requirements on cleanliness are quite exacting for wood to be painted and this, of course, affects the choice of preservatives for certain wood products. On the other hand, users of some products, such as crossties, prefer a tarry surface to retard weathering and the accompanying checking.

Some chemicals might give good decay protection, but are barred from use by their corrosive action on steel pressure-treating equipment. The corrosion of metal fasteners in wood has some bearing on the choice of preservatives.

The health hazard offered by preservatives must be given special consideration in some cases. While all preservatives are toxic, some leach at such a low rate that they may be used to treat wood flumes for conducting potable water. Some preservatives are more irritating to the skin than others. This needs to be considered in choosing a preservative for wood items such as picnic benches and stadium seats. Treated wood generally presents no serious hazard to livestock coming into contact with it, although cases have been reported of toxic effects on cattle that licked treated fence posts, or horses that chewed treated lumber.

Phytotoxicity is generally of minor importance in the outdoor use of treated wood items, such as fence posts, that are contacted by field crops. However, damage to certain plants, especially seedlings, has resulted from the use of treated wood in greenhouses. Here again proper preservatives should be selected.

Oil-type preservatives have no apparent effect on the strength properties of wood, but the process of injecting them into wood may cause a measurable loss of strength; it is therefore necessary to control the treating factors, especially the temperature. Some waterborne preservatives have an unfavorable effect on strength, especially if the treated wood is used in arid regions or in locations in industrial plants where temperatures above normal are prevalent.
Waterborne preservatives may have some fire-resistant effect, although it is small in the amounts generally used for protection against decay. Wood freshly treated with oil-type preservatives tends to be somewhat more flammable than untreated wood. As the more volatile constituents evaporate, the fire hazard from the preservative becomes negligible under most use conditions.

The odor of wood treated with some preservatives may constitute a problem if this wood is used in close proximity to food. Nonvolatile preservatives are therefore recommended for such situations.

VI. OIL-TYPE PRESERVATIVES

Two classes of oil-type preservatives are in wide use: (1) Distillates of coal tar, designated as creosote and varying in composition, which may be used without modification or blended with cheaper oils such as petroleum oils or undistilled coal tar; and (2) solutions of an oil-soluble toxicant in a carrier such as a petroleum oil having properties suitable for the wood product being treated. The most important toxicant presently in use is pentachlorophenol.

Oil-type preservatives have certain advantages and disadvantages in common. Their traditional use has been for wood products that are used in ground contact and do not have high requirements for cleanliness. These products include crossties, telephone and utility poles, piling, lumber, timbers such as bridge timbers, and fence posts. Since they comprise approximately 90% of the wood treated annually, oil-type preservatives are of considerably greater industrial importance than waterborne preservatives. A relatively recent development in oil-type preservatives is the use of liquefied petroleum gas as a solvent for a toxicant such as pentachlorophenol. This will be discussed later.

The low water solubility of oil-type preservatives is an important factor in their wide use for contact with the ground or water. Their permanence under these conditions has been demonstrated by long experience. The oily surface, typical of the treated wood, may be a drawback in some cases but a distinct advantage in others because it retards abrupt changes in moisture content with accompanying weathering and checking. A further advantage of these preservatives is that significant amounts of water need not be introduced during treatment, thus avoiding dimensional changes and grain raising. If the wood is well seasoned before treatment, and the preservative penetrates reasonably well, the danger of checks extending into the untreated interior will be greatly minimized.
11. FUNGICIDES IN WOOD PRESERVATION

A. Creosote

The Manual of the American Wood-Preservers’ Association, in defining the term ‘creosote,’ states that “as used in the wood-preserving industry coal-tar creosote, or ‘creosote,’ denotes a distillate of coal tar produced by high-temperature carbonization of bituminous coal; it consists principally of liquid and solid aromatic hydrocarbons and contains appreciable quantities of tar acids and tar bases; it is heavier than water and has a continuous boiling range of at least 125°C beginning at about 200°C.”

Creosote was the first preservative used by Bethel, who invented the pressure process for treating wood in 1838. Although the use of certain other preservatives has increased steadily during the past several decades, creosote remains the most important preservative in terms of quantity of wood treated. This is because it is highly effective and reasonably priced, and its shortcomings, such as lack of paintability, are relatively unimportant for the majority of wood products treated.

Creosote is distilled from coal tar, which is collected during the carbonization of coal. There are several processes for carbonizing coal; the essential feature is that coal is heated in an enclosed space from which air is excluded. The resulting hot gases are cooled whereby the higher boiling compounds are condensed to a highly complex liquid called tar. This contains in solution many compounds that are solids in pure state.

The composition of the tar varies with several factors of which temperature is the most important. The time during which the gases are exposed to the maximum temperature range is also important. When coal is carbonized at a relatively low temperature, for example, 500° to 600°C, the resulting tar contains relatively high amounts of aliphatic hydrocarbons and oxygen-containing compounds that are feebly acidic and are designated as tar acids. As the temperature of carbonization is increased, there is an increase in the percentage of aromatic hydrocarbons at the expense of aliphatic or saturated cyclic hydrocarbons and tar acids.

Low-temperature processes have never had much commercial success in the United States, and most domestic coal tar is produced in large coke ovens at temperatures over 1000°C. The primary product is metallurgical coke. Prior to the widespread use of electric lighting, much municipal gas was produced from coal in small retorts, called horizontal retorts. Operating temperatures were similar to those now used in coke ovens.

In England, as in the United States, much high-temperature coal tar
is produced in coke ovens. However, there is also a large production of tar by plants that use vertical retorts for producing municipal gas. They are operated at intermediate temperatures. Compared with high-temperature tar, vertical-retort tar is lower in aromatic hydrocarbons and higher in saturated hydrocarbons and tar acids.

It is convenient to visualize the distillation of coal tar as a one-step process; the distillate up to approximately 200°C is collected and sold as "light oil" while the distillate from approximately 200° to 400°C is sold as creosote, with the undistilled residue sold as coal-tar pitch. In actual practice, many fractions are collected. Some of the lower boiling fractions are processed for the recovery of materials such as naphthalene and tar acids which bring a higher price than creosote. The processed cuts and higher boiling cuts are then blended to produce creosotes of varied distillation patterns.

The relation between the composition of coal-tar creosote and its effectiveness as a preservative has been the subject of many investigations, but nevertheless many questions about it remain unanswered. The subject is very involved. Not only is creosote a complex and variable material, but the performance of any preservative is influenced by many factors, especially: (1) the retention level and distribution in the wood; (2) properties of the wood that affect the rate of loss of preservative, for example, size, shape and anatomical structure; and (3) conditions of exposure that likewise affect the rate of loss of preservative and also determine the type and activity of organisms that come into contact with the wood.

At one time, there was considerable difference of opinion regarding the role of tar acids in the preservative action of creosote. Bateman and Henningsen (1925) tested agar emulsions of modified creosote inoculated with a wood-destroying fungus. They showed that the hydrocarbons were the most important constituents, and that the removal of tar acids and tar bases (nitrogen-containing compounds) produced little change in effectiveness. This was later confirmed by Rhodes and Erickson (1933) and by other investigators. Field tests by Blew (1964) on stakes treated with high-temperature coal-tar creosotes before and after the removal of tar acids showed negligible differences. However, laboratory studies on low-temperature tar creosote by Duncan (1952) and Baechler (1953) showed that for such material the tar acids are essential constituents, and that the residual oil remaining after their removal is far less effective than the whole oil.

Aside from the ratio of the main classes of compounds, and considering only a creosote consisting virtually of aromatic hydrocarbons, the boiling range of a creosote is of obvious significance since it may affect
both toxicity and permanence. Fortunately for the user, experience has shown that good results are attainable with creosotes that vary quite widely in distillation range. This may be partly explained by the broad generalization that as the percentage of high-boiling constituents increases, the permanence also increases; but the toxicity decreases. The opposing nature of these changes, even though they may not balance each other exactly, tends toward a leveling effect on creosote performance. The relation between any single property, for example, percentage of distillation residue, and the effectiveness of a creosote, seems to follow the shape of a rounded plateau rather than a sharp peak. It is now generally accepted that extremes in any property should be avoided, but the approximate boundaries of the plateau corresponding to any property are still subject to differences of opinion.

B. Pentachlorophenol

While a number of the preservatives now in use were discovered by trial-and-error experiments before the nature of decay was understood, pentachlorophenol may be considered a product of modern research. Its potential usefulness was revealed by studies at the Forest Products Laboratory on relations between the chemical constitution of benzene derivatives and their toxicity to a wood-destroying fungus. In the form of sodium salts dissolved in water, they were first used in the early 1930's in dip treatments to prevent the staining of southern pine lumber. The high effectiveness of such treatment can be demonstrated in a short time in contrast to preservative treatments that require many years for their evaluation.

Carswell and Hatfield (1939) called attention to the high toxicity of pentachlorophenol to fungi and insects along with its physical properties that indicated permanence under use conditions. The dip treatment of window sash and other millwork, using solutions of water repellents and fungicides, began slowly in the early 1940's and grew rapidly thereafter.

The first field tests on pentachlorophenol as a wood preservative were started in 1936. Results were very encouraging. A shortage of creosote supplies developed during the postwar period and several newer preservatives, including pentachlorophenol, were used by wood-treating companies, especially for pole treatments. Its use gradually increased, and now it is second only to coal-tar creosote in volume of wood treated annually. Its early promise has been confirmed by numerous service tests. During the past decade or more, a number of papers have appeared dealing with various aspects of the pressure treatment of wood
with solutions of pentachlorophenol in petroleum. These have been published in the Proceedings of the American Wood-Preservers' Association, the Forest Products Journal, and other journals. A complete review will not be attempted here.

Early field tests by a number of investigators (Hatfield and Sokorn-but, 1955; Meyer and Gooch, 1956; Baechler and Roth, 1962) showed that the performance of pentachlorophenol was influenced by the nature of the carrier. McNight (1957) showed that the effect of pentachlorophenol on a white-rot fungus was independent of the swelling characteristics of the carrier, but against a brown-rot fungus, this compound was more effective in a carrier that produced swelling of the wood, indicating cell-wall penetration. Baechler and Roth (1962) analyzed stakes treated with pentachlorophenol in various types of petroleum, and exposed to the soil for 12 years. While the loss of pentachlorophenol from the below-ground sections tended to be greater with the lighter oils, the difference did not fully account for differences in performance. They concluded that the toxicity of the petroleum was an important factor.

In the early uses of pentachlorophenol, it was observed that when a light volatile solvent such as mineral spirits was used, the chemical tended to migrate to the surface as the solvent evaporated. This was known as blooming and was objectionable not only because it promoted loss of chemical but also because pentachlorophenol is irritating to the skin. A number of so-called anti-blooming agents were developed to overcome this difficulty, and their use became routine whenever low-boiling carriers were used. They are high-boiling materials with a high solvency for pentachlorophenol. While the selection of the carrier made it possible to achieve relatively clean surfaces in pressure treatments, difficulties often were encountered in painting the wood. Blew and Panek (1964) discussed this problem and pointed out the factors involved, such as nature of carrier, retention, and species of wood. Hudson (1953) developed a method of cleaning the surface of the wood by the use of solvent vapors.

Goodwin and Hug (1961) and Henry (1963) described a pressure-treating process using liquefied gas as the solvent, most of which is recovered after the pressure cycle, leaving the wood clean and paintable. This process has been received with great interest and has already come into commercial use at several plants.

Because of the low viscosity of the lighter petroleum carriers, such solutions are well adapted to nonpressure treatment of wood items to be used under conditions of moderate decay hazard. The most impor-
tant application of such treatments is the dip treatment for millwork. Generally a water repellent is added to the carrier along with the pentachlorophenol.

Snoke (1954) has discussed benefits to be obtained by the addition of approximately 2% pentachlorophenol to creosote. This has become an important preservative for the treatment of telephone poles.

C. Other Oil-Soluble Toxicants

A fairly large number of oil-soluble toxicants have been tried as wood preservatives, and some have achieved limited commercial acceptance. Chlorinated compounds, other than pentachlorophenol, have been used in antispastain dip treatments, and to some extent in the dip treatment of window sash.

Of the metallic salts of organic acids, copper naphthenate has enjoyed the widest use—mainly in brush and dip treatments. During the creosote shortage following World War II, however, its use in the pressure treatment of poles rose to an annual consumption of 2 1/2 million lb. It has been used to treat wood boat hulls and exterior planking where it is believed to offer some protection against boring as well as fungi. Zinc naphthenate offers the advantage of a light color but is less effective than copper naphthenate and, therefore, has found little use. Phenyl mercury oleate has been used to some extent in dip treatments of window sash. McNight and Merrall (1958) found that in soil-block tests copper abietate retarded attack by Poria monticola. Other copper compounds such as lanolate, oleate, tallate, resinate, and stearate have been tried, but apparently with little success.

Organotin compounds have given good results in European field tests. In the United States, Fahlstrom (1958), using the soil-block method, found several compounds of this kind to have high toxicity toward three wood-destroying fungi.

Goldfarb (1962) pointed out the potential usefulness of solubilized copper 8-quinolinolate, especially for specialty uses; it is the only specific formulation permitted by the U.S. Food and Drug Administration for the treatment of wood which may come into contact with food.

VII. WATERBORNE PRESERVATIVES

A. Zinc Chloride

In the early part of this century, zinc chloride was the most important preservative for creosotes. It owed this position to its low cost,
compared with coal-tar creosote, which was recognized as being more effective. As the cost of raw ties increased and as the delivered cost of creosote decreased, the use of zinc chloride for this purpose declined. However, it continued to be a leading preservative for lumber treatments for many years. Straight zinc chloride is now used in negligible amounts and is no longer a standard of the American Wood-Preservers' Association (A.W.P.A.).

**B. Chromated Zinc Chloride**

Drefahl and Bescher (1939) reported that, of a wide range of materials they tried as additives to improve various properties of zinc chloride, sodium dichromate proved to be the best. They recommended a ratio of approximately 81.5% zinc chloride to 18.5% sodium dichromate. Following the publication of favorable results by field tests on treated stakes, the acceptance of this mixture increased. For many years, it has been one of the most widely used waterborne preservatives. A modification, consisting of the addition of copper chloride and known as copperized chromated zinc chloride, was formerly recognized in the standards of the American Wood-Preservers' Association.

**C. Fluoride-Chromate-Arsenate-Dinitrophenol Mixtures**

Two mixtures of this type are in wide use. They vary somewhat in composition. The abbreviation FCAP (type A or type B) appears in specifications for treated wood products.

These preservatives were developed in Germany. Consumption in this country has gradually increased along with an increase in the use of treated lumber in housing. They are also used in other lumber items such as roof timbers in wet-process industrial buildings and railroad car decking. Despite the fact that they are only moderately resistant to leaching, they give fairly good protection to wood in ground contact. They have been used to some extent in the treatment of posts, especially when cleanliness and paintability are desired. For such use, however, they are being replaced by more permanent formulations to be discussed later. Over 4,700,000 lb of these preservatives were used in pressure treatments in 1963.

**D. Borax and Boric Acid**

Borax and boric acid individually have been used to some extent in treatments of wood. A mixture of the two is used on a large scale for
the treatment of lumber in Australia, and even more so in New Zealand where it is approved by government specifications. It is applied mainly by diffusion methods, and the principal objective is control of the Lyctus beetle.

In laboratory tests, borax and boric acid have shown good effectiveness against wood-destroying fungi, but these results have not been substantiated by field tests. The discrepancy is believed to be due to lack of resistance to leaching, but the low toxicity toward termites is a contributing factor. These chemicals are ingredients of several formulations for decreasing the flammability of wood. Their fungitoxicity is believed to be an additional advantage.

E. Acid Copper Chromate

Acid copper chromate consists of a mixture of copper sulfate and sodium dichromate to which a small amount of chromic acid may be added. It was patented in England in 1928. The addition of dichromate has two effects. It acts as a corrosion inhibitor, and it leads to the formation of a relatively insoluble copper compound in the treated wood. Copper sulfate by itself is toxic to many microorganisms including most wood-destroying fungi. However, it is corrosive to steel and is therefore unsuitable for pressure processes which are invariably carried out in steel equipment. A mixture of copper sulfate and dichromates (Sodium or potassium) is noncorrosive and may therefore be applied in conventional pressure equipment.

The insolubilizing effect is based on the tendency of certain wood constituents to reduce hexavalent chromium compounds. This is accompanied by a decrease in acidity which results in the formation of an insoluble copper chromate of indefinite composition. The speed of the reaction between wood and hexavalent chromium depends upon several factors of which pH and temperature are the most important. Because of the temperature effect, pressure treatments with preservatives containing chromate are generally made at temperatures not exceeding 120°F. The use of relatively cold solutions promotes penetration by retarding precipitation, and also reduces the formation of sludge in the treating solution. Field tests on wood treated with this preservative have been generally favorable; it is covered in A.W.P.A. specifications. Occasional failures of wood, apparently well treated with this mixture, have been assumed to be due to the presence of certain fungi tolerant to copper.
Several proprietary formulations answering this description are being promoted in various parts of the world, and their importance in wood preservation is increasing. The first patent covering them was a British patent, issued in 1934 to S. Kamesan of Dehra Dun, India. A typical treating solution was described as consisting of 1 part arsenic pentoxide (As$_2$O$_5$·2H$_2$O), 3 parts copper sulfate (CuSO$_4$·5H$_2$O), and 4.5 parts potassium dichromate (K$_2$Cr$_2$O$_7$). It was first used on a large scale in the United States for treating southern pine telephone poles. An extensive study of the preservative was made, with results being published by McMahon et al. (1942) and by Lumsden and Hearn (1942). The service records on poles treated in the early 1940's have been excellent. Certain production problems have retarded the wider use of this type of preservative in treated poles. The increased recent use of it has been mainly for treating other products such as fence posts and lumber to be used under conditions favorable to leaching. The fact that the preservative becomes insoluble in the wood has removed this type of waterborne treatment from the traditional restriction to above-ground use. The formula of Kamesan with certain modifications being permitted is covered in an A.W.P.A. specification adopted in 1953. It is designated as Chromated Copper Arsenate, Type A. A second formulation was developed in Sweden and was adopted in A.W.P.A. specifications in 1964. It is designated as Chromated Copper Arsenate, Type B and covers a mixture of the oxides of copper, arsenic, and chromium.

G. Chromated Zinc Arsenate

This preservative, also patented by the same Swedish company, is a mixture of zinc sulfate, sodium dichromate, sodium arsenate, and arsenic acid. It was formerly a standard preservative of the American Wood-Preservers' Association. While it has given fairly good results, it is less effective than the copper formulations discussed in the preceding paragraph.

H. Ammoniacal Copper Arsenite

This consists of copper hydroxide and arsenic trioxide dissolved in dilute ammonium hydroxide. As the ammonia evaporates from the treated wood, copper arsenite is deposited in the wood. It has a high resistance to leaching, and treated wood exposed to wet conditions has
given excellent performance. Its development was discussed by the inventor, A. Gordon (1947).

1. Miscellaneous Waterborne Preservatives

A number of water-soluble compounds—including salts of copper, nickel, lead, mercury, chromium, arsenic, and fluorine—have been tried and, although some have given good results in some instances and have received some commercial acceptance, their wide adoption has been hampered by certain practical shortcomings.

Copper sulfate is still used in Europe in the Boucheerie process, to be described later. The steeping of wood in mercuric chloride solution was patented in England in 1838. For many years, the process was used on a limited scale in the United States but has been discontinued. Some salts, such as zinc sulfate and sodium silicofluoride, have found some use in other countries. Sodium pentachlorophenate is used chiefly in antistain-dip treatments of lumber. When used in pressure treatments, the penetration tends to be poor; the acidity of the wood converts it to pentachlorophenol which is insoluble in the water being used as the carrier. Smith et al. (1956) reported favorable results in treating the wood with copper formate, and then heating it to induce a fixation of copper by the cellulose.

VIII. Relation Between Chemical Constitution and Toxicity to Wood-Destroying Fungi

Of the various aspects of wood preservation, this is perhaps most closely related to the general subject of fungicides. It will therefore be discussed more thoroughly than it would be in a practical treatise on wood preservation.

The killing concentration of a chemical toward a given organism will vary with the conditions of the test. Also, the relative order of the toxicity of two chemicals may vary with the test organism used. Generalization regarding the toxicities of chemicals are therefore subject to many exceptions. Papers by Cowling (1957) and others show the relative tolerance of different organisms.

The toxicity of inorganic salts toward wood-destroying fungi generally follows their toxicity toward other microorganisms. Bateman and Baechler (1927) reported on tests made on inorganic salts by an agar-base method with the wood-destroying fungus Fomes annosus. This
technique leaves much to be desired as a practical test of wood preservatives because the test organism, although a wood destroyer, is not grown on wood. Furthermore, no indication of permanence is obtained. However, it yields surprisingly precise results on chemically stable compounds of low volatility; thus, it is well suited to investigations of relations between chemical constitution and toxicity. Of the cations, the alkaline and the alkaline earth metals tended to be low in toxicity while the less basic metals such as iron, nickel, zinc, cadmium, cobalt, manganese, and lead varied from moderately toxic to very toxic. Two noble metals, silver and mercury, were highly toxic as was copper which approaches the noble metals in its characteristics. Nickel was surprisingly high in toxicity. Among the anions or acidic elements, only fluoride of the halides was toxic. Borates, arsenates, and chromates were toxic. Little or no toxicity was shown by the sodium salts of many of the oxoacids, e.g., sulfate, nitrate, chlorate, tungstate, uranate, and vanadate. The mild toxicity shown by sodium phosphate could be attributed to its effect on pH. While sodium sulfate was relatively inert, sodium sulfite was quite toxic, probably because of its reducing activity. Similarly, some of the toxic effect of sodium chromate probably was due to its oxidizing action.

Baechler and Roth (1956), using the soil-block method which is believed to give more reliable indications of effectiveness than the agar method, obtained threshold values for some salts used in modern waterborne preservatives (Table II).

Baechler and Bateman (1936) and Baechler (1939, 1947), using the agar-base method with the wood-destroying fungus Madison 517 as the test fungus, studied relations between chemical structure and toxicity of organic compounds. Certain general principles were found to hold for both aliphatic and aromatic compounds. Exceptions to these rules were few, and were most likely to be found among compounds whose properties presented difficulties in the determination of toxicity.

Insofar as comparable data were obtained on both aromatic and aliphatic compounds, including some unpublished data, the following groups when substituted for hydrogen in either class of compound increased toxicity: alkyl, phenyl, halogen, nitro, aldehyde, and a double bond. The hydroxyl, carboxyl, sulfonic, amide, keto, and nitrile groups decreased toxicity. The amino group increased the toxicity of aliphatic compounds but decreased the toxicity of aromatic compounds.

The effect on toxicity produced by replacing hydrogen with different groups is shown in Table III. Toxic concentrations are on the basis of moles per liter. If they were given on a percent-by-weight basis, differ-
<table>
<thead>
<tr>
<th>Species of wood</th>
<th>Fungus</th>
<th>Isolation number</th>
<th>Copper sulfate (lb/ft²)</th>
<th>Zinc chloride (lb/ft²)</th>
<th>Sodium arsenate (lb/ft²)</th>
<th>Sodium benenate (lb/ft²)</th>
<th>Sodium fluoride (lb/ft²)</th>
<th>Sodium dichromate (lb/ft²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pine</td>
<td>Lentinus lepideus</td>
<td>(534)</td>
<td>0.19</td>
<td>0.32-0.42</td>
<td>0.016</td>
<td>0.077-0.12</td>
<td>0.084-0.13</td>
<td>0.35-0.46</td>
</tr>
<tr>
<td>Pine</td>
<td>Lentinus ulmarius</td>
<td>(517)</td>
<td>0.30-0.42</td>
<td>0.32-0.47</td>
<td>0.23-0.35</td>
<td>0.077-0.12</td>
<td>0.16-0.19</td>
<td>1.3-1.6</td>
</tr>
<tr>
<td>Pine</td>
<td>Poria monticola</td>
<td>(698)</td>
<td>0.31-0.33</td>
<td>0.62-1.36</td>
<td>0.068-0.084</td>
<td>0.037-0.078</td>
<td>0.16-0.18</td>
<td>2.8-4.4</td>
</tr>
<tr>
<td>Oak</td>
<td>Fomitopsis versicolor</td>
<td>(697)</td>
<td>0.054-0.11</td>
<td>0.32-0.71</td>
<td>0.28-0.35</td>
<td>0.11-0.18</td>
<td>0.84-1.4</td>
<td>0.87-1.1</td>
</tr>
<tr>
<td>Oak</td>
<td>Lentinus ulmarius</td>
<td>(517)</td>
<td>0.16-0.24</td>
<td>0.15-0.35</td>
<td>0.16-0.20</td>
<td>0.11-0.18</td>
<td>0.16-0.30</td>
<td>1.6-1.8</td>
</tr>
<tr>
<td>Oak</td>
<td>Poria monticola</td>
<td>(698)</td>
<td>0.32-0.73</td>
<td>0.63-1.01</td>
<td>0.046-0.098</td>
<td>0.045-0.064</td>
<td>0.15-0.25</td>
<td>1.7-2.3</td>
</tr>
</tbody>
</table>
TABLE III

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Toxicity (mole/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Butyl alcohol</td>
<td>CH₃·CH₂·CH₂·CH₂OH</td>
<td>0.135</td>
</tr>
<tr>
<td>1, 3-Butyl glycol</td>
<td>CH₃·CH₃·CH(OH)</td>
<td>0.11</td>
</tr>
<tr>
<td>sec-Butyl carbinol</td>
<td>CH₃·CH(CH₃)·CH₂OH</td>
<td>0.0635</td>
</tr>
<tr>
<td>2-Ethyl n-butyl alcohol</td>
<td>CH₃·CH₂·CH(CH₂)·CH₂OH</td>
<td>0.018</td>
</tr>
<tr>
<td>2-Amino n-butyl alcohol</td>
<td>CH₃·CH₂·CH(NH₂)·CH₂OH</td>
<td>0.0156</td>
</tr>
<tr>
<td>2-Nitro n-butyl alcohol</td>
<td>CH₃·CH₂·CH(NO₂)·CH₂OH</td>
<td>0.0145</td>
</tr>
<tr>
<td>n-Propyl alcohol</td>
<td>CH₃·CH₂·CH₂OH</td>
<td>0.387</td>
</tr>
<tr>
<td>3-Phenyl n-propyl alcohol</td>
<td>CH₆H₅CH₂·CH₂·CH₂OH</td>
<td>0.01</td>
</tr>
<tr>
<td>Allyl Alcohol</td>
<td>CH₃·CH·CH₂OH</td>
<td>0.0002</td>
</tr>
<tr>
<td>2, 3-Dichloropropyl alcohol</td>
<td>CH₃·CHCl·CH₂OH</td>
<td>0.0266</td>
</tr>
<tr>
<td>Sodium n-valerate</td>
<td>CH₃·CH₂·CH₂·CH₂COONa</td>
<td>0.193</td>
</tr>
<tr>
<td>Sodium levulinate</td>
<td>CH₂·CO·CH₂·CH₂COONa</td>
<td>0.304</td>
</tr>
<tr>
<td>Sodium propionate</td>
<td>CH₃·CH₂·COONa</td>
<td>0.25</td>
</tr>
<tr>
<td>Sodium succinate</td>
<td>COONa·CH₃CH₂COONa</td>
<td>0.71</td>
</tr>
</tbody>
</table>

ences would generally be in the same direction but would be of different magnitude.

Some idea of the relative effect of eight groups may be obtained from Table IV. It shows the toxic concentrations of eight compounds that may be regarded as derivatives of normal butane.

The position of a group in the molecule has a bearing on the intensity of the effect that the group may have on toxicity. As a rule, proximity to another group restricts the typical effect of a group. For example, when a second hydroxyl group is added to phenol, its toxicity-reducing

TABLE IV

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Toxicity (mole/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Butyramide</td>
<td>CH₃·CH₂·CH₂CONH₂</td>
<td>0.33</td>
</tr>
<tr>
<td>Sodium butyrate</td>
<td>CH₃·CH₂·CH₂COONa</td>
<td>0.29</td>
</tr>
<tr>
<td>Butyronitrile</td>
<td>CH₃·CH₂·CH₂CN</td>
<td>0.15</td>
</tr>
<tr>
<td>Butyl alcohol</td>
<td>CH₃·CH₂·CH₂CH₂OH</td>
<td>0.135</td>
</tr>
<tr>
<td>Butyl chloride</td>
<td>CH₃·CH₂·CH₂CH₂Cl</td>
<td>0.025</td>
</tr>
<tr>
<td>Butyraldehyde</td>
<td>CH₃·CH₂·CH₂CHO</td>
<td>0.012</td>
</tr>
<tr>
<td>Butylamine</td>
<td>CH₃·CH₂·CH₂CH₂NH₂</td>
<td>0.011</td>
</tr>
<tr>
<td>Butyl bromide</td>
<td>CH₃·CH₂·CH₂CH₂Br</td>
<td>0.001</td>
</tr>
</tbody>
</table>
effect will be greatest in the para position and least in the ortho position; accordingly hydroquinone is less toxic than catechol and resorcinol is intermediate in toxicity. When a group increases toxicity, the effect is also greater in the para position; and thus p-dichlorobenzene is more toxic than o-dichlorobenzene. Obviously, it is not possible to generalize about the relative toxicity of ortho and para compounds. Statements to this effect that have appeared in the literature definitely do not apply to toxic effects of organic chemicals on wood-destroying fungi.

There is a similarity between aliphatic and aromatic compounds as to the effect of the position of a substituted group on the toxicity of the molecule. When two hydrogen atoms attached to the same carbon atom in an aliphatic compound are replaced, the substituted groups do not always show their full effects. There is not much difference in the toxicities of sodium mono-, di-, and trialkylphosphates, despite the strong effect that chlorine substitution generally has on toxicity. Again, there is little difference in the toxicities of dimethylcarbinol \([\text{CH}_3 \cdot \text{C} \cdot \text{CH}_3 \cdot \text{OH}]\) and monomethylcarbinol \([\text{CH}_3 \cdot \text{CH} \cdot \text{CH}_3 \cdot \text{CH}_2 \text{OH}]\). The effect of the position of a group is illustrated in Table V, which shows the toxicities of n-butyl alcohol and its four methyl derivatives.

Invariable relations between theoretical structure and toxicity could not be expected to exist in certain types of compounds. It is well known that the chemical behavior of a group may be affected by the configuration of the molecule of which it is a part. Since toxic effect is a manifestation of chemical activity, the toxic effect of such a group must in turn be influenced by molecular structure. The keto group \((\text{C}=\text{O})\) may be chosen to illustrate this point. In compounds containing this group, there is a tendency for a hydrogen atom to migrate to the oxygen from an adjacent carbon atom with the attendant formation of an

\[\text{CH}_3 \cdot \text{CH} \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{OH} \rightarrow \text{CH}_3 \cdot \text{CH} \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{OC}-\text{CH}_3\]

<table>
<thead>
<tr>
<th>Compound</th>
<th>Formula</th>
<th>Toxicity (mols/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Butyl alcohol</td>
<td>(\text{CH}_3 \cdot \text{CH}_3 \cdot \text{CH}_3 \cdot \text{OH})</td>
<td>0.135</td>
</tr>
<tr>
<td>Methyl propyl carbinol</td>
<td>(\text{CH}_3 \cdot \text{CH} \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{OH})</td>
<td>0.088</td>
</tr>
<tr>
<td>sec-Butyl carbinol</td>
<td>(\text{CH}_2 \cdot \text{CH} \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{OH})</td>
<td>0.0655</td>
</tr>
<tr>
<td>Isobutyl carbinol</td>
<td>(\text{CH}_3 \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH})</td>
<td>0.0566</td>
</tr>
<tr>
<td>n-Amyl alcohol</td>
<td>(\text{CH}_3 \cdot \text{CH} \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{OH})</td>
<td>0.0454</td>
</tr>
</tbody>
</table>
Both tautomeric forms of the molecule exist simultaneously in an equilibrium mixture, which generally contains only small amounts of the unsaturated alcohol. If the point of equilibrium were constant so that the percentage of the highly toxic unsaturated compound were always the same, the effect of the keto group on toxicity might be expected to be constant. However, because of the effect of the surrounding structure on the point of equilibrium, the toxicity of tautomeric compounds is hard to predict. Nitro derivatives also exhibit tautomericism as do aldehydes and cyanides. Halogen-substituted compounds show some tendency toward an internal rearrangement that consists of an exchange of positions of a halogen and some group within the molecule.

Despite this tendency of some types of compounds to deviate partly from their accepted molecular structure, most relations between chemical constitution and toxicity are fairly consistent. In families of compounds that are chemically stable, a relation may be shown between molecular weight and the toxic concentration. Baechler (1939) showed a very regular relation of normal alcohols, except that the lower members of the series were more toxic than would be required by theory. This was explained as being due to the phenomenon of osmosis which would cause a movement of water from the living cell to the surrounding solutions and intensify the truly toxic effect of the chemical which depends upon a reaction between the chemical and some vital constituent of protoplasm. The osmotic effect would be negligible at the low concentrations of the higher alcohols at which the organism is killed but at the high concentrations required of the lower alcohols, the osmotic effect is probably a significant factor in the total effect. The logarithmic relation between the molecular weights and toxic concentrations of the normal alcohols is shown in Fig. 1.

IX. THE EFFECTIVENESS OF WOOD PRESERVATIVES

The general subject of the effectiveness of wood preservatives will be covered only briefly.

A. Relation Between Test Method and Results

The evaluation of wood preservatives is a fairly complex subject, and has been discussed in many papers. An article by Colley (1953) contained 129 literature references. Published data on the effectiveness of wood preservatives contain many discrepancies and contradictions. This
is due, in part, to the lack of precision of most methods, but is also due to the difference in results that may be obtained in testing the same material by different methods.

Of the laboratory methods for evaluating preservatives, the agar-block method is commonly used in Europe. The treated block is placed above a pure culture of a wood-destroying fungus growing on nutrient agar. The soil-block method is standard in the United States. A strip of
untreated wood called a feeder block is first placed on wet soil in a bottle, and the entire assembly is steam sterilized. The feeder block is then inoculated with a pure culture and, after a vigorous growth has developed, the weighed treated block is placed on the decaying feeder block. After a fixed period of exposure, usually 12 weeks, the treated block is removed, conditioned to its original moisture content, and again weighed. The loss in weight indicates the extent of decay. The minimum retention of a chemical required to prevent decay is designated as the threshold retention. The treated blocks may be subjected to artificial weathering in the laboratory before exposure to the fungus, thus providing an index of the preservative's permanence.

The method has not been used long enough to show how well the results correlate with the results obtained on the commercial-sized material exposed to actual use conditions. It appears to be a very useful tool for research. With refinements from further experience, it may yield a reasonably reliable indication of the actual value of a new preservative.

This method was first described by Florov and Popov (1933) in Russia. It was developed independently in the United States by Leutritz (1939). Duncan (1958) and coworkers published a series of articles on the refinement of the method which have been published in one report. The method is outlined in AWPA Method M10 (American Wood-Preservers' Association, 1963) and in ASTM Standard D1413-61 (American Society for Testing and Materials, 1961).

During the past several decades, a number of papers have contained data obtained by the soil-block method. They have been published mainly in the Annual Proceedings of the American Wood-Preservers' Association and in the Forest Products Journal. There are some disagreements even when the same kind of wood, treated with the same chemical, is exposed to the same fungus, but as a rule the results are reasonably consistent. The report by Duncan (1958) contains data on a variety of materials injected into several species of wood and exposed to different species of fungi.

B. Field Tests on Stakes and Posts

These tests differ from actual service tests because they are confined to a relatively few specimens exposed in a small area that provides reasonably uniform conditions of soil, rainfall, temperature, and other conditions affecting the rate of biological attack. With a smaller amount of test units, greater care may be exercised in the selection of
the wood, in the control of retentions, and in the periodic inspections. Standard M7-55 of the American Wood-Preservers' Association (1955) outlines a method for conducting field tests with stakes having a cross-section of either \( \frac{3}{4} \) by \( \frac{3}{4} \) inch or 2 by 4 inches. Stakes of southern pine sapwood are most commonly used. A.W.P.A. Standard M8-56 (American Wood-Preservers' Association, 1956) gives directions for tests with round posts.

Comparisons of wood preservatives in field tests on stakes and posts have been published by Blew (1964), Blew and Kulp (1965), Leutritz (1964), Lumsden (1964), Colley et al. (1962), Rennerfelt (1963), Bescher and Kepfer (1946), and others.

C. Results of Service Tests

The American Wood-Preservers' Association, for many years, maintained standing committees with instructions to assemble service records on different wood items. The reader is referred to the reports of these committees for detailed information, published in annual A.W.P.A. proceedings prior to 1967.

As might be expected, the service life of treated wood varies widely depending on numerous factors, including: nature of preservative, amount retained by the wood, depth and uniformity of retention, species of wood, climatic factors and soil conditions that affect the rate of loss of preservative, and also the species of fungi encountered, mechanical wear, and others. Service records showing an average life of over 50 years for treated wood in areas favorable for decay are not uncommon. Unfortunately, good treating practices are not always followed, so that examples of disappointing results are also found in service records.

X. CONVENTIONAL RETENTIONS OF WOOD PRESERVATIVES

The term "retention," as commonly used in wood preservation, corresponds to the term "loading," that is often used in the application of fungicides to other materials. Retentions of wood preservatives are expressed on the basis of pounds of preservative per cubic foot of wood. The value is an average for a treated charge or lot. It is recognized that individual pieces in the same charge vary considerably, and that in each piece there generally is a concentration gradient from the outer to the inner zones. An untreated core of varied size exists in round material of species that have heartwood resistant to treatment. Sawn material may
have heartwood portions containing little or no preservative. This constitutes a shortcoming of pressure-treated wood, especially in a species that has nondurable heartwood.

Until recent years, it was common practice to determine retentions by measuring the volume of liquid preservative, before and after treatment, in a so-called working tank. The difference in preservative on hand was assumed to be the amount absorbed by the wood. This system was subject to several sources of error. There is a decided trend toward determining retentions by the assay of cores taken from representative pieces. Generally, some arbitrary length of each core is taken, depending on the kind of wood being treated. The result of the analysis does not show average retention. However, it is considered a good index of the quality of treatment when coordinated with other factors, such as penetration.

The retention of preservative that will be required to yield some given length of service cannot be accurately predicted. It will depend upon many factors that may be grouped roughly under the nature of the wood treated (species, size, etc.), and conditions of exposure. The engineer, in specifying a treatment, is guided by experience, and generally takes into account economic factors such as the initial cost of the structure, cost of replacements, probability of obsolescence, and others. Despite the complexity of the subject, there is generally fairly good agreement among those experienced in wood preservation in regard to the most economical retentions to specify for a given situation.

For the benefit of those not familiar with wood preservation, a list of the retentions of standard preservatives recommended in Federal Specification TT-W-571g (U.S. General Services Administration, 1961) is given in Table VI.

XI. METHODS OF APPLYING PRESERVATIVES

An untold number of inventors have devised methods for treating wood. The reader interested in a thorough discussion is referred to textbooks on wood preservation such as those by Hunt and Garratt (1953) and Van Grouw et al. (1951). The separate on Wood Preservation from the Wood Handbook (U.S. Forest Products Laboratory, 1955) is a further source of information. Many articles on treatment methods, and the many details affecting the results, have appeared in the Proceedings of the American Wood-Preservers' Association and in the Forest Products Journal.

Wood-treating methods may be divided into two classes, pressure and
pressure. In pressure methods, the wood is enclosed in a vessel, and the liquid preservative is injected under considerable hydrostatic force. Non-pressure methods do not utilize artificial pressure, the preservative being applied by dipping, soaking, brushing, or spraying. A third, somewhat distinct from others, is called the thermal method. It consists of heating the wood to expel air, and then allowing the wood to cool in the liquid whereby a partial vacuum forms in the internal spaces. Although movement of the liquid is due to atmospheric pressure, the process is not classed among the pressure processes.

To obtain maximum retention of preservative in the pressure process, the wood may first be subjected to a vacuum to evacuate the wood cells before being impregnated. This is called the full-cell process, and is generally used for the treatment of marine piling which require high retentions for protection against wood-boring animals. Much wood for land use is treated by one of the so-called empty-cell processes. In the Rueping process, air is injected into the treating cylinder to create a greater-than-atmospheric pressure within the wood. The cylinder is then filled with preservative in such a way that the injected air is trapped in the wood. The pressure is then increased to force preservative into the wood until gage readings indicate that a desired "gross" retention has been obtained. After the pressure is released, the cylinder is drained. The compressed air in the wood expands to expel some of the preservative. The recovered preservative is called the kickback, and the final retention is termed "net" retention or merely "retention." In this process, it is possible to increase the depth of penetration obtained with a given net retention.

The Lowry process differs from the Rueping process in that no initial air pressure is applied. The air normally present is compressed during the pressure cycle and produces a certain amount of kickback when pressure is released.

When oil preservatives are applied by soaking methods, the wood should be well seasoned to provide air spaces into which the oil may move by capillarity and by hydrostatic pressure; the latter, in some cases, seems to have an appreciable effect on the pieces that are most deeply submerged. Oils of low viscosity are preferable. The results vary greatly with the species being treated. The sapwood of pines may be treated quite effectively by this method; the variability in results and the difficulty of controlling retentions have impeded any extensive use of the method on a commercial basis.

