Mode of Action and Selectivity of Fungicides

by HUGH D. SISLER* and NANCY N. RAGSDALE†

ABSTRACT
Various agricultural fungicides are considered with particular reference to mode of action and selectivity. First-generation fungicides are broad-spectrum surface protectants that are usually multi-site biochemical inhibitors. This group includes inorganic copper compounds, quinones, dithiocarbamates, phthalimides, chlorothalonil, and triphenyltin. Problems of fungal resistance have seldom been encountered in the use of these compounds. Second-generation fungicides, which include the benzimidazoles, carboxanilides, acylanilines, and ergosterol biosynthesis inhibitors, act as surface protectants and also penetrate plant tissue to eradicate or suppress established infections. These compounds usually act at a single or limited number of sites and, therefore, some have encountered problems with fungal resistance. Third-generation protectants are frequently not fungitoxic in vitro, but interfere specifically with fungal parasitic systems or accentuate host defense mechanisms. Unique new developments in plant protection are likely to involve chemical regulation of these parasitic or defense systems. The third-generation protectants, aluminum tris-O-ethyl phosphonate, probenazole, and the melanin biosynthesis inhibitors, have already proved to be effective in practice. Experience thus far indicates that effectiveness of individual compounds within this category may be limited to specific hosts, pathogens, or host-pathogen combinations.

INTRODUCTION
Many different types of chemicals that act by various mechanisms are used to control fungal diseases of plants. Over the past century, chemical protectants

*Department of Botany, University of Maryland, College Park, Maryland 20742; and †Cooperative State Research Service, U.S. Department of Agriculture, Washington, D.C. 20250.
have evolved from simple fungicides acting as surface protectants to systemic fungicides and finally to nonfungitoxic, systemic compounds that suppress fungal pathogenicity or accentuate natural plant resistance systems. Nevertheless, most older fungicides are still in use, having been only partially displaced by newer compounds with more sophisticated mechanisms. This essay discusses some of the more important fungicides from several categories, with particular reference to mechanism of action and selectivity. Unfortunately, space does not permit a consideration of all compounds in use or a complete literature citation for those that are discussed.

SURFACE PROTECTANT FUNGICIDES

Prior to 1965, practically all chemicals used to control fungal diseases lacked the stability or biochemical specificity necessary for selective eradication of fungal invaders within the host tissues. Disease control, therefore, was based essentially on surface protection by fungicides that penetrated poorly into plant tissue or that were readily detoxified if they did penetrate. Such surface protectants can be regarded as first-generation fungicides.

Inorganic fungicides. Historically, the first chemicals used as plant protectants were inorganic in nature. From the latter part of the 19th century until 1940, fungal diseases were controlled primarily by elemental and lime sulfur and by insoluble preparations of copper, such as Bordeaux mixture and copper oxide.

Although it was initially believed that fungitoxicity of elemental sulfur is due to the H₂S resulting from fungal action on sulfur, it was later shown that sulfur is more toxic than H₂S and apparently is the primary toxicant (Horsfall 1956).

Elemental sulfur interferes with energy production by intercepting electrons on the substrate side of cytochrome c in the mitochondrial electron transport system.
Organic fungicides. The era of organic fungicides began with the introduction of the quinone chloranil in 1940 (Horsfall 1956). Shortly thereafter the dialkyldithiocarbamates, thiram, ziram (Fig. 15.1) and ferbam, and the ethylenebisdithiocarbamates, nabam and zineb (Fig. 15.2) became available. McCallan (1967) stated “the dithiocarbamates are, without question, the most important and versatile group of organic fungicides yet discovered.” This statement probably remains valid in 1983; however, relative significance of the dithiocarbamates has declined considerably as other types of fungicides have come into use.

Other major fungicides developed before 1970 include captan, folpet (Fig. 15.3) and captafol, chlorothalonil, dichlone, and triphenyltins (Fig. 15.4), and the surface-active agents glyodin and dodine. All of the aforementioned organic fungicides are useful only as surface protectants.

A number of these fungicides are highly reactive with thiol groups of proteins and low molecular weight metabolites (Sisler and Ragsdale 1981; Kaars Sijpsteijn 1982) and, therefore, affect biochemical activity in fungal pathogens at a variety of sites. Compounds acting by this mechanism include chloranil, dichlone, nabam, maneb, zineb, captan, folpet, captafol, and chlorothalonil.

The dialkyldithiocarbamates are known to inhibit a multitude of enzymes...
178 Mode of Action and Selectivity of Fungicides

(Sisler and Ragsdale 1981); therefore, fungitoxicity probably involves concurrent inhibition of enzymes at several sites. The pyruvate dehydrogenase reaction is one of particularly high sensitivity to dialkyldithiocarbamates (Kaars Sijpesteijn 1982).

Dodine and glyodin are surface active agents used to control a limited spectrum of fungal pathogens. Dodine affects fungal membrane permeability (Brown and Sisler 1960) and probably inhibits a variety of enzymes as well (Sisler and Ragsdale 1981). Although cationic agents, such as dodine, must be regarded as relatively nonspecific inhibitors, a twofold to fourfold increase in resistance in Venturia inaequalis (Cke.) Wint. to dodine has developed in orchards where the fungicide was used continuously for ten years (Gilpatrick 1982). The fungicide is no longer of any value for controlling scab in these orchards.