Brushing and short dips generally lead to low retentions and shallow penetration, especially into side grain. Such treatments, however, have
<table>
<thead>
<tr>
<th>Preservative</th>
<th>Commodity</th>
<th>Use conditions</th>
<th>Retention (lb/ft³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creosote</td>
<td>Ties</td>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Poles, small</td>
<td>Moderate</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Poles, large</td>
<td>Severe</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Poste</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Piles, long</td>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Piles, marine, Douglas-fir</td>
<td>Land or fresh water</td>
<td>12</td>
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<tr>
<td></td>
<td>Piles, marine, southern pine</td>
<td>Coastal waters, moderate boror hazard</td>
<td>17⁵</td>
</tr>
<tr>
<td></td>
<td>Lumber</td>
<td>Coastal waters, severe boror hazard</td>
<td>20⁶</td>
</tr>
<tr>
<td></td>
<td>Lumber</td>
<td>Coastal waters, severe boror hazard</td>
<td>20⁷</td>
</tr>
<tr>
<td></td>
<td>Lumber</td>
<td>Ground contact</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Lumber</td>
<td>Not in ground contact</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Poles</td>
<td>Ground contact</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Lumber</td>
<td>Not in ground contact</td>
<td>6</td>
</tr>
<tr>
<td>Pentachlorophenol⁵ (5% solution in petroleum oil meeting A.W.P.A. Standard P9)</td>
<td>Poles</td>
<td>Moderate leaching conditions</td>
<td>1.00</td>
</tr>
<tr>
<td>Acid copper chromate</td>
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<td>Not in ground contact</td>
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</tr>
<tr>
<td>Fungicide</td>
<td>Posts</td>
<td>Lumber</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Ammonium copper arsenite</td>
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<td>0.50</td>
<td></td>
</tr>
<tr>
<td>Chromated copper aziridine</td>
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<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Chromated zinc oxide</td>
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<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Fluoride-chromated arsenate-zinc</td>
<td>0.30</td>
<td>0.35</td>
<td></td>
</tr>
</tbody>
</table>

* As determined by many of other 2 inches of borings.  
* As determined by many of other 3 inches of borings.  
* Including supplementary treatments of borings.  

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**11. FUNGICIDES IN WOOD PRESERVATION**
been shown to give substantial protection to wood used where only a moderate decay hazard exists. The 3-minute dip of window sash in preservative and water-repellent materials dissolved in volatile carriers has been a standard of the millwork industry for many years. Verrall (1961) Scheffer et al. (1963), Browne and Zabel (1955), and others have discussed this subject.

Another type of non-pressure treatment of considerable commercial importance is the application of preservative pastes to the groundline area of poles that have started to decay. This subject has been discussed by a number of investigators including Panek (1960) and Leutritz and Lumsden (1962).

Diffusion treatments, as the name implies, depend upon the diffusion of water soluble chemicals into the moisture held in green wood. The amount of preservative and its depth of penetration are, of course, related to the concentration and nature of the chemicals as well as to the immersion period. However, the results that may be obtained under favorable conditions are also governed by the species being treated. As in the soaking of dry wood in oils, the results attainable are good on some species but poor on others. Much lumber for above-ground use is treated by diffusion in New Zealand and Australia. In the United States, annual statistics are not available on the amount of wood treated by diffusion; apparently, it is quite small.

Chemicals introduced by diffusion tend to be leached from the wood under wet exposure. Baechler and Roth (1964) described a two-stage diffusion process, designed to deposit insoluble toxics in the wood, and reported excellent results on some species.

The selection of a treating process and a preservative is governed largely by the severity of exposure and requirements other than service life, such as cleanliness. However, since the ultimate objective of the user of treated wood is to reduce annual maintenance costs, economic aspects are of obvious importance. The economics of wood preservation have been discussed in numerous articles in the Proceedings of the American Wood-Preservers' Association and elsewhere. The subject is reviewed by Hunt and Garratt (1963).

References


11. FUNGICIDES IN WOOD PRESERVATION


11. FUNGICIDES IN WOOD PRESERVATION


I. INTRODUCTION

Development of a candidate fungicide into a commercial plant protectant requires a detailed knowledge of the physicochemical properties of the compound, the interactions it must undergo with its various environments, and how its properties can be adjusted by formulation to achieve optimum performance. The first of these requirements can be met by determining the water solubility, vapor pressure, photochemical stability, hydrolysis rates, chemical reactivities with metabolites, and related properties of the pure and technical grade compounds. If the formulator does not have access to this information he will be working at a severe disadvantage. Yet in the early days of phytopharmacy it was customary to develop formulations empirically with only a rudimentary knowledge of the compound involved. The second requirement referred to above presupposes a basic understanding of how the compound will interact with its environment during application, weather-
ering, and the period during which it must ward off plant disease. It is
easy enough to produce a formulation in the laboratory which has good
appearance, and wets and disperses well. Yet such materials often fail
in the field. Therefore, interactions with the environment must be
stressed. Finally, it is necessary to know how optimum properties can
be achieved by regulation of particle size and selection of adjuvants
such as diluents, wetting agents, spreading agents, stickers, and sus­
pending agents.

It is customary to regard formulation as an art rather than a science.
This is no more true than to say that organic chemistry is an art since
a certain amount of empirical experimentation is required. The formu­
lation of fungicides requires judicious application of the principles of
phytopathology, surface chemistry, and physical organic chemistry to­
gether with an indefinable amount of practical know-how in order to
achieve good results.

This chapter is not a guide to formulation. This topic by itself would
fill a separate treatise. Instead, it is a review of the interactions fun­
gicides undergo with their environments during application and the con­
trol of plant disease. From this, the general requirements for a success­
ful fungicide formulation will emerge.

II. THE ACTORS

Before considering the interactions which fungicides undergo with
their environments it is first necessary to consider separately the roles
of the individual actors—fungicides, adjuvants, plant surfaces, and fun­
gus spores. In a more detailed review covering systemic and soil fungi­
cides it would be necessary to include information on plant physiology,
soil chemistry, and soil microbiology. The interactions of fungicides
with the soil environment have been described elsewhere (Burchfield,
1960) and will not be repeated here (see also Chapter 13).

A. The Fungicides

Plant fungicides are usually protectants rather than eradicants.
Therefore, it is necessary that they be present on plant surfaces as
finely divided particulate matter in order to prevent invasion by the
fungi. For this reason, they are usually formulated as wettable pow­
ders which are dispersed in water prior to application. Permeation of
CHEMICAL AND PHYSICAL INTERACTIONS

the plant tissue is neither necessary nor desirable as is the case with systemic insecticides and herbicides. In fact, formulation in oil bases renders many fungicides highly phytotoxic. Therefore, emulifiable concentrates need not be considered here. Fungicides are conveniently classified into four groups based on their affinities for water. These groups include hydrophobic compounds, hydrogels, cationic fungicides, and water-soluble materials. The requirements in formulating these groups differ somewhat.

1. Hydrophobes

Compounds belonging to this class are sparingly soluble in water and some of them are water-repellent. They can be conveniently divided into three subgroups: inorganic, metallo-organic, and organic compounds. The inorganic subgroup comprises some of the older fungicides such as sulfur, cuprous oxide, and tribasic copper sulfate. Metallo-organic compounds which are used as fungicides include the zinc and iron salts of dimethyldithiocarbamic acid (ziram and ferbam), salts of ethylenebisdithiocarbamic acid (maneb and zineb), copper 8-hydroxyquinolinolate, and organomercury compounds. Well-known organic fungicides include thiram, captan, phalan, dichlone, Dyrene, and Daconil. These compounds are usually applied to plant surfaces as aqueous suspensions; dusts are seldom used.

2. Hydrogels

Hydrogels are freshly precipitated inorganic or metallo-organic salts which are prepared in situ by mixing their precursors. For example, Bordeaux mixture is prepared by mixing a solution of cupric sulfate with a suspension of lime, and tank-mixed zineb can be prepared by adding zinc sulfate solution to a solution of sodium ethylenebisdithiocarbamate. Although these compounds are essentially water insoluble, they possess good suspendibility and form tenacious films on plant surfaces because they are highly hydrated as initially prepared. However, on aging, crystal growth takes place, and their desirable properties are lost. In fact, 10–100 Bordeaux mixture which has been allowed to age in the spray tank is much less tenacious than fixed copper fungicides, although the freshly prepared hydrogel is superior in this respect (Burchfield and Goenaga, 1957b). Despite the good physical properties of freshly prepared hydrogels, their use as fungicides is declining because of the inconvenience in preparing them, and the supplanting of
bordeaux and Burgundy mixtures by organic compounds. However, a method has been developed by which hydrogels can be conveniently prepared in situ in specially designed mixing nozzles (Stull and Morrow, 1963). In the case of bordeaux, the copper sulfate comes out of one tank, the lime out of another, and the two are mixed in the nozzle as they emerge from their separate supply reservoirs. In this way the hydrogel is prepared and applied at the same time. This system can also be used for the preparation of inverse phase emulsions which are reported to reduce spray drift (Kelly, 1962).

3. Cationics

Cationic fungicides in common use include glyodin (2-heptadecyl-imidazoline) and dodine (dodecyl guanidine). Both of these compounds possess hydrophobic and hydrophobic groups. They are basic in character because of the proton affinities of the nitrogen atoms in the imidazoline ring and the guanidine group, respectively. A preparation containing one quart of glyodin in 100 imperial gallons of water is reported to have a surface tension of 52 dynes/em compared to a value of 74 dynes/em for a suspension of formulated captan at a concentration of 2 lb/100 gal (Maxwell et al., 1965).

4. Hydrophils

A few fungicides are water-soluble compounds which form insoluble spray deposits on aging. These include lime sulfur and nabam. Lime sulfur, prepared by digesting elemental sulfur with aqueous calcium hydroxide, is a complex alkaline polysulfide which carbonates on aging. Although nabam per se is highly water soluble, on exposure to air it forms an insoluble precipitate of ethylenethiourea, ethylenethiourea-monosulfide, and polymers with the concomitant evolution of CS₂ and H₂S (Thorn and Ludwig, 1954; Ludwig et al., 1954).

B. The Adjuvants

The adjuvants commonly used in formulating fungicides include inorganic diluents such as clays and calcium carbonate, surface-active materials that serve as wetting and suspending agents, spreader-stickers, and various materials intended to modify fungitoxicity or phytotoxicity. The proper qualitative and quantitative selection of these materials can greatly influence the performance of the fungicide in the field. These materials are in general not highly fungitoxic or phytotoxic.
12. CHEMICAL AND PHYSICAL INTERACTIONS

by themselves. However, their interactions can often produce striking results.

1. Diluents

The diluents used most frequently in formulating fungicides are finely divided minerals. They are employed to extend the product, as grinding aids, and may, to a minor extent, improve the coverage of plant surfaces by the fungicide. In one instance, the results of a research program made it possible to double the effectiveness of a fungicide already on the market by reducing particle size. Therefore, the manufacturer diluted the fungicide with an equal weight of an inexpensive mineral and maintained the same cost per pound for the formulation. This was a profitable enterprise. In other cases diluents can serve a useful purpose by making it possible to apply small quantities of fungicidal dusts and still obtain reasonably uniform distribution. Often, the incorporation of a mineral diluent will make it easier to grind fungicides to small particle size in hammer mills and micropulverizers. Sometimes, the particle size of the fungicide is influenced by the nature of the diluent used during grinding (Gullstrom and Burchfield, 1948). Minerals can also improve the distribution of pesticides by retarding the contraction of spray droplets on the plant surface. The advancing contact angle of the droplet on the leaf surface is not altered (Fogg, 1948). On drying, however, the solution remains spread as menisci between the particles on the leaf surface. This results in better coverage. Of course the fungicide particles themselves contribute to this effect.

Diluents used in pesticide formulation are available commercially in various mesh sizes and are usually sold under trade names. Attapulgite clays, which are hydrous aluminum magnesium silicates, are marketed under the names Carrielay, Florex, Floradin, and Pulgit. Montmorillonite clays, hydrous aluminum silicates, are sold as Bentonite, Pike’s Peak Clay, and Volclay. Calcium carbonate, Acricite (hydrated aluminum silicate), vermiculite (hydrated magnesium aluminum iron silicate), talle (hydrated magnesium silicate), diatomite (amorphous opalin silica), friamite (complex acidic potassium aluminum silicate), and pyrophyllite (hydrdrous aluminum silicate) are also often used.

It is a risky business to select diluents randomly. Although ostensibly inert, many of them possess high base-exchange capacities and can interact with fungicides. As will be seen later, this often results in degradation of the active ingredients, resulting in loss of fungitoxicity and sometimes an increase in phytotoxicity.
Surfactants are added to fungicide formulations primarily to insure that the powders are easily wettable and are well dispersed in the spray tank. However, the effects they have on spray deposition and coverage of plant surfaces are often equally important. Sometimes the secondary effects are good; often they are bad. Surface-active agents most commonly in use are of three types: anionic, cationic, and nonionic. The anionic surfactants are usually sodium salts of sulfonic or carboxylic acids containing a long aliphatic or aromatic side chain. The hydrocarbon moiety is lipophilic and the ionic moiety is hydrophilic; this results in orientation of the surfactant at organic-aqueous interfaces, thus accounting for their activities. Cationic surfactants are similarly designed except that the ionic moiety is usually an amino or guanidino group bearing a positive charge. Anionic and cationic surface-active agents can seldom be used in the same formulation without causing precipitation of complexes which effectively removes both materials from solution (Addison and Furmidge, 1956). Nonionic surfactants usually consist of a poly(ethylene oxide) or poly(propylene oxide) chain terminated by a hydrocarbon group. When the hydrocarbon chain contains 10 carbon atoms (cetyl) the optimum molecular ratio appears to be about 6 moles of ethylene oxide to 1 mole of cetyl alcohol. Aqueous solutions of nonionic surface-active agents of the polyethylene oxide type tend to be mildly cationic in nature; therefore they are not entirely compatible with anionic agents. However, nonionic and cationic surfactants are entirely compatible, and when properly balanced can confer valuable properties on the formulation. Also, cationic surfactants possibly promote deposition of fungicides on plant surfaces since they are positively charged while most leaf surfaces are presumed to be negatively charged. This electrostatic interaction could promote selective deposition of the fungicide on the plant.

The principal physical effects produced by surfactants on spray solutions and suspensions are reduction in surface tension, reduction in interfacial tensions, and reduction in the contact angles of spray droplets with planar surfaces.

Contact angle (θ) is defined by

\[ \gamma \theta = \frac{W_s - \gamma_v}{\gamma_l} - 1 \] (1)

where \( \gamma_l \) is the surface tension of the liquid, \( W_s \) the work of adhesion, and \( \gamma_v \) a quantity that can be determined from the Gibbs adsorption
isotherm. The contact angle is critical in determining whether or not a spray suspension will spread uniformly on the surface of a plant. A contact angle approaching zero indicates that the liquid is attracted to the surface by forces as high as the internal forces of cohesion, so that the spray droplets tend to flatten and form thin films. Conversely, a contact angle approaching 180° indicates that wettability is so poor that the droplets do not adhere to the surfaces. Actually, these extreme values are never reached, the contact angle of water on wax being only about 100°.

Contact angles can be measured either as advancing or receding angles. Advancing angles are measured as soon as the spray droplet is placed on a planar surface and has just begun to spread. Receding angles are measured when the droplet is beginning to diminish in size due to the evaporation of the aqueous phase. When the difference between the receding contact angle and the advancing contact angle is large the system is said to exhibit considerable contact angle hysteresis. The addition of surface-active agents to a fungicide spray almost always reduces both the advancing and receding contact angles on all surfaces. The relative amounts by which each angle is reduced depend on the nature of the surfactant, its concentration, and the texture of the leaf surface.

Furmidge (1962) has derived an equation which makes it possible to relate contact angles and interfacial tension to measured retention of sprays. A reduced form of this equation is given by

\[ F = \theta_r \gamma_{av} (\cos \theta_a - \cos \theta_r) / \rho^{1/2} \]  

where \( F \) is the calculated retention factor, \( \theta_r \) is the receding contact angle, \( \theta_a \) is the advancing contact angle, \( \gamma_{av} \) is to a first approximation the arithmetic mean of \( \theta_a \) and \( \theta_r \), \( \gamma_{av} \) is the air-liquid surface tension measured at a slowly expanding surface, and \( \rho \) is the density of the liquid phase. By use of this equation it was possible to correlate the results obtained on the retention of sprays containing various surfactants on artificial surfaces (Fig. 1).

It must be emphasized here that the retention referred to is the retention at the point of runoff. When a surface is sprayed with small drops of liquid these accumulate until a critical spray load is reached. When this load is exceeded, the droplets tend to coalesce and run off the surface, providing it is held in a non-planar position. Obviously the deposit accumulated prior to runoff cannot be affected by surfactants if rates of deposition are equal. However, the presence of a surfactant

will usually reduce the spraying time required to reach runoff, thus reducing total deposition. When runoff occurs, part of the pesticide is carried away with the aqueous phase, leaving only that portion of it behind which is bound to the plant surface in the retention film.

Equation (2) can be used to predict the behavior of surfactant solutions on plant surfaces when sprayed to runoff. The effect of increasing the concentration of cetylpyridinium chloride on the retention factor for various leaf surfaces is shown in Fig. 2. In most cases the deposit decreases with an increase in surfactant concentration. This decrease is particularly pronounced over the low concentration range. This behavior is common to all leaf surfaces which show a considerable contact angle hysteresis with water alone; that is, where tan α is large. In the case of rubber leaves (curve A) the addition of a small amount of cetylpyridinium chloride can produce a considerable increase in the retention at runoff. This occurs because rubber leaves show very little contact angle hysteresis with water alone; that is, the difference between tan α and θ is very small. The addition of surfactant decreases θ much more rapidly than θ, so that the contact angle hysteresis is increased, resulting in increased retention. Since the values of both θ and θ decrease as the concentration of surfactant increases, the retention passes through a maximum and then decreases. This behavior is found
3. Spreaders-Stickers

Spreaders-stickers are materials which are added to fungicide formulations in order to increase the spreading of spray droplets on plant surfaces and render the residues more tenacious. As the name implies, these materials should have mild surfactant properties and at the same time possess enough adhesion to fungicide particles and plant surfaces to improve the tenacity of the residue. Materials which have been used for this purpose include rubber latex, alginites, agar, gelatin, casein, lime casein, linseed oil, and various synthetic resins such as polyvinyl acetates, polyvinyl chloride, and coumarone resins.

4. Modifiers

Modifiers are compounds which are added to pesticide formulations to enhance biological activity or in some cases to reduce phytotoxicity. Since these compounds are rather specific with respect to the pesticide
with which they interact, it is difficult to make any general statements concerning them. In one instance it was found that sodium bicarbonate enhanced the nematocidal action of 3,4-dichlorotetrahydrothiophene-1,1-dioxide. It was demonstrated that the bicarbonate caused the in situ dehydrochlorination of the pesticide to the highly unstable and transient thiophene-1,1-dioxide which was proved to be the biologically active compound. Somers (1956) demonstrated that polyvinyl chloride increased the fungitoxicity of cupric oxide to Alternaria tenuis. He speculated that the surface-active properties of his preparation increased the dispersability of the copper oxide. Conversely, lime casein inhibited fungitoxicity by competing with the spores for copper.

Calcium carbonate has been shown to reduce the phytotoxicity of captan and Dyrene. Possible reasons for this are discussed in a later section of this chapter.

C. The Plant Surfaces

Extreme morphological differences exist between the surfaces of various species of plants which undoubtedly influence the deposition and retention of fungicide sprays. These include variations in the geometry and positioning of the fruit and foliage, differences in wax content and its distribution on surfaces, and differences in the pubescence of the leaves. Moreover, major differences may occur between plants of the same species depending upon age, positions of the leaves, and location in the field. All of these differences interact to project a sometimes erratic picture of the performance of fungicide formulations.

1. Geometry

The retention of fungicide sprays will vary depending upon the angle of inclination of leaves. For example, vertical leaves such as those of grasses and cereal crops will retain much less spray than large flat leaves which are positioned at low angles or horizontally. In the former cases retention is low, in part because of the fact that the droplets easily roll off the steeply inclined surfaces. The amount of spray intercepted by the plant will also depend upon the leaf area and the arrangement of the leaves with respect to overlapping. The angle of incidence of the spray is an important factor. In some instances, fruit may be shielded from the fungicide spray by a dense canopy of foliage. The influence of angle of inclination on spray retention has been shown
in experiments in which individual soybean leaves were held by their petioles with the leaf lamina horizontal or inclined 45° (Ennis et al., 1951). The horizontal leaves retained about twice as much spray as did those which were inclined. Retention also varies depending on whether the upper or the lower leaf surface is exposed to the spray. The upper surfaces of the leaves of some plants completely repel water droplets, while the lower surfaces can be wet quite easily. Intensive cultivation and the locations of individual plants within the community will often determine which leaves and surfaces are likely to come into contact with spray droplets.

Surface irregularities of leaves can effect retention in two opposing ways. First, they may promote premature runoff from some points of the leaf surface before runoff conditions are reached in others. This will tend to reduce retention. Second, the irregularities may form physical barriers to runoff so that spray liquid collects in depressions of the leaf surface. This tends to increase the measured retention. As a result of these irregularities and associated factors, spray retention tends to be irregular.

2. Hydrophobicity

Some leaves such as those of potato, tomato, tobacco, cotton, and castor bean are wet readily by water suspensions containing little or no surfactant. Others such as cabbage, pea, banana, and rubber are water-repellent. These differences arise from the amounts and distributions of the wax deposits which overlie the cuticle. Electron microscopy and X-ray diffraction studies have shown that external wax deposits are a special feature of the surfaces of the leaves and herbaceous stems of a large number of plant species (Schieferstein and Loomis, 1956; Juniper and Bradley, 1958). These deposits overlie a continuous cuticle and can exhibit various forms. Some of these resemble extruded columns or ribbons, even though the actual process of formation does not appear to take place through channels extending through the cell walls and cuticle. In other cases, wax deposits are rodlike, granular, or may form a random network. The net result is the formation of a surface containing irregular projections of wax. These projections either repel spray droplets completely, or the droplets balance on the wax projections and dry without coming into contact with the cuticle.

Surface wax is evidently formed only during and shortly after the period of leaf expansion. With aging and weathering much of the wax disappears until only faintly raised layers remain. Consequently, older
leaves tend to wet better than young leaves or those which have just completed expansion.

Wax structures are quite uniform over the surface of one side of a leaf, except for variations which occur in the vicinity of the stomata where the wax is more sparsely distributed. Variations in wax content and distribution can vary with respect to weathering, damage in the field, and other environmental conditions such as light intensity. Resistance to damage and weathering evidently differs between plant species. Attenuation of the wax structures is accompanied by a corresponding increase in wettability of the plant surfaces.

3. Pubescence

The wettability of leaf surfaces is also governed by the presence of leaf hairs. These can either repel or bind spray droplets depending upon their numbers and distributions. Thus the tropical floating weed fern *Salvinia auriculata* repels water because the upper surface of the leaf is densely covered with hairs having outer ends composed of loops of hydrophobic material (Adam, 1958; Hartley, 1960). A spray droplet landing on the upper surface of the leaf touches only a small portion of the leaf area. The air gaps between the hairs increase the contact angles of the droplets to such an extent that the drops roll off. This is an extreme case that would be seldom encountered in an application of fungicides.

Retention of sprays can be promoted if leaf hairs are readily wetted. In these cases the hairs form an irregular network which tends to hold the aqueous phase and enable it to penetrate through to the cuticle. This is illustrated by the work of Ennis et al. (1951) who compared the retention of sprays by a variety of soybean having pubescent leaves, with a variety of the same plant having glabrous leaves. The pubescent leaves retained 1.5 to 2 times more spray than did the glabrous leaves.

4. Variability

It has been mentioned previously that spray retention differs between plant species because of geometry, hydrophobicity, and pubescence of the leaves. This is illustrated by the work of Blackman et al. (1958). They sprayed the leaves of a number of species of plants at the same physiological age at a rate of 116 ml per square meter of ground area. Surfactants were not used. Sunflower and *Brassica alba*
(which possess broad, horizontally arranged, easily wetted leaves) re­tained much greater amounts of the spray than did the pea which has a waxy water-repellent surface, and barley which has narrow verti­cal and water-repellent leaf blades (Table I). Linseed, which possesses a combination of easily wetted broad cotyledons combined with narrow waxy leaves at the physiological age at which the test was conducted, was intermediate in the amount of spray that was retained. Alto­gether, there was in 8-fold difference in the amount of spray retained depending upon the plant species. Spray retention also varied markedly with stage of growth. Thus *Brassica alba* retained 2½ times more spray when treated at the two-leaf stage than it did at the three- to six-leaf

<table>
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<tr>
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<td>Linseed</td>
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<td>Pea</td>
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<td>Barley</td>
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</table>

*Blackman et al., 1958.

stage (Blackman et al., 1958). This is probably due to the fact that the ratio of cotyledon surface to total surface is greater at the two­leaf stage. The cotyledons retain spray readily, but the drops tend to remain discrete. This allows for a large buildup of spray droplets. By contrast, the true leaves are very easily wetted so that runoff occurs and a thin surface film is formed. Thus in this case the ratio of cotyle­don area to true leaf area plays a determining role.

Changes in angle of inclination during growth can also influence spray retention. Thus barley plants accumulate more spray per unit­area at the 22- to 26-leaf stage than they do at the three-leaf stage. This is probably due to the fact that the leaves become bent during growth so that the horizontal portions of the leaf surfaces do not shed spray droplets as readily.

As noted in the foregoing section, the wax formations which occur on the surfaces of many plants are attenuated during weathering. This can also contribute significantly to increased wettability.
5. Exudation

Fluids excreted by leaves by means of guttation, gland secretion, or movement through stomata can modify fungicidal action. These fluids contain inorganic salts, carbohydrates, amino acids, and other intermediary metabolites. Consequently, the chemical environments at leaf surfaces do not correspond to those found at artificial surfaces (polyvinyl acetate, for example) which have been employed in many fungicide evaluations. In some instances, the presence of metabolites at leaf surfaces could stimulate the germination of fungus spores, thus promoting infection. In others, naturally occurring metabolites might increase the fungitoxicity of the spray deposit by mobilizing the active ingredient so that it can diffuse to the fungus spores more readily. For example, amino acids and certain Krebs-cycle acids such as citrate are good chelating agents. These could conceivably form chelates with copper-containing fungicides which would be more mobile than the parent compounds. The possibility exists that dithiocarbamates such as ziram, ferbam, manebe, or zineb could participate in similar interactions.

Fungicides such as the s-triazines are known to react readily with metabolites containing amino and thiol groups to form nontoxic derivatives (Burchfield and Storrs, 1956). Consequently, it is possible that amino acids present in plant exudates could detoxify these materials as they became solubilized from the particulate phase at the plant surface. This would tend to limit the range over which individual fungicide particles can inhibit spore germination. Similar interactions could be proposed for other active halogen compounds such as captan, phalan, and dichlone. However, no experimental evidence is available to support this. While many possibilities exist for chemical interactions at plant surfaces to enhance or suppress the action of fungicides, very little concrete evidence is available.

D. The Invaders

Although the morphology, dissemination, and germination of fungus spores have been described adequately in this treatise, brief mention of these subjects will be made here since they are part of host-protectant-pathogen interaction. Fungus spores may be either sexual (ascospores, for example) or asexual, as illustrated by conidiospores. Sporulation occurs at various times of the year to maintain the continuity of the species. In size, most spores vary between 3 and 40 μ in length.
CHEMICAL AND PHYSICAL INTERACTIONS

and between 2 and 25 μ in width and are variable in shape (Table [I]). Dispersal of the spores from fructifications to new potential loci of infection usually takes place by wind or water. Some spores are coated with a mucilage which favors their dispersal by insects and limits their dispersal in dry air. After dissemination, airborne spores are carried to new locations by wind. Spores can also be disseminated by water. This mechanism is most important in spreading spores on individual plants, thus resulting in multiple infections. Water dispersal is effective in increasing the size of intensely infected areas early in epidemics since it ensures that all susceptible foliage within the area becomes infected.

Most fungus spores produce infection by direct penetration of the plant cuticle. Following germination, hyphae grow across the host surface. Appressorium formation takes place and hyphal elongation ceases. During this process the developing fungus becomes attached to the host surface. Penetration of the tissue usually occurs either through the formation of a fine infection peg which grows through the

<table>
<thead>
<tr>
<th>Species</th>
<th>Mean length (μ)</th>
<th>Mean width (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Penicillum expansum</em></td>
<td>3.3</td>
<td>3.0</td>
</tr>
<tr>
<td><em>Mycobecticium verrucaria</em></td>
<td>7.0</td>
<td>2.7</td>
</tr>
<tr>
<td><em>Ustilago maydis</em></td>
<td>8.2</td>
<td>7.7</td>
</tr>
<tr>
<td><em>Monolinia fructicola</em></td>
<td>12.0</td>
<td>9.0</td>
</tr>
<tr>
<td><em>Glomerella singulata</em></td>
<td>15.5</td>
<td>5.9</td>
</tr>
<tr>
<td><em>Tilletia caries</em></td>
<td>20.3</td>
<td>18.7</td>
</tr>
<tr>
<td><em>Uromyces phaseoli</em></td>
<td>25.7</td>
<td>21.3</td>
</tr>
<tr>
<td><em>Erysiphe polygoni</em></td>
<td>37.9</td>
<td>19.5</td>
</tr>
</tbody>
</table>

*McCallan, 1958.*

plant cuticle or by means of enzymic action on the cell wall. Once infection takes place, there are few fungicides which will eradicate it. Consequently, a successful protectant fungicide must be able to prevent spore germination or inhibit hyphal growth before the appressorium is formed.

Some infective agents, most commonly bacteria and fungal zoospores, can enter plants through the stomata. In the case of some zoospores, evidence exists that they are guided to the stomata by a directional stimulus. The compound causing the directional movement is evidently water soluble and is probably secreted by the plant. Hence
it can be seen that the interactions involved in infection and infection prevention are extremely complex.

III. THE INTERACTIONS

Various interactions which take place between fungicide sprays, plant surfaces, and the invading fungi are described in this section. Factors which must be considered include the aerodynamics of deposition, wetting of the plant surface, spreading of spray droplets, tenacity of the fungicide deposit, and finally the capacity of the fungicide deposit for protecting plants from infection. Some of these interactions have been investigated exhaustively; all we know about others is based on speculation. Much conflicting evidence appears in the literature on the merits of low- and high-volume spraying, and the value of surfactants and other adjuvants. Probably most of the experimental evidence is valid. However, substantial differences exist between various fungicides, adjuvants, and plant surfaces. These differences tend to be magnified when various components of the system interact. Therefore, it is often dangerous to compare results from seemingly parallel experiments when a new actor is assigned an old role.

A. Deposition

Deposition is the process during which the fungicide mixture in the spray tank is broken up into small droplets and deposited on plant surfaces. Deposition consists of two primary processes; the impact of the droplets on the surfaces, and their retention and spreading to build up a spray deposit. These will be considered separately.

1. Impact

Protectant fungicides are usually applied to fruit and foliage as aqueous sprays, less often as dusts. To be able to settle on the plants, the droplets (or dust particles) must have enough momentum to overcome repulsive forces which exist near surfaces. These may be electrostatic in nature or they can arise from convection currents caused by temperature differentials between the surfaces and the surrounding atmosphere. Furthermore, the high velocity air streams used to propel concentrate sprays or dusts tend to glide around plant surfaces. Consequently, the particles carried by them must have enough momentum to strike the plant surfaces instead of being carried away by the deflected
air current. Momentum is the product of mass times velocity; hence when the particles are extremely minute, they must be projected at high speeds to penetrate these barriers. However, direct impingement of droplets on the surfaces accounts for only part of the fungicide deposited. Much of it misses the main targets and eventually settles on the plants by gravity. Consequently, droplet size cannot be too small, for the limiting velocity a spherical object can attain on falling through still air is given by Stokes’s law

\[ v = \frac{2\pi \eta (d - d_0)}{9\eta} \]

where \( v \) is the limiting velocity, \( d \) is the density of the fungicide, \( d_0 \) the density of air, \( r \) the radius of the particle, \( g \) the acceleration of gravity, and \( \eta \) the viscosity of air. Calculations made from this equation show that a particle with a radius of 10 \( \mu \)m and a density of 2 gm/cm\(^3\) would reach a limiting velocity of 2.3 cm per second and have a momentum of \( 1.9 \times 10^{-8} \) gm/cm/second. This is about the minimum value that would enable it to penetrate the barriers created by repulsive forces at the plant surfaces.

Overcoming the repulsive forces at surfaces is not the only problem in the deposition of small droplets, for calculations from Stoke's law show that a particle 100 \( \mu \)m in diameter with a density of 1 will fall at a rate of 30 cm/second in still air, while a 1 \( \mu \)m particle has a limiting velocity of only 10 cm/hour. Thus, air currents may tend to carry smaller droplets away from the area of application faster than they can settle on the plant surfaces.

In conventional high-volume sprays applied at rates of 75 to 300 gal per acre, drop size is likely to be of the order of 0.5-3 mm. If properly formulated, these large drops easily acquire enough momentum to drench the leaves thoroughly. However, in concentrate spraying, as little as 1 to 15 gal of liquid per acre may be used to distribute the same amount of chemical. Drop size must therefore be reduced dramatically to achieve adequate coverage of the foliage. For application of concentrate sprays from the ground with air-blast equipment, optimum radius is 15 to 40 \( \mu \)m, while for aircraft application it is about 35 to 70 \( \mu \)m (Potts, 1958). While this size range is considerably above the minimum required to overcome the high repulsive forces at the surfaces, it is sufficiently small so that high velocities must be imparted to the particles to carry them to their destinations. Yeomans and Rogers (1953) state that the maximum distance a particle can move in a direction parallel to the ground is directly proportional to its initial
velocity and to the square of its radius. Consequently, very small particles will be stopped much more readily than large ones. For example, a droplet with a radius of 50 \( \mu \) and an initial velocity of 112 mph will travel 150 cm, while a 5 \( \mu \) droplet ejected at the same speed will be stopped after penetrating only 1.5 cm of air. Therefore, small drops must be carried by air currents moving at high velocities to obtain good deposition of concentrate sprays.

Impact of a spray droplet on the surface does not always mean that fungicide is deposited; occasionally small droplets will rebound without wetting the surface, and often the liquid tends to coalesce to form large globs which run off without leaving any deposit. Furmidge (1959) considers a spray droplet to be an elastic sphere which, at the moment of impact, possesses kinetic energy. Some of this energy is transmitted to the surface on which the impact takes place and most of the remainder is absorbed initially as compressive strain energy. This is then transformed into (1) kinetic energy which causes the droplet to flatten radially from the point of impact and (2) surface energy which increases as the droplet flattens owing to the increase in the surface area of the liquid-air and liquid-solid interfaces. The resultant forces on the droplet will be partially in opposition and the fate of the droplet will depend largely upon the point at which equilibrium is reached.

When the surface energy is low and/or the kinetic energy is high, disintegration of the droplets will occur with splashing. In all other cases, once the energy causing the deformation has dissipated, the surface forces produce a contraction of the droplets. In extreme cases this will cause the droplets to bounce off the surface of the plant. This phenomenon will occur only when the initial impact energy and the surface energy are high. This phenomenon has been demonstrated on tea leaves by Brunskill (1956) and on banana by Burchfield and Goenaga (1957b). Otherwise the initial deformation followed by the contraction sets up an oscillation of the droplet. This oscillation will continue until all kinetic energy has been dissipated if no runoff occurs. When the droplet comes to rest, the surface forces will be in equilibrium as given by the following equation

\[
V_{sa} = \gamma_{sl} + \gamma_{la} \cos \theta
\]

where \( V_{sa} \) is the solid-air interfacial tension, \( \gamma_{sl} \) the solid-liquid interfacial tension, \( \gamma_{la} \) the liquid-air interfacial tension, and \( \theta \) is the angle of contact of the liquid on the solid.

As \( \gamma_{sl} \) becomes small compared to \( V_{sa} \), \( \theta \) will also become small. Under
these conditions retraction of the droplet will be less so that a greater area of solid is covered by the liquid. The incorporation of a surface-active agent in the fungicide spray will reduce the values of $\gamma_{1}$ and $\gamma_{2}$ in the equilibrium condition. However, its effect on the very rapid process of impinging is limited by the absorption rate of the material to the two interfaces involved. As noted above, several cases have been described in which changes in surface activity have modified the impact. In the example described by Brunskill (1956), tea plants were sprayed with methanol-water mixtures possessing different surface tensions. It was found that very little of the spray (250 $\mu$ and 350 $\mu$ droplets) was retained until the surface tension was reduced below 60 dynes per centimeter (Fig. 3). In the example described by Burchfield and Goenaga (1957b), it was demonstrated that the surface-active agent Triton X-114 improved the impact of 10-10-100 bordeaux mixture. When banana leaves were attached to a rotating drum and sprayed with the fungicide in the absence of Triton, deposition was so slow that after seven revolutions of the drum a total of only 1 mg
per square centimeter was accumulated. By contrast, a deposit containing 5 mg of Cu per square centimeter was built up from a spray mixture containing 200 ppm of Triton X-114. Many of the spray droplets from the formulation which did not contain Triton were observed to hit the leaf surface and bounce off like minute rubber balls, but the mixtures containing the surfactant wet the leaves uniformly. Thus a 5-fold improvement in initial deposit was obtained as a result of adding the surfactant. It is probable that similar effects would be observed on other hydrophobic plant surfaces such as those of cabbage, rubber, and soybean. However, the impact on beets, tobacco, and cotton would probably not be improved.

Somers (1957) states that the advancing contact angles of water drops on the upper surfaces of leaves of potato, bean, and laurel were 35°, 49°, and 81°, respectively, and that their wettability decreased in that order. Generally, the impact is poorest on plants with smooth waxy surfaces, and best on those with nonwaxy rough or moderately hirsute leaves.

2. Deposit Buildup

   If successful impact is assumed, buildup of the spray deposit depends upon a variety of factors. In low-volume spraying it is essential that the spray fluid be broken up into the smallest droplets possible consistent with reasonable impact properties in order to obtain maximum coverage of plant surfaces. This requires use of an efficient surfactant to obtain maximum spreading. The area covered by each spray droplet will be determined by its intrinsic spreading ability and its energy on impact. Intrinsic spreadability can be determined by measuring the area of spread of droplets in the absence of impact forces (Evans and Martin, 1935). Spreadability is best when the advancing contact angle is low. However, during evaporation of the aqueous phase the volume of the droplet becomes smaller. As its perimeter recedes, the spray material may be carried back with the fluid, thus rendering the surface covered by fungicide somewhat smaller than would be judged by the area of the droplet at maximum spread. This effect is at its lowest when both the receding contact angle and the difference between the advancing and receding contact angles are low. Therefore, the coverage obtained with low-volume sprays is usually improved by use of surface-active agents since these reduce both contact angles and the hysteresis effect.

In high-volume spraying, the amount of protectant deposited on the
plant is proportional to the volume of spray deposited and the concentration of fungicide in the spray until the point of runoff is reached. When this occurs the droplets coalesce and liquid runs off the surface, carrying fungicide with it. The amount of fungicide on the surface immediately after runoff is therefore usually less than the amount just prior to the time at which runoff occurs. Therefore, the longest runoff time consistent with good coverage is desirable. Moilliet and Collie (1951) considered that an intermediate degree of wetting will tend to cause higher deposition on sprayed surfaces than either complete wetting or complete nonwetting owing to the greater adhesion of droplets of intermediate contact angle and interfacial tension. However, optimum wetting properties in each individual case would be impractical to achieve because of the variations that occur in the wettability of different species of plants and for the surfaces of the same plants at different ages.

The use of surfactants in high-volume fungicide sprays decreases the volume of spray required to obtain runoff and consequently decreases deposits. For example, Cupples (1941) found that improved wetting properties led to lower fungicide deposits on apples. Similarly, Maxwell et al. (1965) found that the initial deposit of lead arsenate on apple was reduced from 10.7 mg to 5.5 mg when Triton B 1956 was added to the formulation and spraying was continued to the point of runoff (Table III). Somers (1956) showed that spray deposits of Burgundy mixture, Bordeaux mixture, and cuprous oxide were not increased when laurel leaves, bean leaves, and cellulose acetate films were sprayed beyond the point of runoff. In these experiments, runoff occurred below 1 second and spraying was continued for 30 seconds. This work also demonstrated that low concentrations of anionic, cationic, and nonionic surfactants slightly lowered the amount of deposit at runoff.

At low-gallonage applications, the beneficial effects of surface-active materials are retained without sacrificing deposit buildup. Thus, Swales and Williams (1956) report that inclusion of nonionic surfactants in lime sulfur and ferbam concentrate spray mixtures improved their effectiveness for the control of apple scab. This may have resulted in part from improved distribution of the fungicide on the leaf surfaces, but it is also likely that overall deposition may have been increased because of improved impact properties. This is suggested by the work of Pielou and Williams (1962), who compared the buildup of carbaryl on cherry leaves using high- and low-volume sprays with and without a spreader-sticker (Table IV). With the low-volume application, 3.3
TABLE III

<table>
<thead>
<tr>
<th>Additive</th>
<th>Amount</th>
<th>Surface tension (dynes/cm)</th>
<th>Initial deposit (mg)</th>
<th>Loss of As after 1 hour rain</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>88</td>
<td>10.7</td>
<td>37</td>
</tr>
<tr>
<td>Captain</td>
<td>2 lb/100 gal</td>
<td>74</td>
<td>9.1</td>
<td>42</td>
</tr>
<tr>
<td>Glyodin</td>
<td>1 qt/100 gal</td>
<td>52</td>
<td>5.0</td>
<td>34</td>
</tr>
<tr>
<td>Triton B 1050</td>
<td>0.25 lb/100 gal</td>
<td>49</td>
<td>5.5</td>
<td>36</td>
</tr>
</tbody>
</table>

*a Maxwell et al., 1965.
b Arsenate was applied at rate of 3 lb/100 gal.

mg of carbaryl per square centimeter of leaf surface was deposited with the spreader-sticker and 2.5 mg per square centimeter in its absence, thus indicating that the adjuvant increased deposition of the spray. However, when spraying was continued beyond runoff, (high volume) the deposit was 1.8 mg per square centimeter with spreader-sticker and 3.1 mg per square centimeter without it. Evidently the surface-active properties of the formulation resulted in premature runoff with the high-volume application and improved impact with the low-volume application. Consequently, the kinds and amounts of surfactants to use will depend upon the type of application.

Other things being equal, the amount of fungicide deposited on a plant surface should be directly proportional to its concentration in the spray tank. However, indirect proportionality has been reported.

TABLE IV

<table>
<thead>
<tr>
<th>Type of application</th>
<th>With spreader-sticker</th>
<th>Without spreader-sticker</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-volume (16 lb/100 gal)</td>
<td>3.3</td>
<td>2.5</td>
</tr>
<tr>
<td>High-volume (1 lb/100 gal)</td>
<td>1.8</td>
<td>3.1</td>
</tr>
</tbody>
</table>

* Pielou and Williams, 1962.
Thus Rich (1954) found that the amount of bordeaux mixture deposited on leaves of beans and celery tended to reach a limiting value as the concentration in the spray tank was increased, while zineb deposits increased linearly with concentration in the range studied. Rich pointed out that bordeaux particles are positively charged—a fact which might cause them to be attracted to negatively charged leaf surfaces by electrokinetic forces. As the unoccupied sites on the foliage surfaces became reduced in number, rate of deposition would tend to decrease. These results seem inconsistent, since zineb particles are negatively charged and thus should be deposited with less rather than greater efficiency than bordeaux. However, the concentration range studied was not the same for the two fungicides, so more evidence is required. The most reasonable explanation for this phenomenon is that deposit was directly proportional to concentration up to the point of runoff. When this condition was finally exceeded, bordeaux continued to be deposited at a slower rate, the runoff water being slightly poorer in copper than the impinging spray droplets. Thus, deposition might not cease abruptly when runoff is reached, but continue awhile beyond it until the capacity of the leaves for retaining fungicide is exhausted. This could not occur when runoff is very rapid and insufficient time is allowed for the fungicide to become attached to the leaves. It has not been established whether or not electrokinetic affects are involved in this process. However, in view of the finding by Somers (1957) that negatively charged surfactants such as sodium dioctyl sulfosuccinate decreased deposit buildup, a reinvestigation of this problem would be of great interest.

The nature of the leaf surface greatly influences deposit buildup when spraying is carried out to the point of runoff. This is illustrated by Fig. 4, in which measured spray retention in milligrams per square centimeter is plotted against the retention factor $F$, calculated from surface tensions and contact angles by Eq. (2). Leaves of blackberry and currant showed a satisfactory linear relationship between the calculated and observed values. However, it should be noted that these lines would intercept the ordinate at values greater than zero. This indicates that the leaves contain irregular depressions which form physical barriers. These tend to reduce runoff because spray liquid collects in them. The data obtained on banana and rubber leaves show that the relations between calculated and observed retentions were non-linear, and in most cases were lower than predicted by Eq. (2). This probably arises from poor impact because of the hydrophobic nature of these surfaces.

B. Persistence

Persistence is a term used to describe the longevity of a pesticide deposit on a surface. All pesticide deposits tend to decline more or less rapidly, and in fact it is necessary that they do so in order to meet Food and Drug Administration residue tolerances at time of harvest. Disappearance of spray deposit in the field is ascribed to a number of mechanisms. These include chemical and photochemical deterioration, sublimation, erosion by wind and rainfall, and finally, attenuation of the deposit by growth of the plant and resulting expansion of the fruit and foliage surfaces.

1. Decomposition

Fungicides are likely candidates for chemical decomposition through photolysis since they are applied as finely divided powders with high specific surfaces. Thus they could easily dissolve in moisture films at foliage surfaces where they could be acted upon by sunlight. The earth's atmosphere absorbs much of the harder radiation. Approximately 40% of the total solar energy reaching the ground level is in the 4000–7600 A (visible) region of the spectrum, while 5% or less is below 4000 A. Consequently, only those compounds which absorb light in the visible or near ultraviolet regions are likely to be affected seriously.
The most important group of fungicides known to undergo photolysis are the quinones (Burchfield and McNew, 1950). In aqueous solution, \( p \)-benzoquinone yields hydroquinone and a product believed to be a dimer when exposed to light of wavelengths less than 5770 Å. The quantum yield is 0.505. The efficiency of the photochemical process decreases stepwise as chlorine atoms are introduced into a molecule. Thus the quantum yield for chloranil is only 0.095. Despite this low efficiency, aqueous solutions of it are very unstable unless stored in the dark.

The threshold wavelength region for the photolysis of quinones decreases with decreasing oxidation potential. Thus chloranil with a potential of 0.73 V and a threshold region in the neighborhood of 5770 Å should decompose more readily when exposed to light than dichlone with an oxidation potential of 0.3 V. Measurements of the decomposition rates of the two compounds in dioxane-water solutions exposed to sunlight indicate that dichlone is the more stable compound. This agrees with practical experience, for although chloranil is a good seed protectant, it has poor persistence on foliage, while dichlone is an effective fungicide in both applications.

Sometimes sensitivity to ultraviolet radiation can be reduced by formulation. Thus the deterioration of rotenone deposits can be retarded by the use of carbon black (Jones et al., 1933), while antioxidants such as hydroquinone are good stabilizers for pyrethrins (Bell and Kato, 1956). Antioxidants probably inhibit photolysis by forming free radicals of their own which can terminate chain reactions. They would not be effective for inhibiting reactions with quantum efficiency much less than unity, since these are self-terminating.

Fungicides which are esters, amides, or contain reactive halogen atoms or nitro groups often hydrolyze in water to yield products which are inert or possess modified biological activity. This may be one of the principal avenues through which some pesticides are attenuated by chemical reaction on plant surfaces. Stabilities in aqueous media vary enormously, even between compounds having the same mode of action. Thus tetraethylpyrophosphate, a powerful esterase inhibitor, hydrolyzes to ethanol and inorganic phosphate with great rapidity, while compounds with similar function such as malathion and parathion are considerably more stable.

Fungicides known to hydrolyze in aqueous solution include captan, phalan, Dyrene, dichlone, and FDNB (1-fluoro-2,4-dinitrobenzene). Generally, these reactions are believed to proceed through the replacement of a halogen by a hydroxyl ion. Daines et al. (1957) found that
captan decomposes slowly at pH 7 and instantaneously in the presence of sodium hydroxide. Actually, decomposition is rapid even in neutral solution. Burchfield and Schechtman (1958) showed that captan has a half-life of only 2.5 hours in aqueous buffer at pH 7. However, it persists much longer than this on foliage because of its low water solubility and due to the fact that only material in true solution can hydrolyze. Nevertheless, it is an unstable compound toward hydrolysis compared to fungicides such as Dyrene and FDNB which have half-lives of about 20 days under similar conditions (Burchfield and Storrs, 1956, 1957b).

Many fungicides are potentially capable of reacting with naturally occurring plant metabolites. These include amino acids, peptides, and other compounds containing nucleophilic functional groups. Reactions take place most often through addition or substitution mechanisms. Fungicides that can undergo addition reactions include some quinones, N-aryl maleimides, and compounds with double bonds $\alpha-\beta$ to carbonyl groups. These chemicals can react with free amino and thiol groups as follows:

\[
\text{HC-} + \text{RSH} \rightarrow \text{HC-} + \text{RSH} 
\]

Fungicides that can participate in true substitution reactions include dichlone, chloranil, Dyrene, pentachloronitrobenzene, captan, phalan, FDNB, nitrochlorobenzenes and naphthalenes, and compounds with aliphatic alogens. They differ enormously in stability toward hydrolysis and reactivity with various metabolites. Yet their overall reactions are very similar, as exemplified by the combination of an $s$-triazine (Dyrene) with a thiol:

\[
\text{Cl} \quad \text{N} \quad \text{N} \quad \text{Cl} + \text{RSH} \rightarrow \text{Cl} \quad \text{N} \quad \text{N} \quad \text{Cl} + \text{RSH} 
\]

The degree to which these reactions are responsible for attenuating spray deposits is unknown. However, such reactions are undoubtedly important in the stability of these compounds in soil as indicated by the fact that they decompose in this environment much more rapidly than in aqueous solution (Burchfield, 1959).
2. Volatility

Fungicides can also disappear from the infection court by sublimation. The air in direct contact with small spherical particles of volatile compounds is saturated with vapor, and the rate of exchange of molecules between the solid and its surrounding shell of vapor is very rapid, compared to the rate at which vapor can diffuse away into the air. Consequently, rate of evaporation is determined in part by rate of diffusion through the surrounding air and is given by

$$\frac{-dm}{dt} = \frac{4\pi r D M p}{RT}$$

where \(\frac{-dm}{dt}\) is the rate of weight loss of a single particle, \(r\) the radius of the particle, \(D\) the diffusion coefficient of the compound, \(M\) its molecular weight, \(p\) its vapor pressure, \(R\) the gas constant, and \(T\) the absolute temperature. Thus the molecular weight of the compound and its diffusion coefficient, as well as vapor pressure, influence rate of dissipation by sublimation. It is noteworthy that the weight loss of small particles is proportional to their radii and not to their surfaces.

Thatcher and Streeter (1925) have shown that sulfur deposits are attenuated by sublimation. Later Miller and Stoddard (1957) found that o-chloronitrobenzene had strong fumigant action, pentachloronitrobenzene moderate fumigant action, and captan and chloranil weak fumigant action when tested against four species of fungi in closed containers. Thiram and other related compounds were ineffective. This suggests that sublimation might be an important factor in the disappearance of some of these compounds from the infection court and be negligible in others. Decker (1957) regards sublimation to be highly important in determining the rate of residue loss of insecticides. He points out that persistence is directly proportional to the logarithm of the time of exposure under conditions where losses caused by wind, rain, and plant growth are negligible. Furthermore, persistence of insecticides on foliage decreases in the order DDT > methoxychlor > toxaphene > dieldrin > chlordane > heptachlor > aldrin > lindane, which is the order of increasing volatility. Since most of these compounds are stable chemically, sublimation appears to be the only reasonable way to account for their disappearance.

3. Tenacity

Fungicides can be dislodged from plant surfaces mechanically by wind and rain. Thus Somers and Thomas (1956) found that wind alone reduces cuprous oxide deposits by more than 50% after 27 days exposure,
while Byrdy et al. (1957) report significant reductions in the toxicity of DDT residues after exposure for 5 minutes to air currents with a velocity of 3.5 meters/second. Rain removes spray deposits considerably faster than wind, but in the field the action of wind is more prolonged. After rapid initial loss, commonly called sloughing, the logarithm of the percent of fungicide remaining on the foliage is proportional to inches of rain (Fig. 5.). This is true for both hydrogels such as bordeaux mixture, and for particulate fungicides. Burchfield and Goenaga (1957b) found that 43% of the cuprous oxide deposited on banana leaves was removed by the first quarter inch of rain (Table V). However, the next

![Graph showing the relationship between percent deposit retained on banana leaves and inches of rain for 10-10-100 bordeaux mixture formulated with Triton X-114 (Burchfield and Goenaga, 1957b).](image)

**FIG. 5.** Relation between the percent deposit retained on banana leaves and inches of rain for 10-10-100 bordeaux mixture formulated with Triton X-114 (Burchfield and Goenaga, 1957b).

**TABLE V**

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Percent Cu retained after inches of rain</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>10 0 0 0 0</td>
</tr>
<tr>
<td>600 ppm of rain soap</td>
<td>14 10 8 6 4</td>
</tr>
<tr>
<td>200 ppm of Pluronic F-68</td>
<td>36 29 26 18 22</td>
</tr>
<tr>
<td>200 ppm of Tween 20</td>
<td>56 41 33 29 23</td>
</tr>
<tr>
<td>200 ppm of Triton X-114</td>
<td>82 85 81 74 65</td>
</tr>
</tbody>
</table>

* Burchfield and Goenaga, 1957b.
quarter inch removed only 4% and succeeding treatments removed only about 1-2% per 0.5 inch of rain. Similar results were obtained with cuprous oxide on artificial surfaces (Sommers and Thomas, 1956), suggesting that 30-80% of most fungicides is deposited in forms that have very poor residual properties. Pond and Chisholm (1958) reported that 26-63% of the amount of DDT on potatoes was lost during 24-hours' weathering in the field, so it appears that as much as one-half of the value of the chemicals used for plant protection is expended with small return.

One reason for this high initial disappearance of fungicide may be unfavorable particle size distribution. This was shown by the work of Somers and Thomas (1956) who demonstrated that copper oxide particles over 5 μ in diameter had very little tenacity to artificial surfaces (Fig. 6.). It is probable that the tenacity of fungicides increases with decreasing particle size because of higher specific surface. This allows for a greater total area of contact between the fungicide and the plant surface. Since the total force of adhesion is the product of the contact area and the force per unit area, it is evident that increasing the former automatically results in an increase in total force of adhesion. Furthermore, small particles should resist dislodgement better since they are lighter in weight. Thus it is possible that the high initial losses experienced on weathering arise from a rapid and complete loss of large particles, while the tenacious part of the residue consists of small particles. Ground powders contain many more small particles
than large ones, but the large ones usually contribute most to the weight. Thus, one particle with a radius of 10 μ would weigh as much as 1000 particles with radii of 1 μ. Therefore, if particle size is the predominant factor in regulating tenacity, a high initial rate of loss should occur as large particles are removed, followed by a slower rate of loss of the remainder of the deposit, as is found in practice.

Primary particle size of the fungicide alone probably does not account for all of this effect, because aggregates of small particles can be formed which could behave like large particles in weathering tests. Thus Gullstrom and Burchfield (1948) found it necessary to use a 0.25% solution of dispersing agent to deflocculate dichlone to the point where the particles would sediment individually rather than in groups. This is far in excess of the amounts of these agents that would be included in commercial fungicide formulations. It is likely, therefore, that most materials are aggregated to a greater or lesser extent in the spray tank and that these agglomerates are carried over onto the plants. Further evidence for the occurrence of interactions between particles can be adduced from the findings of Somers and Thomas (1956) that the tenacities of copper fungicides increased with decreasing initial deposit. This agrees with the early observation of Turner and Woodruff (1948), who suggested that particle-surface adhesion governs the tenacity of sparse spray deposits while particle-particle cohesion becomes important with increasing deposit. This is equivalent to saying that aggregates have lower tenacity than discrete particles. Assuming random distribution of the fungicide on the plant surface, lateral associations between particles would be negligible since scale drawings have shown that dichlone can control early blight when most of the surface is unoccupied by particles (Burchfield and McNew, 1950). However, in practice, distribution of primary fungicide particles is probably far from random, owing to the presence of aggregates in the spray tank.

Tenacity is also influenced by the spreading properties of the spray on foliage. Thus, bordeaux mixture applied to the waxy leaves of bananas collects as discrete droplets at the leaf veins (Burchfield and Goenaga, 1957b). On drying, these form small friable pellets of bordeaux which are easily sloughed off mechanically by the first rain. The addition of a nonionic surfactant to the mixture reduces interfacial tension so that the droplets spread. The deposit that is formed is exceedingly tenacious, even in the presence of three times the amount of surfactant required for good spreading. Tenacity increases with increasing effectiveness of the surfactant. Thus the retention obtained
with Triton X-114 is noticeably superior to that obtained with Phuronics F-68 (Table V). Similar results were obtained with copper oxide deposits on banana leaves, so presumably these results hold for particulate fungicides as well as hydrogels (Table VI). Presumably good spreading results in the formation of smaller and fewer aggregates during drying. However, the use of large amounts of surfactants may lead to premature runoff in high-gallonage applications, and the foliage may be more easily rewet by rain. Many workers believe that surfactants lead to high losses during periods of rainy weather. Fajans and Martin (1938) showed that the addition of surface-active agents reduced the tenacity of cuprous oxide when applied to artificial surfaces. In support of this view, Linskens (1951) has demonstrated that the wettability of leaves treated with surface-active agents remained increased for more than 4 weeks after treatment. However, this is not supported by all of the available data. Thus the experiments of Maxwell et al. (1965) showed that Triton B-1956 reduced the deposition of lead arsenate when apples were sprayed to run-off, but that tenacity was not reduced (Table III). Also, it is apparent that the adhesion of cuprous oxide particles to banana leaves is improved by surfactants (Table VI). It is, therefore, evident that the influence of surfactants on tenacity is determined by a number of interacting factors: fungicide, surfactant, rate of application, and leaf surface.

### Table VI

**Effects of Various Adjuvants on the Tenacity of Cuprous Oxide to Banana Leaves**

<table>
<thead>
<tr>
<th>Adjuvants</th>
<th>Percent Cu retained after inches of rain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>None</td>
<td>26</td>
</tr>
<tr>
<td>Binderine flour</td>
<td>22</td>
</tr>
<tr>
<td>Corpus oil</td>
<td>32</td>
</tr>
<tr>
<td>Triton X-114</td>
<td>57</td>
</tr>
<tr>
<td>Triton X-114 plus National sticker</td>
<td>55</td>
</tr>
</tbody>
</table>

* Burchfield and Goenaga, 1957b.

### 4. Plant Growth

In the foregoing sections it has been seen that losses of pesticides can occur through chemical decomposition, sublimation, and weather-
ing by wind and rain. These are real losses. However, apparent losses can be caused by plant growth. In some cases these losses can be as serious as those caused by disappearance of the fungicide from the surface environment. Taschenberg et al. (1963) have studied the disappearance of spray deposits of DDT, methoxychlor, Perthane, ethion, and diazinon from Concord grapes. Their experiments were designed to measure both the actual and apparent attenuation of the pesticide residue (Table VII). They concluded that growth is an important factor in attenuating spray deposits, but that its effect is somewhat limited in the case of grapes because the berries have attained from 70-75% of harvest weight at the time of the third application of a three-spray schedule. Also, during the latter half of the growing season the large leaves and the method of training vines on a trellis tended to protect the pesticide deposit on clusters from the erosive effect of rainfall and also limited exposure of clusters to sunlight. Nevertheless, even under these favorable conditions, apparent losses due to growth are sizeable. Measurements of residues made after 56 days from application showed that the apparent loss due to growth was 25% of the initial deposit as compared to a 34% actual loss due to weathering. Thus under these very favorable conditions the attenuation caused by growth was of the same order of magnitude as the actual loss. This result would be accentuated on rapidly growing surfaces. However, it should be mentioned that the actual loss was lower than would be encountered on a fresh spray application. These measurements were made after the third application of pesticide spray. Major sloughing off had already occurred and the residues from the two previous sprays were probably very tenacious.

**TABLE VII**

<table>
<thead>
<tr>
<th>Days after application</th>
<th>Total residue per 100 berries (mg)</th>
<th>Weight of 100 berries (gm)</th>
<th>Actual loss (%)</th>
<th>Loss due to growth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1100</td>
<td>250</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>1130</td>
<td>260</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>28</td>
<td>939</td>
<td>319</td>
<td>16</td>
<td>21</td>
</tr>
<tr>
<td>42</td>
<td>949</td>
<td>335</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td>56</td>
<td>739</td>
<td>339</td>
<td>34</td>
<td>25</td>
</tr>
</tbody>
</table>

Taschenberg et al., 1963.
C. Biological Activity

The usefulness of fungicides and the effectiveness with which they are formulated must be evaluated by measuring biological activity. Intrinsic fungitoxicity is a function of each individual chemical compound and the organism against which it is tested. Consequently, each case must be treated separately. It is possible to modify intrinsic fungitoxicity by formulation, however, so a few generalizations can be made on this subject.

Phytotoxicity or injury to the host plant is often a serious problem with experimental and commercial fungicides. This occurs because most fungicides are relatively nonspecific in their modes of action. They function by disrupting cell membranes through surface effects, precipitating enzymes and other macromolecules, or reacting indiscriminately with amino acids, peptides, and other intermediary metabolites. This situation exists because fungus spores and mycelia are relatively undifferentiated organisms which do not possess specialized structures such as chloroplasts, nerve tissue, or erythrocytes which can be acted upon by toxicants selectively. Hence most fungicides are general protoplasmic poisons which can be as injurious to the host as to the invader, given the opportunity. However, the host is usually protected by a position effect, since fungicide particles are deposited on the cuticle of the plant where they cannot readily permeate the vascular system or cell membranes.

1. Fungitoxicity

Deposition of a fungicide on a plant surface is not by itself sufficient to insure the utmost utilization of its capacity for controlling disease. It must be distributed so that the maximum number of potential loci of infection is protected. Wilcoxon and McCallan (1931) found that there were significant differences in toxicity between sulfur dusts having different particle sizes when they were compared on an equal weight basis, the more finely divided dust being able to inhibit spore germination at lower doses. When the dusts were compared on the basis of an equal number of particles per unit area, there was no major difference in toxicity. This illustrates the importance of good coverage in protecting surfaces and stresses that the number of particles used is most critical when they are distributed randomly on the plant surface. However, the sizes of the individual particles cannot be neglected entirely, for this would imply that small particles containing minute amounts of fungicide would be as effective as large ones for preventing infection...
by the parasite. This supposition may be approximately correct within a limited range, but must be invalid when the particles become extremely small. This is shown by the findings of Burchfield and McNew (1950), who measured the capacity of different particle-size fractions of dichlone to control early blight of tomatoes in greenhouse tests. When particle size was reduced from a mean radius of 24.5 to 0.45 μ only 1/40th as much dichlone was required to maintain disease control (Fig. 7). Fungicidal efficiency, therefore, was not directly proportional to the number of particles on the surface. Instead, a limiting value was approached at small particle sizes, indicating that capacity for controlling disease was proportional to the logarithm of the number of particles per unit area of surface, or

$$\frac{1}{G} = m \log_{10} \frac{3G}{4\pi r^2 d} + q$$

where G is the weight of fungicide per unit area required to control the disease, d its density, r the mean particle radius, and m and q are empirical constants.

This equation suggests that two basic factors are involved in the protection of surfaces by fungicides. These are: (1) that the number of particles necessary to insure that each potential locus of infection is
given nominal protection is constant and independent of the dose; and
(2) that on subdivision of a constant weight of material the rate of
change of fungicidal effectiveness is inversely proportional to the num­
ber of particles present at the time of the change. The first of these
assumptions is based on the supposition that the fungicide is randomly
distributed on the plant surface. If this occurs, a small number of large
particles will lead to superabundance of chemical in some localities and
little or none in others, while subdividing the material will tend to
equalize coverage at various loci of potential infection.

Equation (6), as well as the practical results of many workers,
shows that improvement in efficiency of utilization by reducing particle
size soon reaches a limiting value beyond which it is uneconomical to
go. Thus the effectiveness of dichlone in protecting tomatoes from early
blight was increased by only 25% on decreasing mean radius from 0.81
to 0.5 μ while the specific surface of the powder, which is related to the
work required for comminution, was almost doubled.

Furthermore, it suggests that weak fungicides which can be used at
high doses because of low cost are not likely to be improved as much by
extreme subdivision as compounds with high intrinsic toxicity. A ma­
terial with very low toxicity would have to be applied at such high
doses to obtain any disease control at all that the surface would be
saturated with respect to coverage at comparatively large particle
sizes. Conversely, when intrinsic toxicity is very high, great care must
be taken to distribute the smaller amount of material in such a way
that all of the potential infection loci are protected. Thus compounds
which must be used at relative doses of 100, 10, and 1 units because of
differences in intrinsic toxicity would give equivalent coverage at mean
particle radii of 3.2, 1.6, and 0.7 μ, respectively.

Even in greenhouse tests coverage is never so complete as would be
predicted from particle size alone, since sprays are deposited on leaves
unevenly. Often the droplets coalesce and runoff occurs, leaving differ­
ent amounts of fungicide on the tips and veins of leaves than found on
the edges and interiors of the blades. Field applications are even more
spotty, and this condition is likely to be aggravated in deposits formed
from low-gallonage concentrate sprays where as little as 15% of the
plant surfaces may be coated with fungicide. In these cases, disease
control in unprotected areas may be achieved through redistribution
by dew and rain so that physical factors other than particle size become
important (Rich, 1954). Thus Björling and Sellgren (1957) found that
rain treatments improved the protection of potato foliage by bordeaux
mixture against infection by Phytophthora infestans when the fungi-
cide was applied as small droplets in large volumes, while deposits formed from large drops applied at high volumes were not affected significantly. They ascribed this improvement to the local redistribution of the fungicide by weathering. However, they found that the effects of rain on zineb deposits were erratic. Improvements in disease control were recorded in a few tests, but more often deterioration of the protective power of the residue occurred. Perhaps this difference rose in part from the fact that bordeaux mixture is much more tenacious than particulate fungicides and that the effects of redistribution were not overshadowed by a high overall loss in residue (Burchfield and Goenaga, 1957b). Furthermore, the dosage response curve of bordeaux mixture is exceedingly flat, so disease control would not be seriously reduced in protected areas by the removal of sizable amounts of fungicide. The redistribution of comparatively small amounts of copper in previously unprotected spots might result in more effective disease control in these locations. Thus the overall result might be favorable despite a net loss of fungicide. However, this behavior would not be expected from compounds having very steep dosage response curves and poor tenacity, since control in protected areas might be decreased sharply without commensurate improvements in other localities owing to the relatively small amount of toxicant redistributed.

Butt (1955) investigated the movement of captan deposits on pear leaves and obtained no evidence to show that it was translocated in leaf tissue. He assumed that its fungicidal action was exerted through water layers linking the captan to the spores. Photomicrographs of captan deposits in water drops suggested that particles of the fungicide may become detached from the leaves and be redeposited near the periphery of the original residue, thus resulting in an expansion of the protected area. Presumably this process would be carried out more efficiently by gentle dews than by driving rains since the latter would be expected to result in an overall loss of the spray deposit with negligible opportunities for redistribution of dislodged particles and aggregates.

Contrary to the finding of Butt, Napier et al. (1957) present evidence which they interpret to mean that captan exerts systemic action in the protection of broad beans against Botrytis fabae. They found that sprays applied to the dorsal surfaces of leaves resulted in significant reduction in the number of lesions found on the ventral surfaces, although complete disease control was never achieved. Furthermore, treatment of the first leaves of bean resulted in reduction of the infection incidence of the second and third leaves. While diffusion of
captan through leaves via dorsal and ventral stomata and the loosely organized structure of the spongy parenchyma is possible, translocation through greater distances in the vascular tissue of the plant is unlikely, owing to the extremely high instability of captan in aqueous media (Burchfield and Schechtman, 1958). It should be noted that the bean plants used in these experiments were incubated in a moist chamber for 18 hours to provide conditions suitable for infection by the pathogen. Thus the fungicide may have been redistributed in small moisture droplets conveyed to unprotected areas by convection currents. It must be considered that some metals have high enough vapor pressures to kill spores by fumigant action in enclosed spaces. Examples from the history of plant pathology show that similar cases of “action at a distance” have been misinterpreted. Therefore, experiments which show that protectant fungicides can be redistributed by translocation within the plant must be evaluated critically before final acceptance.

As shown above, fungicides must be ground to small particle size to obtain adequate coverage of plant surfaces. However, extreme subdivision may lead to poor persistence when the compound is volatile or can react chemically at the plant surface. The total surface of fungicide per unit area of leaf or fruit is

\[ A = 3 \frac{G}{\pi d} \]  

where \( A \) is the surface, \( G \) the weight of fungicide per unit area, \( d \) its density, and \( r \) the particle radius. Thus the surface of fungicide exposed to weathering is ten times greater at a particle radius of 0.5 \( \mu \) than at 5 \( \mu \). Burchfield and McNew (1950) suggest that a compromise between coverage and surface exposed might be reached by selecting a particle radius near where disease control is achieved at minimum total surface of fungicide. Theoretical calculations based on data obtained on dichlone show this to be

\[ r = \left( \frac{3}{8mdk^{2/3}v^{1/3}} \right)^{1/3} = 4.9 \mu \]  

where the constants are the same as those of Eq. (6). This relationship is illustrated in Fig. 8. It is based on the fact that when particle size is large, specific surface is small. However, so much material is required to give disease control that the total surface of the fungicide per unit area of plant is large, showing that it is not being used efficiently. As particle radius is reduced, coverage improves faster than specific surface increases, so that the total surface of fungicide required for control of the disease decreases. Finally, when the surface nears sat-
uration with particles, the effect of increased specific surface predominates, and the total surface of fungicide per unit plant area required for disease control increases. The radius of 4.9 μ given by Eq. (8) is the minimum of the curve shown in Fig. 8.

When dichlone formulations having mean particle radii of 1.5 and 3.6 μ were evaluated for capacity to control tomato early blight, fungicidal efficiency diminished by only 30% when the plants were held in the greenhouse for one week between spraying and inoculation. At radii less than 1 μ, 80% of the protective value of the fungicide was lost, and at radii of 8.4 μ and above it disappeared altogether. Presumably the total failure of the larger particle size preparations to control the disease arose from poor tenacity even in the absence of rain. Sulfur has also been shown to have impaired residual properties at very small particle size, but fixed coppers do not, possibly because they are chemically stable and have negligibly low vapor pressure (Horsfall, 1956). Thus the optimum size distribution for good persistence is regulated by the intrinsic chemical and physical properties of the fungicide.

Disease control also diminishes on the erosion of spray deposits by rain. Thus when captan and maneb were applied to tomato plants at
spray concentration of 16 to 250 ppm, and the plants exposed to various amounts of artificial rain prior to inoculation, disease control varied in an orderly way with respect to concentration of fungicide and amount of rain (Burchfield and Goenaga, 1957a). At low doses of captan, disease control was lost more rapidly than at high doses presumably because the fungicide was present in an excess in the latter cases (Table VIII). It is interesting to note that the disease control obtained at 125 and 50 ppm of captan after 2 inches of rain was about the same as found at 16 ppm without rain. This suggests that about 85–95% of the fungicide was lost during weathering. Similar results were obtained with maneb, although the general level of effectiveness was considerably higher since this material is superior to captan for the control of early blight. Thus at 125 and 50 ppm good control of the disease was obtained after all rainfall treatments, presumably because an excess of fungicide was present initially, and a fraction of it was tenacious enough to stand weathering. However, when the spray concentration was reduced to 16 ppm, disease control was lost rapidly with increasing rain.

Disease control was not directly proportional to tenacity since the

<table>
<thead>
<tr>
<th>Inches of rain</th>
<th>0</th>
<th>0.25</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 ppm</td>
<td>66</td>
<td>49</td>
<td>34</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>32 ppm</td>
<td>86</td>
<td>78</td>
<td>63</td>
<td>62</td>
<td>45</td>
</tr>
<tr>
<td>63 ppm</td>
<td>86</td>
<td>77</td>
<td>67</td>
<td>71</td>
<td>63</td>
</tr>
<tr>
<td>125 ppm</td>
<td>95</td>
<td>90</td>
<td>85</td>
<td>68</td>
<td>50</td>
</tr>
<tr>
<td>250 ppm</td>
<td>99</td>
<td>99</td>
<td>96</td>
<td>83</td>
<td>62</td>
</tr>
</tbody>
</table>

* Burchfield and Goenaga, 1957.
slopes of the dosage response curves as well as the amounts of fungicide per unit per leaf area must be taken into consideration. However, it should be noted that 63 ppm of captan without rain gave about the same control as 125 ppm after exposure to 0.5 inch of rain showing that about one-half of the fungicide was lost by this mild treatment.

Since 50% or more of the spray deposits formed from particulate fungicides are sloughed off under mild weathering conditions, it seems likely that compounds of this class are not being utilized to their full efficiency in the field.

However, the use of stickers does not appear to provide an answer to this problem. Agar, linseed oil, lime casein, polyvinyl acetate, coumaron resins, rubber latex, and polyvinyl chloride were all found to increase the retention of cupric oxide to artificial surfaces on exposure to rain (Somers, 1956). However, bioassay against Alternaria tenuiss showed that of these compounds, polyvinyl chloride and polyvinyl acetate were the only adjuvants that did not decrease the fungitoxicity of the copper oxide. Field tests against potato blight revealed that only the agar formulation gave better control than cupric oxide alone, and this was significant only at the 5% level. Laboratory bioassay revealed that agar, coumaron resin, and linseed oil lowered the fungitoxicity of copper oxide while lime casein suppressed its toxicity completely. It is probable that an impervious coating forms on the surface of the fungicide particles which prevents the diffusion of the toxicant to the spores. This performance is characteristic of most materials which have been evaluated as stickers; they often improve tenacity, but usually at the expense of fungitoxicity.

2. Phytotoxicity

Injury to the host plant is often a limiting factor in the use of fungicides. This must be expected, since most chemicals used as plant protectants can react with components of protoplasm, chelate essential metals, or accumulate at vital biological interfaces. They are less specific in their action than insecticides, herbicides, and medicinal chemicals, and are thus likely to be phytotoxic if they can penetrate the cuticularized tissue protecting the host. Thus compounds such as dichlone, which otherwise can be used safely on many plants, will cause severe burning when formulated in oils. Presumably the fungicide dissolves in the hydrocarbon which enables it to permeate the leaf tissue more efficiently.

Sometimes the difference between a safe fungicide and a phytotoxic compound is determined by the length or nature of a side chain. In a
study of imidazoline derivatives Wellman and McCallan (1946) found that optimum fungitoxicity was obtained when the alkyl side chain substituted in the 2-position of the heterocyclic ring contained 17 carbon atoms, but when it contained 11 carbon atoms the compound was phytotoxic. Similarly, Schuldt and Wolf (1956) showed that derivatives of 2,4-dichloro-6-anilino-s-triazine are good protectant fungicides, while Koopman and Daams (1958) found that similar triazines containing alkyl groups in place of aryl groups are herbicides.

Some of these differences in specificity of action may arise from changes in uptake and solubility relationships. This is illustrated below for an s-triazine series (Burchfield and Storrs, 1957a).

Thus, compound (I) is not accumulated rapidly by fungus spores, and is ineffective for the control of early and late blights of tomato. Its solubility in water is about 100 ppm and it injures the test plants. Substitution of a methyl group in the ortho-position of the benzene ring (II) reduces solubility to 60 ppm and results in improved fungitoxicity, while a chlorine atom in this position (III) enhances its properties still further. The solubility of Dyrene (III) in water is only 10 ppm and it is a far better fungicide than (I). Furthermore, it is not phytotoxic to the test plants under conditions where (I) produces severe injury. Since both compounds react with the same metabolites and compete for the same sites within fungus spores it is likely that they have the same mode of action (Burchfield and Storrs, 1957a).
Therefore, the difference in phytotoxicity between these compounds may arise from a difference in solubility and hence in rate of movement. Compound (I), being ten times more soluble in water than (III) might dissolve faster in moisture films and move into the host plants through the stomata. Water seems to be implicated in the movement of these compounds, since Dyrene causes necrotic flecking and defoliation of pepper plants when they are incubated in a moist chamber for 24 hours before being placed in a greenhouse. Plants sprayed with Dyrene and transferred directly to the greenhouse bench were unaffected. The leaves were dropped while still turgid by disintegration of the abscission layer, so evidently this compound can be translocated to some extent under extreme conditions of humidity. However, solubility cannot be the only factor governing phytotoxicity, since captan is at least as soluble as Dyrene in water, and it is considerably safer for use on apples for the control of scab. An explanation might be sought in the fact that the half-life of captan in aqueous buffer at pH 7 is only about 2.5 hours, compared to about 22 days for Dyrene, so that their longevity in the aqueous phase differ by a factor of more than 200. While captan might diffuse far enough to reach fungus spores or localized regions within the plant tissues, it would not have the range of penetration of Dyrene because of its shorter life. Thus, the intermeshed effects of water solubility, diffusion coefficient, and hydrolysis rate, in combination with intrinsic biological activity, might help to explain why some compounds are phytotoxic and others are not.

It is interesting that injury caused by both captan and Dyrene can be reduced by formulation with calcium carbonate. Both compounds produce hydrochloric acid on hydrolysis, and in the case of captan the acidity might become high enough to burn the plants (Daines, et al. 1957). The presence of the carbonate would tend to minimize this through neutralization of the acid with the formation of CaCl₂ and CO₂. Daines et al. (1957) showed that kaolinite, which has a low capacity for binding acids, aggravates injury. However, Dyrene hydrolyzes so slowly that the concentration of HCl at the plant surfaces is never likely to be very high. It is possible that the calcium carbonate safens the fungicide by accelerating its decomposition rate in the aqueous phase so that the range over which it can diffuse is limited.

The hydrolysis products of Dyrene are not fungitoxic. However, in some cases the breakdown products of fungicides are more injurious than the original compounds. Thus, aqueous suspensions of dichlone formulated with some attapulgite clays slowly became deep red in color and are phytotoxic to higher plants. Presumably the red compound is
2-hydroxy-3-chloro-1,4-naphthoquinone. Although it is a weak fungicide compared to dichlone, it can probably permeate leaf tissues more readily because of the higher water solubility conferred on it by the hydroxyl group.

Examples where fungicides yield decomposition products with a basically different type of biological activity are known. Thus, 1-fluoro-2,4-dinitrobenzene is probably toxic to spores because it can participate in substitution reactions with metabolites such as amino acids and proteins, while its hydrolysis product, 2,4-dinitrophenol, uncouples phosphorylation from oxidation. Pentachloronitrobenzene produces pentachlorophenol on hydrolysis. Both these compounds are good fungicides but have different areas of application, the former being used in soil and the latter for the protection of wood. These observations suggest that the margin of safety which separates a fungitoxic dose of chemical from a phytotoxic dose might be narrowed perilously if interactions in the infection court can convert a compound which is predominantly fungicidal to one with herbicidal properties.

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12. CHEMICAL AND PHYSICAL INTERACTIONS


CHAPTER 13

Fungicides in the Soil Environment*

DONALD E. MUNNECKE

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*I would like to commemorate this effort to my friend, William Moje, who died in 1964 just as his all-too-brief career was reaching the fruition of his careful and extensive preparatory efforts.
DONALD E. MUNNECKE

I. INTRODUCTION

It is the purpose of this review to discuss the fate of fungicides in soil and the principles and practices of treatment of soil as they affect the success or failure of fungicides in practice. Attempts will be made to correlate the experience gained by researchers in allied fields.

Within the last two decades a striking increase has occurred in the use of soil fungicides; for example, 2 million acres of cotton land were treated with soil fungicides in 1963 (Johnson et al., 1963). This increase is continuing and it may be the most dramatic development in agricultural fungicides.

Unfortunately, much of the research on soil fungicides has been rather empirical. The period of transition from empirical to more experimental studies began about 1950 as emphasized by Fuchs (1952) when he stated that the research on chemical disinfection was just at the beginning of its development. Fuchs (1952), Newhall (1955), and Hall and Clegg (1949) have adequately summarized the work before this period. This view, however, was not entirely shared by McNew (1953) who made the rather pithy comment that research on fungicides in general had a great weakness because scientists were working in a vacuum. Fortunately, the "vacuum" has been gradually overcome so that there is now a considerable body of knowledge on soil fungicides and much more on the related pesticides. There is still much more to be done, as was pointed out by the President's Science Advisory Committee (1963) which stressed that research efforts must be extended beyond an empirical approach to more fundamental studies. They further stipulated that such research should be stimulated and encouraged by various governmental agencies. Let us hope that this stimulation is promulgated!

The following review articles were helpful in the preparation of this chapter. An annotated bibliography for the period 1944–1963 (Anonymous, 1964) on pesticides in soil and water is useful for general pesticide research. For insecticide research, the articles by Ebeling (1963) and Lindgren and Vincent (1962) are excellent. A recent book on herbicides edited by Audus (1964) is outstanding. Also available are the following excellent reviews which are based on the problems relevant to soil fungicides from different viewpoints: Bollen, 1961; Burchfield, 1960; Domsch, 1964; Goring, 1962; Kreutzer, 1963; Kendrick and Zentmyer, 1957; Martin, 1963; Moore, 1960; Priest, 1963; Vaartaja, 1964.

In this chapter the pesticides will frequently be referred to by their common names, as shown in Table I.
13. FUNGICIDES IN THE SOIL ENVIRONMENT

### TABLE I

<table>
<thead>
<tr>
<th>Text name or abbreviation used</th>
<th>Active chemical</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fungicides</strong></td>
<td></td>
</tr>
<tr>
<td>Captan</td>
<td>n-trichloromethylmercaptto-4-cyclo-hexene-1,2-dicarboximide</td>
</tr>
<tr>
<td>Dexon</td>
<td>p-Dimethylaminobenzensulfonyl chloride</td>
</tr>
<tr>
<td>Dichlor (Phygon)</td>
<td>2,3-Dichloro-1,4-naphthoquinone</td>
</tr>
<tr>
<td>Ferbam</td>
<td>Ferric dimethyldithiocarbamate</td>
</tr>
<tr>
<td>Lanstar</td>
<td>1-chloro-2-nitropropane</td>
</tr>
<tr>
<td>MMDD (Pacogen)</td>
<td>Methylmercury dicyandiamide</td>
</tr>
<tr>
<td>Mykone</td>
<td>3,5-Dimethyltetrahydro-1,3,5-2H-thiadiazine-2-thione</td>
</tr>
<tr>
<td>Naham</td>
<td>Diethanol ethylene dithiocarbamate</td>
</tr>
<tr>
<td>NaDTC</td>
<td>Sodium dimethyldithiocarbamate</td>
</tr>
<tr>
<td>NTC</td>
<td>Ethylmercury phosphate</td>
</tr>
<tr>
<td>PCNB</td>
<td>Pentachloronitrobenzene</td>
</tr>
<tr>
<td>Scarsen</td>
<td>2-Chloro-4-(hydroxymercuri) phenol</td>
</tr>
<tr>
<td>Thiram</td>
<td>Tetramethylthiuram disulfide</td>
</tr>
<tr>
<td>Vapam</td>
<td>Sodium N-methylthiuram disulfide</td>
</tr>
<tr>
<td><strong>Herbicides</strong></td>
<td></td>
</tr>
<tr>
<td>Chloropropin</td>
<td>Trichloroacetate methane</td>
</tr>
<tr>
<td>CG</td>
<td>Carbon disulfide</td>
</tr>
<tr>
<td>D-D</td>
<td>1,3-Dichloropropene-1,2-dichloropropene mixture</td>
</tr>
<tr>
<td>EDB</td>
<td>Ethylene dibromide</td>
</tr>
<tr>
<td>MIT</td>
<td>Methyl isothiocyanate</td>
</tr>
<tr>
<td>Telon</td>
<td>1,3-Dichloropropene</td>
</tr>
<tr>
<td><strong>Insecticides</strong></td>
<td></td>
</tr>
<tr>
<td>Diuron</td>
<td>3-(3,4-Dichlorophenyl)-1,1-dimethylea</td>
</tr>
<tr>
<td>Mepron</td>
<td>3-(B-Chlorophenyl)-1,1-dimethylea</td>
</tr>
<tr>
<td>Simazine (chlorozine)</td>
<td>2-Chloro-4,6-bis(ethylamino)-3-triazine</td>
</tr>
<tr>
<td>2,4-D</td>
<td>2,4-Dichlorophenoxyacetic acid</td>
</tr>
<tr>
<td><strong>Isonicotinoids</strong></td>
<td></td>
</tr>
<tr>
<td>Aldrin</td>
<td>1,2,3,4,10,10-Hexachloro-1,4,4a,5,6,6a-hexahydro-1,4-endo,8,8a-dimethanophthalene</td>
</tr>
<tr>
<td>Chlordane</td>
<td>1,2,4,5,6,7,8,8a-Octachloro-4,7-dimethanophthalene</td>
</tr>
<tr>
<td>DDT</td>
<td>1,1,1-Trichloro-2,2-bis(p-chlorophenyl)ethane</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>1,2,3,4,10,10-Hexachloro-1,4,4a,5,6,6a-octahydro-1,4-endo,8,8a-dimethanophthalene</td>
</tr>
<tr>
<td>Endrin</td>
<td>1,2,3,4,10,10-Hexachloro-1,4,4a,5,6,6a-octahydro-1,4-endo,8,8a-dimethanophthalene</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>1,2,3,4,6,7,8,8-Heptachlor-3a,4,6,6a-tetrahydro-4,7-methanindene</td>
</tr>
<tr>
<td>Lindane</td>
<td>γ1,2,3,4,5,6-Hexachlorocyclohexane</td>
</tr>
<tr>
<td>Phorate (Thimet)</td>
<td>Diethyl ethylthiophosphoramidate</td>
</tr>
</tbody>
</table>

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II. THE FATE OF FUNGICIDES IN SOIL

When considering the fate of chemicals in soil, generalization from laboratory observations to field experiences is often difficult. A most extreme example may be, as Hemwall (1962) acknowledged with regard to the use of computer calculations of the behavior of fumigants in soil, that laboratory experiences may not fit practical field observations. Yet, generalizations must be made so that a basic core of knowledge can be developed for future progress. Vaartaja (1964) made a number of such generalizations regarding soil fungicides. Fourteen commonly used non-fumigant fungicides were successfully used 27 times to control diseases, unsuccessfully 14 times, and with "inconsistent" results 20 times. Such compilations of empirical trials are not meaningful, and Vaartaja pointed out that inconsistencies may be due to excessive errors, wrong concentrations, and ignorance or neglect of factors modifying action of the toxicant. Another problem of generalization is that results obtained from experiments with chemicals that may produce the same toxicant are not always similar under the same conditions. For example, high soil moisture increases release of methylisothiocyanate (MIT) from soil treated with Mylone, but it decreases release of MIT from soil treated with Vapam. Obviously, conclusions about one fungicide and soil moisture may be false with regard to the other.

A. Research Methods Used in Studying the Fate of Fungicides in Soil

Assays of fungicidal behavior have been made on the basis of: (1) response of mycelium or spores of test fungi buried in treated soil (Chinn and Ledingham, 1962; Corden and Young, 1962; Domsch and Schieke, 1960; Klotz et al., 1959; Munnecke and Ferguson, 1953; Newhall, 1958; Zentmyer, 1955); (2) response of spores in agar media to diffusates directly from soil (Munnecke, 1958a), or from extracts from fungicide-treated soil (Richardson, 1954); (3) response of fungi to various vapors from treated soil (Corden and Young, 1962; Jacks and Smith, 1952; Munnecke et al., 1962); (4) disease reaction of susceptible plants in infested soil treated with fungicides (Domsch, 1958a; Richardson, 1960; Richardson and Munnecke, 1964a; Reinhart, 1960); (5) infestation of agar media in treated soil (Mauer et al., 1962); (6) chemical analyses (Ashley and Leigh, 1963; Barchfield, 1959; Hughes, 1959; Jurinak and Inouye, 1953; K. Kotter et al., 1961; Moje et al., 1964; Munnecke et al., 1962; Turner, 1962; Turner and Corden, 1963; Willenbrink et al., 1961); or (7) combinations of one or more of the above (Domsch, 1956, 1958b,d; Mauer et al., 1962; Munnecke et al., 1962).
13. FUNGICIDES IN THE SOIL ENVIRONMENT

No single technique is suited for all purposes, which makes generalization difficult. Bioassays closely measure actual fungitoxicity and, if the assay is rigidly standardized, particularly with respect to the quantity and genetic stability of the test material used, reproducible results are obtained.

The effect of spore number on fungicide response is well known, but the age of culture used for inoculum is not. With Pythium ultimum (Munnecke and Moore, 1963) a pronounced lag, which increases with increase in age of mycelium, occurs before growth resumes after transferring mycelium. When mycelia of various ages were exposed to carbonyl sulfide (COS) gas, cultures arising from old mycelia were more susceptible than cultures arising from young mycelia. Probably, old inoculum was more susceptible because the toxicant acted longer on individual cells before new cells were formed. Thus, the age of inoculum may be critical—even grossly misleading—and, if not controlled, lead to unreproducible results.

Some disadvantages of bioassays are that they do not measure effects that may occur after the fungicide is no longer detectable. Also, assays depend upon water or gas diffusion and a fungicide may possibly be useful or active in the soil without having these properties. However, this is not likely.

The advantage of using plant-disease response to assay fungicides is that it most closely approximates field conditions. It is time-consuming, requires considerable space, and is difficult to make quantitative, however.

Chemical assays are the most quantitative, and most researchers rely upon them when possible. Often they must be used in conjunction with other methods to determine the various processes occurring in the soil.

In studying soil fungicides the inherent complexities of soil itself must be considered. In leaching studies, for example, consideration must be made of the persistence of the compound in soil, sorption, microbial breakdown, chemical breakdown, volatilization, uptake by plants and other organisms, etc. These factors affecting the fate of fungicides in soil will be discussed further.

B. Factors Affecting the Penetration of Soil

1. Sorption

Several excellent reviews are available on the process of sorption, the word best suited to describe the overall disappearance of fungicides in soil due to adsorption at interfaces or dissolving in free water or organic matter: Burchfield (1960) and Kreutzer (1963), fungicides; Goring
DONALD E. MUNNECKE

(1962), fumigants; Hartley (1964) herbicides; and Ebeling (1963), insecticides.

Since the soil particulate matter provides sorptive surfaces, it has been the basis of many studies. If a compound is adsorbed on a surface in a monomolecular layer, it should fit the Langmuir isotherm equations. In soils, S-shaped isotherms have been obtained from fumigants, and the Brunauer, Emmett, and Teller (BET) equations have been applied to these cases, based on the assumption that the divergence from linearity is due to multimolecular adsorption due to action of van der Waals' forces (Jurinak and Volman, 1957). The BET equations have been widely used, although Burchfield (1960) stated that the basic theory is unsound.

The roles of clay and organic matter content on sorption have been studied in detail. It is now generally accepted, except in very dry soils where clay content and specific surface are the principal factors regulating sorptive capacity, organic matter is most important. It is especially critical at field capacity (Call, 1957b). Hartley (1964) believed that clay has "preoccupied" most researchers' minds, stating that it is not important with large molecules, especially uncharged ones. This was not to say that the clays were of no import, however. Other reviewers have come to the same, albeit less succinct, conclusions (Goring, 1962).

The effect of moisture content of soils on sorption has led to some interesting research. Wade (1954) showed that ethylene dibromide (EDB) was rapidly sorbed by dry soils, but that the amount sorbed dropped off sharply at moisture contents above approximately 15% and rose very slightly as a moisture content of 60% was reached. Later Wade (1955) showed that sorption isotherms of soils held at less than 50% relative humidity were sigmoidal and corresponded to BET equations, but above 50% the curves did not fit the equations. Jurinak and Volman (1957) also fitted sorption isotherms data for EDB to the BET calculations and assumed that van der Waals' forces were operative after a monolayer was formed. Call (1957e) showed that EDB was displaced from sorption by water in three stages. At low humidities water molecules more successfully competed for adsorption sites and displaced the EDB molecules. At higher humidities (10–20%) when the formation of a monolayer of water was complete, a greater number of water molecules were required to replace each molecule of EDB and probably EDB was sorbed onto the sorbed water. Then solution of EDB in soil water did not become important until the sorbed water films attained the properties of bulk water which probably occurred at field capacity. Also, Call (1957b) studied the sorption of EDB on 20 different soils held at moisture contents corres-
ponding to field capacity. He expressed the data as the sorption coefficient (slope of the isotherms) and found correlations with surface area, organic matter content, moisture content, and less closely with clay content. The last three properties also correlated with each other. On the basis of moisture levels at field capacity the sorption coefficient could be predicted with reasonable accuracy from moisture content alone. Further, the correlation of sorption coefficients of the various soils indicated that clay content and specific surface were the principal factors regulating sorptive capacity of dry soils; organic matter was critical at field capacity.

Frissel (1961) pointed out that many workers used dry clays in their studies and thus gave little evidence of what may happen in wet systems in natural soils. He pointed out in greater detail than had Call that montmorillonite clays have an expanding lattice, whereas the illites and kaolinites have a nonexpanding lattice when wetted by water. Thus, when dry, only the edges of the lattice serve as sorption sites for herbicides, but when the lattice expands upon hydrating, even large molecules like EDB may penetrate within the lattices. These sites are once again obscured by water films as the moisture content rises, however. The swelling of clays, penetration of the lattices by herbicides and subsequent drying, trapping the molecules viselike, may explain the long persistence of some compounds in dry soil.

The pH of soil as well as the charge on clays affects the dissociation of pesticides and thus is very important in soil processes. Crafts (1962) pointed out that cationic compounds in moist soil are immediately affected by the base-exchange complex akin to the rapid immobilization of ammonia by soil. The same effect occurs with quaternary nitrogen compounds. Anionic and nonpolar compounds are not so affected by soil pH, although they may be precipitated as insoluble compounds of soil bases. Frissel (1961) concluded that at low pH all compounds are adsorbed strongly, at slightly basic conditions anionic substances are adsorbed negatively, and nonionic compounds are moderately adsorbed. The pH effect was shown strikingly with the adsorption of chlorazin, trietoxine, and simazin on sodium montmorillonite. Sorption was very pronounced below pH 4, decreasing greatly to pH 9. At pH 8 the amount adsorbed was approximately one-half that adsorbed at pH 6. Munnecke and Martin (1964) found a similar pH effect with three soils treated with methylisothiocyanate (MIT). As soil pH was increased, the amount of MIT released increased linearly, although the slopes of the three varied.

When herbicides are adsorbed as ions they are present in a diffuse
layer. Generally, non-Coulombic forces play an important role in addition to Coulombic forces. The adsorption of nonionic organic compounds as a result of van der Waals' or other non-Coulombic forces will be important only if these compounds have a large dipole moment or an aromatic structure (Frissel, 1961).

2. Volatilization

Volatilization is dependent upon the inherent volatility of the compound, temperature, dilution with biological materials, and general restraint in diffusion caused by soil (Goring, 1962). Obviously, compounds with high vapor pressures potentially may penetrate field soils best, providing that the gas is not allowed to escape into the air. For example, at 20°C the vapor pressure of methyl bromide is 1380 mm, but for chloropicrin it is only 20 mm (Youngson et al., 1962). Consequently, it is useless to use methyl bromide without covering the soil. Frequently, the effectiveness of compounds with lower vapor pressures (such as chloropicrin, MIT, and carbon disulfide) may be enhanced by tarping. Several ingenious cultural operations have been devised to help control loss by volatilization to the air. One involves injecting half of the dose deep in the soil and allowing the fumigant to diffuse for a time. Then the soil is plowed so that the soil which had been on the surface is placed at the bottom. The second half of the dose is injected to kill the remaining organisms. A modification has been made with soils that have the ability to sorb large quantities of fumigant, such as chloropicrin. In this case only one injection is made. After a few days the surface soil is inverted by plowing and the surface is compacted by rollers to restrict gas emission. The principle involved here is that the gas which is sorbed deep in the soil releases enough gas after the plowing to kill those organisms which survived initially on the surface. Other operations designed to restrict loss by volatilization include irrigating or packing the surface with a heavy roller after injection of the fumigant. Most of these operations depend upon too many fortuitous occurrences and control has varied from excellent to poor, and with the advent of cheaper means of applying tarps, they are no longer so widely used.

In laboratory assays sometimes volatilization obscures the manner in which a compound penetrates soil. For example, Corden and Young (1962) assessed fungicides using small cans and claimed that drenchability was more closely associated with volatility than with water solubility. This appears to be an oversimplification and may apply to small closed containers; however, in the field water solubility probably is more important.
3. Leaching

The transport of toxicants in soil water depends upon the solubility of the toxicant in the soil solution, the nature of soil colloids, the geometry of the soil solid phase, and the rate of liquid flow (Hartley, 1964).

The percolation and solvating action of water as it passes through soil is of greatest importance with long-lasting pesticides such as the chlorinated hydrocarbons. Lichtenstein (1958) showed that 60–95% of the initial application of aldrin, lindane, and DDT was recovered in the top 3-inch layer of soil after 17 months. After 3 years the lindane and aldrin had moved downward in silt loam soil. This problem is not so pronounced with soil fungicides, since they are lost by volatilization or by chemical or biological breakdown. With the so-called nonvolatile compounds, leaching is important. Munnecke (1961) studied these in large-diameter cylinders and found that persistence of water-soluble compounds, such as nabam and MMDD are greatly affected by leaching. The less soluble materials such as thiram, captan, ferbam, and PCNB were less easily leached, but their movement was affected to a greater degree than is explainable solely on the basis of water solubility.

With monuron, the solubility of the herbicide in soil is apparently much greater than it is in the water content of the soil alone (Hartley, 1964). The reason is that the enormous soil surface provides sorption surfaces which allow the uptake of more herbicide in molecularly dispersed form than its water content alone would accept. Thus, the solubility of the herbicide itself in pure water may no longer be a significant factor. Also, the rate of flow of water through a soil is as important as the total amount of water. The rate of solution of herbicide particles is important also, being slowest from porous granules with herbicide adsorbed to it, more rapid when they are held as surface coating on a soluble adhesive, and most rapid when granule matrix disintegrates or dissolves in water.

Hartley (1964) also pointed out that leaching studies in columns of soil may not be typical of activity in natural soil because the flow is too much by mass action. In natural soils water movement is by unsaturated flow and thus soil columns do not reflect the equilibria that exist between soil water and soil granular interiors.

4. Diffusion

Excellent reviews have been prepared on diffusion (Goring, 1962; Hartley, 1964) and several papers are pertinent for this very abbreviated discussion (Call, 1956, 1957a,b,c,d).
Diffusion is the main factor affecting penetration by fumigants such as methyl bromide, and it is more important than water downflow. Most researchers agree that gases move through soil by diffusion and not by mass flow. With water-soluble compounds, such as Vapam and Mylone, whose activity depends upon volatile breakdown products, air diffusion and water flow are inexorably linked. In all cases soil moisture content affects diffusion greatly because of the physical blocking of free air space by water, a condition which thus hinders diffusion.

The rate of diffusion of a gas is influenced by molecular weight, temperature, presence of codiffusing gases, continuity of air spaces, and distribution of fumigant between air, water, and solid phases of soil. This later distribution is influenced by temperature, moisture, air space, clay, and organic matter (Goring, 1962). The rate of diffusion is important and it may explain the report by Youngson et al. (1962) who noted that factors that tend to increase diffusion of chloropicrin increased control, whereas factors decreasing diffusion of methyl bromide increased control. Also, addition of peat moss to soil enhanced depth of control by methyl bromide probably by decreasing the rate of diffusion.

Hartley (1964) pointed out that diffusion is very important in transport of molecules over distances up to 1 mm from a surface of a solid whose presence retards flow processes. He emphasized that air diffusion may be important with herbicides which are of low volatility because a substance partitioning even as much as 5000 times in favor of water from air would diffuse more rapidly in the air spaces of dry soil than in a solution of water-logged soil. For a substance partitioning even as much as 50,000 in favor of water, air diffusion may be more important.

Some provocative theoretical considerations of soil fumigation were prepared by Hemwall (1962) using a theoretical model of a nondecomposing fumigant. He expressed the factors affecting fumigation in mathematical terms, and by varying single factors and using a computer to calculate the effect he drew the following unusual conclusions. The optimal properties of a fumigant are low reactivity with the soil, low diffusibility, low vapor pressure, low adsorbability, and high water solubility. Optimum soil properties are low organic matter content, low adsorptive capacity, low air space, and low water content. As Hemwall stated, some of these conclusions are greatly in variance as to what has been found in the field or in other laboratory experiences. For example, he considered that if the rate of diffusion is decreased, the time of contact of the fumigant with organisms would be increased and hence the kill would be increased. It seems that this is an oversimplification since the corresponding factors may be just as important; i.e., reducing the rate would tend also to de-
crease penetration, and thus decrease the kill. Another of his conclusions was that increasing Henry’s law ratio (increasing ratio of fumigant in water to air) increases efficiency of kill. However, this would at the same time decrease the amount available to diffuse deeper in soil. Also, he stated that as air space is decreased, effectiveness is increased, based upon the premise that retardation of rate of movement is favorable. My impression is that this method is valuable, but difficult to put to practical use, since it grossly oversimplifies the problem by isolating one factor as though it were independent of the others.

5. Other Factors

Other factors affect penetration of soil by pesticides. Natural soil heterogeneity may be obscured by constant cultivation so that subsoil variations may be undetectable. Plow sole, subsurface clay layers, wet subsoil, clods, and pockets of undecayed plant parts, often block penetration of fungicides, and make disease control difficult when the pathogens are located deep in the soil. Fortunately, the majority of plant pathogens are in the upper 6 inches of the soil, although with a fungus such as *Armillaria mellea*, penetration to 6 ft or more may be necessary.

Substances may be used to prevent penetration of compounds below a desired depth. Hartley (1964) placed activated charcoal in bands below the placement of herbicides for this purpose. Charcoal was effective, but special adsorptive clays were not.

As a general rule, the more photolabile, oxidizable, volatile, or soluble a compound is, the larger the particle needs to be if persistence is desired. Munnecke (1961) found that captan particles 1 μm in diameter penetrated soils better than particles 14.5 μm in diameter. There is a limit to the size that particles may be made because of processing and stabilizing problems. For example, McNew (1953) showed that when Phygon is used as a foliage spray, particles less than 3–5 μm did not persist long enough to be effective. Probably the same relationship would apply in soils.

C. Factors Affecting Persistence

Fungicides may be lost from soil by leaching, microbial breakdown, chemical destruction, sorption, photoinactivation, evaporation, and sometimes by growing plants.

1. Leaching

Essentially the same factors affecting leaching in relation to penetration apply to persistence and these need not be discussed further here.
2. Microbial Breakdown

Spanis et al. (1962) found that Semesan was inactivated by isolates of *Penicillium* and *Aspergillus* and MMDD by several *Bacillus* spp. The inactivating organisms of one fungicide were unable to inactivate the other fungicide. They postulated that inactivation occurred in a three-step process: (1) an initial sorption of toxicant by the organisms; (2) metabolism of the toxicant; and (3) possible utilization of the decomposed toxicant for growth. Spanis (1963) amplified the work on MMDD breakdown and proposed that the *Bacillus* spp. act on the fungicide at the cytoplasmic membrane surface. Further binding to the membrane may be facilitated by a guanidine-membrane attachment, perhaps resulting in an innocuous mercury-sulfo complex. The report of the inactivation of organomercury fungicides by sulfur in groundwood made from salt-water-stored pulpwood is significant. Russell (1960) reported that pulpwood logs stored in fresh water or water of low salinity were protected from fungal attack by treatment with phenylmercuric acetate or phenylmercuric acetate-oxine, but logs stored in sea water were not. In salt water storage logs gradually accumulate mercury-inactivating organosulfur compounds, collectively referred to as thiolignin, which arise from the reduction by sulfate-reducing bacteria of sulfates adsorbed from the sea. Hydrogen sulfide liberated by these bacteria combines chemically with the lignin in the tissues forming organosulfur compounds which have great affinity for mercurial fungicides and render them biologically inert. This report is mentioned since the soil may act in a similar manner where organic materials may have been combined with active sulfur groups.

3. Loss by Chemical Breakdown

Chemical breakdown in soil may be partially obscured, but usually it is a first-order rate reaction. It is affected by temperature, soil moisture, soil humus, and soil pH which may be the most important. For example, at pH 4 the half-life of simazin is 10 years, but at pH 2 it is 40 days (Hartley, 1964). Munnecke (1958b) found that captan was stable in soil of pH 5.7 for over 65 days, but it is well known that under alkaline conditions it is quite unstable. Hanson and Nex (1953) found that nearly all EDB had decomposed in an alkaline soil in 172 days, but only 9% had decomposed at pH 5 and 50% had decomposed at pH 5.8.

The chemical breakdown of mercury fungicides in soil has led to apparently conflicting results. Booer (1944) postulated that organic mercury compounds reacted by base exchange with soil clays to form an intermediate compound. The intermediate subsequently gave a dialkylmercury or
diphenylmercury and a mercury clay compound. The dialkymercury would escape into the atmosphere while diphenylmercury would accumulate in the soil. Metallic mercury would result from degradation of the clay mercury complex eventually being rendered innocuous by formation of mercury sulfide. These results were not confirmed in later studies by Kimura and Miller (1964).

Kimura and Miller (1964) using their new analytical techniques, determined the residual mercurials in soil and in the atmosphere above treated soil. The objective was to elucidate the degradation in soil and to determine whether or not biological inactivation and mercury evolution occur together. Puyallup sandy loam was dried, and intimately mixed with phenylmercury acetate (PMA), ethylmercury acetate (EMA), methylmercury dicyandiamide (MMDD), or methylmercury chloride (MMC) to make a concentration of about 11 μg mercury per gram of soil. Autoclaved and nonautoclaved soils held at various moisture capacities were used. They determined the concentration of: the parent compounds in the soil and in air above the soil; ionic mercury in soil; and total mercury. The three organomercurials differed with respect to degradation in soil and their mode of loss from soil. PMA was degraded in soil and was lost as metallic mercury vapor. EMA also was degraded mostly as mercury vapor, but trace amounts of the parent compound were detected in the air. In contrast, MMDD and MMC were not broken down to mercury vapor and their loss from soil was due almost entirely to the volatility of the organomercury compound. Further, they reported that most of the mercurials remaining in soil could be extracted undegraded by using mild reagents, indicating that the mercurial fungicide persisted in soil for a considerable time. From these results they assumed that biological inactivation and mercury evolution do not necessarily occur together.

Several things concerning the paper seem to be significant although the authors did not emphasize them. With PMA and EMA, the two compounds which were degraded in soil, a pronounced increase in amount of mercury vapor from nonautoclaved soil compared to autoclaved soil was shown. By estimation, with PMA after 20 days, approximately 2.9 times more mercury vapor was produced from nonautoclaved as compared to autoclaved soil, and with EMA after 40 days approximately 1.7 times more was produced in the nonautoclaved soil. Yet, essentially the same products were recovered from both autoclaved and nonautoclaved soils. This would support the assumption that microbial as well as nonmicrobial breakdown might be the same. Also, with the MMDD and MMC experiment an autoclaved series was not included so it is not really known what the role
of organisms is. Finally, the authors commented that the cumulative vapor for methylmercury compounds appeared "abnormally low" which they ascribed to surface compaction due to watering and to impervious pots. The low values could also be due to lack of organisms in the soil capable of breaking down the compounds. Consequently, it does not seem valid to ascribe differing mechanisms of breakdown in soil by the action of organisms as contrasted to other means.

4. Loss by Other Means

Sorption, generally speaking, reduces persistence, but a compound may be sorbed in a dried soil particle and thus resist degradation for a long time. When finally the soil is wetted the compound may desorb, thus accounting for some long-period persistence.

External environmental factors affect persistence. Insecticides disappear more rapidly from the soil surface than within the soil itself (Edwards, 1964). Wind and water erosion obviously are main factors, but high temperatures and strong sunlight combine to make surface breakdown more rapid. Sunlight adversely affects monocroton, diuron, and simazine (Sheets, 1962) and Dexon (Hills and Leach, 1962).

Relatively little is known concerning loss of fungicides by cropping. The fact that sodium dimethylthiocarbamate (NaDDC) (Dekhuijzen, 1961, 1964) is taken up by plants from soil indicates that this may be very important. Herbicide loss by cropping is considerable (Hartley, 1964). The pickup and translocation of systemic insecticides by plants has long been known, but many nonsystemic insecticides, such as lindane, dieldrin, heptachlor epoxide, aldrin, and heptachlor were shown by Lichtenstein and Schutz (1960) to be picked up by pea roots and translocated to the tops of the plant.

Ordinarily, loss of pesticides from soil by evaporation to the air is usually not great. With persistent compounds which are retained by soil near the surface this may be a big factor. Hartley (1964) pointed out that from a wet soil supplied with 200 tons of water per acre per month and held at 15°C, a herbicide having a molecular weight of 10 times that of water but one-third of the diffusion rate in air, and a vapor pressure of about $10^{-4}$ mm Hg could disappear at the rate of 24 lb per acre per month.

III. FACTORS AFFECTING FUNGICIDAL EFFICIENCY

Kreutzer (1963) stated: "It is surprising that a soil toxicant ever gets to the site of action and remains there in sufficient quantity to do anything."
A. Basic Susceptibility or Resistance to Specific Fungicides

It is a basic principle that the concentration of a toxicant often determines whether it is biocidal or biostatic in action. Consequently, it is imperative to know whether or not a fungicide is intrinsically capable of killing the pathogen involved.

1. Adaptation to Tolerate Fungicides

In general, resistance to fungicides in nature by plant pathogenic fungi has not developed (Priest, 1963). A most notable exception was the discovery (Harding, 1959) that *Penicillium italicum* developed permanent resistance to biphenyl used in citrus fruit decay control. This phenomenon has been discussed by Eckert in Chapter 9, so it will not be considered here.

Endo (1963) suspects that golf greenskeepers who tend to change fungicides regularly have unwittingly done so because resistant strains of the pathogen have been developed. Golf greens should be among the first places that resistant fungi would develop, since the host is a perennial and many applications of fungicides are applied for continuous periods.

The probability that soil fungi may develop resistance to fungicides is high since soil fungi are so intimately associated with fungicides. Thus, combined with the established facts that fungi may be trained to adapt to fungicides in vitro (Parry and Wood, 1958, 1959; Brook and Chesters, 1957; Grover and Moore, 1961; Elsaid and Sinclair, 1964), and that differences in tolerances exist between various isolates of fungi (Rose and Hamlin, 1961; Shatla and Sinclair, 1963; Sinclair, 1960; Thomas, 1962), make the probability almost a certainty. Another point is that as fungicides are developed that have a more specific action and presumably do not have as wide a range of toxic action, more resistance is apt to be found (Sisler and Cox, 1960).

Ashworth and Amin (1964) noted that *Aspergillus niger* was protected from the toxicity of MMDI by adding glutathione and cysteine, whereas *R. solani* and *P. ultimum* were not similarly protected. Also, Spanis (1963) was able to offset mercury toxicity to Bacillus spp. by adding -SH donors to the medium. Ashworth and Amin analyzed mycelial mats and found that *A. niger* contained 100 μg/gm dry weight of -SH; *R. solani*, 9 μg, and *P. ultimum*, no measurable amounts. They proposed that the mercury tolerance of *A. niger* was due to a pool of intracellular—
SH that is free of protein which protects enzyme systems by forming complexes with mercury as it is taken up by the thallus. Spani et al. (1962) found that their isolate of A. niger was capable of breaking down one mercurial, Semesan, but not MMDD. Since Ashworth and Amin worked with only one isolate of A. niger, it seems advisable that more isolates of the fungus be used to determine whether the --SH content is fortuitous or actually a real cause of the resistance.

Casida (1964) pointed out several facts dealing with insecticides which are pertinent. Insect resistance does not always extend to closely related compounds. The level of resistance may be greater to a compound to which the population has never been exposed than to the compound used in a selection program. Also, resistance is not always monofactorial.

2. Relation of Moisture and Susceptibility of Organisms

Dry seeds, insects, and fungus spores held at low relative humidity are much more resistant to toxicants than if exposed at high humidities. The reason frequently given is that the metabolic activity of the organism is higher, and hence more susceptible, to the toxic material. This may not always be the case because it may be related as much to sorption, membrane passage, and solution of the gas at the membrane-air interphase.

Munnecke et al. (1959) exposed dried spores of Alternaria solani to methyl bromide gas at various relative humidities. At very low humidities it was impossible to kill the spores even with concentrations as high as 20% methyl bromide by volume. The susceptibility of the spores rose sharply with increasing humidity but fell off slightly above 90% relative humidity. No explanations were given for the phenomenon. In unpublished studies (1958) I found that there appeared to be a close correlation between the amount of bromine ions sorbed by the spores and the relative humidity at which the spores were exposed. In another series the spores were held in an atmosphere of nitrogen, water vapor, and methyl bromide, and the spores were as sensitive as they were in air. These observations may indicate that the susceptibility of the spores is directly related to uptake, not necessarily to spore metabolism.

In more recent unpublished studies (1964) I have found that dry A. solani spores are completely unaffected by high concentrations of dimethylamine gas. If turgid, and presumably with a certain amount of free water around them, they are very sensitive to dimethylamine. Probably this is related to an extreme rise in pH due to absorption of the gas by the water in and around the spores. When dry, the gas is not sorbed and the spores are unaffected.

Miller (1962) listed five processes involved in membrane passage: (1)
mass flow through pores (2) diffusion (which requires no specific membrane-permeating material relationship) (3) facilitated diffusion (specific structural relationship between membrane and material) (4) active transport (requires relationship of membrane to material and metabolic energy) and (5) pinocytosis. Owens and Miller (1957) earlier noted that fungicides accumulate in the cytoplasmic particles of fungi. Because of this it would appear that the effect of moisture content or relative humidity is related more to penetration and permeation of the spores and mycelia than to metabolism.

Humidity conditions affect the reaction in soil, and it has been shown (Barlow and Hadaway, 1956) that insecticides are more toxic at high relative humidities because less is sorbed by soil than under conditions of low humidity.

B. Obscuring Effects of Recontamination

Natural soil contains a multitude of organisms in continuously changing equilibria which form a biological buffering system that tends to prevent any one segment of the population from gaining ascendancy. When this buffer is modified by fungicides, the buffer capacity is greatly reduced, and fungicide treatment may be completely obscured by the subsequent reintroduction of a parasite. Ferguson (1958) elegantly showed this relationship which is reproduced in Table II. He treated a naturally infested gladiolus field with methyl bromide gas and measured disease response of plants grown from planting stock obtained from various sources. When pathogen-free stock was used, the best treatment was obtained from the soil which had not been treated with methyl bromide. This phenomenon of the obscuring of effectiveness of soil fungicides by reintroduced pathogens is now well known, but it was not always appreciated in the past. Results from research should be scrutinized with this factor in mind, however.

C. Effect on Other Organisms

The effect of fungicides and other toxicants on soil organisms has been extensively studied (Bollen, 1961; Domseh, 1963b, 1964; Martin, 1963). Usually the more effective the fungicide, the more severe is its effect on the associated microorganisms in the soil. Domseh (1959, 1960a,b) studied the action of fungicides in relation to the general effect on the
TABLE II

DISEASE RATING OF GLADIOLUS CORMS ILLUSTRATING PROPORTIONATE INCREASE IN EFFECTIVENESS WITH INCREASING TREATMENT RATES WITH PATHOGEN-FREE STOCK, AND ERRATIC RESULTS WITH INFESTED STOCK

<table>
<thead>
<tr>
<th>Type of planting stock</th>
<th>Pounds of methyl bromide per 100 sq ft</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease-free corms</td>
<td>0 1 2 3 4</td>
</tr>
<tr>
<td>Hot-water treated corms</td>
<td>1 2 3 4 5</td>
</tr>
<tr>
<td>Grower's infested corms</td>
<td>5 1 4 2 3</td>
</tr>
</tbody>
</table>

* After Ferguson (1958).

fungus flora. He did not find any great differences in the susceptibility of bacteria, streptomycetes, or soil fungi to six fungicides. He divided the fungi into tolerant, semitolerant, and intolerant groups in response to nabam, Vapam, captan, TMTD, allyl alcohol, and an arsenical compound.

Fungicides are effective against soil-borne plant pathogenic bacteria (Munnecke and Ferguson, 1960) and after treatment with Vapam, methyl bromide, or chloropicrin the nitrifying bacteria are almost eliminated, and, on standing, ammonia accumulates. The ammonia level may build up to phytotoxic amounts as commonly occurs with steamed soils. The transitory effect may be offset by additions of nitrate fertilizers shortly after transplanting, to balance the ammonia nitrogen nutrition. In a few weeks the nitrifying organisms become reestablished in the soil and the normal nitrogen cycle is resumed (Baker, 1957).

The efficacy of a treatment may be affected by the subsequent planting. Epps et al. (1964) showed that after fumigation with methyl bromide if the fumigation were followed with a nonhost crop, the subsequent soybean cyst nematode population was low; if followed by a host crop the nematode population at harvest time was even higher than that of the control.

**D. Interaction of Dose, Time of Exposure, and Inoculum Potential**

The effectiveness of a toxicant is directly related to its concentration and the duration of exposure, providing that the concentration of infectious materials is the same and that it is uniformly susceptible to the toxicant. In soil this is not usually the case, making the inoculum potential of the soil the third great variable in effectiveness of a toxicant. Several researchers have attempted to formulate these relationships in meaningful
FUNGICIDES IN THE SOIL ENVIRONMENT

13. Fungicides in the Soil Environment

Domisch (1958a) proposed that fungicide effectiveness may be compared on the basis of the ratio of the concentration in parts per million applied to soil, and the number of days before half of the original activity is gone. Thus, a 10:1 ratio would be obtained with a fungicide having a half-life of 50 days with an initial concentration of 500 ppm.

Richardson and Munnecke (1964b) summarized the difficulties in determining effective fungicide dosage in relation to inoculum concentration in soil and described a quantitative relationship between the fungicide dosage required to control a soil-borne disease and the concentration of mycelial inoculum in soil.

Parallel linear dosage-control curves were obtained which indicated that the logarithm of the fungicide dosage at the 50% control level increases proportionately with the logarithm of the inoculum density. This linear relationship provides a basis for evaluation of the activity of fungicides against various pathogenic fungi in soil. Thus, the amount of fungicide required to control a soil-borne disease is proportional to the degree of infestation of the soil by the pathogen. By using the technique it is possible to compare the effectiveness of various fungicides against a single test organism or the relative sensitivity of different pathogens to the same fungicide.

A study by Bald and Jefferson (1956) resulted in some interesting observations pertinent to this. With increased doses of Vapam on corms treated with New Improved Ceresan (NIC), incidence of bacterial scab of gladiolus fell, rose, and fell again. They explained their results as being due to a combination of residual activity of NIC combined with higher dosages of Vapam, causing failure or erratic development of a protective microflora in the rhizosphere of growing plants. These results and explanations were similar to those reported by Gibson (1953) earlier.

Also, they fitted their data to a Poisson distribution series. This showed that to obtain infection of 50% of the susceptible plants it would take 60 infectious particles, but to raise it to 99% would take 400 particles. In natural soil fumigations, soil heterogeneity would tend to extend the range of doses that are less than 100% lethal. In a highly infested soil only an efficient fungicide is worthwhile.

E. Disease Severity Affected by Chemicals
Not Considered Actively Fungicidal

Many insecticidal and herbicidal chemicals can potentially affect plant diseases caused by soil- or airborne pathogens. Richardson has shown that insecticides and herbicides in soil affect seedling damping-
off caused by *Helminthosporium sativum* (1957); tomato early blight caused by *A. solani*; and tomato wilt caused by *Fusarium oxysporum f. lycopersici* (1959). Even when applied to seeds they affect damping-off of peas caused by *P. ultimum* and *R. solani* (1960).

Some of the 19 chemicals tested by Richardson resulted in increased fungus infection, some had little effect and some reduced infection. With early blight, soil applications of lindane increased leaf infection approximately 50%, whereas treatments with aldrin decreased it nearly 70% (Richardson, 1959). The effects on *Fusarium* wilt were not as pronounced, although lindane definitely resulted in increased wilt. In soil infected with *P. ultimum* or *R. solani*, seeds treated with lindane, aldrin, or dieldrin were more vulnerable to damping-off than untreated seeds, whereas seeds treated with combinations of insecticides and thiram were better protected than those treated with fungicides alone (Richardson, 1960).

Richardson and Miller (1960) pointed out that previous attempts to demonstrate fungitoxicity of insecticides by sowing spores of various fungi on culture media treated with these compounds had given negative results. Inhibition of mycelial growth had been demonstrated, however. These authors showed bimodal dosage response curves of *Fusarium*, *Rhizoctonia*, *Pythium*, and *Mucorina* to several insecticides, particularly to lindane.

Of interest is the anomaly that *Pythium* was greatly inhibited (75% reduction) by 25 ppm lindane in agar, but when lindane was used as a seed treatment much greater damping-off due to *Pythium* resulted. Perhaps lindane is phytotoxic but when combined on the seed with thiram the phytotoxicity is offset. Then the fungitoxicity of lindane to the fungus may come into play, thus accounting for the enhancement of thiram fungitoxicity.

The reason why lindane stimulated infection in early blight of tomato and aldrin inhibited it may be due to the ability of plants to translocate these compounds. Lichtenstein and Schulz (1960) found that lindane was translocated to the tops of pea plants, but only the epoxides of aldrin, heptachlor, and dieldrin were translocated. The difficulty in reconciling these findings with Richardson's is that lindane is fungistatic (to the four fungi listed—unfortunately the reaction of *A. solani* was not listed), yet early blight was stimulated by the presence of lindane. Perhaps this supports the view that lindane is actually subphytotoxic and pea seeds are more susceptible to damping-off or tomato leaves are more susceptible to blight due to its presence. Also, it is possible that the in vitro fungitoxicity, being only fungistatic, does not greatly affect the disease interactions. The whole relationship of insecticides and herbicides to disease is unclear and needs further study.
Peculiarities have been reported involving cotton treated with the systemic insecticide, phorate. Erwin et al. (1959, 1961) showed that in the greenhouse phorate reduced damping-off, but in the field it predisposed cotton to damping-off, predominantly caused by *Pythium*. The insecticide reduced the stand count, but this could be offset by applying Ceresan or captan in a manner similar to the experimentation of Richardson with lindane. Ranney (1964) confirmed the phytotoxicity of phorate to cotton and reported that combinations of hexachlorophene-captan with either phorate or Di-Syston resulted in increased disease loss. The treatments alone resulted in only a slight degree of abnormal root growth. These reports, combined with one by Hacskaylo and Stewart (1962), definitely showed that phorate is phytotoxic and they indicate that increased damping-off is due to host predisposition initiated by the presence of the insecticide.

Most nematocides, such as D-D mixture and EDB are slightly fungitoxic, but considerable disease control has been obtained which cannot be directly attributable to nematode control, even though nematodes and fungus diseases are often closely linked. Very little is known about the effects of herbicides on the ecology of soil microflora and plant fungus diseases. Millikan and Fields (1964) reported that various herbicides in nutrient solution were toxic to *P. ultimum*, *R. solani*, *Fusarium culmorum*, and *Trichoderma viride*. The reactions varied with the fungi and the herbicides.

These very brief citations indicate that some aspects of disease control with compounds not fungicidal per se need further study.

**F. Translocation in Plants of Fungicides Applied to Soil**

This subject is discussed fully elsewhere in these volumes, but mention should be made of the work of the Dutch researchers (Dekhuijzen, 1964; Kaars Sijpesteijn and Kaslander, 1964; Kaars Sijpesteijn et al., 1963) who have amplified the original discovery made by Dekhuijzen (1961) in which three fungitoxic compounds were recovered in cucumber seedling foliage after NaDDC was supplied to the roots. This phenomenon may be more widespread than hitherto thought and many compounds added to the soil actually may be acting at different sites than those originally exposed. Previously, reports have been made that soil treatments may be used to control such foliage diseases as mint rust by dinitroamine, *Corticium* rot of endive by PCNB, *Sclerotium* stem rot of bean by dichlone or PCNB, *Botrytis* of strawberries by a preharvest dust, and snapdragon rust by *p*-aminobenzoicloxynitromethylamide (Campbell, 1956;
Hassebrauk, 1952), but they were difficult to reproduce. Cremlyn (1961) pointed out that compounds, such as amino acids, can diffuse out of plants into the soil, to affect the rhizosphere and the natural soil ecology and thus provide a degree of root protection. The phenomenon by which large foreign molecules gain entrance and move through plants deserves further investigation by soil fungicide researchers.

6. The Increased Growth Response Phenomenon (IGR)

Frequently, better plant growth, called IGR (increased growth response), is obtained in fungicide-treated soils, even in the absence of pathogens. IGR is frequently explained as being due to the inhibition of unrecognized root parasites, to release of nutrients (mostly nitrogen), or to the chemical itself (Martin, 1966). Domsch (1963a) indirectly attempted to discover whether or not IGR was due to the suppression of semipathogenic fungi by studying the effects of 25 saprophytic soil fungi on growth of five test plants. Some fungi were inhibitory, some were stimulatory, but the total was algebraically zero for plant response to the whole group. He concluded that "semiparasitic" potencies of fungi are unlikely to cause marked inhibition in plants unless a saprophyte becomes dominant. Possibly fungi similar to the ones he studied could survive chemical treatments and be responsible for IGR. If a species classified above as an "inhibitor" survived, decreased plant growth would ensue but the reduction probably would be ascribed to an inefficient control of the known pathogen. If a "stimulator" survived, however, the response would be noted as an IGR. Such contingencies deserve further experimentation.

Fungicide-treated soil may occasionally have an unfavorable ratio of ammonium to nitrate nitrogen and subsequent plantings may be abnormal, as already noted (Baker, 1957). Not all plants are susceptible to this toxicity, and it is possible that some plants may thrive in the presence of the different nitrogen balance. This factor may explain some IGR also.

Sometimes IGR is due to control of associated parasites, such as nematodes. Kreutler (1958) has discussed these relationships in detail in conjunction with fungus pathogens.

A complex growth response has been reported by English and DeVay (1964). A 20-50% loss may occur on young plantings of stone fruit trees due to a disease complex variously called "gummosis, sour sap, or bacterial canker." The cause is obscure, but Pseudomonas syringae has been implicated as the causal agent for part of the disease syndrome. Nematodes, viruses, and environmental factors have been discounted as primary agents in the disorder. Frequently with sandy soils treated with D-D, Telone, or chloropicrin, the disease on peaches was strikingly controlled.
and much better tree growth occurred, although the trees were not immune to subsequent inoculation with P. syringae. The IGR and reduction of canker development is not understood. The authors suggested that it is due to an increased availability of essential elements, but that it is unlikely to be due to a direct response against the pathogenic bacteria because of the great diversity of the response-inducing chemicals used. De-Vay et al. (1962) attempted to determine whether a translocatable substance from D-D or three of its isomers was involved, but none was found.

The quantity of soil nutrients and their utilization by plants may be altered. Smith (1963) reported that treatment of Hawaiian soils with methyl bromide or D-D resulted in improved utilization, but no uptake, of iron by pineapple; phosphorus content increased also, but uptake was reduced.

A decreased growth response sometimes occurs in treated soils. Frequently it can be ascribed to chemical residues (such as bromine from methyl bromide), unbalanced nitrogen nutrition, and excesses of various metal elements from soil. One of the most perplexing is that reported by Martin et al. (1963) on citrus. When certain citrus soils were treated with any agent, chemical or physical, which effectively "sterilizes" the soil, a very haphazard and difficult-to-reproduce stunting of citrus plants resulted, lasting a few weeks to a year. It was associated with an inhibition of phosphorus absorption and reduction of copper and zinc intake, although extractable soil phosphorus was high. Plants were stunted, phosphorus was depleted in the leaves, but other ions were taken up, and sodium and boron occasionally reached toxic levels in the tissues. The explanation is unknown, but they postulated that an inhibition of organic ion absorption may have occurred, possibly produced by an organism which becomes dominant following partial sterilization.

II. Anomalies

A number of anomalous responses have been reported in which disease control could not be directly attributed to the fungicide treatment. In a field infested with Streptomyces scabies, Oswald (1954) added sulfur as indicated in Table III and measured soil pH and the yield of potatoes for 4 years. Most of the increase in yield appeared to be directly attributable to the acid conditions induced by sulfur. It was not absolutely related to pH since the effectiveness of the treatment dropped abruptly in 1952, even though the pH (4.7–4.9) was not appreciably higher than the previous 2 years when maximum yields were obtained. Obviously, other factors were in action, but the data have not been further elucidated.

Gibson (1952, 1956) found that low dosages of ethylmercury phosphate resulted in an increased incidence of damping-off of pine seedlings due
TABLE III

| Treatment          | pH  
|-------------------|------
| No treatment      | 7.2  | 6.7  | 6.3  | 6.1  | 5.9  | 26   | 70   | 56   | 11   |
| Sulfur, 2500 Ib/acre applied Feb. 1948 | 6.1  | 3.9  | 4.3  | 4.4  | 4.7  | 78   | 100  | 184  | 263  | 53   |
| Sulfur, 1500 Ib/acre applied Feb. 1948, 1000 Ib/acre applied Sept. 1948 | 6.5  | 4.3  | 4.6  | 4.8  | 4.9  | 70   | 100  | 156  | 277  | 96   |

* From Oswald (1954).

b Average of five monthly readings.

C 100-lb sacks per acre.

He could not attribute the results to a phytotoxic response nor to enhanced pathogen growth and speculated that it was due to inhibition of the normal soil organisms which presumably act as inhibitors of the parasites. No experimental evidence was presented to support the suppositions, however. Later Gibson et al. (1961) found that PCNB controlled Rhizoctonia damping-off but increased Pythium damping-off of pine seedlings. Isolates of Penicillium paxilli were found in association with the infested soil and pure cultures were inhibitory to the Pythium on agar media. They suggested that the increase in damping-off of Pythium was due to the inhibition of P. paxilli by PCNB although it was not further detailed. They noted that in two undescribed tests results of the addition of P. paxilli to infested soil were inconclusive, stating that such studies would be an unlikely practical extension of their work. Unfortunately no other details have been reported.

IV. SPECIFIC DETAILS REGARDING COMMERCIALLY USED FUNGICIDES

A. Fungicides Whose Activity Depends upon Volatility without Degradation of Parent Molecule

1. Methyl Bromide

Methyl bromide, a broad-spectrum toxicant, is one of the standards of comparison for new fungicides, except against Verticillium, on which it is
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relatively ineffective (Munnecke and Ferguson, 1953). It is applied as a gas beneath a cover and its activity is dependent upon gaseous diffusion. It is quite soluble in water (1.341 gm/100 gm; Haight, 1951), which affects its action.

Usually methyl bromide is most successful when the treated organisms are moist, but the role of moisture has not been completely elucidated. As mentioned earlier, dry A. solani spores exposed at very low humidities are almost impossible to kill, but at higher humidities kill is readily effected (Munnecke et al., 1959). I found (1958) that dry analytical grade filter paper did not impede the flow of methyl bromide, but if saturated with water it stopped passage of the gas for several minutes, and the original rate of passage was not reached for 10–12 minutes. This behavior may explain the drop-off in efficiency experienced at high-moisture conditions, but not the inefficiency under very dry conditions.

An exception to the rule that methyl bromide is more effective in moist rather than dry soils has been found with field fumigations against A. mellea. Undoubtedly, this is related to the great depths of penetration necessary for success against Armillaria.

2. Chloropicrin

Chloropicrin is the other standard by which soil fungicides may be compared. It is used frequently when Verticillium is a problem. It is a powerful lacrimator, although its vapor pressure is similar to water. It is insoluble in water, and water is frequently used to confine the gas to soil by wetting the surface layer of soil. With the advent of cheap methods of covering soil with tarps, the water seal method is used less frequently.

3. Methyl Isothiocyanate (MIT)

Various isothiocyanates have been implicated in fungicide action and to utilize this property MIT has been formulated dissolved in xylol (Trapex), or in D-D mixture (Vorlex). MIT, like chloropicrin, is often considered highly volatile, whereas it, too, has almost the identical vapor pressure of water. Initial reports (Pieroh et al., 1959) of the behavior of MIT as a nematocide probably were too inclusive. They claimed that soil type does not affect its effectiveness; it acts independently of soil moisture (based upon comparisons of air-dried versus nearly saturated soils); it is more effective than Vapam used in comparable doses, and it may be used effectively as a fumigant at temperatures near freezing. Such sweeping generalizations are unlikely, especially in regard to previous experiences with other chemicals. C. Kotter (1963) found that parasitic soil fungi were more susceptible than saprophytic fungi to the material. Munnecke et al.
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(1962) found that it was quite toxic as a gas to *Rhizoctonia* and *Pythium* mycelium and *Myrothecium verrucaria* spores. K. Kotter *et al.* (1961) showed that MIT was quickly decomposed in compost and loam soils, only slightly in peat, and almost not at all in sand. Breakdown was faster in nonsteamed than in steamed soils, indicating the occurrence of biological and chemical breakdown. It is understandable why sand (pH over 9) had so little effect, but it is unusual that more MIT was not sorbed in the peat (pH 3.9), since Munnecke and Martin (1964) have shown that MIT is highly sorbed at low pH values in several soils. Willenbrink *et al.* (1961) examined the uptake of MIT by tomato plants transplanted into compost soil treated with MIT 23 days previously and concluded that there was no measurable uptake of MIT by roots. This report appears to have slight value since it is highly unlikely that any MIT would be left in soil at the time of their planting.

B. Fungicides Whose Activity Depends upon Degradation of Parent Compounds to Form Volatile or Water-Soluble Toxicants

1. Vapam

Vapam, a good fungicide, has often given sporadic control in the field. Probably such vagaries have been responsible for the relatively large amount of research that has been done with the compound.

One of the first reports that the effects of Vapam in soil extended farther than the actual wetted soil was reported by Linden and Schieke (1957). They proposed that a volatile product was involved and recommended that treated soil be covered with a tarp to enhance Vapam activity. Initially, there was doubt that MIT was the actual fungitoxic breakdown product, although it was theoretically feasible (van der Kerk, 1959; Thorn and Ludwig, 1962), but numerous investigators, beginning with a short report by Hughes (1959), have shown that MIT is a main fungitoxic gaseous component. Lambe (1960) studied Vapam in sandy loam soil (pH 6.2) and found evidence for formation of MIT from Vapam as well as from Mylone. When he determined the toxicity of MIT, Vapam, and Mylone to *Verticillium* on a comparative basis, as shown in Table IV, MIT was not as efficient as the other two compounds. These data may indicate that MIT is not solely responsible for Vapam action, or that some of the MIT was lost through volatilization. The dosage response curves for the three compounds were very similar, indicating a common toxicant to *Verticillium*. Unfortunately, MIT was not actually identified in these experiments.

Munnecke *et al.* (1962) determined the fungitoxicity of volatile effluents
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from columns of soil (50% peat moss, 50% Oakley loamy sand, pH 5.9) treated with Vapam, Mylone, or MIT. Vapam, Mylone, and MIT produced similar effects, and the release of MIT and the fungitoxicity of the effluent air stream were closely correlated. This was especially noticeable when the logarithm of rate of production of MIT for a 24-hour period was plotted against the logarithm of the time after application of fungicides to the soil. The authors cautioned against making sweeping generalizations from their limited observations, especially that MIT was the only toxicant involved. These findings were similar to those of Lambe and tended to confirm his suppositions for a common toxicant based upon dosage responses.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mol/l)</th>
<th>ppm</th>
<th>Inhibition (%)</th>
<th>LD₅₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mylone</td>
<td>1 x 10⁻⁴</td>
<td>16.2</td>
<td>97.3</td>
<td>10.0</td>
</tr>
<tr>
<td>Vapam</td>
<td>1 x 10⁻⁴</td>
<td>12.9</td>
<td>97.6</td>
<td>7.1</td>
</tr>
<tr>
<td>MIT</td>
<td>1 x 10⁻⁴</td>
<td>7.3</td>
<td>61.1</td>
<td>7.0</td>
</tr>
</tbody>
</table>

* After Lambe (1960).

Wedding and Kendrick (1959) pointed out that thiuram sulfides, isothiocyanates, thiourea, H₂S, CS₂, and ionized or undissociated dithiocarbamic acids had all been implicated at one time or another, but that isothiocyanates were increasingly selected as the toxic substances from dithiocarbamates under aerobic conditions. They studied the effect of Vapam, MIT, and elemental sulfur on the production of ¹⁴CO₂ from glucose by the mycelium of R. solani using Warburg respirometry. The method of inhibition of fungus respiration by MIT and Vapam was different. Vapam caused a pronounced increase in permeability of the mycelium, whereas MIT or sulfur did not affect it. Like Lambe, they found that survival of R. solani was greater in MIT solutions than in Vapam. Wedding and Kendrick concluded that isothiocyanates are not the primary toxicants involved in the fungicidal effect of the dithiocarbamates. They discussed various possibilities of differences in metabolic pathways being the cause of the differences of the two compounds. As an alternative to the toxicity being due to MIT, they stressed the possibility that dithiocarbamate ions are the toxicant and that they react with cellular materials directly. The ions may react with some sulfhydryl constitu-
ent of cell membranes to physically change the membrane, altering the
permeability, resulting in either loss of essential cell constituents or entry
of the toxicant, which then may act as an enzyme poison within the cell.
No evidence was given for these suppositions, however. The authors ac­
knowledge that the differences in the effects of Vapam and MIT may
have been due to their experimental approach, the relative solubilities,
or the effort of pH or other factors on differential rate of breakdown.
These points remain a major criticism of their work.

Turner (1962) and Turner and Corden (1963) showed that several path­
ways of breakdown of Vapam may occur in dilute aqueous solutions. An
oxidative reaction occurs at pH 9.5 which yields MIT and sulfur. Under
acid conditions a nonoxidative process may yield CS$_2$, H$_2$S, $N,N'$-
dimethylthiuram disulfide (DMTD), methyamine, and MIT. They also
discussed the side reactions that may occur with the breakdown products
resulting in the formation of MIT from combinations of methyamine
with CS$_2$ in the vapor phase, combination of MIT with Vapam to form
DMTD; and MIT and methyamine to form $N,N'$-dimethylibisourea. In
the unpublished continuation of our previous study (Moje et al., 1964)
we have found that Vapam also produces carbonyl sulfide, as well as
MIT, depending upon the pH of the soil. For example, in soil at pH
2.3 only carbonyl sulfide was detected; at pH 3 and pH 5 both carbonyl
sulfide and MIT were detected, but at pH 7 only MIT was found. Thus,
more than one gaseous toxicant is involved in the breakdown of Vapam.

Not all of the breakdown products are beneficial, since Gray and
Streim (1962) found that $N,N'$-dimethylibisourea was a phytotoxic residue
in certain soils and in sand after fumigation. It had formed during stor­
age and it was necessary for the manufacturers to add a stabilizer to their
commercial preparations to prevent its formation.

The factors influencing breakdown of Vapam in the soil were elabo­
rated by Turner (1962), Turner and Corden (1963), Gray (1962), Mun­
necke et al. (1962), and Ashley et al. (1963), who essentially agreed on
the details. The rate of breakdown of Vapam is increased by high tem­
perature, low moisture, and high pH. It is not correlated with the pres­
ence of organisms, although it is more rapid in soil than in dilute solu­
tions. Gray (1962) reported that the type and constituency of soils and
various clays greatly affected the amount of Vapam decomposition.
Ashley and Leigh (1963) found that the presence of copper, iron, and
manganese increases the release of MIT from aqueous solutions. Al­
though they did not make the statement, probably the same ions in
soil would likewise affect release of MIT.

The relationship of soil moisture is particularly interesting especially
in relation to Mylone breakdown. Investigators have found that the effectiveness of Vapam decreases as soils become increasingly wetted (Madel et al., 1962; Schickle, 1962; Turner, 1962). An explanation for this fact was offered by Turner (1962) who postulated that it is due to a decrease in oxidation in the oxidative breakdown pathway and also because Vapam is less fungitoxic under anaerobic conditions. The observations of Freed et al. (1962) concerning herbicides is pertinent. The ratio of solute as vapor to solute in aqueous phase is a constant, so that with a given amount of a substance as vapor in soil, increasing the amount of moisture will reduce the amount in the vapor state. With Vapam breakdown being a nonhydrolysis reaction, this relationship may apply particularly in high-moisture-content soils.

2. Mylone

Although Mylone is more complex than Vapam, it is readily decomposed in soil and MIT is released freely. Perhaps the most striking difference between it and Vapam is that Mylone breakdown is hydrolytic and is favored by increasing soil moisture. Torgeson et al. (1957) identified MIT, formaldehyde, H₂S, and monomethylamine as some of the Mylone breakdown products in soil, but none when used alone was sufficiently fungicidal to inhibit Pythium sp. in soil. Unfortunately, they did not specify how they tested the materials and further reports were not forthcoming.

The breakdown of Mylone in soil has been presumed to be as follows (Anonymous, 1957). Step 1, Mylone hydrolyzes to form formaldehyde and methylvamine methylthiocarbamate (I) under the catalytic effect of soil minerals and soil microorganisms. Step 2, (I) further breaks down to monomethylamine (II), H₂S, and MIT. Since (II) and H₂S are very reactive they probably react with some of the formaldehyde to form methylaminomethanol (III) or dimethylaminomethane (IV). Step 3, Another reaction may proceed simultaneously or separately and triformaldehyde may yield CO₂, NH₃, SO₂, and water. At the same time MIT may react with water to yield CO₂, H₂S, and methylvamine. The methylvamine may further breakdown to CO₂ and ammonia. The fact that the original compound may yield so many potentially reactive and fungistatic compounds may explain why field response with this compound has been so variable.

In our studies (1964) we have attempted to trap the gases in the atmosphere above soil treated with Mylone using liquid nitrogen and analyzing the gases with an infrared spectrophotometer and have indications that other reactions may occur in soils at pH 3; for example, carbonylsulfide is formed. Of all of the products described above we have detected only
MIT, carbonyl sulfide, and carbon dioxide from the atmosphere above
the soil. The other products, if formed, presumably are sorbed by the soil
or are in solution in soil water.

The amount of MIT released is proportional to the concentration of
Mylene mixed in the soil and the rate of release is dependent upon vari­
ous factors (Munnecke et al., 1962; Munnecke and Martin, 1964). MIT
release from soil treated with Mylene increases with temperature and
soil moisture up to approximately 80% of saturation. It increases with
soil pH (pH 2.3-6.5), but falls off slightly as higher values up to pH
7.7. The release of pure MIT from soil is affected by soil pH similarly.
Increase in clay or peat content of soil decreases the amount of MIT re­
leased. MIT formation and release probably are independent of soil
microorganisms with the soils tested.

C. Nonvolatile Fungicides Whose Activity Presumably Depends
upon Intimate Contact with the Fungus

These fungicides may have a considerable vapor activity but their effec­
tiveness depends upon being in intimate contact with the fungus in the
soil. Many were first used as foliage protectants or as seed treatments
and were pressed into use as soil fungicides with the hope that they might
be effective, or at least, that they would not be harmful to the plants
around which they were applied. As a result there are many reports of
members of this group being used for control of soil-borne diseases, but
with relatively little success. It is this group which has suffered the most
from empirical and haphazard studies.

1. Compounds with Specific Activity toward Certain Fungus Groups

While there has been rather intensive searching for chemicals which
have specific toxicity for the fungi which are pathogenic to plants, only
PCNB and Dexon have been commercially successful.

PCNB has been used to control Rhizoctonia, particularly on cotton in
the United States. Kreutzer (1963), a leading advocate of the desirability
of developing specific toxicants, has summarized this phase very well.
He noted that PCNB and a close relative, tetrachloronitrobenzene, spe­
cifically attack a relatively narrow list of plant fungus pathogens
belonging to the following genera: Plasmopara, Rhizoctonia, Sclero­
tinia, Sclerotium, Betula, Streptomyces, Rhizopus, Macrophomina, Peni­
cillium, Trichoderma, and Olpidium. It is ineffective against members
of the following genera: Pythium, Fusarium, Colletotrichum, Verticillium,
Typhula, and Tilletia. Strecker (1957) reported that PCNB is unique in
13. FUNGICIDES IN THE SOIL ENVIRONMENT

that as a vapor to fungus spores it is nonspecific, whereas to mycelium it is selective.

Eckert (1962) studied the fungistatic (to *Rhizoctonia* and *Pythium*) and phytotoxic (to beans and cucumber) properties of 19 substituted nitrobenzenes and deduced some relationships between physiochemical properties and biological activities of the group. Although exceptions were noted, progressive chlorination decreased water solubility, enhanced fungistatic activity toward *Rhizoctonia* but not *Pythium*, and decreased phytotoxicity. He discussed the relationship of biological activity and water solubility and decided that most of the nitrobenzenes are "structurally nonspecific" inhibitors.

Very little vapor activity has been shown with PCNB. Richardson and Munnecke (1964a) used a bioassay technique to determine quantitatively the fungistatic effects of vapors from PCNB applied to soil in a closed container. Although the vapor pressure of PCNB is low (5.0 × 10⁻⁵ mm Hg) very flat dosage response curves for *Pythium*, *Rhizoctonia*, and *Trichoderma* were obtained over a range of concentrations of 125 to 1000 ppm in soil. They suggest that saturation of the atmosphere occurs before fungicidal concentration of vapor is attained. This may indicate that the vapor phase of toxicity is not great in the soil. It is of interest that of 13 fungicides studied only PCNB suppressed *Trichoderma* growth more than *Pythium* or *Rhizoctonia*. In assays of continuously moving air over treated soil no toxicity had been detected in a previous study (Munnecke *et al.*, 1962).

Dexon has been introduced recently as a soil drench or as a seed treatment to control diseases caused by the "water mold," usually *Pythium*, *Phytophthora*, and *Aphanomyces*. It is specific in its activity, being relatively ineffective against most other plant pathogenic fungi.

The photodecomposition of Dexon may be a reason why some investigators have reported that it is not fungitoxic *in vitro*. When an aqueous solution is exposed to sunlight its yellow color fades, becomes colorless, then develops a pink color which becomes purple and eventually dark brown. Hills and Leach (1962) found that in full sunlight the decolorization times varied from as little as 10 seconds (10 ppm) to 6 hours (1000 ppm). The color changes reflected a photochemical reduction of the diazonium group, evolving nitrogen, and an oxidation followed by polymerization of the reaction products. The loss of toxicity upon exposure to light suggested to them that the Dexon molecule, rather than a breakdown product, is the toxicant involved in disease control. Slopes of dosage response curves of *Pythium* and *Rhizoctonia* to Dexon were not significantly different (ED₉₀: *Pythium*, 9.6 ppm; *Rhizoctonia*, 93 ppm). The difference in posi-
tion of the curves indicated that Dexon may have the same site of action for both fungi. They explained the selective action of Dexon as being due to a relatively greater uptake of Dexon by Pythium.

Dexon is translocatable in plants (Hills, 1962). Sugar beet seedlings were treated with Dexon–14C and a translocated chemotherapeutic was detected in the tops. Indications were obtained that xylem and phloem transport occurred, and that at least four unidentified substances were translocated. Despite the fact that little Dexon as such was recovered from seedlings, the results suggested to Hills that Dexon was translocated to hypocotyls, contributing to the disease control. The case would have been stronger had he been able to conclusively demonstrate Dexon in situ.

Considerable work has been done on the biochemistry of Dexon, although only short abstracts have appeared in print. Tolmsoff (1962a,b) reported that Dexon blocks the oxidation of reduced nicotinamide adenine dinucleotide (NADH), but the succinic acid pathway of respiration is not inhibited. It is effective against mitochondrial preparations from Pythium, but not from sugar beet, or Rhizoctonia. Rhizoctonia and sugar beets contain a mitochondrial system that decomposes Dexon in the presence of NADH, but Pythium lacks the decomposing system and actually stabilizes Dexon solutions. The basis for the biological specificity of Dexon has not been elucidated.

2. Compounds with Nonspecific Fungicidal Activity

This category contains mostly the successful foliage fungicides and seed treatments, although a few have been specifically developed for use in soil. As a result of their low volatility and often low solubility these compounds have been used mostly as soil drenches made up as aqueous suspensions or as solutions. Frequently, they are mechanically mixed into soil by tilling after they are applied, or they are placed in the furrow with the seed during sowing.

Compounds containing mercury have frequently been effective in soil treatments. Although some of the vapors from soil are fungitoxic, the vapor pressures are very low, as shown in Table V (Phillips et al., 1959). The significance of vapor toxicity has been noted with several of the compounds, but most of the effectiveness is due to direct contact with the fungus.

Perhaps the most successful mercurial used as a soil fungicide is MMDD. Ark and Sibray (1954) reported the rather startling news that very dilute concentrations (1:10,000 in water) of MMDD were able to control damping-off caused by Pythium and Rhizoctonia. Further, seeds of many plants were safely planted in MMDD-treated soil 24 hours
after application. They implied that the volatility of the compound increased its effectiveness.

The fungitoxicity of the vapors from MMDD is noteworthy. When Munnecke et al. (1962) mixed MMDD in soil and passed a slow-moving stream of air over the surface, they found that the effluent was extremely fungicidal to *Pythium* mycelium and *Myrothecium* spores even after 216 hours but not to *Rhizoctonia* mycelium. The resistance of *Rhizoctonia* was noted and it was pointed out that strains of *Rhizoctonia* resistant to other mercury compounds have been found, which may indicate a generalized resistance of *Rhizoctonia*. Also, MMDD was reportedly not particularly effective in controlling *Rhizoctonia* in the field.

### Table V

<table>
<thead>
<tr>
<th>Compound</th>
<th>Vapor pressure at 35°C (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methymercury chloride</td>
<td>$2.30 \times 10^{-4}$</td>
</tr>
<tr>
<td>Mercury (gray powder with talc)</td>
<td>$4.0 \times 10^{-4}$</td>
</tr>
<tr>
<td>Mercapto chloride</td>
<td>$1.40 \times 10^{-5}$</td>
</tr>
<tr>
<td>Ethylmercury dicyandiamide (tech)</td>
<td>$3.0 \times 10^{-5}$</td>
</tr>
<tr>
<td>Methymercury dicyandiamide (tech)</td>
<td>$6.5 \times 10^{-3}$</td>
</tr>
<tr>
<td>bis-Ethylmercury phosphate</td>
<td>$1.8 \times 10^{-3}$</td>
</tr>
<tr>
<td>Phenylmercury acetate</td>
<td>$17.0 \times 10^{-6}$</td>
</tr>
</tbody>
</table>

* From Phillips et al. (1959).

The susceptibility of *Pythium* and *Rhizoctonia* to MMDD vapors was reversed when the fungi were held on agar plates above treated soil in a closed system (Richardson and Munnecke, 1964a). Dosage response curves showed that in the static environment the ED50 for growth retardation for *Rhizoctonia* was approximately 15 ppm, but for *Pythium* it was approximately 40 ppm. The reasons for the differences in response is not known.

The fact that the vapors of MMDD are so toxic even though the vapor pressure is only $6.5 \times 10^{-5}$ mm Hg (Table V) again indicates the care that is necessary to assess the importance of volatility in fungitoxicity.

The water solubility of MMDD makes it particularly interesting and potentially useful. The effect of repeated leachings with excess quantities of water to soil in columns over a period of 15 days was investigated (Munnecke, 1961). Although water flowed freely through the loamy sand columns, considerable amounts of MMDD remained in the upper inch of the column after five daily washings. After 5 days MMDD activity
was higher in the 4-inch level of the soil column than in the top layer. No explanation was given, but it may be due to the fact that the bottom of the column had not drained as completely as the top. The effect of leaching is related to microbial breakdown which occurs with MMDD, as discussed earlier.

Since MMDD is such an effective fungicide; that it is soluble in water, but yet is retained by soil; that it has considerable vapor action; and that it has had such a long period of success as a seed treatment; it would seem to indicate that this compound deserves wider commercial use than it has at present.

Other mercurials have been used in the soil. Semesan, which appeared to have some promise as a soil drench, is rarely used today. However, it is interesting because it is rapidly degraded by fungi in the soil (Spanis et al., 1962). Although an extensive survey was not made, P. notatum, A. niger, and T. viride became adapted to very high concentrations of Semesan in soil and subsequently become increasingly capable of breaking it down.

The dithiocarbamates have been used as soil fungicides without much success, but considerable research has been done with nabam and thiram which will be discussed further.

Nabam was one of the first to be used since it is soluble in water, easy to apply, and possibly may act systemically (Stoddard, 1951). Unfortunately, nabam has little actual toxicity to soil fungi but it has some intriguing properties that have stimulated considerable research.

Several volatile materials are produced from nabam, especially from soil. As reviewed by Thorn and Ludwig (1962) and Moje et al. (1964), hydrogen sulfide and carbon disulfide are produced in the vapor above aqueous nabam solution, but neither accounts for the fungitoxicity. Ethylenediamine has also been interpreted as being present. Cox et al. (1951) accounted for the high fungitoxicity of vapor to fungus spores suspended in water above nabam solutions by assuming that the amine and carbon disulfide recombined in the spore drop to form the toxic dithiocarbamate. Although ethylenedichloroacetate (Kev, 1959) has been implicated in nabam breakdown as a potential toxicant (Kev, 1959), it has been difficult to demonstrate. Weed et al. (1953) considered that the chief volatile fungitoxic product of nabam breakdown is not carbon disulfide, ethylenediamine, or ethylenedichloroacetate.

When nabam-treated soil was assayed using a continuously replenished supply of air (Munnecke et al., 1962), the effluent vapors were not toxic to bioassay fungi, and only copious quantities of carbon disulfide were detected. Rate curves were plotted of the breakdown of nabam as measured
by the release of carbon disulfide and they indicated that undetected in­
termediary compounds were formed. Various gas solvents were unsuccess­
fully used to attempt to detect (by ultraviolet spectroscopy) nabam, ethylenethiuram monosulfide, or an isothiocyanate. Also, only carbon disulfide was obtained from dilute nabam aqueous or ethanol solutions suspended on glass wool instead of soil.

However, when plate cultures of fungi were exposed above nabam­
treated soil in closed containers a pronounced vapor toxicity was meas­
ured (Richardson and Munnecke, 1964a). Inhibition of mycelial growth
of *R. solani* was greatest from acid soils, decreasing steadily as pH in­
creased as follows: pH 3.3, 75% inhibition; pH 5.4, 40%; pH 6.6, 35%; pH 7.7, 25%. In continuation of these Moje et al. (1964) found, using a
liquid nitrogen gas trap and infrared spectrophotometry and gas chroma­
tography techniques, that in very acid soils carbonyl sulfide, carbon disul­
fide, carbon dioxide, but no isothiocyanates were produced. The carbonyl sulfide vapors were quite toxic to mycelial growth of *P. ultimum* on potato dextrose agar, having an ED₅₀ of $6.7 \times 10^{-5}$ moles per liter.

The problem is far from being solved. Nabam produces a number of
gaseous products, several of which—carbonyl sulfide, ethylenediisothio­
cyanate, and probably an amine recombined with carbon disulfide—are apt to be a fungitoxicant.

The instability of nabam in soil probably accounts for its lack of suc­
cess as a soil fungicide. When a 1000-ppm solution of nabam was added to
soil, approximately half of its activity was lost due to nonbiological factors in the first 24 to 48 hours after application (Munnecke, 1958b). Even though nabam is relatively unstable in soil and it is soluble in water, it may be retained in soil for surprisingly long times. For example, in UC­
type mix, detectable amounts were present at both the top and bottom of a 4-inch column after seven flushings of water applied over 22 days (Mun­
necke, 1961). When applied to Oakley loamy sand without peat moss (half of the UC-mix) the fungicide activity was practically gone after the third leaching. Other factors pertaining to nabam activity have been discussed in other sections of this treatise.

Thiram has been used with moderate success for damping-off control
(McKee, 1950), and it has been reported to be somewhat systemic
(Volger, 1959). Some of the first work on the persistence of fungicides and
relationship to both the parasitic organisms and the normal microflora
was done with thiram by Richardson (1954). He found that thiram
persisted in sandy soil for over 2 months but disappeared from compost
soil within 1 week. Thiram treatment resulted in an increase in bacteria
and decrease in fungi. Thiram treatment rendered soil more difficult to
infect with *P. ultimum* and also prevented a natural increase in the population of damping-off organisms. This protection for seedlings persisted longer than did the fungicide in the soil.

Thorn and Richardson (1962, 1964) pointed out some interesting reactions that may occur with thiram and emphasized the care that must be taken with the dithiocarbamates to ascertain exactly what is being studied. Ferbam in aqueous solutions at low pH values (below pH 6) yields an ultraviolet absorption spectrum like that of thiram. With time the change to thiram is intensified. Also, they only got a “typical” ferbam curve when they used acetonitrile as the solvent. Because the solubility of ferbam in water is nil, some researchers used acetone as a solvent which, these authors found, reacts with ferbam to form thiram. As a result, they believe that ferbam actually may act as thiram and any accessory benefits derived from its use may be due to the presence of iron which may act as a chelating mechanism.

It remains to be shown that ferbam is converted to thiram in soil. Ferbam was very stable in the UC mix, pH 5.7, and decay was independent of the presence of microorganisms (Munnecke, 1958b). Although thiram was not used in parallel experiments, Richardson’s experience (1954) that thiram was rapidly broken down in soil may indicate that ferbam is not necessarily always converted to thiram in soil.

Captan has been used extensively in furrow applications or mixed with the soil, but relatively little has been published on factors affecting it in soil. It remained essentially unchanged for 65 days in both sterile and non-sterile UC mix, but it is unlikely that it would persist so long in alkaline soil (Munnecke, 1958b). It did not produce toxic vapors in soil held either under static conditions (Richardson and Munnecke, 1964a) or under continuously aerated conditions (Munnecke et al., 1962). The size of the captan particle directly affected the depth of penetration in soil. In the UC mix in columns a small quantity of captan was carried down 4 inches, but most remained in the top 1-inch layer. The concentration distributions remained essentially unchanged after 10 leachings over an 18-day period. Probably most of the insoluble captan was held at the surface and that portion in solution was carried to the bottom of the column and was responsible for the low level of activity reported there. With repeated leaching, more of the material at the surface was solubilized and was carried downward, replacing that which had been leached from the bottom layers. When applied to Oakley loamy sand, the stratification between the top and the bottom was not as pronounced, and captan was washed from the soil in approximately seven leachings.

Attention should be drawn to several new compounds which may have
commercial success, although little has been published about them. Allyl-
idene diacetate (2-propene-1,1-dioildiacetate) is reported to be water sol-
uble and is effective when applied around living plants (Sciaroni and
McCain, 1963). Another group reported as being potentially useful are
the N-phenylmaleimides and related compounds (Torgeson et al., 1963).
Finally, lanstan, although volatile and unstable in soil, has been intro-
duced for control of cotton diseases and it may present some interesting
problems, because its molecular configuration is so different.

Munnecke (1961) formulated a few principles on the behavior of non-
volatile soil fungicides. Fungicides which are applied as solutions will
penetrate soils farther than fungicides applied as suspensions. Fungicides
will penetrate coarse soils farther than fine-textured soils. Sphagnum peat
moss in soil will restrict the passage of fungicides considerably, probably
by sorptive processes. The clay fraction in soils restricts the passage of
fungicides more than the coarse fractions. The depth of penetration in soil
is related to the rate of percolation of water through the soils; the faster
the rate, the farther the penetration. Fungicides will penetrate dry soils
farther than wet soils. The penetration ability of a soil fungicide applied
as a suspension may be enhanced by formulating the fungicide out of
smaller particles. Solution fungicides may be leached more readily from
soil than suspension fungicides. Although solution fungicides are leachable
from soil, they may be remarkably persistent and be retained by soils af-
fter many flushings with water. To evaluate the factors affecting movement
of fungicides in soil, the persistence behavior in the absence of leaching
must be known. The generalizations are admittedly broad and many may
not be universally applicable, but they are presented with the aim of stim-
ulating further thought and experimentation.

V. Future of Soil Fungicides and Research

A. Prospectus

The use of soil fungicides has been slow, but it is progressing very rap-
idly. Wilhelm (1965), a pioneer in innovations with soil-fungicide research
has recently pointed out that Verticillium is controlled on a large scale by
fumigation with mixtures of methyl bromide and chloropicrin under
polyethylene tarps. Presently, in California he estimated that 4000 to 5000
acres are treated annually. The task has grown to the extent that one oper-
ator alone uses 4000 miles of 13-ft wide tarps yearly. But the point is not
especially that fumigation has reached such a large scale in California,
but rather than as a result of the field control of diseases, the necessity
for primarily breeding crops resistant to root diseases has been abrogated and now plant breeders may concentrate on increasing the yield and quality. The other vital point is that cultural practices have been changed as a result of the application of fungicides, and better results have been obtained.

Along with the expanding concept of the use and benefits of chemical soil treatments has been a change in the concept of what the chemicals should be called upon to do. Rarely does one presently consider that field treatments are eradicative. Instead, the concept is like that long accepted in combating foliage pathogens; i.e., treatments are designed to suffice for a relatively short period of time, and when necessary, they are repeated. Another concept is that tree sites or in-row application, rather than broadcast application, may be successfully employed, thus reducing costs tremendously. As Kreutzer (1960) pointed out, less effort will be expended to treat large soil masses in the future and more effort will be made to develop chemicals that have a specific action against parasites and yet support the sustaining microorganisms in the soil. Thus, it seems obvious that there is ample room for greater research emphasis in this expanding field of plant pathology.

B. Research

It is difficult to determine where future research in the field of soil fungicides will develop. Many of the deliberations at the various meetings of the Conference on Control of Soil Fungi (which held its tenth meeting in San Diego in 1964) have dwelt on this, and I am indebted to many of the participants for some of the thoughts expressed here.

1. Why Do Fungicides Fail?

I have tried to point out the interactions that occur between plant, soil, fungus, and fungicide which either directly or indirectly may answer this question. The ultimate reason for studying fungicides is to learn better how to control diseases, and a corollary is to determine why sometimes they do not do so. Many obvious reasons for failure of the fungicides have been pointed out; i.e., excessive soil moisture, restricted gaseous diffusion, lack of penetration in heavy soils, temperatures too high, and the like. But there are many areas remaining to be studied.

2. How Do Fungicides Kill Fungi in the Soil Environment?

Although we know a considerable amount about the mechanism of action of fungicides in general, we know practically nothing about action of
fungicides in the soil environment. In many cases it is uncertain just what stage of the organism is affected by the chemical. The problem of inoculum potential and fungicide efficiency is pertinent here too. As Bald and Munnecke (1962) noted, when inoculum was diluted the soil environment changed so that more infections occurred at low concentrations of inoculum than were to be expected based on chance alone. The explanation seemed to be that there was a simultaneous dilution of antagonistic fungi from the noninfested soil. Also, fungi introduced on the seed complicated the analysis. The formula of Bald and Jefferson (1956) for the relation between fumigant dosage and infection of test plants grown in treated soil is pertinent in this regard. The quantitative relationship between inoculum density and effective fungicide dosage in a soil environment described by Richardson and Munnecke (1964b) provides a basis for comparing either the effectiveness of various fungicides against single test organisms or the relative sensitivity of different pathogens to the same fungicide. Such means of measurement may be useful in future research on mechanisms of fungicide action on the soil.

3. How Can Satisfactory Control be Obtained Without Leaving Harmful Residues in the Soil or in Plant Products?

This has been of such recent concern that it needs little elucidation. Fortunately, soil fungicides have not been implicated as being of great import, although probably they will be a problem as more are used and developed. The mechanism by which fungicides are biologically decayed is still unknown, and only a few of the many thousands of organisms in soil have been implicated in the process. Further, there has been little correlation with research with other soil pesticides. Here, certainly, is a fertile field for research.

A somewhat related problem would be to discover why fungi develop tolerance to fungicides so readily in vitro, while in the field they do not.


Methods of formulation of the active constituents have been devised which facilitate their application by incorporating them in adjuncts such as vermiculite, talc, and silicones. However, the deliberate formulation of fungicides to be released at varying rates so that a sustained concentration of the material may be obtained has had little development. Miles et al. (1962) reported that they could markedly increase the usefulness of 2,2-dichlorovinyl (dimethyl phosphate), which is volatile and decomposes in water, by incorporating it in montan wax, which slowed the release of the
chemical. Although this was not done in soil, it seems practical to do with soil fungicides.

5. How May Fungicides Be Applied around Living Plants?

This may be one of the most pressing but least investigated problems dealing with soil fungicides, yet the problem of the perennial plant that becomes infected by root parasites is of great importance. Compounds have been developed to rid the soil of insects and nematodes around living plants, but a commercially successful one for controlling soil fungi has not, as yet been developed. Little or no research has been done to investigate the potential differences in response of roots in soil and fungi in soil to various compounds to aid in formulating chemicals for this use. The fact that roots take up more compounds than formerly realized and that many fungicidal compounds are changed to other fungicidal compounds in the host after uptake, may indicate that it is possible to supply innocuous compounds to the roots of plants, and that the host may then change them to fungitoxicants in situ. The possibility that a combination of treatments may be used, one to the top of the plant and the other to the soil has appeal as a fresh approach to the problem. These and other approaches warrant further investigation. In this respect Rombouts et al. (1963) made an appropriate comment when they deplored the “gloomy pessimism” that substances applied to soil will be either too rapidly sorbed or too rapidly deactivated by organisms to be effective systemic fungicides.

6. How Can Effectiveness of Fungicides Be Improved by Cultural Practices?

Various techniques have been developed for controlling soil-borne plant pathogens. The deep-plowing method to control parasites in the upper portion of the soil profile has been combined with fumigant treatments (Dallimore, 1955). This relatively simple and cheap technique may be used to greatly enhance the effectiveness of compounds which act as gaseous diffusants. Another report (Watson, 1964) combining crop residues, flooding, and anaerobic fermentation to attempt to rid soil of Pyrenochaeta terrestris indicates the practices which may be used in combination with soil treatments. The inoculum level of the pathogen may be greatly reduced and the fungicides applied to apply the coup de grace, or the process may be reversed and the various cultural treatments be used after fungicides.

The experience with many biological control situations has been that a radical upset in the existing ecological equilibrium is necessary before an antagonist may be successfully introduced or stimulated to increase in the environment to maintain a suitably high population to either control or
eliminate the unwanted organisms (Baker and Snyder, 1965). With re­
searchers active in the field of biological control of insects the present
concept generally is that there must be integration of both chemicals and
predators for maximum effectiveness. This approach needs more pursu­
ing with soil fungus diseases. Also, a fertile approach would be to try to
develop soil organisms which are resistant to a given fungicide, and also
antagonistic to soil pathogens. In such a case it may be possible to keep
the population of antagonists at high levels in soil by frequent applications
of a fungicide.

Another example of how ingenuity and knowledge of the physical be­
havior of fungicides in soil may be used in new and effective ways is the
report by Wilhelm (1963) that it is possible to plant melon seeds and apply
chloropicrin simultaneously for control of Pythium damping-off. Wilhelm
knew that seeds are relatively resistant to chloropicrin, that chloropicrin
acts on the fungi when the seeds are dormant, and that the gas would be
dissipated by the time the seeds were in the susceptible stage. The applica­
tion of these facts allowed the use of this very efficient fumigant in this
novel way, which shows that old materials can be used in new ways by in­
genious investigators.

7. Can More Specific Fungicides Be Developed?

One of the main objectives of future soil fungicide research is to obtain
and develop fungicides which have specific activity toward the relatively
few organisms in the soil which are harmful to man's activities. This cer­
tainly has appeal and should be pursued vigorously. However, Corden and
Young (1962) and others have pointed out that this objective may not be
too feasible and that general-action biocides may remain the answer.
Since most success has been obtained from the general purpose fungicides,
such as methyl bromide and chloropicrin, it seems logical that future em­
phasis should not desert these old friends. Also, since so much is known
about the general biocide compounds, it may be equally feasible to attempt
to use them to greater advantage and in cooperation with other techniques.

8. Is It Feasible to Develop Standardized Assay Techniques for
Comparing Soil Fungicides?

The American Phytopathological Society set up parameters against
which foliage fungicides were compared under standard conditions. No
such standards have been proposed for soil fungicides, and it is difficult
for one investigator to compare his results with those of others. Some
methods are good for detecting volatile but not the nonvolatile chemicals,
no common test organism is used, few techniques relate inoculum poten­
tial to effectiveness, no fungicide standard of comparison has been accepted, no standard soil has been used, and the like. It is imperative that more cooperative effort be made to establish some standard tests. It should be possible to devise as test substrates a soil or group of soils (such as has been done in horticulture with the UC-mix) that are reproducible around the world. Several representative fungi, for example, *Pythium, Rhizoctonia, Fusarium, Verticillium*, and *Sclerotium* could be standardized and methods developed to produce a uniform inoculum potential. Several assays may be standardized to determine the effectiveness of the chemicals applied to the soil and related to a standard chemical. Results may be expressed in mathematical or graphical terms so that evaluations may be made readily. When such parameters are set, we will be able to more readily utilize the knowledge gained by all of the researchers and many of the present chaotic conditions can be avoided.

VI. Summary

Soil fungicide research and development has come a long way since its first empirical beginnings. At times one may look with envy at the advances made in associated fields, notably with herbicides, but much of the lag in plant pathology is due to the relatively few people actually concerned. As population pressures increase, intensification of agriculture will likewise increase, as will soil fungicide research. I hope that in this treatise I have stimulated further research activity. I realize that soil fungicide research is fraught with difficulties and inherent "booby traps" but it seems to me that the failures and difficulties add zest to the research, and the successes, when finally obtained, are that much sweeter to the recipient. Let us hope that the rise of knowledge of soil fungicides approaches the exponential rate so common in other fields of science today.

References


13. FUNGICIDES IN THE SOIL ENVIRONMENT 551


Dekhuijzen, H. M. (1964). The systemic action of dimethyldithiocarbamates on cucumber scab caused by Cladosporium cucumerinum and the conversion of these compounds by plants. Neth. J. Plant Pathol. 70, Suppl. 1-16.


13. FUNGICIDES IN THE SOIL ENVIRONMENT


Linden, G., and Schicke, P. (1957). Untersuchungen über die Fungizide und Herbizi­


Thomas, W. D., Jr. (1962). Reaction of biotypes of *Rhizoctonia solani* to different fungicides. Phytopathology 52, 396 (abstr.).

Tollmsoff, W. J. (1962a). Biochemical basis for biological specificity of Dexon (p-dimethylaminobenzene diazo sodium sulfonate) as a fungistat. Phytopathology 52, 705 (abstr.).

Tollmsoff, W. J. (1962b). Respiratory activities of cell-free particles from Pythium ultimum, Rhizoctonia solani and sugar beet seedlings. Phytopathology 52, 755 (abstr.).


A fungicide that remains on the surface of the plant to which it is applied has certain limitations in that it cannot eradicate a pathogen established within the plant tissue, does not protect areas missed during the spraying or produced subsequently, and cannot readily be used to protect roots from invasion by soil-borne pathogens. The limitations of nonmobile fungicides have long been realized and much work has gone into a search for chemicals capable of being translocated from their site of application to other parts of the plant. Many such chemicals have now been discovered and are mentioned in the reviews of...
A. Müller (1926), Stoddard and Dimond (1949), Horsfall et al. (1951), Horsfall and Dimond (1951), Brian (1952a,b), Wain (1952, 1953), Byrde and Ainsworth (1958), Dimond (1959), Dimond and Horsfall (1959), Howard and Horsfall (1959), Livingston and Hilborn (1959), Oort and van Andel (1960), Cremlyn (1961), Kaars Sijpesteijn (1961a,b), and Wade (1963).

The majority of these systemically active chemicals must be absorbed and translocated to some extent but, as will be discussed shortly, measurement of systemic activity gives only a very limited idea as to the degree of uptake of the chemical and the extent of its translocation.

Although one of the earliest fields of plant physiology to be studied, translocation has been, and to some extent still is, one of the most difficult and controversial. One difficulty often encountered in translocation studies is the estimation in the plant tissues of the chemical whose movement is being studied. Most of the early work was concerned with inorganic ions which were relatively easy to detect and estimate. Since then other compounds have been studied whose presence in the plant may be detected either by visual inspection of their morphological effects or by simple assay procedures; such compounds include viruses, growth-regulating substances, antibiotics and radioactively labeled minerals, carbon assimilates, and certain herbicides. These studies have thrown considerable light upon the pattern of uptake and translocation of chemicals in plants, various aspects of which have been discussed in a recent monograph (Crafts, 1961). Studies on the translocation of fungicides, as distinct from their systemic activity, are not numerous. Antibiotics have received much attention owing to their translocatability and systemic activity and the fact that their presence in plant tissues can often be detected and estimated by bioassay procedures. The uptake and translocation of these and other antifungal materials has been discussed by Brian (1952b, 1954), Crowdy and Prater (1955a), Zaumeyer (1958), Crowdy (1959), Goodman (1959, 1962a,b), Howard and Horsfall (1959), Mitchell et al. (1960), and Dekker (1963).

Most of the earlier studies were performed with antibiotics then commercially available. Although these had been developed for pharmacological purposes and were antibacterial rather than antifungal, work with these materials will be included in this discussion since it throws light upon the movement of antibiotics in general; furthermore, certain antibacterial antibiotics, notably streptomycin, have been shown to exert systemic activity against fungal pathogens (Bonde, 1953; K. O. Müller et al., 1954). Some of the antifungal antibiotics such as cyclo-
heximide and griseofulvin, which were discovered later, proved to have
systemic activity and their uptake and translocation have been studied
in some detail (see Ford et al., 1958; Brian, 1960; Rhodes, 1962).

B. Detecting and Measuring the Movement of
Chemicals in Plants

Various methods of assessment have been employed to follow move­
ment of chemicals from the point of application. Their relative merits
and their usefulness as evidence of translocation will now be briefly
considered.

1. Systemic Activity

As already mentioned, most of the evidence for the movement of
fungicides within plants is based on an ability to reduce disease symp­
toms at a point removed from the site of application. Such observations
are of practical value but they do not necessarily indicate translocation
of the molecule per se. One would expect systemic action to involve
penetration of the plant surface and movement to a site of action and,
for a systemic compound possessing in vitro activity, this site of action
may well be the site of infection. Many chemicals which are systemically
active, however, have little in vitro activity and may produce their ef­
fect by altering the host metabolism. In these cases the site of action
is unknown and need not necessarily be the site of infection. Another
possibility is that the chemical becomes transported to the infection
site but is then unable to reduce disease symptoms because it is an­
tagonzed by plant constituents. It is clear then that while detection
of systemic activity shows that the applied compound is absorbed into
the plant body it gives no information as to its fate and precise move­
ment within the plant.

2. Phytotoxic Symptoms

When a fungicide applied through roots or locally to aerial parts
causes visible symptoms of plant toxicity, then the distribution and
intensity of these symptoms gives some indication as to the movement
of the chemical within the tissues. Localization of toxicity around the
application site implies a lack of mobility (see e.g., Edgington and
Dimond, 1964). Such observations can only provide a qualitative meas­
ure of uptake and translocation and a further complication is that
damage caused to the plant tissues may modify the uptake pattern of
the chemical.
3. Analysis or Assay of Plant Extracts

Clearly the only direct way to measure the uptake and movement of a chemical within the plant is to determine the concentration of that chemical in different parts of the plant body. For chemicals which are fungitoxic in vitro a logical method is to submit the plant tissues to bioassay. The simplest method is to place the intact or macerated plant organ onto an agar plate seeded with a sensitive organism and after incubation look for development of an inhibition zone (e.g., Mirzabekyan, 1955; Wallen and Millar, 1957). A more quantitative method, widely used with antibiotics, is to express the plant sap or extract the tissues with solvents and assay the extract, together with known amounts of the test chemical, using a plate diffusion method. In this method it is usually assumed that the activity observed in the assay arises from the presence in the tissue of the unaltered compound although it could be due to other products produced by metabolism. The presence of the chemical is more certain when bioassays are made against a range of test organisms and the activity spectrum of the pure chemical and the tissue extract are then compared. Even more precise identification is possible by including strains of assay organisms that are resistant to the test chemical or, better still, which have been trained to require the test chemical as an essential nutrient (Mitchell et al., 1954; Gray, 1956). Specific growth effects on the assay organism, such as the curling effect of griseofulvin on the hyphae of Botrytis allii (Brian et al., 1946), give a more precise identification than unspecific toxicity. Chromatography of the plant tissue extract followed by bioassay of the chromatograph and comparison of \( R_f \) values with those of the authentic chemicals has been employed (e.g., Dekhuijzen, 1961b) as a means of identifying the nature of antifungal chemicals in plant tissues.

Chemical detection of the applied fungicide within the plant is more specific than bioassay and is the only method applicable to the many chemicals which have no in vitro fungicidal activity. A disadvantage of chemical means of detection is that usually the plant extract must be highly purified. This is usually achieved by chromatography of the extracts. Paper chromatography, with recognition of compounds on the paper by application of chromogenic reagents or fluorescence in the ultraviolet, is a usual procedure, \( R_f \) values being compared with those of authentic compounds (e.g., Dekker, 1961b). Colorimetric estimations of sulfonamides in plant tissue extracts were made after precipitation.
of the proteins with trichloroacetic acid (Crowdy and Rudd-Jones, 1956a).

The final proof of translocation lies in the extraction from untreated tissues of the applied chemical and its identification by physiochemical methods. Such a procedure is usually tedious and has rarely been attempted although Crowdy et al. (1955) have successfully isolated pure griseofulvin and chloramphenicol from the shoots of root-treated broad bean plants. Gravimetric and spectrophotometric estimations of these antibiotics agreed well with the values obtained by an agar plate diffusion bioassay.

4. Use of Radioactive Tracers

An important method for studying the translocation of chemicals involves the use of chemicals labeled at one or more positions in the molecule with radioactive elements. The distribution of radioactivity throughout the plant body is then assessed either by producing autoradiographs from pressed, freeze-dried plants or by more elaborate techniques. The method is sensitive but, since it only measures the distribution of the radioactive element and not necessarily the intact molecule, chromatography or some other technique must be applied to confirm the chemical identity of the radioactive material present in the plant. As yet, tracers have only been applied in a few instances (e.g., Lemin and Magee, 1957; Somers and Richmond, 1962) in studies on the uptake of fungicides.

Using the various methods outlined above, the uptake and movement of fungitoxic chemicals applied to seeds, roots, cut shoots, stems, and leaves of plants has been studied. Some of this work will now be discussed.

II. UPTAKE AND MOVEMENT OF CHEMICALS

A. Following Application to Seeds

Observations on the effect of seed treatment with chemicals have indicated uptake of certain compounds into the seed and from there into the developing seedling. Some of the chemicals reported as acting in this way are listed in Table 1.

In most studies, movement into the seed was inferred from the effect of treatment upon a pathogen carried within the seed tissues. The usual procedure was to treat infected seeds with a solution of the chem-
TABLE 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Seed</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acriflavin</td>
<td>Pea</td>
<td>Wallen and Skolko (1950, 1951)</td>
</tr>
<tr>
<td>Rimostatin, pimaricin</td>
<td>Pea, bean, beet</td>
<td>Dekker (1955, 1957); Oort and Dekker (1960)</td>
</tr>
<tr>
<td>Myrothecin</td>
<td>Wheat</td>
<td>Rangaswami (1956)</td>
</tr>
<tr>
<td>Actidione, filipin, endomycin</td>
<td>Maize</td>
<td>Krüger (1906a)</td>
</tr>
<tr>
<td>Various</td>
<td>Tomato</td>
<td>Krüger (1966b)</td>
</tr>
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<td>Helixin B</td>
<td>Oats, barley</td>
<td>Leben and Arny (1952); Leben et al. (1953)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>Oats, barley</td>
<td>Lockwood et al. (1954)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Cucumber, tomato, bean</td>
<td>Smith (1949); Starr et al. (1961); Ritskowsky et al. (1955); Woollams (1957)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Barley</td>
<td>Paulus and Starr (1951)</td>
</tr>
<tr>
<td>GS 1</td>
<td>Pea, squash, maize, bean, cucumber</td>
<td>Ark and Dekker (1958); Dekker and Ark (1959)</td>
</tr>
<tr>
<td>Various</td>
<td>Bean</td>
<td>Hildreth and Starr (1959)</td>
</tr>
<tr>
<td>Penicillin, streptomycin, gramicidin</td>
<td>Tomato</td>
<td>Mirzakhabiyev and Menkov (1955)</td>
</tr>
<tr>
<td>Aureomycin</td>
<td>Swede</td>
<td>Sutton and Bell (1954)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Various</td>
<td>Mehta and Arny (1954)</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyridine-2-thiol-N-oxide</td>
<td>Pea, bean</td>
<td>Kars Sijpesteijn et al. (1958); Dekker et al. (1958)</td>
</tr>
<tr>
<td>Dithiocarbamates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dithiocarbamates</td>
<td><em>Pinus silvestris</em></td>
<td>Volger (1956a,b, 1959)</td>
</tr>
<tr>
<td>Captan</td>
<td>Pea</td>
<td>Waller and Hoffman (1959)</td>
</tr>
<tr>
<td>Copper and copper complexes</td>
<td>Pea</td>
<td>Wain and Wilkinson (1945)</td>
</tr>
<tr>
<td>Nickel salts</td>
<td>Wheat</td>
<td>I. Hoffman et al. (1962)</td>
</tr>
<tr>
<td>Mercury and sulfur</td>
<td>Various</td>
<td>Vir and Bajaj (1963)</td>
</tr>
<tr>
<td>Organomercurials</td>
<td>Oat</td>
<td>Arny and Leben (1954)</td>
</tr>
</tbody>
</table>

ical, wash them, and then plate them out on agar, or plant them into soil and observe the incidence of the pathogen as compared with that in untreated seed. Commonly the seeds of only one or two species were used but some compounds such as streptomycin, vancomycin, and GS1 have been shown to penetrate the seeds of a number of different species. Both Grimmer and Mach (1955) and Dekker (1957) obtained evidence that penetration occurs much more readily into damaged than intact seeds.
In some studies (e.g., Dekker, 1957; Dekker and Ark, 1959), seed tissues internal to the seed coat have been bioassayed and antibiotic activity established. Other studies (Krüger, 1960b; Klimiecinska and Pound, 1961) have detected activity in the washed seed coats of treated seeds but failed to detect any in the inner tissues. Wain and Wilkinson (1945) made detailed studies on the distribution of copper within treated pea seeds and found large amounts in the testa and embryonic radicle. By contrast, the inner cotyledonary tissues and the embryonic plumule contained very little. Copper complexes were taken up in smaller amounts and were less concentrated within the testa.

Many compounds appear unable to penetrate into seeds in effective quantities. Timonin (1946) found patulin to be ineffective against loose smut of barley and of the eleven antibiotics tested by Krüger (1960a) against Diplodia zeae only three were markedly active. Wallen and Skolko (1951) found only two of six antibiotics tested effective against Ascochyta pisi infections; one of these, actidione, had previously been reported as inactive against this disease (Wallen et al., 1950). Compounds of similar structure may differ in their effectiveness for Dekker et al. (1958) found that both 2-pyridine-thiol-N-oxide (PTO) and 2-pyridyl-N-oxide isothiourea hydrobromide were active in controlling internal pathogens in pea seed; the carboxymethyl derivative and the copper chelate of PTO, however, were inactive. It is likely that the volatility of PTO assists in seed penetration as it does with some of the organomercurials (Arny and Leben, 1954).

The ability to eradicate a seed-borne pathogen depends upon the depth of the pathogen within the tissues. Most Helminthosporium species are not deeply situated within the seed and can be controlled by the volatile organomercurials (Arny and Leben, 1954). Others such as Ascochyta pisi are situated more deeply within the seed and chemicals controlling them must therefore penetrate further into the tissues.

Other evidence for seed penetration is available: for example, I. Hoffman et al. (1962) found that grain from wheat plants sprayed with nickel salts contained this element and, since a proportion could not be removed by three water washings, it was concluded that some nickel had entered the seed.

Evidence of the transport of seed-absorbed chemicals into the developing seedling has been obtained both by a reduction in the disease susceptibility of seedlings grown from treated seeds (Volger, 1959a; Williams, 1957; Rich, 1966a) and by bioassay of the sap expressed from such seedlings (Wallen and Hoffman, 1959; Volger, 1960; Krüger, 1966b). Vir and Bajaj (1963) have detected radiometric mercury and
sulfur in seedlings grown from treated cereal seeds. In most of these studies, however, it is difficult to decide whether the chemical moved directly into the seedling from the seed tissues or whether it was leached out from the testa and entered through the roots of the developing seedling.

B. Following Application to Roots

Many chemicals have been detected in the shoots after application to plant roots. Others have given indications of such movement by the appearance of toxicity symptoms in the leaves or by their effect on the development of leaf-inoculated pathogens. Since the number of such chemicals reported in the literature is large only those which have been detected chemically or by bioassay are included in Table II.

TABLE II
SOME COMPOUNDS SHOWING EVIDENCE OF ABSORPTION BY PLANT ROOTS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Inorganic</td>
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</tr>
<tr>
<td>Salts and chelates of nickel, zinc, copper, chromium, and aluminum</td>
<td>Mustard, tomato</td>
<td>DeKock and Mitchell (1957)</td>
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<td>Lithium salts</td>
<td>Tomato, celery</td>
<td>Kent (1940, 1941)</td>
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<td>Zinc salts</td>
<td>Various</td>
<td>Tombasin and Webb (1955, 1960)</td>
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<td>Zinc chelates</td>
<td>Bean</td>
<td>Kastington et al. (1962)</td>
</tr>
<tr>
<td>Ruthenium-106, Cerium-141</td>
<td>Various</td>
<td>Nishita et al. (1961)</td>
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<td>Organic—Antibiotics</td>
<td></td>
<td></td>
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<tr>
<td>Bulbineformin</td>
<td>Pigeon pea</td>
<td>Vasudeva et al. (1963); Pritam Singh et al. (1965)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>Cucumber</td>
<td>Prammer (1955)</td>
</tr>
<tr>
<td>Chloromycetin</td>
<td>Tomato</td>
<td>Klemmer et al. (1955)</td>
</tr>
<tr>
<td>Chlorotetracline (aureomycin)</td>
<td>Bean</td>
<td>Blankenhard and Diller (1951)</td>
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<td>Chlorotetracline (aureomycin)</td>
<td>Tomato</td>
<td>Klemmer et al. (1955)</td>
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<tr>
<td>Cycloheximide and derivatives</td>
<td>Wheat</td>
<td>Walker and Millar (1967)</td>
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<td>Dihydrostreptomycin</td>
<td>Bean</td>
<td>Staryk and Mitchell (1963)</td>
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<td>Eriaücin</td>
<td>Broad bean</td>
<td>Oort and Dekker (1960)</td>
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<td>Griesofulvin</td>
<td>Lettuce, oat</td>
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<td>Wheat</td>
<td>Various</td>
<td>Sticker (1954)</td>
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<td></td>
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<td>Crowley and Pramer (1955b)</td>
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14. UPTAKE AND TRANSFORMATIONS BY PLANTS

TABLE II—(Continued)

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<th>Compound</th>
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<tbody>
<tr>
<td>GS 1</td>
<td>Broad bean</td>
<td>Crowdy et al. (1955)</td>
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<td></td>
<td>Broad bean, tomato</td>
<td>Crowdy et al. (1956)</td>
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<tr>
<td>Rice</td>
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<td>Subba Rao (1957)</td>
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<td>Bean</td>
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<td>Cucumber</td>
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<td>Ark and Dekker (1958)</td>
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<tr>
<td>Mycothricin</td>
<td>Tomato, cucumber</td>
<td>Raagawarni (1958)</td>
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<tr>
<td>Oxytetracycline (terramycin)</td>
<td>Various</td>
<td>Krasilnikov (1952)</td>
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<td>Penicillin</td>
<td>Cress</td>
<td>Winter and Willeke (1951A); Winter (1952)</td>
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<td>Ristocetin</td>
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<td>Streptomycin</td>
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<td>Cress</td>
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<td>Tomato, potato</td>
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<td></td>
<td>Various</td>
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<td></td>
<td>Peanut</td>
<td>Dye (1956a)</td>
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<td>Streptothricin</td>
<td>Bean</td>
<td>Gray (1958)</td>
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<td>Thiolutin</td>
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<td>Vancomycin</td>
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<td>Sulfanilamide</td>
<td>Broad bean</td>
<td>Crowdy and Rudd-Jones (1965a,b)</td>
</tr>
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<td>Sulfanamides</td>
<td>Sugar beet</td>
<td>Hills (1962)</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>Cucumber</td>
<td>van Andel (1962)</td>
</tr>
<tr>
<td>Captan</td>
<td>Peach</td>
<td>Wallews and Hoffman (1969)</td>
</tr>
<tr>
<td>Sodium dimethylthiocarbamate</td>
<td>Broad bean</td>
<td>Schemes and Richmond (1962)</td>
</tr>
<tr>
<td>(NaDDC)</td>
<td>Cucumber</td>
<td>Dekkuijzen (1964)</td>
</tr>
<tr>
<td>Carboxymethyl DDC</td>
<td>Cucumber</td>
<td>Dekkuijzen (1964)</td>
</tr>
<tr>
<td>l-Methyl-carboxymethyl DDC</td>
<td>Cucumber</td>
<td>Dekkuijzen (1964)</td>
</tr>
<tr>
<td>NaDDC alamine</td>
<td>Various</td>
<td>Dekkuijzen (1964)</td>
</tr>
<tr>
<td>N-carboxymethylthiocarbamides</td>
<td>Broad bean</td>
<td>Heyns (1964)</td>
</tr>
<tr>
<td>Procaine hydrochloride</td>
<td>Cucumber</td>
<td>Dekker (1961a,b)</td>
</tr>
<tr>
<td>Pyridine thiub-Vcacin (Mn salt)</td>
<td>Various</td>
<td>Durpoux et al. (1961)</td>
</tr>
<tr>
<td>6-Azauracil</td>
<td>Cucumber</td>
<td>Dekker and Oert (1961)</td>
</tr>
</tbody>
</table>
Many studies record only the presence or absence of the test chemical in the shoot and whether or not those absent in the shoot were present within the root tissues was not determined. More detailed investigations record the amount of the chemical present in different plant organs following root application enabling a distribution pattern to be determined.

Many inorganic ions are fungitoxic in vitro (see McCaLLan and Wilcoxson, 1934; Somers, 1961; Carter and Wain, 1964) and a number of inorganic salts, notably those of copper, cadmium, zinc, and lithium, have been reported to have systemic antifungal activity (see Carter and Wain, 1964). Numerous studies made on the root uptake of micro-nutrients and, more recently, of products of nuclear fission, have shown that many fungitoxic cations are absorbed by plant roots. W. O. Robinson and Edgington (1945) detected thirteen highly fungitoxic elements in plants grown in unamended soils. Some were only detected in trace amounts but copper and zinc were both found to be present in concentrations up to several hundred parts per million. DeKock and Mitchell (1957) found that mustard and tomato plants grown in culture solution readily absorbed nickel, zinc, and copper but took up only traces of chromium and aluminum. Work with radioactive tracers has indicated that tomato plants absorb and translocate zinc and selenium (Langston, 1956) and that very small amounts of ruthenium and cerium are taken up by some plants (Nishita et al., 1961). Kent (1940, 1941) studied the uptake of lithium by wheat and celery plants and found the element to be translocated to the leaves although it did not appear to be mobile within the shoot tissue while Toralinson and Webb (1959, 1960) showed that zinc accumulates within the leaves of soil-grown turnip, cabbage, swede, cucumber, chrysanthemum, and gooseberry plants following root application.

It would appear from the foregoing that root uptake and translocation occurs quite readily for many fungitoxic ions though not all are so mobile in plants. Only very small amounts of mercury were taken up by plants grown in soils treated with mercuric oxide and calomel (Pickard and Martin, 1961; Pickard et al., 1963) or phenyl mercuric acetate (R. G. Ross and Stewart 1962), though this finding could be due to the low solubility of these compounds.

Some metals are more readily taken up in a chelated form (see Wallace, 1956). DeKock and Mitchell (1957) found that the addition of certain chelating agents to the culture solution enhanced the uptake of chromium and aluminum although the uptake of nickel, zinc, and copper was reduced. EDTA and some of its analogs have been shown to in-
crease both the root uptake and root to shoot translocation of zinc (Essington et al., 1962).

Much literature exists on the movement of antibiotics in plants. Many of these compounds are capable of moving into the shoots of plants following root application. Anderson and Nienow (1947) reported that streptomycin could be detected in the shoots of soybean plants following root application and this led to many other studies on the movement of this antibiotic. It appears to be mobile in most plant species although Crowdy and Pramer (1955b) could not detect it in the leaf sap of broad bean plants following root application. They found that under similar conditions cabbage and wheat plants took up small amounts into their leaves while the leaf sap of tomato plants accumulated a concentration higher than that of the treating solution. Pramer (1954) detected a very slow accumulation of streptomycin within the lower leaves of broad bean. It would thus appear that the mobility of streptomycin within the plant body varies from species to species. Streptomycin applied to the soil apparently fails to enter the shoot in measurable quantities (Mitchell et al., 1953, 1954; Maier, 1960) possibly due to inactivation or breakdown of the chemical within the soil. Root uptake from water or sand cultures may differ considerably from that of the test compound added to soil. With tomato plants Crowdy and Pramer (1955b) demonstrated a concentration gradient of streptomycin from the base of the shoot to its apex. Similarly, Dye (1956a) found such a gradient in peach seedlings. K. O. Müller et al., (1954) reported that there was little evidence of mobility of streptomycin in tomato and potato plants once root treatment was stopped.

Other antibiotics have not been studied over such a range of test plants as has streptomycin; nevertheless, variations in their mobility in different plant species has been demonstrated. Both Krasilnikov (1952) and Blanchard and Diller (1951) detected chlortetracycline in the shoots of root-treated plants although it was not detected in the sap of decapitated cucumber seedlings nor was oxytetracycline (Pramer, 1953). Both Krasilnikov (1952) and Klemmer et al. (1955), however, found oxytetracycline to be mobile in their test plants.

Chloramphenicol applied to the roots appears in cucumber seedlings (Pramer, 1953), broad bean and tomato plants (Pramer, 1954), and tomatoes (Klemmer et al., 1955) and, indeed, Crowdy et al. (1955) have isolated pure chloramphenicol from broad bean shoots following root application. Pramer (1954) found that although initially there was a concentration gradient from the base to apex of the shoot, 72 hours after treatment stopped this gradient was completely reversed.
Klemmer et al. (1955) found that chloramphenicol became concentrated in the leaves rather than the roots and stems of tomato plants. Crowdy and Pramer (1955b) demonstrated that the leaf sap of treated broad beans contained a concentration of chloramphenicol approaching that of the treatment solution although the amounts present in the leaf sap of tomato, cabbage, lettuce, and wheat were considerably lower. Another readily mobile antibiotic is vancomycin. This water-soluble amphoteric compound (Mehta et al., 1959) enters the roots of a number of plants and may reach a concentration higher than that applied in treatment. Movement to and accumulation in the shoot is also rapid, leading to a concentration which can exceed that of the roots. Boyle and Price (1963) have shown that when vancomycin is added at weekly intervals to the roots of tomato plants, the concentration in the crown of the plant rapidly reaches a high level; this gradually declines during the subsequent 7 days and rises rapidly again after the next application. The roots showed a similar fluctuation except that the peak concentrations were much lower but, by contrast, the leaves showed only a slow increase over the entire experimental period.

For most of the antibiotics showing in vitro fungitoxicity, e.g., phleomycin (Smale et al., 1961), F-17 (Mitchell et al., 1959), P-9 (Hagborg et al., 1961), all that is reported is their ability to reduce foliar disease following root application. The antifungal antibiotic, griseofulvin, however, has been studied in some detail. The activity of this compound against leaf pathogens following root application has been noted for a number of plant species (Stubbs, 1952; Rhodes, et al., 1957). Brian et al. (1951) detected griseofulvin by bioassay in the leaves of lettuce seedlings growing in a water culture containing the antibiotic and in guttation droplets from root-treated oat seedlings growing both in water culture and in soil. Stokes (1954) bioassayed guttation drops from wheat seedlings in a study on the effect of environmental factors on griseofulvin uptake and transport. Griseofulvin was detected in these drops 48 hours after treatment commenced and the concentration tended to increase with both time of treatment and the concentration of the solution applied. Crowdy and Pramer (1965b) detected small amounts (less than 1 \( \mu g/ml \)) in the leaves of broad bean, tomato, cabbage, and wheat plants treated at their roots. Crowdy et al. (1955) isolated pure griseofulvin from the shoots of broad beans and, using a spectrophotometric method of estimation, obtained a balance sheet for griseofulvin applied to the roots of these plants for 3 days. They found that approximately one-third of the chemical applied to the roots was taken up into the plant.
and that of this nearly half was present in the roots, a quarter in the shoots, and a quarter was unaccounted for, presumably lost in metabolism. In a similar study of uptake and distribution within broad bean and tomato plants, Crowdy et al. (1956) found that expressed sap contained only low quantities of the chemical. They used chloroform extracts of the tissues for bioassay and showed that the roots of both species rapidly accumulated griseofulvin. In tomato plants, roots were the region of maximum accumulation, moderate amounts being present in the stem and both upper and lower leaves. By contrast, broad bean plants showed maximum accumulation in lower leaves with some in the top leaves and no accumulation in the stem. When root treatment stopped, griseofulvin present in the roots of beans moved rapidly to the leaves but no such movement occurred in tomato plants. Movement into the shoot of rice seedlings appears to be more rapid than that found for broad beans (Subba-Rao, 1957).

Starzyk and Mitchell (1963) found that cycloheximide, another antifungal antibiotic, was readily taken up from culture solutions by the roots of bean plants and rapidly transported to leaf and stem tissues. When root treatment was stopped, levels in both root and stem fell to virtually zero within 4 days but they remained high in leaves for considerably longer. Earlier, Wallen and Millar (1957) had found that this antibiotic rapidly entered existing stem and leaf tissue of wheat seedlings following root applications, but that it was not transported into new growth.

Among synthetic organic fungicides the most detailed studies on uptake and translocation have been made on sulfonamides. Hassebrauk (1938) was the first to report on their systemic antifungal activity. Rudd-Jones and Wignall (1955) demonstrated their uptake into plants by detecting sulfanilamide in the leaves of broad bean plants treated at the roots. These studies were extended to include a wider range of sulfonamides by Crowdy and Rudd-Jones (1956a) who estimated these chemicals in the roots, stems, and leaves of root-treated broad bean plants and detected all the test compounds in these organs. Sulfanilamide accumulated quickly in the leaves and to a lesser extent in the roots and stems. The other compounds accumulated preferentially in the roots often to a higher concentration than in the treatment solution, although sulfaguanidine was only poorly taken up. Both the rate and extent of root to shoot movement varied widely, thus only 4% of the root absorbed sulfathiazole but 75% of sulfanilamide was translocated.

Root applications of captan (N-trichloromethylthiocylohex-4-ene-
1:2-dicarboxyimide) have been reported to reduce the incidence of foliar pathogens (see Somers and Richmond, 1962). Wallen and Hoffman (1959) detected very small quantities in the leaf of root-treated pea seedlings although Somers and Richmond (1962), using 32P-labeled captan, have shown that broad bean plants treated with captan through the seeds never contained more than 0.09 ppm in their shoots. Fawcett et al. (1958a) examined the systemic antifungal activity of a series of aryloxythiotrichloromethanes and although captan proved inactive in their tests, as it did in those of Grossmann (1959), several other compounds were active in reducing infections of Alternaria solani on tomato foliage following root application.

Dithiocarbamates have proved valuable protectant fungicides and there are reports of their movement within plants. Van Raalte (1954) showed that TMTM (tetramethylthiuram monosulfide) was translocated in segments of potato petiole but that NaDDC (sodium dimethyldithiocarbamate) and TMTD (tetramethylthiuramdisulfide) were not. This led to the view that the antifungal dithiocarbamate ion was not readily translocated per se but that related chemicals capable of producing it in the plant might be. Pluijgers (1959) later found that many of the dithiocarbamates which he tested protected cucumber seedlings from attacks of scab following root application. Grossmann (1957) failed to show any significant effect of root applications of NaDDC or TMTD on the development of leaf pathogens and the bleeding sap from treated plants was not fungitoxic. By contrast, Darpoux et al. (1958b) found the sap of root-treated plants of a number of species to be fungitoxic in vitro. By bioassaying chromatograms of the sap from cucumber seedlings treated at the roots with NaDDC it has been demonstrated (see Dekhuijzen, 1964) that NaDDC ions are taken up by the seedlings but are then converted into various derivatives, some of which are themselves fungitoxic. It was also established that sodium diethyldithiocarbamate is similarly taken up and metabolized. Quantitative studies on one of the NaDDC metabolites, NaDDC alanine, showed this chemical to be rapidly absorbed by the roots of cucumber seedlings and moved to the hypocotyl and cotyledons. When root treatment ceased, NaDDC alanine moved from the root and cotyledons into the developing first leaf though some was leached into the nutrient solution and some was metabolized. The compound was also shown to be taken up by French bean, broad bean, and tomato plants where the highest concentrations were found in the stem and there was a tendency for it to accumulate in younger more than older leaves. S-carboxymethyl dimethyldithiocarbamate (G33), although inactive in
vitro, shows systemic activity (van Raalte et al., 1955), and van der Kerk (1956) suggested that this compound was translocated and decomposed in situ to give dithiocarbamate ions. Small quantities of the compound and its breakdown products were detected in the shoots of root-treated cucumber seedlings (Dekhuijzen, 1954) although these concentrations were too low to account for its systemic activity. Evidence was also obtained for the uptake by cucumber seedlings of 1-carboxyethyl and 1-methyl-1-carboxy-ethylidimethylthiocarbamates and other compounds of the type (CH₃)₂N·C(S)·S·(CH₂)n·COOH. Heyns (1964) has demonstrated, chromatographically, the uptake of certain N-carboxymethyl dithiocarbamates by seedlings of broad bean.

In contrast to these detailed studies on N,N-dialkylthiocarbamates, little attention seems to have been paid to the possible movement of the N-alkylthiocarbamates and the related ethylenebisdithiocarbamates. Grossmann (1957) observed no systemic activity of ethylenebisdithiocarbamates when applied to the roots of tomato plants although both sodium N-methyldithiocarbamate (Zentmyer and Enspalter, 1957) and disodium ethylenebisdithiocarbamate (Stoddard, 1951) show some systemic activity.

Pyridine-2-thiol-N-oxide is not readily transported from roots to shoots in plants (Kenaga and Kiesling, 1957; Rombouts and Kaars Sijpesteijn, 1958; Kaars Sijpesteijn et al., 1958). Some of its derivatives would seem to be more mobile, however, for its manganese salt was detected in the foliage of tomato, bean, and cucumber plants following root application (Darpoux et al., 1958a), and Ristich and Cohen (1961) found certain of its alkyl-oxygen and thio-ether derivatives to have systemic activity in cucumber and tomato plants.

Other fungitoxic chemicals have been shown to be taken up by roots and moved into the shoot. Hills (1962) obtained evidence that labeled Dexon (p-dimethylaminobenzenediazodium sulfonate) was taken up by sugar beet seedlings; the labeled material, however, was not detected in appreciable amounts on chromatograms of extracts of the tissues. 6-Azauracil shows systemic activity in cucumber plants following root application (Dekker, 1962) and has been detected chromatographically within the aerial tissues of treated plants (Dekker and Kort, 1964). Van Andel (1962) found that sap expressed from the hypocotyls of cucumber seedlings root treated with DL-p- or DL-m-fluorophenylalanine was active in vitro. Some substituted triazoles (Koopmans, 1960; Elings, 1962), chloronitrobenzenes (Grossmann, 1958), and alkyl mercaptotetrahydropyrimidines (Niekoll et al., 1961) have all shown activity against leaf pathogens following root application, but their up-
take and translocation has not been studied. Some nitroso-pyrazoles have shown similar activity (Crowdy and Wain, 1951; Stubbs, 1952) as have both 8-hydroxyquinoline and its salts (e.g. Crowdy and Davies, 1952). The latter compounds possess a wide range of systemic activity (see Pluijgers, 1959) and are highly fungitoxic in vitro; their systemic effects against vascular pathogens may, however, be connected with fungal toxin inactivation (Zentmyer, 1954).

Many chemicals reported as having systemic activity following root application have no in vitro activity and are thought to exert their effects by modification of the host metabolism. Such compounds include certain heterocyclic compounds (Davis et al., 1954), 2-mercaptobenzothiazole derivatives (Davis and Dimond, 1952; Dimond and Davis, 1953), substituted arylx- and arylthioalkane-carboxylic acids (Fawcett et al., 1955a, 1957), growth-regulating substances (see Horsfall and Dimond, 1957), amino acids (van Andel, 1958), phenyl thioureas (Kuipers Sijpesteijn and Pluijgers, 1962), thioureas (Fuchs and Bauermeister, 1958), and sym-dichlorotetrafluoroacetic acid (T. C. Allen and Freiberg, 1964). The extent to which most of these chemicals are taken up and translocated is not known but certain growth-promoting substances are known to be readily translocated within plants (see van Overbeck, 1956; Mitchell and Linder, 1957) and it is very likely that the closely related but nongrowth-regulating aryloxy acids studied by Fawcett et al. (1955a) are translocated in a similar manner. Amino acids, too, are known to be taken up by plant roots (see D. E. Wright, 1962). A systemic chemical of low in vitro fungitoxicity that has been detected in the leaves of root-treated plants is procaine (Dekker, 1961a,b).

C. Following Application to Cut Shoots

As indicated in the previous section, many chemicals after entering a plant through the roots ascend the stem and enter the leaves. Certain compounds can also enter the cut ends of stems and pass upwards into the leaves. Some of these chemicals are listed in Table III.

Many studies of this type of uptake have been made with antibiotics and particularly with streptomycin which has been shown to be taken up by cuttings of apricot, peach, Pyracantha, carnation, chrysanthemum, broad bean, and tomato. However, Atkinson and Ark (1956) found three tetracycline antibiotics to move more readily than streptomycin both in carnation and Pyracantha cuttings while Charles (1953) found that when cut petals of cherry laurel were placed in solutions of
TABLE III

SOME COMPOUNDS SHOWING EVIDENCE OF ABSORPTION INTO CUT SHOOTS

<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptomycin, chloramphenicol</td>
<td>Broad bean, tomato</td>
<td>Pramer (1954)</td>
</tr>
<tr>
<td>Streptomycin, oxytetracycline</td>
<td>Chrysanthemum</td>
<td>R. S. Robinson et al. (1954)</td>
</tr>
<tr>
<td>Streptomycin, griseofulvin</td>
<td>Apricot, peach</td>
<td>Morokawa (1955)</td>
</tr>
<tr>
<td>Streptomycin, tetracyclines, neomycin</td>
<td>Pyraminda</td>
<td>Ako and Ark (1956)</td>
</tr>
<tr>
<td>Neomycin</td>
<td>Cucumber, peach</td>
<td>Dye (1956a)</td>
</tr>
<tr>
<td>Terramycin</td>
<td>Potato</td>
<td>Bode (1954)</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Cherry laurel</td>
<td>Charles (1953)</td>
</tr>
<tr>
<td>Helixin</td>
<td>Tomato</td>
<td>Leben and Keitt (1952)</td>
</tr>
<tr>
<td>Teiximycin</td>
<td>Tomato</td>
<td>Stessel et al. (1953)</td>
</tr>
<tr>
<td>Mycothricin</td>
<td>Tomato, cucumber</td>
<td>Rangaswami (1956)</td>
</tr>
<tr>
<td>Phenyl mercuric acetate</td>
<td>Apple</td>
<td>R. G. Ross and Stewart (1962)</td>
</tr>
<tr>
<td>w-(X,N-Dimethylthioformamidyloxy)-</td>
<td>Broad bean</td>
<td>Garraway and Wain (1962)</td>
</tr>
<tr>
<td>alkane carboxylic acids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Carboxymethyl dithiocarbamate</td>
<td>Broad bean</td>
<td>Heyns (1964)</td>
</tr>
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</table>

Penicillin or streptomycin the former could be detected within the leaves after 33 minutes whereas streptomycin was not detectable after 4 hours. Pramer (1954) found a difference in the distribution of streptomycin in the cut shoots of different species. Cut shoots of broad bean accumulated a fairly high concentration in their lower leaves but none at the apex whereas tomato cuttings, although showing a similar gradient, contained the antibiotic in all regions. By comparison, chloramphenicol showed a much more uniform distribution within the cut shoots of both species.

Certain antifungal antibiotics have also been reported to be taken up by cut shoots. Leben and Keitt (1952) found that helixin was absorbed by tomato cuttings to a limited extent. Cuttings of this species apparently also absorb quantities of toximycin since a solution in which cuttings were allowed to stand for 2 days showed a lower activity than that of the original solution (Stessel et al., 1953). Rangaswami (1956) detected a fairly rapid uptake of mycothricin by cut shoots of tomato and cucumber with a preferential accumulation in the stem rather than the leaves. There have been fewer studies on the cut shoot uptake of compounds other than antibiotics. R. G. Ross and Stewart (1962) detected mercury within cut shoots of apple allowed to stand in solutions
of phenyl mercuric acetate although much of the element did not appear to be present as the original compound. The distribution of phytotoxicity symptoms within cut shoots of broad bean cuttings allowed to stand in solutions of various inorganic salts (Carter, 1963) suggests that some fungitoxic inorganic ions e.g., tellurite, copper, nickel, are taken up and migrate to the leaf tissues while others, e.g., cerium, osmium, are mainly localized at the stem base. By chromatography of plant extracts, Garraway and Wain (1962) detected the presence of certain dithiocarbamic acids and their β-oxidation products in cut shoots of broad bean which had been immersed in solutions of these compounds. A similar technique was used by Heyns (1964) who studied the uptake of some N-carboxymethyl dithiocarbamates by broad bean cuttings and detected all the applied chemicals in both stems and leaves.

D. Following Application to Stems

1. Intact Stems

In 1948 Hampton showed that application of streptomycin- or penicillin-soaked pads to crown galls tissue arrested development of the disease thus indicating that these antibiotics can penetrate into galled stem tissue. In their studies on streptomycin movement in bean plants, Mitchell et al. (1952, 1953, 1954) applied lanolin paste containing the antibiotic to the stem. Rapid entry of the antibiotic occurred, as detected by bioassays and pathogen inhibition, and a maximum concentration was reached locally within 3 days. Movement upward from the site of application was also detected. Maier and Horner (1957) used a similar technique in applying streptomycin to stems of hops and again rapid entry and upward movement was detected. Following the application of streptomycin to the surface of tobacco stems, Hidaka and Murano (1956b) detected the antibiotic in epidermis, phloem, and pith, but not xylem.

Antibiotics other than streptomycin, e.g., streptothricin (Gray, 1958), are also absorbed and translocated upwards when applied to stems. F-17 also appears to move in this manner since following application to the base of the stems of bean plants it reduced rust infection on the primary leaves (Preston et al., 1956; Mitchell et al., 1959). The synthetic therapeutant, N-phenyl-N'-3-sulfolanyl-hydrazine (Evans and Saggers, 1962) likewise protected leaves against rust when cotton wool soaked in a solution of this compound was wrapped around the base of the stem of bean plants.
The direct penetration of the periderm of mature tree trunks would also seem to occur since Strong and Cation (1940) found that sprays of sodium dinitrocresylate had eradicant activity against cedar rust galls and Beckman (1959) reduced Ceratostomella infection of inoculated elm trees by painting a band of 2,3,5,6-tetrachlorobenzoic acid in an oil carrier onto their trunks. More direct evidence of bark penetration is provided by the observations by Lemin et al. (1960) and Weir (1963) that following the application of cycloheximide in oil to the trunks of white pine and Douglas fir, respectively, the antibiotic could be detected in the needles.

2. Wounded Stems

In attempts to introduce chemicals into mature trees, the chemical is sometimes applied to wounds made in the trunk and this method is often successful although P. Hoffman (1952) found it less effective than root application. Early work on the control of wilt diseases (Caroselli and Howard, 1942; Zentmyer and Horsfall, 1943) showed that injections of certain chemicals such as quinones, pyrogallol, nitrophenol, and 8-quinolinol, would reduce disease symptoms. A commonly employed technique is to bore a number of tangential holes through the sapwood cylinder of the trunk and place the chemical within these boreholes either in gelatin capsules (e.g., Dunegan and Wilson, 1953; Goodman and Johnston, 1957) or as a dry powder (Tarjan and Howard, 1953; Beckman, 1958). Following such applications, streptomycin has been observed to move into the foliage of apple (Goodman and Johnston, 1957), peach (Dunegan and Wilson, 1953), and pear (Ark and Alcorn, 1956) trees. Terramycin injected into peach trees also apparently moved into the foliage (Dunegan et al., 1953). Synthetic chemicals including benzothiazolyl-2-thioglycolic acid and 8-quinolinol benzoate, have also been applied as dry powders placed in boreholes and have reduced foliar disease (Tarjan and Howard, 1953; Beckman, 1958). Helton and French (1962) and Helton and Harvey (1963) fed solutions of fungicidal compounds into boreholes made in the scaffold branches of plum trees and noted the movement of the chemicals by tissue bioassay and the distribution of toxicity symptoms. They found that the antibiotics phytostreptin, phytosactin, and mycostatin were only slightly absorbed while cycloheximide and its semicarbazone derivative, although only absorbed to a limited extent, were fairly mobile. Pyridine-thiol-N-oxide was about as readily absorbed as cycloheximide and a thiourea hydrochloride derivative was more mobile. The presence of these chemicals could not, however, be determined in the tis-
In contrast, 8-quinolinol salts, especially the benzoate, were taken up very readily, translocated in large quantities and could be detected in the tissues by bioassay. A somewhat different method of application was used by Kuc et al. (1957) who fed phenylthiourea and α- or α-phenylalanine into the leaf petioles of apple shoots and in this way increased the resistance of the shoot to Venturia inaequalis. Tsao et al. (1960) pricked internodes of tomato plants and wrapped paper soaked in the antibiotic B-74 around the wounded area. Following this treatment the susceptibility of leaves above the treated area to A. solani was reduced.

E. Following Application to Leaves

Most fungicides are applied in the field as foliar sprays and their capacity for absorption into leaves is therefore important. Many chemicals can enter a sprayed leaf and some of them are mobile not only within the treated leaf, but can move out into the stem and be transported both upward and downward in the plant. The evidence for these movements is usually based on changes in disease susceptibility; detection of the chemical within the tissues is not as common as with root uptake. Furthermore, in leaf application experiments, local movement into and within the treated leaf has been mainly studied while with root application, the investigation has been concerned with long-range movement from root to shoot.

The simplest evidence for leaf absorption is the ability of the chemical to eradicate an established leaf infection because, with the notable exception of the powdery mildews, nearly all pathogens penetrate into the leaf tissues during the infection process. Other methods used to detect leaf uptake include the inoculation or bioassay of leaves from which the surface residue of the spray has been washed off or from which the sprayed epidermis has been stripped. Movement within the leaf has been detected either by spraying one leaf surface and inoculating the other or by applying the chemical to only the proximal or distal half of the leaf and then inoculating or bioassaying the untreated half. Export from a treated leaf has been detected by the bioassay or inoculation of plant tissues remote from it.

Studies on the uptake of plant mineral nutrients by leaves (see Wittwer, 1957; Bukovac and Wittwer, 1957) have shown that many inorganic ions including fungitoxic ones such as zinc (Wallihan and Heymann-Herschberg, 1956) are absorbed and some, such as phosphate, are readily transported. Ruthenium, although readily taken up
by the aerial parts does not appear to redistribute within the plant (Tukey et al., 1961). Cupric ions are also known to be absorbed by leaves. Arman (see Arman, 1957; Arman and Wain, 1960) found that cupric ions were absorbed readily by apple leaves dipped into copper sulfate solution. It was also found (Arman, 1957) that if one of a pair of lower leaflets of broad bean seedlings is immersed in copper sulfate solution, then copper is taken up by the immersed leaflet and translocated to other regions. The extent of this movement depends upon the concentration of the treatment solution—migration into the opposite leaflet occurs most readily, followed by upward movement in the stem. Movement downward from the treated leaf occurred less readily and only when high concentrations were applied. A copper–EDTA complex applied in the same manner appeared to move more readily. Evidence was obtained (Arman and Wain, 1963) that this and other copper complexes are absorbed as such by apple leaves. M. Allen (1964) has also demonstrated that apple leaves take up copper when immersed in copper sulfate solution; the rate of uptake was found to vary during the immersion period although a similar pattern of uptake was shown for leaves dipped momentarily in copper sulfate solution (M. Allen, 1965). Nickel salts have been shown to have an eradicant action against cereal rusts and tea blister blight (see Table IV) and thus are presumably capable of penetrating the plant surface. Mercury, at least in the form of phenyl mercuric salts, also appears to be absorbed from foliar sprays. Some of these salts are known to possess eradicant properties (e.g., Howard and Sorrell, 1943) and analyses made on the fruits and leaves of coffee bushes (Pickard and Martin, 1959) and apple trees (Pickard and Martin, 1990, 1961; Stewart and Ross, 1966) indicate that movement occurs from sprayed foliage to tissues which develop subsequently. R. G. Ross and Stewart (1962) produced further evidence of this type, but found that movement to unsprayed mature leaves did not occur and that spur leaves immersed in a solution of phenyl mercuric acetate took up mercury but did not transfer it to shoots. Pickard and Martin (1962) showed that phenyl mercuric acetate moved from sprayed broad bean foliage chiefly into stem and petioles but also in small amounts into roots, seeds, and pods. Evidence has been obtained of movement from potato foliage to the tubers (Pickard and Martin, 1962; Smart, 1964) although the amounts of transported mercury were always low and probably insufficient to induce disease resistance.

Antibiotics, and especially streptomycin, have been widely used in leaf-uptake investigations and the papers listed in Table IV indicate


<table>
<thead>
<tr>
<th>Compound</th>
<th>Plant</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Inorganic</td>
<td></td>
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<tr>
<td>Ruthenium-116</td>
<td>Tomato</td>
<td>Tukey <em>et al.</em> (1961)</td>
</tr>
<tr>
<td>Zinc-68</td>
<td>Citrus</td>
<td>Wallihan and Heymann-Herschberg (1956)</td>
</tr>
<tr>
<td>Copper salts and chelates</td>
<td>Apple, broad bean</td>
<td>Arman (1957); Arman and Wain (1960, 1961); M. Allen (1964, 1965)</td>
</tr>
<tr>
<td>Nickel salts</td>
<td>Rye</td>
<td>Keil <em>et al.</em> (1968); Forsyth and Peterson (1969a,b); Venkata Rama (1963)</td>
</tr>
<tr>
<td>Phenyl mercuric salts</td>
<td>Coffee, apple, broad bean, potato</td>
<td>Pickard and Martin (1959, 1960), 1961, 1962; Stewart and Rose (1960); R. G. Ross and Stewart (1962); Smart (1964)</td>
</tr>
<tr>
<td>Organic—Antibiotics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anisomycin</td>
<td>Bean</td>
<td>Zaumeyer (1957)</td>
</tr>
<tr>
<td>Antimycin A, blastistatin, blastomycin</td>
<td>Broad bean</td>
<td>Misato <em>et al.</em> (1956)</td>
</tr>
<tr>
<td>B-74</td>
<td>Tomato</td>
<td>Tso <em>et al.</em> (1960)</td>
</tr>
<tr>
<td>Chlorothiamine</td>
<td>Various</td>
<td>Gray (1956)</td>
</tr>
<tr>
<td>Cycloheximide and</td>
<td>Wheat</td>
<td>Livingston (1953)</td>
</tr>
<tr>
<td>derivatives</td>
<td>Cherry, bean</td>
<td>Hamilton and Sokoloski (1953)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Starzyk and Mitchell (1963); Khs and Frentek (1964)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hamilton <em>et al.</em> (1956); Starzyk and Mitchell (1963); Khs and Frantek (1964)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Preston <em>et al.</em> (1956); Mitchell (1959)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stibbe (1952); Davis and Rothrock (1955); Bostrom <em>et al.</em> (1957)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin</td>
<td>Bean, hops, peach, tobacco, tomato, cucumber, Coleus, Selten purpureum</td>
<td>Gray (1955a,c, 1956, 1958); Goodman and Dowler (1958); Ogawa <em>et al.</em> (1960); Maier (1960); Dye (1960); Hidaka and Murawo (1956a,b); Muresbizkyan and Mekhova (1965); Ark and Wilson</td>
</tr>
</tbody>
</table>
the wide range of species which can absorb this antibiotic through their leaves. Dye (1956b) showed that streptomycin applied to the lower surface of peach leaves protected the upper surface from infection but he could not demonstrate distal to proximal movement within the leaf nor vice versa. Mitchell et al. (1953, 1954) revealed proximal to distal
movement in leaves of bean plants but not in the reverse direction. Differences have also been found in the extent to which streptomycin is exported from treated leaves. Mitchell et al. (1953, 1954) could detect no movement out of the leaves of bean plants and Gray (1955b) found no evidence of downward movement in the stem. However, Gray (1955a) showed that while streptomycin did not move out from sprayed leaves, it moved upward in the stem, but not downward, if the leaf was immersed in a solution of the antibiotic. He later found that the amine and oxime derivatives were better distributed following leaf immersion than was the parent compound (Gray, 1958). Maier (1960) reported that in hop plants, streptomycin will move upward from the point of application and reach a higher concentration in the growing tip than in intermediate leaves, but again no downward movement could be detected. On the other hand, Dowler and Goodman (1958) have obtained evidence for the downward movement from treated leaves of Coleus.

The antifungal antibiotics cycloheximide and griseofulvin, are also known to be absorbed by plant leaves. Hamilton and Sokolnik (1953) reported an eradicant activity for cycloheximide against cherry leaf spot and Hamilton et al. (1956) showed that cherry leaves took up the oxime derivative and new growth was protected against disease. The semicarbazone and acetate were also active but cycloheximide itself showed little systemic action. More detailed studies involving bioassay of plant extracts were made by Starzyk and Mitchell (1963) who found that cycloheximide moved from a sprayed primary leaf of bean plants into the other plant parts. In sunflowers, movement was largely to the growing apex but no movement could be detected in cucumber, cowpea, or corn plants. Uptake into both bean and cherry leaves from a surface spray deposit was enhanced by high humidity, but bioassays of leaf tissues revealed only low concentrations either because of rapid translocation away from the leaf or binding and breakdown within the leaf. Both Stubbs (1952) and Rhodes et al. (1957) obtained evidence of uptake of griseofulvin by leaves although root applications always appeared more effective. Davis and Rothrock (1956) showed that application of griseofulvin to one surface of a tomato leaf would protect the other from infection by A. solani, and Boothroyd et al. (1957), using the $^{32}$Cl-labeled compound, also obtained evidence of movement from treated tomato leaves although the extent to which this movement occurred was greatly influenced by environmental conditions. Other antifungal antibiotics have shown systemic properties following leaf application. Studies on the antibiotic
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F-17 (Preston et al., 1956; Mitchell et al., 1959) have shown that applications to the upper surface of bean leaves protected the lower surface from infection; movement within the leaf occurred in a proximal to distal direction but not vice versa. Phleomycin shows similar systemic activity in bean plants (Smale et al., 1961). B-74 applied to the lower surface of tomato leaves protected the upper from infection. It was found that proximal to distal movement within the leaf occurred and there was also evidence of an upward movement from the treated leaves to give disease protection in the leaves above (Tsao et al., 1960). Movement from one leaf surface of bean leaves to induce disease resistance in the other occurs with alogynycin and anisomyacin (Zaumeyer, 1957). Davis et al. (1960) and Hagborg et al. (1961) showed that the antibiotic P-9 can eradicate established rust infections on wheat leaves and move within the leaf, this movement being proximal to distal at lower concentrations; at higher concentrations movement in both directions occurred.

Gray (1955a) studied the movement of a number of antibiotics out of the sprayed leaf. He found both pleocidin and streptothricin to be translocated upward and downward within the stem while other antibiotics were immobile. When leaves were immersed in a solution of the antibiotic, however, penicillin, streptomycin, neomycin, vinyycin, streptothricin, and pleocidin moved upward in the stem; the last two also moved downward. Misato et al. (1958) performed experiments in which median leaves of broad bean plants were treated with paraffin-glycerine pastes containing antibiotics. Antimycin A, blastcidin, and blastmycin were shown to move both upward and downward but no movement was detected for humidin, eurocidin, or actidione. Those antibiotics mobile in broad bean were also found to be mobile in rice seedlings.

Certain commercial protectant fungicides and other antifungal synthetic chemicals appear to enter into and be exported from treated leaves as shown by evidence of disease reduction. Captan applied to the dorsal surface of broad bean leaves increased the resistance of the ventral surface of the treated leaf, and also leaves above and below, to chocolate spot infection (Napier et al., 1957). The compound also appeared to move up from sprayed lower leaves of cucumber plants to protect the leaves above from attacks of scab; Zinc behaved similarly (Rich, 1956b). Corbaz (1962) found evidence of a limited systemic activity of NaDDC in tobacco plants, and Pluijgers (1959) showed that if cucumber seedlings had one cotyledon dipped several times into solutions of a number of dithiocarbamates the resistance of the plants to scab was increased. Dodine would also appear to undergo
limited movement in apple foliage for in addition to its eradicant ac-
tivity against scab, it will protect the upper surface of apple leaves
when applied to their lower surface (Hamilton and Szkolnik, 1958;
Jepson, 1961). It does not appear to be translocated, however, from
apple foliage to developing fruits (Curry, 1962). When the first fully
expanded leaf of tomato plants was immersed in suspensions of vari­
ous 1-aryl, 3,5-dimethyl-4-nitrosopyrazoles for 36 hours, some of the solu­
tion was absorbed and some disease resistance was conferred upon
other leaves (McNew and Sundholm, 1949). Pyridine-thiol-N-oxide ap­
ppears to be fairly mobile following foliage applications for Sander
and Allison (1956) reported that after dipping the primary leaf of
cucumber into a solution of this compound, homogenates of the root
tissues and other plant organs possessed antifungal activity. Rom­
bout and Kaars Sijpesteijn (1958) and Kaars Sijpesteijn et al.
(1958) report upon similar tests but they found that roots of untreated
cucumber plants could also show antifungal activity. They observed,
however, that treating one cotyledon of a cucumber seedling could pro­
tect the other against scab. When one leaflet of the middle pair on
broad bean plants possessing three pairs of leaflets was painted on one
surface with this chemical, or its carboxymethyl derivative, not only
was the untreated surface of the leaflet protected from chocolate spot
infection but some effect was noticed on the twin leaflet and a still
smaller effect upon the leaves below. The fumigant activity shown by
PTO was not considered to account for all the observed systemic ac­
tivity. Crowdy et al. (1958) have well demonstrated that certain sulfo­
amides, p-aminobenzoic acid, and sulfanilic acid are taken up by leaves
of wheat dipped into solutions of these compounds although there were
considerable differences in the degree of uptake. Dekker (1961b) used
chromatography of tissue extracts to show that cucumber leaves
sprayed with protein hydrochloride absorbed but did not translocate
this compound. When one leaf of the plant was immersed in a solution
for 2 days, protein was detected in large amounts in the shoot both
above and below the treated leaf; very low concentrations were present
in the roots. Hills (1962) autoradiographed sugar beet seedlings one
leaf of which had been treated with drops of 14C-labeled Dexon. He
found radioactivity to be concentrated in the vascular system and the
growing point as well as in the treated leaf. MacLennan et al. (1963)
showed that α-aminoisobutyric acid when infused into a lower leaf of
an apple shoot moves out and preferentially accumulates in younger
leaves at the tip. Kirkham and Hunter (1964) also used apple shoots
and dipped the three leaves below the youngest fully expanded leaf
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in a suspension of isobutyl-o-coumarate. This ester, together with coumarin and traces of coumaric acid, was detected in leaves above those treated. After 6 days the highest concentration of the ester was present in the fifth leaf above those treated.

Studies on the eradicant activity of a wide variety of chemicals (e.g., Hotson, 1953; Livingston, 1953; Klos and Fronek, 1964) have shown that many can apparently enter leaf tissues. Some of these chemicals, both active and inactive fungitoxicants in vitro, are included in Table IV.

III. FACTORS AFFECTING UPTAKE AND TRANSLLOCATION

It is clear from the preceding survey that many compounds move from one plant organ to another and that for a few of these compounds such as streptomycin, sulfonamides, and griseofulvin, a quantitative picture of their uptake and distribution has been obtained. Such distribution patterns, however, are not static and are the result of interactions between the plant and the chemical and external environment. Changes in any of these factors will affect the pattern of uptake.

A. The Plant as a Variable Factor

Because of the agricultural importance of systemic action generally, it is necessary to have an understanding of the mechanisms underlying intake and transport of ions and molecules. Much attention has been given to the basic factors which influence these phenomena. Important among these are the anatomy of leaves, stems, and roots and the structural and chemical characteristics of cuticle, epidermis, and phloem and other parts of the plant which influence intake and transport (Mitchell et al., 1960).

The form in which the substance is presented to the leaves is also important. For efficient uptake, an even film of the material making excellent contact with the leaf surface is clearly to be preferred to large aggregates of crystals which incompletely cover the leaf. In agreement with this it is found that such properties as the particle size of the material applied, and whether surfactant materials or oils are used to aid dispersion and spreading, are all concerned in determining how much of the chemical will be taken up by the plant.

Of particular importance, too, is the nature of the plant surface; the cuticle of the leaf, stem, and fruit, for example, varies considerably from plant to plant. In most cases there are layers of cutin impregnated with wax. The chemical nature of the cutin layer as a whole is ex-
tremely complex, but its properties are important since it presents a barrier through which the applied ions or molecules must pass. With an organic substance such penetration is related to its lipophilic properties, such as its solubility in cuticular wax. If the organic molecule is too polar with a high affinity for water and polar substances, it will not, in general, penetrate readily through the cuticle; on the other hand, a completely nonpolar compound may accumulate in the wax and not pass through. Once within the plant, other barriers exist, such as the protoplasmic membranes of cells through which both ions and molecules must penetrate to reach their site of biological action.

In the case of a physiologically active organic acid, which can be applied either as the weakly dissociated acid or its highly ionized salt, it is usually found that the former is more active than the anion. The key to this different biological effect of molecule and ion is the protoplasmic membrane. These membranes are complicated in structure. It has been suggested that they contain a double film of orientated phospholipid ions as well as other materials which are lipophilic. Alternatively, the membrane is envisaged as a lipoid barrier made up of steroids, glycerides, free fatty acids, and phosphatides bounded on each side by a thin film of protein. The arrangement of these constituents is such that free positive and negative charges arise where certain of the lipoid materials interact with the free –NH₂ and –COOH groups present in the protein molecules (Davson and Danielli, 1952).

Both of these concepts, it will be noted, provide for ionic groupings within the membrane and it is probably the positive charges that impede the penetration of a charged organic anion. An undissociated acid, on the other hand, does not suffer this hindrance and might, therefore, be expected to penetrate such a membrane more readily and thereby produce a greater biological effect. Since there is evidence that the protoplasmic membrane contains hydrophilic as well as strongly lipophilic groupings, the balance between lipophilic and hydrophilic character of a biologically active molecule is also a factor in regard to its ease of penetration.

But while lipophilic properties are of importance in this connection, a systemic molecule must also be translocated within the plant. For this to occur the molecule must be "polar"; it must either itself have sufficient water solubility to move within the transpiration stream, or be capable of combining with some soluble plant constituent which will carry it in solution within the plant. Water solubility is, in fact, often a limiting factor in systemic activity.
As was seen earlier, plant species differ in the extent to which they translocate a given chemical. Mobility depends upon the ability of the chemical to avoid local accumulation at permeability barriers the nature and extent of which will differ from one plant species to another (e.g., Kamimura and Goodman, 1964a). Within a single species translocation will vary according to the site of application. For most compounds root uptake occurs more readily than leaf uptake and gives a more uniform distribution but for a few (e.g., pyridine-thiol-N-oxide; Rombouts and Kaars Sijpsteijn, 1958), the converse is true. Application to different areas of the same organ, especially a leaf, can also produce different patterns of uptake, as was shown by Dunegan and Wilson (1956). It must be remembered that both the structure and physiology of a plant are not constant but change throughout its ontogeny. This will lead to observations such as those of Beckman (1959) and Arman and Wain (1960) that a chemical applied at different stages in the development of a plant does not produce the same results.

B. The Chemical as a Variable Factor

The physicochemical properties of a fungicidal molecule will influence its translocatability as was noted above in connection with the dissociation of acidic compounds. Many studies on comparative translocation have employed compounds of widely differing and complex structures, such as antibiotics; other studies have related structure to systemic activity rather than translocation. In a few groups of compounds such as sulfonamides (Crowdy and Rudd-Jones, 1955a) and griseofulvin analogs (Crowdy et al., 1959b), attempts have been made to study the relationship between structure and ease of translocation. Since the chemical moves within the plant in aqueous solution and yet relatively nonmobile lipids are also present, its lipid/water partition might influence its mobility, a factor which seems to operate in some griseofulvin analogs (Crowdy et al., 1959b). Another important factor in restricting the movement of a chemical is its degree of adsorption onto binding sites many of which appear to be negatively charged. Crowdy and Pramer (1955a) pointed out that readily translocated antibodies were either neutral or acidic, anomalous results being obtained with basic or amphoteric compounds. It has been shown (e.g., Chapman, 1951; Edgington and Dimond, 1960a, 1961) that the charge on a chemical affects its binding to cellulose and plant tissues. Furthermore, similarities have been noted between the movement of chemicals within
As already indicated, the form in which a fungicide is applied will affect its movement as is illustrated by the differences in uptake of chemicals when applied as a solution surrounding a leaf and when applied as a spray (Gray, 1955a; Dekker, 1961b). Larger aggregates are less likely to penetrate the plant and it has been noted with, for example, seed uptake studies (Krüger, 1960a,b; Dekker, 1957), that slurries and dusts are less efficient than solutions.

C. The Environment as a Variable Factor

The properties of both the plant and, to a lesser extent, the fungicide, can be modified by changes in the external environment. Climatic conditions such as temperature, light, and oxygen supply affect the metabolism of the plant and there is evidence that some processes involved in the absorption of chemicals by both roots (Crowdy et al., 1956; Crowdy and Rudd-Jones, 1956a) and leaves (Kamimura and Goodman, 1964b) are metabolically controlled. Transpiration rates also appear to influence the uptake of chemicals (Crowdy et al., 1956; Crowdy and Rudd-Jones, 1956a).

The environment can affect the chemical too, since alterations in the pH of its solution can affect its degree of ionization and thus its uptake. For foliar applications, the rate at which the solvent evaporates is important and it has been shown that high atmospheric humidity can enhance leaf uptake (e.g., Starzyk and Mitchell, 1963). When applied to soil-grown plants the soil itself can exert a great influence upon uptake. Soils can adsorb certain antibiotics (e.g., Siminoff and Gottlieb, 1951; Gopalkrishnan and Jump, 1952; Martin and Gottlieb, 1952) and degrade them either by pH effects or microbial decomposition (Jefferys, 1952; J. M. Wright and Grove, 1957). Inorganic compounds, such as those of mercury (Booer, 1944), are also changed within soils. Some soil-adsorbed chemicals may still be taken up by plants for although they do not diffuse out into aqueous media (e.g., Siminoff and Gottlieb, 1951) the adsorbed material may still be biologically active (Pramer and Starkey, 1962) and transferred to the roots without free solution being involved (Jenny, 1951).

Many environmental factors are not readily controlled but in some cases other chemicals have been applied with the therapeutant (see Goodman, 1959, 1962a,b) with the aim of assisting its entry into the plant. In certain cases, organic solvents such as ethanol (Leben et al.,
acetone (Wallen and Hoffman, 1959; Milborrow, 1963), or chloroform (Darlington and Barry, 1965) have assisted penetration. Humectants, too, have aided leaf uptake (Gray, 1955b, 1956; Rich, 1956b) but this is not always so (e.g., Ark and Wilson, 1956). Surfactants, although often used to aid wetting of the leaf surface, do not necessarily assist uptake and may in fact decrease it (Gray, 1956). Goodman and Dowler (1958) found that certain growth-regulators also enhanced the uptake of streptomycin by leaves. Cations such as potassium, calcium, and thorium, by competing for binding sites, have been shown to reduce the adsorption of cationic therapeutants by soil clays (e.g., Ark and Alcorn, 1956b) and by plant tissues (Alcorn and Ark, 1956; Goodman, 1959; Edgington and Dimond, 1960a, 1964).

IV. TRANSFORMATIONS OF FUNGICIDES WITHIN PLANTS

So far in this review the amount of fungicide present within an untreated part of a plant has been considered solely in terms of the rate and extent of its translocation from the site of application. A further factor determining the local concentration of the applied chemical is the extent and speed with which it is metabolized by the plant. When exposed to the action of plant enzymes many fungicides will be changed, but as yet there is only limited information available on this topic, a point stressed in the review by Kaars Sijpesteijn and Kaslander (1964).

Although decomposition of some organic fungicides might arise through simple chemical degradation in the new environmental conditions within the plant, most transformations are likely to be enzymic in nature. By such action it is possible for the fungicide to be catabolized by oxidases, reductases and hydrolases, modified by enzymes such as transaminases, anabolized into conjugates such as β-glucosides, or perhaps be incorporated into plant assimilates.

As was the case with translocation studies, much of the evidence for the breakdown of fungicides within the plant body is indirect in nature. Changes in the degree of disease resistance induced in treated plants over a period provides some indication of breakdown. When such changes are measured on only one organ of the plant, one must allow for the possibility of translocation from that organ to other parts of the plant. Andersen and Rowell (1962) attempted to put this type of measurement on a quantitative basis by treating wheat leaves with a dosage of the test chemical that gave 90 to 95% disease control.
24 hours after treatment. Surface residues were then removed by washing and the leaves inoculated with rust at different intervals of time. They expressed their results in terms of a "duration index" which was the time in hours required to produce a 50% decrease in disease control. Index figures ranging from 31 for nickel chloride to 283 for cycloheximide semicarbazone were obtained.

The detection of a chemical in the tissues a given time after its application provides a qualitative measure of its persistence which, for any given chemical, may vary widely from one plant species to another.

Streptomycin appears to be stable in most plant tissues although breakdown in hop plants seems to be fairly rapid (Maier and Horner, 1957). There is evidence that this antibiotic can persist in chrysanthemum cuttings for 8 weeks (R. S. Robinson et al., 1954), in tobacco leaves for 5 weeks (Hidaka and Murano, 1956b), and in carnation cuttings for 33 days (Alcorn and Ark, 1956). Its persistence in some trees appears to be even more prolonged; thus, after application to boreholes in the trunk it was detected in the foliage of apple trees after 4 months (Goodman and Johnston, 1957) and in pear trees after 1 year (Ark and Alcorn, 1956).

The persistence of a chemical can also vary from organ to organ in the same plant. Silvulescu et al., 1963, found the persistence of antibiotics within tomato plants to be greater in their roots than their leaves, a difference which they attributed, in part, to the less intense and complex metabolism of the roots. It is interesting to note in this connection that chlorotetracycline has been observed to persist for 9 months in dried beet seed (Sutton and Bell, 1954).

Other antibiotics, on the whole, are less persistent than streptomycin. Prescott et al. (1956) report a half-life of only 24 hours for cycloheximide in ripe cherry fruits although it persisted longer in the foliage and could be recovered from wheat leaf tissue 5 weeks after treatment (Wallen and Millar, 1957). It also appears to be fairly stable in seedlings of white pine (Lenin and Thomas, 1961) although Starkey and Mitchell (1963) did not find it to persist in the tissues of bean and cherry leaves. Crowdy (1957) reported a logarithmic decay of griseofulvin in broad bean tissues with an estimated half-life of 4 days while Dekker and Ark (1958) found the antibiotic CS 1 to be inactivated in orange rind, sap of bean and cucumber plants, and the seeds of various legumes. Little inactivation took place, however, in the sap of Oxalis and seeds of cucurbits and maize. It was suggested that in-
activation, at least in orange rind, was brought about by the action of cytochrome oxidase.

Studies upon synthetic organic fungicides have indicated a wide variation in their stability within plants. Captan has been detected within pea seeds 9 weeks after treatment (Wallen and Hoffman, 1959) whereas Dokker (1961b) found procaine hydrochloride to be rapidly broken down by cucumber tissue.

While it is relatively simple to demonstrate the disappearance of a chemical it is more difficult to identify the breakdown products. Certain derivatives of the compounds, e.g., streptomycin oxime and amine (Gray, 1958), cycloheximide acetate (Leman and Magee, 1957), the carboxymethyl derivative of pyridine-thiol-N-oxide (Rombouts and Kaars Sijpesteijn, 1958), which are less active in vitro than the parent compound, are just as active systemically and this may indicate that a conversion to the parent compound has occurred within the plant. Studies on the fungicidal activity of fifteen esters of 2-methyl-4,6-dinitrophenol (DNC) (Bates et al., 1962) showed that aromatic esters had a low in vitro fungitoxicity. The benzoic ester was only weakly active against mycelial growth and in preventing spore germination but as a protectant fungicide against _B. fabae_ on broad beans it was very effective. It was established that DNC benzoate is stable in light, under conditions of high humidity (see Fig. 1) and in the presence of fungal spores, but it was shown that some degree of hydrolysis occurs on the leaves of broad bean to yield the parent cresol (see Fig. 2). Phytotoxic levels of DNC were not reached but it was considered that sufficient was produced to account for the observed protectant activity of the benzoic ester.

As already indicated, a number of aryloxyalkanecarboxylic acids have been shown to possess systemic fungicidal activity (Crowdy and Wain, 1951; Fawcett et al., 1955a, 1957). Derivatives of these compounds such as the nitriles, amides, primary alcohols, aldehydes, and esters have been shown to be converted to the parent acid within the plant (see Wain, 1958) and certain nitriles may undergo α-oxidation (Fawcett et al., 1955b, 1958b; Taylor and Wain, 1959). The acids themselves may be subjected to β-oxidation within the plant tissues (Synerholm and Zimmerman, 1947; Wain and Wightman, 1954) and chromatographic analysis of tissue extracts has shown that α-(N,N-dimethylthiocarbamoylthio)alkanecarboxylic acids likewise undergo β-oxidation within the tissues of wheat and broad bean (Garraway and Wain, 1962). This type of breakdown has also been reported by
Fig. 1. Electrochromatogram of DNC and its benzoic ester and of these substances after incubation on glass slides in the greenhouse (G.H.) or humidity cabinet (H.C.). (From Bates et al., 1962.)

Fig. 2. Electrochromatogram of extracts of broad bean plants which have been sprayed with DNC or its benzoic derivative and held in the greenhouse. (From Bates et al., 1962.)
Pluijgers and van der Kerk (1961) and the liberation of dimethylthiocarbamate ions from such acids is known to occur in some plants (Dekhuijzen, 1964). There is also evidence (Mitchell and Brown, 1946; Mitchell et al., 1960) that the movement of certain aryloxy acids, when applied to leaves, is associated with the flow of sugars produced in photosynthesis, usually towards sites of high metabolic activity. This indicates a reaction of the aryloxy acid with sugar in the plant, whereby the solubility of the acid would be increased.

Hills (1962) chromatographed sap from sugar beet seedlings treated with labeled Dexon and found little $^{14}C$ at the $R_f$ of Dexon; in addition, the presence of four other labeled compounds was noted. The identity of these breakdown products was not ascertained but the work of Tolsmaoff (1962) indicates that they might be products of oxidation. Curry (1962), using labeled dicycine, found that in apple tissues the guanidine group is split off. Dekker and Oort (1964) chromatographed the sap of cucumber plants treated with 6-azauracil and detected this compound together with larger amounts of 6-azauridine, indicating a ready coupling with ribose within the plant. C. Ross (1964) studied the fate of azauracil added to cocklebur (Xanthium pensylvanicum) and found that while much remained unmetabolized, some was converted in 6-azauridine or 6-azauridine-5'-phosphate and some was incorporated into RNA.

Rudd-Jones and Wignall (1955) reported that sulfanilamide becomes acetylated in broad bean plants. In further work (Crowdy and Rudd-Jones, 1958) changes occurring in sulfanilamide applied to the roots of broad bean and wheat plants were studied both qualitatively and quantitatively. They found that acetylation of sulfanilamide occurred at both the N-1 and N-4 positions and N-4-acetyl sulfanilamide was itself acetylated at the N-1 position. In the roots of broad beans acetylation was very vigorous, 30% of the sulfanilamide entering being acetylated; in the stems and leaves deacetylation was the dominant reaction. Crowdy et al. (1958a) used spectrophotometric methods to detect the breakdown of some griseofulvin derivatives within broad bean plants though not all of the degradation products were identified.

The bioassay of chromatographed sap has been used to study the metabolism of some dithiocarbamates within plant tissues. This work has been described in detail by Dekhuijzen (1964) and summarized by Kaars Sijpesteijn et al., (1963) and Kaars Sijpesteijn and Kaslander (1964). Dekhuijzen (1961a,b) reported that sap from cucumber plants treated with NaDDC showed three fungitoxic areas when chromatographed, none of which had the $R_f$ of NaDDC itself. Since then Mas-
aux (1963) has demonstrated that when chromatographed, sap from *Begonia* plants treated with labeled TMTD showed areas of radioactivity at the \( R_t \) values of the fungitoxic compounds detected by Dekhuijzen (1961a,b). Kaslander et al. (1961) reported that following treatment with NaDDC, three fungitoxic compounds having the same \( R_t \) values as those present in treated cucumber seedlings (Kaslander et al., 1961) were present in the tissues of broad bean and two were present in potato tuber tissue. One of the potato metabolites was identified as the \( \beta \)-glucoside and the other, which was present in small quantities, was later shown to be the alanine conjugate (Kaslander et al., 1962). This alanine derivative was shown to undergo a nonenzymic conversion to thiazolidine-2-thione-4-carboxylic acid which was not fungitoxic. Dekhuijzen (1964) has suggested that these alanine and glucoside derivatives are interconvertible within plant tissues; he also obtained evidence that in cucumber seedlings \( N, N \)-diethyl dithiocarbamate gave rise to similar derivatives. Carboxymethyl-DDC (G33) and some related dithiocarbamates were also found to be converted into DDC-alanine and DDC-glucoside following the production of dimethyl-dithiocarbamate ions by enzymic action. Van der Kerk (1961) had previously reported that G33, within broad bean tissues, undergoes demethylation followed by ring closure and the formation of \( N \)-methyl rhodanine.

Although the possibilities of inorganic fungicides becoming changed within the plant are more limited, chelation, for example, might occur. The fact that similar levels of zinc within the leaves of a number of plant species confers resistance to powdery mildew upon some but not upon others (Tomlinson and Webb, 1959, 1960), suggests that the element may be present in a different form in different species. Again, Bowen et al. (1962) have obtained evidence that a number of inorganic ions applied to tomato plants were not present within the tissues entirely as simple ions and R. G. Ross and Stewart (1962) found that after apple shoots were treated with phenyl mercuric acetate they contained mercury, only 7% of which was acetone-soluble.

The possible use of systemic chemicals for the protection of plants against fungal diseases raises the question whether the natural immunity of plants toward many fungi is dependent on the presence of protective chemicals present within their tissues. Fungicidal substances have in fact been isolated from a number of plants. Several such compounds, of very high fungicidal activity, are present in the shoot and root tissue of broad bean seedlings (Wain, 1959). These substances, which are receiving intensive study in our laboratory, may prove to
be "natural" systemic fungicides, though it must not be assumed that they will necessarily possess adequate stability to survive and move within the tissues of other plants and confer protection against diseases to which these are susceptible. Nevertheless, such investigations on naturally occurring fungicides provide a useful approach to the problems of systemic protection of plants against disease.

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14. UPTAKE AND TRANSFORMATIONS BY PLANTS


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In the earlier chapters the accent has been primarily on the effect of the fungicide on the fungus. There is, however, an increasingly important complimentary aspect of fungicide research—the control exerted on the fungicide by the environment. It is with the microbial aspects of this subject that this chapter is concerned.

Bacteria, yeasts, and fungi differ widely in their reaction to foreign chemicals and with the increasing use of synthetic organic pesticides, the need for fuller information on their breakdown by microorganisms is becoming more and more urgent. Although few microbial degradation studies have been carried out with antifungal compounds there is al-
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Ready a mass of information concerning the breakdown of herbicides, perhaps not surprising since substances like 2,4-D (2,4-dichlorophenoxyacetic acid) were among the first pesticides found to be susceptible to soil bacteria. Because some herbicides have fungicidal relatives, or indeed are fungicidal in varying degree in their own right (particularly in regard to systemic action), and since many of the principles involved in breakdown are very relevant, such compounds will be included in the following discussion.

II. BACTERIAL DEGRADATION

That bacteria exist which are capable of utilizing paraffinic and aromatic hydrocarbons as the sole source of carbon was recognized early from the work of Tausson (1928) and others. Much subsequent work has been devoted to investigations of metabolic pathways and Evans (1956), Fuhs (1961), Rogoff (1961), and Trecani (1962) have provided useful reviews.

A. Phenols

Although the outstanding qualities of phenols as bactericides and fungicides are well known, there are some bacteria which can grow vigorously in concentrations of phenols as high as 0.1%. Such an organism was a Vibrio sp., shown by Evans and Happold (1939) to be capable of utilizing phenol as the sole energy source with the formation of catechol (I) and an acid, later identified by Kilby (1951) as β-oxoadipic acid (II). The first clue to the immediate precursors of this metabolite was furnished by Hayashi and Hashimoto (1950) who isolated and purified an enzyme from Pseudomonas sp. which converted catechol (I) to cis-cis-muconic acid (III), as had been originally postulated by Tausson (1928) for benzene. The stereospecificity of the enzyme preparations from such typical soil organisms as Vibrio 0/1 and Pseudomonas fluorescens strains A3/8 and A3/9 was demonstrated by Evans and Smith (1951) who showed that only the cis-cis-muconic acid was metabolized. Evans et al. (1951) also found that the same enzyme converted γ-carboxymethyl-α,β-butenolide (IV) to β-oxoadipic acid (II), and a C2—C2 split of this acid by intact cells was demonstrated by Kilby (1951). Acetic and succinic acids, as coenzyme A adducts, can then enter the main terminal respiratory cycle of the cell. The final scheme (Evans et al., 1951) is shown below:
Evidence for the utilization of p-cresol by a strain of *Pseudomonas* sp. with the successive production of p-hydroxybenzyl alcohol, p-hydroxybenzaldehyde, and p-hydroxybenzoic acid was obtained from simultaneous adaptation experiments by Dagley and Patel (1955). p-Hydroxybenzoic acid, which had earlier been isolated from a p-cresol culture medium by B. S. W. Smith et al. (1952), is then converted to protocatechuic acid, the degradation of which to β-oxoadipic acid being perhaps less well established than the catechol pathway (Gross et al., 1956; Ribbons and Evans, 1959, 1962).

Although the effect of nitrophenols on cell respiration is well known (Simon, 1963), and 2-methyl-4,6-dinitrophenol (DNOC) has found widespread use as a herbicide, relatively little is known about their bacterial degradation. The earliest observation appears to be that of Erikson (1941) who found that certain strains of *Micromonospora chalcea* isolated from lake mud were able to utilize 2,4,6-trinitrophenol and trinitroresorcinol. The first information on decomposition products was furnished by Simpson and Evans (1953) in a brief communication. They isolated two *Pseudomonas* spp. from soil capable of decomposing o- and p-nitrophenols, respectively, and found that by adaptation criteria, the corresponding aminophenols were not steps in the metabolic pathway. The detoxication mechanism was believed to be an oxidative elimination of the nitro group with subsequent oxidation of the dihydric phenol so formed by the known metabolic pathway (Evans et al., 1951).

Simpson and Evans (1953) also isolated another bacterium from soil.
which was able to utilize 2,4-dinitrophenol with the production of 
$\mathrm{NO}_2^-$, and nitrite formation also took place on perfusion of soil with 
chloramphenicol which contains a p-nitrophenyl moiety.

Gundersen and Jensen (1956) using a soil enrichment process, ob­
tained a strain of Corynebacterium simplex which was able to use 
DNOC as the sole carbon and nitrogen source. The nitro group in the 
para-position appeared to be an essential point of attack, because al­
though p-nitrophenol, 2,4-dinitrophenol, and picric acid were similarly 
metabolized, ortho- and meta- substituted phenols such as o- and m-
nitrophenols, 2,5- and 2,6-dinitrophenols were not. 2-Butyl-4,6-dinitro­
phenol (structure of the alkyl group unspecified) and 3,5-dinitroanhy­
cyclic acid were also unattacked which suggests the operation of a steric 
factor in view of the fact that the organisms were still viable after 3 
weeks. By contrast Douros and Reid (1956) showed that strains of 
both Pseudomonas aeruginosa and Pseudomonas putida were able to 
grow readily with 2-sec-butyl-4,6-dinitrophenol as the sole carbon 
source.

B. Phenoxyalkanecarboxylic Acids

1. 2,4-Dichlorophenoxyacetic Acid (2,4-D)

The first report of the breakdown of 2,4-D in soil appears to have 
been given by Nutman et al. (1945) although the first detailed study 
of microbial breakdown was carried out by Audus (1949). He showed 
that while a Bacterium globiforme strain grown with 2,4-D as the sole 
carbon source could decompose both 2,4-D and 4-chloro-2-methyl­
phenoxyacetic acid (MCPA) but not 2,4,5-trichlorophenoxyacetic acid 
(2,4,5-T), the same organism adapted to MCPA successfully metabolized 
all three herbicides. Other workers have isolated different species of soil 
bacteria capable of utilizing 2,4-D or MCPA as sources of energy and 
explored the metabolic pathways. Evans and Smith (1954) made the 
rather tenuous suggestion that a hydroxy acid produced from 2,4-D in a 
culture of a gram-negative motile rod might be the 6-hydroxy acid, while 
Audus and Symonds (1955) working with liquid cultures of Bacterium 
globiforme claimed two unidentified metabolites. The fact that a yellow 
Corynebacterium sp. obtained from soil by Rogoff and Reid (1956) 
could oxidize 2,4-dichlorophenol in addition to 2,4-D raised the possi­
bility that this might be concerned in the metabolic pathway. How­
ever, Steenson and Walker (1957) using a 2,4-D-decomposing strain of 
Achromobacter concluded that since neither 2,4-dichloro-6-hydroxy-
phenoxycetic acid nor 2,4-dichlorophenol was oxidized, neither was a likely metabolite. Later, following manometric experiments with adapted cultures of *Flavobacterium peregrinum* and *Achromobacter* sp., some doubt arose about the validity of this conclusion, although it was recognized that there could well be metabolic differences between species. They then suggested that the initial stage of 2,4-D breakdown was oxidation to 2,4-dichlorophenol followed by chlorine-hydroxyl replacement to give 4-chlorocatechol. Because the adapted organisms could oxidize either 4-chlorocatechol or catechol the replacement stage was to some extent suspect, but the failure to oxidize 3,5-dichlorocatechol (V) provided strong support for rejection of 2,4-dichloro-6-hydroxyphenoxycetic acid as an intermediate metabolite.

Although Rogoff and Reid's (1956) detection of an almost quantitative conversion of covalent chlorine to the ionic form made ultimate ring fission certain, there was no indication of how this happened until Fernley and Evans (1959) isolated α-chloromuconic acid (VI), produced in the metabolism of 2,4-D by a soil *Pseudomonas* sp. The formation of this metabolite is particularly interesting because of the survival of the 2-chloro atom which would seem to preclude the participation of 4-chlorocatechol in any breakdown scheme. The loss of the 4-chloro atom is particularly puzzling since Evans and Moss (1957) had earlier reported the isolation of a β-chloromuconic acid (in which the original 4-chloro atom persists) from cultures of the same organism grown in the presence of 4-chlorophenoxycetic acid. The accumulation of this β-chloromuconic acid which resulted when washed cells were incubated with 4-chlorocatechol, however, seems to completely justify the latter as a key compound in the breakdown. Although the final stages of decomposition of 2,4-D have not yet been established, the ultimate products are carbon dioxide, water, and Cl⁻.

The metabolic pathway suggested by Evans and his co-workers is shown on the next page.

2. 4-Chloro-2-methylphenoxyacetic Acid (MCPA)

It seems probable that MCPA is degraded by bacteria in a similar manner to 2,4-D. Using adapted cultures of *F. peregrinum* and an *Achromobacter* sp., Steenson and Walker (1957) found evidence that 5-chloro-o-cresol (VII) was an early metabolite in the breakdown of this herbicide. Gaunt and Evans (1961) obtained a gram-negative soil bacterium which was capable of utilizing MCPA up to a concentration of 0.1% as sole carbon source when grown aerobically in a mineral salts
medium. After the isolation of 5-chloro-o-tolub (VII), α-methyl-α-carboxymethylene-δ-butenolide (VIII) and oxalic acid—all presumed to be intermediates—the pathway outlined below was suggested:

\[
\begin{align*}
\text{OCH}_3\text{CO}_2\text{H} & \quad \text{Cl} \\
\text{OCH}_3\text{CO}_2\text{H} & \quad \text{Cl} \\
\text{OCH}_3\text{CO}_2\text{H} & \quad \text{Cl} \\
\end{align*}
\]

It is interesting that in this metabolic sequence nuclear hydroxylation before demethylation is postulated though no evidence of identification has yet been published for 4-chloro-6-hydroxy-2-methylphenoxycetic acid. Loss of the 4-chloro atom, again apparent later in the sequence, is probably connected with lactone formation.
5. **MICROBIOLOGICAL DETOXICATION**

### S. w-Phenoxyalkane Carboxylic Acids

The breakdown of long-chain homologs of 2,4-D and MCPA by microorganisms, as well as by plants, is now well established. Microbiological attack on the side chain of these compounds takes place in two ways. Thus classic β-oxidation has been demonstrated by Webley et al. (1957) in the degradation of γ-(4-chlorophenoxy)-n-butyric acid by *Nocardia opaca*, β-hydroxyγ-(4-chlorophenoxy)-n-butyric acid being isolated; with γ-(2,4-dichlorophenoxy) and γ-(4-chloro-2-methylphenoxy)-n-butyric acids a slow conversion to the corresponding β-hydroxy acids has also been shown to take place. More recently Taylor and Wain (1962) demonstrated chromatographically β-oxidation of the side chain of the α-phenoxyalkanecarboxylic acid series C₆H₄·O' (CH')ₙ·COOH by *Nocardia coeliaca*, *Pseudomonas* sp., and *Micrococcus* sp. In metabolism experiments using members of altogether six homologous series of chloro-substituted α-phenoxyalkanecarboxylic acids, Taylor and Wain also found evidence for hindrance of β-oxidation by certain types of ring substitution. This phenomenon has also been mentioned by Burger et al. (1962) who isolated a *Flavobacterium* sp. from soil which actively metabolized only those phenoxybutyric acids having no chlorine substituent in the meta-position in the aromatic nucleus. All 3,4-dichloro- and 2,4,5-trichlorophenoxyalkylicarboxylic acids exhibited prolonged persistence in soil, presumably because of this steric factor. Interference with β-oxidation by meta-substituents is a new development and contrasts with previous findings in which ortho-substitution appeared to be the critical factor (Luckwill and Woodcock, 1955; Taylor and Wain, 1962; Fawcett et al., 1959).

An even more interesting breakdown pathway has just been reported by MacRae et al. (1963). A *Flavobacterium* sp. isolated from soil and grown in media containing γ-(2,4-dichlorophenoxy)butyric acid (IX) was shown to metabolize 2,4-dichlorophenol and 4-chlorocatechol without a preliminary induction period. The initial oxidation of the acid was rapid but the rate declined to a level equivalent to that observed for the oxidation of butyric and crotonic acids. More significant still, however, was the paper chromatographic demonstration of ¹⁴C-labeled butyric and crotonic acids in the liquids from the incubation of washed-cell suspensions of *Flavobacterium* sp. with carboxy-¹⁴C-labeled γ-(2,4-dichlorophenoxy)butyric acid as sole carbon source. In addition, the presence of 2,4-dichlorophenol and 4-chlorocatechol was established using paper and gas chromatographic techniques and by a comparison of ultraviolet absorption spectra. The most obvious explanation is
shown in the scheme below, although participation of the β-hydroxybutyric acid (X) and the corresponding crotonic acid (XI) is by inference only. A later paper (MacRae and Alexander, 1963) extended the work when it was shown that the initial step in the degradation of ω-(2,4-dichlorophenoxy)alkylocarboxylic acids by the Flavobacterium sp. also involves cleavage of the ether linkage. The first member of the series—2,4-D—proved exceptional, however, and so the possibility that the first stage in the bacterial degradation of 2,4-D and MCPA may involve a demethylation (and not oxidation as has hitherto been tacitly assumed) is not supported.

The existence of metabolic breakdown involving microbial demethylation is perhaps not surprising, but the problem now posed is why such a pathway fails in the case of the 3,4-dichloro- and 2,4,5-trichlorophenoxybutyric acids, already cited for their stability. The almost complete lack of evidence for this pathway operating in the fungal metabolism of aryloxyalkylocarboxylic acids is also puzzling, although traces of phenol have been reported from phenoxyacetox acid metabolized by A. niger (Bocks et al., 1964) and β-naphtol among the metabolic
products from \( \omega-(2\text{-}naphthoxy)-n\text{-}alkylcarboxylic \) acids using the same fungus (Byrde et al., 1956; Byrde and Woodcock, 1958).

C. Degradation of Antifungal Compounds in Soil

In recent years, fungicides applied as soil treatments have been used with success for the control of soil-borne diseases such as root rot and damping-off. As has already been noted, however, there is little information on the microbiological breakdown of soil fungicides and fumigants, but numerous references to variations in persistence make it obvious that detoxication, albeit often slow, does take place under optimum conditions.

1. Fungicides

In an investigation of the persistence of sodium pentachlorophenate in soil Loustalot and Ferrer (1950) found that toxicity had almost completely disappeared at all levels of application after 1 month in a saturated soil, whereas there was no appreciable inactivation after 2 months in an air-dry soil. An environmental study on thiram by Richardson (1954) is very similar. He found that whereas this fungicide persisted in sandy soil for over 2 months, it was absent from compost soil within 1 week from the time of application. The fact that it was selective in its action against fungi, \textit{Penicillium} and \textit{Trichoderma} spp. being resistant for example, also tends to support the premise of microbiological degradation.

Organomercurial fungicides investigated by Spanis and Munneke (1962) and by Spanis et al. (1962) provide an interesting study in specificity—both biological and structural. Thus, whereas \textit{Bacillus} sp. present in soil were found to inactivate successive additions of various concentrations of cyano(methylmercuri)guanidine at accelerated rates, results which were duplicated in broth culture, methylmercurihydroxide and 2-chloro-4-hydroxymercuriphenol at comparable levels completely inhibited bacterial growth. A mutant of this same bacteria, although tolerant of cyano(methylmercuri)guanidine would not inactivate it. It is interesting too that several \textit{Penicillium} and \textit{Aspergillus} spp., conditioned to withstand normally lethal doses of the fungicide, were able to inactivate 2-chloro-4-hydroxymercuriphenol but had no effect on the cyano compound, although a few \textit{Penicillium} spp. could tolerate low concentrations in the soil.

The breakdown of the fungicides captan, dichlone, 1-fluoro-2,4-dinitrobenzene and 2,4-dichloro-6-\textit{o}-chloroanilino)-s-triazine in a silt
loam soil provides another interesting study in relative stabilities (Burchfield, 1959). It seems certain that this represents true differential microbial degradation for although breakdown was much slower in air-dried soil, stability could not be correlated with hydrolysis rates in aqueous buffer. Doubts would have been removed had control experiments in sterile soil been carried out, or alternatively had soil isolates been made and detoxication studies carried out in vitro.

Among other fungicidal compounds rapidly detoxified when added to soil, allyl alcohol appears to be an attractive nutrient for many bacteria, in particular *P. fluorescens*, *P. putida*, and *Nocardia corallina*. Several soil fungi were also able to use allyl alcohol for growth, notably several strains of *Trichoderma viride* (Jensen, 1959).

### 2. Fumigants

Since many soil fumigants such as carbon disulfide, chloropicrin, and methyl bromide have a partial sterilizing effect on the soil, the resulting reduction in numbers of microorganisms, followed by a slow recovery of resistant species of fungi and bacteria, makes a rather complicated picture. Although very little work has been carried out on the microbial utilization of soil fumigants, their gradual disappearance and the corresponding gradual release of anions such as $\text{SO}_4^{2-}$ (from CS$_2$), $\text{Cl}^-$ (from D-D mixture) and $\text{Br}^-$ (from ethylene dibromide) provide strong circumstantial evidence for microbial decomposition. Nevertheless, although Hanson and Nex (1953) found a very slow decomposition of ethylene dibromide in stored soil, some 91% being destroyed in 172 days, neither they nor Wade (1954) were able to demonstrate microbial degradation conclusively.

There is no evidence either that the release of methyl isothiocyanate from the soil fungicide Vapam (sodium N-methylthiocarbamate) is in any way microbial, soil particles merely serving to increase aeration and so facilitate oxidative decomposition (Hughes, 1960; Turner and Corden, 1963). Methyl isothiocyanate formation from soils treated with Mylone (3,5-dimethyl-1,3,5,2H-tetrahydrothiadiazine-2-thione) was also thought to be probably independent of soil microorganisms (Munnea and Martin, 1964).

### 3. Antibiotics

Since the advent of this class of antimicrobial agent many workers have sought the cause of their inactivation in soil. Thus the inactivation of actinomycin added to soil was attributed by Waksman and Woodruff (1940) not only to adsorption on soil particles but also to
It is clear that the chemical nature of the molecule is an important factor and Siminoff and Gottlieb (1951) felt that a basic antibiotic like streptomycin was effectively inactivated by colloidal soil complexes whereas a neutral compound like chloromycin, although less active in sterile soil than in broth, was rapidly degraded in a nonsterile soil by microorganisms (Gottlieb and Siminoff, 1952). The breakdown of chloramphenicol (chloromycetin) when introduced into actively growing broth cultures, notably *Proteus vulgaris* and *Bacillus subtilis* had earlier been conclusively demonstrated by G. N. Smith et al. (1949) who had isolated and characterized the hydrolysis products—p-nitrophenylserinol and dichloroacetic acid.

Other antibiotics investigated include actidione and clavacin, which are also obviously degraded in a nonsterile soil (Gottlieb et al., 1952), although the former did lose some activity, presumably by physical processes, in a sterile soil. Four others—subtilin, circulin, viomycin, and neomycin—did not inhibit the growth of *B. subtilis* in previously sterilized soil (Martin and Gottlieb, 1955), a situation which thus does not preclude detoxication. Katz and Pienta (1957) found a direct relationship between the size of the inoculum and the extent of decomposition of actinomycin by an *Achromobacter* sp. and also carried out preliminary investigations into the nature of the products. Rapid degradation of the antifungal antibiotic griseofulvin (XII: \( R = Cl \); \( R' = OCH_3 \)) in soil was reported by Wright and Grove (1957). They presented strong evidence that a *Pseudomonas* sp. was responsible, and the same organism was also shown to metabolize two derivatives—dechlorogriseloolvin (XII: \( R = H \); \( R' = OCH_3 \)) and the related amine (XII: \( R = Cl \); \( R' = NH_2 \)). No metabolites containing an intact aromatic ring could be detected spectrophotometrically and the amount of Cl− detected suggested complete breakdown of the antibiotic.

More recently the degradation of various tetracyclines by an ascomycete *Xylaria digitata* has been reported (Meyers and Smith, 1962), disappearance of characteristic fluorescence and bioactivity due to mechanical adsorption being definitely excluded. The case of mitomycin is
probably unique. Produced by the actinomycete Streptomyces caespitosus it is destroyed by the mycelium as soon as relatively anaerobic conditions are reached (Gourevitch et al., 1961).

III. FUNGAL DEGRADATIONS

While bacterial attack on aromatic substances is widespread and usually sufficiently vigorous to cause complete breakdown to carbon dioxide and water, fungi are sometimes much less versatile. Hydroxylation is a well-defined primary feature of fungal attack which in the case of the aryloxyalkanecarboxylic acids goes no further; with other classes of compound, it is merely a prelude to ring fission. Fungal enzymes also bring about some curious manipulations, often involving replacement or shift of atoms or other substituent groups.

A. Aromatic Acids

One of the earliest of such transformations is the production of homogentisic acid (XIII) from tyrosine (XIV) used as the sole nitrogen source (Utkin, 1950). This side-chain shift performed by \textit{A. niger}, \textit{Penicillium notatum}, or \textit{Penicillium chrysogenum} was in complete agreement with the known production of homogentisic acid by the oxidation of tyrosine in \textit{vivo} under the influence of rat-liver tissue, in which a side-chain migration had earlier been proved by Schepartz and Gurin (1949). Homogentisic acid has also been shown to be produced from tyrosine by \textit{Vibrio 0/1} strain (Jones et al., 1952) and a study of the adaptive patterns of this organism by Dugley et al. (1953) indicated that the pathway lay through p-hydroxyphenylpyruvic and p-hydroxyphenyl-acetic acids. It is also the major product apart from oxalic acid identified in the metabolism of phenylacetic acid (XV) by \textit{A. niger}, investigated by Kluyver and van Zijp (1951) using a replacement culture technique.

Interesting variations of the breakdown pathway have been reported by Hockenhull et al. (1952) who demonstrated that \textit{P. chrysogenum} partially degraded the phenylacetic acid side chain to benzaldehyde, and by Isono (1953) who showed that a similar strain produced \textit{o}-hydroxyphenylacetic acid.

This dihydroxylation involved in homogentisic acid formation provides an interesting contrast to the metabolism of aryloxyacetic acids by the same fungus where only monohydroxylation has been observed (see later).
Dihydroxylation has also been observed by Evans (1955) who detected 4,5-dihydroxyphthalic acid (XVI) chromatographically prior to its decarboxylation to protocatechuic acid (XVII) in the oxidative metabolism of phthalic acid by a soil *Pseudomonas* sp.

The utilization of p-hydroxybenzaldehyde, ferulic acid, syringaldehyde, and vanillin as sole sources of carbon by some 61 fungi was studied by Henderson and Farmer (1955). The majority of these organisms brought about ultimate ring fission to some extent, certain *Alternaria* sp. and *Aspergillus* sp. among others bringing about complete disappearance of the benzene ring during the course of the experiment. p-Hydroxybenzaldehyde was most readily attacked, oxidation to p-hydroxybenzoic acid being followed by hydroxylation to protocatechuic acid (Henderson, 1957) before further breakdown. As might be expected, where vanillin was decomposed, ferulic acid was generally metabolized, while syringaldehyde with no free ortho-positions for hydroxylation, was least readily degraded via syringic acid. Several fungi behaved differently when grown on syringaldehyde producing unidentified phenolic metabolites.

Demethylation as a prelude to hydroxylation is often a major feature of the metabolism of various aromatic methoxy compounds by soil fungi. Thus Henderson (1957) found that *Haplographium*, *Hormodendrum*, and *Penicillium* spp. all decompose monomethoxybenzoic acids by way of an initial demethylation, the sequence for the breakdown of anisic acid (XVIII) concluding with hydroxylation to protocatechuic acid prior to complete ring fission. In addition, the *Penicillium* sp. can also affect a carboxyl-hydroxyl replacement forming p-methoxyphenol (XIX). Selective demethylation of veratric acid (XX) has also been
observed with certain Hormodendrum and Penicillium spp. forming vanillic acid (XXI).

B. Phenols and Quinones

2,4-Dinitrophenol (DNP) used extensively in fungicidal preparations for timber preservation has recently been found to undergo detoxification by Fusarium oxysporum (Madhosingh, 1961), which was found to be much more tolerant of DNP than other wood-decaying fungi such as Coprinus micaceus. DNP was reduced to the less toxic 2-amino-4-nitro- and 4-amino-2-nitrophenols when added to the liquid basal medium, and a third metabolite remained unidentified. Further metabolism of the amines by F. oxysporum was indicated, and there was some evidence for the presence of a reversible oxidation-reduction system for aromatic nitro compounds. This microbial reduction of a nitro group contrasts sharply with the elimination of NO₂ as nitrite by Pseudomonas sp. (Simpson and Evans, 1953) and by C. simplex (Gundersen and Jensen, 1956) mentioned earlier. At the other extreme, there is no mention of attack on the nitro groups of 1-fluoro-2,4-dinitrobenzene and pentachloronitrobenzene by F. oxysporum f. cubense in the work of Torgeson (1963), although this may be a reflection of the poor availability of these rather insoluble compounds.

The fungitoxicity of pentachlorophenol is also well known, being used extensively as a wood and textile preservative, although its fungicidal value in crop protection has been limited by its phytotoxicity. Recently Lyr (1963) has reported on certain wood-rotting basidiomycetes such as Trametes versicolor, which by secreting laccase into the cul-
tured medium are able to bring about detoxication of pentachlorophenol. Although no evidence of metabolites was offered, changes in UV spectra, together with the liberation of chlorine and the appearance of colored products all point to the inactivation of phenolic hydroxyl groups. In studies with various chlorinated phenols increased chlorination of the nucleus afforded increased stability to oxidation and enzymic detoxication by laccase and tyrosinase.

The phenolic glucoside phloridzin (XXII) as sole carbon source failed to sustain the growth of the fruit pathogen Venturia inequalis. In the presence of 4% glucose, however, breakdown took place with the formation of the aglycone, phloretin (XXIII), phloretic acid (XXIV), phloroglucinol (XXV), p-hydroxybenzoic acid, and glucose (Holowczak et al., 1962). Phloretin (XXIII) was also too fungitoxic to support growth even in the presence of glucose.

Metabolism of phenols and quinones would be expected by a fungus which normally produces melanin pigments and the results of Rich and Horsfall (1954) are hardly surprising. Forty-two phenols and quinones were tested as fungitoxicants against spores of Stemphylium.
sarcinaeforme and Moniliella fructicola, and mycelial extracts of the two fungi were examined for their ability to oxidize and polymerize the compounds. All but two of the compounds giving color reactions with the mycelial extracts of S. sarcinaeforme (containing a laccase-type polyphenol oxidase) were nontoxic to spores of the fungus and almost all of the compounds which were not visibly changed were toxic, many being shown to be inhibitors of the polyphenol oxidase from the same fungus. The correlation between color formation with the fungal extract and nontoxicity was poorer with M. fructicola, and it was suggested that while this might be due to permeation differences, a more limited substrate range of the polyphenol oxidases is a more likely explanation. Nevertheless it would appear that a reasonable case has been made out for the detoxication of phenols and quinones by these fungi.

C. Aryloxyalkanecarboxylic Acids

Metabolism of these acids by A. niger and other fungi using a replacement culture technique, makes an interesting comparison with the bacterial studies already described. In general, nuclear hydroxylation without subsequent ring fission takes place, although often with surprising results. B-Oxidation, well known under the influence of plant enzymes, appears to be considerably influenced by the nature of the fungal species and very dependent on structural factors which seem to act stereochemically.

1. Phenoxy Acids

a. Phenoxyacetic acid has been found to undergo relatively non-specific hydroxylation under the influence of A. niger (Mulder strain), though the ratio of isomers appears to be somewhat variable. Early work by Byrne and Woodcock (1957) reported that hydroxylation was predominantly para and resulted in a reduction in fungitoxicity of the original acid, while Bocks et al. (1964), using a wild strain of A. niger, found that o-hydroxyphenoxyacetic acid was the main acidic product and claimed that an o-hydroxylase was operative. The demonstration of some dealkylation leading to phenol production by these latter workers is interesting in the light of the results obtained by MacRae and Alexander (1963) with Flavobacterium sp. described earlier. A reinvestigation of the problem with both strains of A. niger, using paper and thin-layer chromatographic techniques, has shown that all three hydroxyphenoxyacetic acids are in fact produced, though the
proportions of m- and p-isomers present are lower with the wild strain than with the Mulder strain (Clifford and Woodcock, 1964). The difference between these findings and the earlier report probably reflects an improved separation technique for hydroxy metabolites, but irregularities due to strain differences should not be excluded.

In contrast to the complete degradation of the side chain which is known to occur in plants, \( \beta \)-phenoxypropionic acid did not undergo \( \beta \)-oxidation under the influence of fungal enzymes, although nuclear hydroxylation produced 2- and 4-hydroxyphenoxypropionic acids. These acids were also the end products from the metabolism of 2-phenoxy-\( \beta \)-valeric acid, final side-chain oxidation again being blocked.

b. \( \beta \)-Chlorophenoxyacetic acid is similarly converted to a mixture of 4-chloro-2- and 4-chloro-3-hydroxyphenoxyacetic acids on incubation with an \textit{A. niger} mycelium (Faulkner and Woodcock, 1961), a striking contrast to the complete degradation of this acid by \textit{Pseudomonas}-type organisms already mentioned (Evans and Moss, 1957).

c. \( \beta \)-Chlorophenoxyacetic acid offers a much more interesting hydroxylation pattern, all possible hydroxy derivatives being formed together with a fifth product which was identified as 2-hydroxyphenoxyacetic acid (Faulkner and Woodcock, 1961). This replacement of chlorine by hydroxyl appears to be the first by fungal species, although this exchange has probably been made by \textit{F. peregrinum} and \textit{Achromobacter} sp. in their metabolism of 2,4-D (Steenson and Walker, 1957).

d. 2,4-Dichlorophenoxyacetic acid metabolism by \textit{A. niger} has also proved most unusual (Faulkner and Woodcock, 1964, 1965). Three phenolic metabolites are produced, of which the major one has been identified as 2,4-dichloro-5-hydroxyphenoxyacetic acid (XXVI) and not the 6-hydroxy isomer often hitherto suggested as a likely 2,4-D metabolite by other workers. Two minor metabolites, separated from the principal metabolite only with difficulty by partition chromatography, are not the 6- and 3-hydroxy-2,4-dichlorophenoxyacetic acids which might have been expected on the basis of the 2-chlorophenoxyacetic acid metabolism results. One has been identified as 2,5-dichloro-4-
hydroxyphenoxyacetic acid (XXVII), the formation of which involves a chlorine shift, but no analogous compound appears to be formed in the metabolism of 4-chloro-2-methylphenoxyacetic acid (MCPA) where the major product is 4-chloro-5-hydroxy-2-methylphenoxyacetic acid; the other is 2,3-dichloro-4-hydroxyphenoxyacetic acid.

e. γ-Aryloxy-n-butyric acids metabolized by A. niger also sometimes furnish unusual results. Thus, although β-hydroxy-γ-(2,4-dichlorophenoxy)-n-butyric acid and 2,4-D have been identified as metabolites from γ-(2,4-dichlorophenoxy)-n-butyric acid, no 5-hydroxy-2,4-D has been found. γ-4-Chlorophenoxybutyric acid, on the other hand, not only gives the corresponding β-hydroxy acid, but also 4-chloro-2-hydroxy and 4-chloro-3-hydroxyphenoxyacetic acids (Faulkner and Woodcock, 1966).

2. Naphthyloxy Acids

In the metabolism of ω-(2-naphthyloxy)-n-alkylcarboxylic acids \( \text{C}_n\text{H}_2\text{O(CH}_2\text{)}_n\text{OOH} \) by A. niger, a highly selective nuclear hydroxylation system was shown to operate (Byrde et al., 1956). Leading exclusively to 6-hydroxy products it takes precedence over β-oxidation, so that the metabolic pathway for γ-(2-naphthyloxy)-n-butyric acid (XXVIII) is as shown below:

![Diagram](image)

Although all the acids in the series having an even number of carbon atoms in the side chain are successively β-oxidized to 6-hydroxy-2-naphthyloxyacetic acid (XXIX) in the presence of A. niger mycelia, the corresponding odd-numbered acids provide another example of β-oxidation blockage, the ultimate product being β-(6-hydroxy-2-naphthyloxy)propionic acid.

Sclerotinia larsna provided a complete contrast to A. niger because nuclear hydroxylation was virtually absent (Byrde and Woodcock,
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1958). β-Oxidation of γ-(2-naphthoxy)-n-butyric and δ-(2-naphthoxy)-n-valeric acids to the corresponding acetic and propionic acids was demonstrated, but the formation of traces of β-naphthol from β-(2-naphthoxy)propionic acid, originally thought to be due to β-oxidation, may well be due to fungal demethylation (c.f., MacRae et al., 1963).

D. ANTIBIOTICS

Cycloheximide (XXX) is a highly potent antifungal antibiotic, the mode of action of which is still being investigated by numerous workers. Its effects on respiration and protein synthesis have been studied in several organisms and some interesting observations were made by Walker and Smith (1952) on its effect on the spore and mycelial respiration rates of Myrothecium verrucaria. They found that inhibition of these rates decreased with time, but whether this was due to a loss in sensitivity or to some detoxication process occurring (indeed whether or not these two are synonymous) still makes interesting speculation only because no further work appears to have been done.

Griseofulvin is active against many important fungal pathogens of plants, and applied through the root system it will protect foliage from infection, even though this protection is somewhat transient because of slow degradation of the compound in plant tissue (Crowdy et al., 1955, 1956). In a study of its uptake and translocation by fungi, Abbot and Grove (1959) used a microspectrophotometric method and bioassay to follow the disappearance of griseofulvin from media in which Botrytis allii and Mucor ramannianus were growing. There was no uptake of the antibiotic by the mycelium of M. ramannianus and no metabolites could be detected, although an unidentified phenolic metabolite was shown to be present in the medium.

An interesting reductive detoxication of an antibiotic by a fungus has recently been reported by Oku and Nakanishi (1964) who found that ascoclitin is converted to the less fungitoxic dihydro derivative
when shaken with microconidia of *Fusarium lycopersici* at pH 7 and 27°C in phosphate buffer.

**IV. TOXICANT INACTIVATION BY METABOLITES**

Following a survey of the literature on the effects of a wide variety of toxicants on various bacteria and fungi, Gottlieb (1957) was able to list some six modes of detoxication. Those involving reaction or complex formation between the toxicant and a normal metabolite are of particular interest in the present context. Thus those fungicides that normally react with —SH groups in the enzymic protein of microbial cells, can also obviously react with amino acids which contain —SH groups. Of such acids cysteine is frequently recorded as reversing the activity of such diverse agents as silver and streptomycin, while the tripeptide glutathione containing cysteine also acts similarly. Excess production of such an amino acid over the normal metabolic requirements would therefore be expected to lead to resistance, and this might well explain some of the sensitivity differences which present some of the most enigmatic aspects of plant disease control.

**A. Sodium Dimethyldithiocarbamate**

Conversely, toxicants containing sulfhydryl groups should be able to react with natural metabolites such as amino acids, and the recent work of Kaars Sijpesteijn et al. (1962) provides an excellent example in sodium dimethyldithiocarbamate (Na DDC). Conversion of this fungicide to γ-(dimethylthiocarbamoylthio)-γ-aminobutyric acid not only by washed cell suspensions of *Saccharomyces cerevisiae*, *Hansenula anomala*, and *Bacterium coli*, but also by mycelial pellets of *Glomerella cingulata*, *A. niger*, and *Cladosporium cucumerinum* forms an interesting contrast to its conjugation with alanine and β-glucose in cucumber seedlings discovered by Dekhuisen (1961) and fully reported by Kaslander et al. (1961, 1962). This appears to be the first recorded case of the transformation of a chemical to an α-aminobutyric acid derivative and although the conjugate is less toxic than the original fungicide, detoxication may only be incidental since it has been shown that liberation of the fungitoxic dithiocarbamate ion is possible in certain circumstances, e.g., incubation with *G. cingulata* pellets.

The formation of aminobutyric acid derivatives was also observed when yeast suspensions were incubated with diethyl-, dipropyl- and dibutyl-dithiocarbamates and with pyridine 2-thiol-N-oxide. New fungi-
toxic transformation products could not be detected, however, in similar experiments with the related —SH compounds—sodium methyldithiocarbamate and disodium ethylenebisdithiocarbamate—nor with the fungicides oxine, captan, dichlone, actidione, and triphenyltin acetate.

An attractive hypothesis for the biosynthesis of these amino acid derivatives, which involves the condensation of Na DDC with serine or homoserine, has been advanced (Ksars Sijpesteijn et al., 1963) and seems very logical.

B. Dichlone

There are several cases of fungicide-metabolite interaction in which, although no positive identification of adducts has been reported, there is good circumstantial evidence for loss of identity of the toxicant. When such interactions interfere with processes essential for living cells, loss of viability may be a natural consequence although this is perhaps of less importance to the present discussion. Such active compounds as chlorinated quinones are thought to act either by such reactions with cell —SH or —NH₂ groups, or by disturbance of electron transport systems. In the light of the former modus operandi, the findings of Owens and Miller (1957) are surely significant. Conidia of Neurospora sitophila were disintegrated by sonic treatment after exposure to dichlone-¹⁴C and the homogenate partitioned by centrifuging. About 75% of the fungicide was found in the supernatant solution, much of it being associated with soluble proteins or other soluble components. These results agreed with other studies by the same workers in which a large proportion of dichlone incubated aerobically with spores of N. sitophila reacted rapidly with substances from the spores to form at least five water-soluble products which were not identified. Since this reaction takes place at concentrations of dichlone well below those required to inhibit spore germination, the temptation to regard this as true detoxication seems justifiable.

In another paper Owens and Novotny (1958) working with intact conidia of N. sitophila found strong evidence that reaction of dichlone (XXXI) with coenzyme A to form compounds such as (XXXII) constituted one important fungitoxic process. This was consistent with previous work in which dichlone was found to inhibit certain enzymes with essential free —SH groups (Owens, 1953) and with known reactions of compounds containing active halogen. Nothing appears to be known about the fate of the modified dichlone molecule (XXXII) although later work confirmed the view that dichlone inactivates ene-
zyme A irreversibly within the range of toxic doses (Owens and Blaak, 1960a).

C. s-Triazines

The fungitoxicity of an s-triazine such as 2,4-dichloro-6-(a-chloroanilino)-s-triazine (dyrene) is also dependent on the presence of active halogen atoms in the molecule. Burchfield and Storrs (1956) showed that this fungicide will react with metabolic intermediates containing amino and sulfhydryl groups, nucleophilic substitution of the triazine taking place. Much of the fungicide which is very rapidly taken up by N. sitophila spores evidently combines irreversibly with protoplasmic constituents because only amounts up to 20 to 25% of it could be recovered on immediate extraction and analysis (Burchfield and Storrs, 1957).

D. Captan

This fungicide also reacts primarily with thiols but the general picture is much more complex than with dichlone. Although the reactions in which —SH groups are oxidized by captan may not be directly concerned with toxicity, it seems likely that they provide a mechanism by which the toxic action is triggered off. Indeed, Richmond and Somers (1962) have queried the importance of the reaction between captan and thiols as a toxicity mechanism and regard it rather as a detoxication process, in which case it falls more within the purview of the present chapter than elsewhere. Sisler (1963), however, claims more than a simple detoxication role for captan “although considerable detoxification probably occurs.” Certainly the transient toxicity of low doses of captan could be so explained, such cases as the slow recovery of metabolism and spore viability of N. sitophila (Owens and Novotny, 1959) and the survival of cells of Saccharomyces pastorianus (Montie and Sisler, 1962) being typical of inductive detoxication.

Lukens and Sisler (1958) first investigated the fungitoxicity of cap-
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...and noted that cysteine, hemocysteine, glutathione, thioglycerol, and coenzyme A all showed appreciable antagonistic action if added before or simultaneously with the fungicide, but failed to reverse toxic effects in cells previously treated. They also reported the significant formation of CS₂ and thiophosgene when captan was added to cultures of yeast cells. In an attempt to elucidate the mode of action, these workers conducted studies with captan and cysteine in vitro. The products of the reaction were found to be cysteine, tetrahydrophthalimide, H₂S, CS₂, and HCl, together with a certain amount of 2-thiathiolinedithione-4-carboxylic acid formed incidentally from cysteine and a thioether fragment. This reaction does not appear to be general, however, since a compound of this type was not produced when captan reacted with glutathione (Owens and Blaak, 1960b).

Two schemes for the breakdown of captan (XXXIII) by thiols seem reasonably grounded. In the first, breakdown of the unstable intermediate (XXXIV) liberates thiophosgene together with a thiol free radical which is the precursor of the disulfide. Interaction of thiophosgene with further thiols forms the trithiocarbonate (XXXV), which may be stable and isolable as that from 4-nitrothiophenol (Owens and Blaak, 1960b), or which may decompose to yield CS₂ and the corresponding monosulfide (Lukens, 1959). The weakness of this mechanism would seem to be that no intermediates of type (XXXIV) have so far been isolated and there is no direct evidence for free-radical participation as yet.
An alternative exchange mechanism involving fission of the N—S bond of captan as the first stage, satisfactorily accounts for the known products of the reaction, and distinction between the two routes does not seem possible in the light of facts presently available.

\[
\text{RS-SCCl}_2 - \text{RSNH} \rightarrow \text{R-S-S-R} + \text{[RS-CCl]}
\]

The formation of thiophosgene has certainly been well established and can be satisfactorily explained, but there is considerable divergence of opinion as to the importance of its claim as a primary toxicant. It may be that its function in trithiocarbonate formation is critical, however, for while the significance of the recent disclosure by Lukens (quoted by Sisler, 1963) that about 14% of the CS₂ released when cells of \textit{S. pastorianus} are treated with captan, originates from cellular sources, is not clear, the irreversibility of the process would appear absolute. It seems inconceivable that a compound of such reactivity as thiophosgene does not make a major contribution to the overall fungicidal activity.

\textit{E. Oxis}

The ability of amino acids to form complexes with heavy metal cations forms the basis of another detoxication mechanism. Thus Zentmayer and Rich (1956) found that the toxicity of copper 8-quinolinate to spores of \textit{A. niger} and to the mycelium of \textit{Botryosphaeria ribis} was reversed by the presence of casein hydrolyzate, L-histidine, or of L-cysteine, presumably by removing the metal as a new amino acid chelate. A similar sequestration of copper has been demonstrated by Byrde et al. (1961). These authors suggested that oxalic acid produced by \textit{A. niger} could remove the metal from copper 6-n-hexyl quinolinolate, thus liberating the fungistatic 6-n-hexyl quinolinol which then interfered with fungal growth. This postulate was strongly supported by the isolation and identification of the complex-breaking factor from the culture filtrate and by the fact that the addition of oxalic acid
to the medium at the time of inoculation markedly affected the growth rate. A slow recovery of growth, although it may be due to a pH effect caused by further oxalic acid production, could in fact represent metabolism of the unchelated quinolinol molecule. In view of other results obtained with \textit{A. niger} using the replacement culture technique, this would be a logical sequence, for which however there is no evidence at present.

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