The primary mechanism of toxicity of triphenyltins in fungi is interference with oxidative phosphorylation (Kaars Sijpesteijn 1982). A similarity of action to that of oligomycin is suggested by the observation that triphenyltin hydroxide (Fig. 15.4)-resistant strains of Cercospora beticola Saac. are also resistant to oligomycin (Chrysayi Tokousbalides and Ginnopolitis 1981). A greater tolerance of Saccharomyces cerevisiae Hansen to triphenyltin hydroxide when growing on a fermentable substrate (glucose) as compared with a nonfermentable substrate (glycerol) also supports the idea that oxidative phosphorylation is the primary site of action (Lancashire and Griffiths 1971). Sensitivity to triphenyltin hydroxide is only threefold to fourfold greater with a nonfermentable substrate, which indicates that it is not a highly specific toxin. Nevertheless, a mutation in a fungal pathogen leading to a small degree of resistance of oxidative phosphorylation may render the fungicide unacceptable as a protectant because of a narrow margin between fungitoxicity and phytotoxicity.

SYSTEMIC FUNGICIDES

Systemic fungicides provide surface protection and also penetrate into plant tissue to provide internal therapy. Internal therapeutants must be relatively stable within plant tissue and exhibit a high degree of selectivity for fungal cells, if phytotoxicity is to be avoided. Usually these “second-generation fungicides” are selective biochemical inhibitors that act at one or a limited number of target sites. This high degree of specificity is a predisposing factor to the development of fungal resistance, a problem which has been encountered in the use of certain systemic fungicides.

Dicarboximides and aromatic hydrocarbons. The dicarboximide fungicides vinclozolin, iprodione, and procymidone and aromatic hydrocarbons, such as chloroneb, quintozene (PCNB), and dicloran (Fig. 15.5), are remarkably similar in their action (Kaars Sijpesteijn 1982). While there may be doubt concerning the systemic activity of some members of this group, chloroneb at least is known to be systemically active (Sisler and Ragsdale 1981). All of the aforementioned compounds cause mitotic instability in Aspergillus nidulans (Eidam) Wint. (Georgopoulos, Sarris, and Ziogas 1979; Kaars Sijpesteijn 1982). Furthermore, fungal mutants resistant to one member of the group typically show resistance to other members as well (Leroux, Fritz, and Greif 1977). The mechanism of action of this group has not been satisfactorily resolved. In a recent study, Kato (1982) showed that the action of several of these compounds resembles that of cytochalasin A, which interferes with motile functions of cells, such as cytoplasmic...
streaming and flagellar movement. However, other mechanisms of toxicity have been proposed; for example, Lyr and Werner (1982) ascribe toxicity of chloroneb to damage of the inner mitochondrial membrane.

Laboratory and field strains of fungi resistant to dicarboximides are frequently reported, but field resistance has not yet proved to be a serious problem (Beever and Byrde 1982).

Carboxanilides. Carboxanilides are systemic fungicides that can penetrate into plant tissue and control fungal diseases when applied as seed, soil, or foliage treatments. Carboxin and oxycarboxin (Fig. 15.6) are derivatives active primarily in controlling Basidiomycetes. Carboxin specifically blocks the transfer of electrons from succinic dehydrogenase (SDS) to coenzyme Q in the Complex II region of the electron transport pathway (Mathre 1971; White, Thorne, and Georgopoulos 1978). Specificity of carboxin for various organisms is usually determined by the sensitivity of the SDS systems to the fungicide. For example, oxidation of succinate by isolated mitochondria of the carboxin-sensitive fungus Ustilago maydis (DC.) Cda. is far more sensitive than isolated mitochondria of tolerant pinto bean (Mathre 1971).

Benzimidazoles. The benzimidazoles (Fig. 15.7) are highly potent systemic fungicides that can permeate into higher plant tissue without producing phytotoxic effects. These fungicides interfere specifically with tubulin polymerization in fungi.

Figure 15.6. Structures of carboxanilide fungicides.
180 Mode of Action and Selectivity of Fungicides

Thiabendazole

Benomyl

MBC (Carbendazim)

Figure 15.7. Structures of benzimidazole fungicides.

As a consequence, mitosis (Davidse 1982; Hammerschlag and Sisler 1973) and various microtubular-dependent processes occurring in the cytoplasm (Howard and Aist 1977) are blocked. Polymerization of alpha and beta tubulin subunits are involved in the formation of microtubules in A. nidulans (Sheir-Ness, Lai, and Morris 1978). Biochemical-genetic evidence indicates that benzimidazoles specifically bind to β-tubulin and thus interfere with microtubule formation. A single gene mutation that alters the affinity of the tubulin binding site for benzimidazoles results in fungal resistance (Davidse 1982). Accordingly, therefore, the high toxicity of these compounds toward certain fungi and their lack of toxicity to resistant fungi, higher plants, and animals is likely to be based on differences in affinity of the tubulin in the various organisms for benzimidazoles. Difference in uptake has been suggested as an alternate tolerance mechanism (Davidse 1982).

These fungicides are not toxic to Oomycetes, which apparently contain tubulin of low affinity to benzimidazoles. Development of fungal resistance has been encountered very frequently in the use of these compounds, an expected phenomenon in view of the single site of action in fungal cells.

Acylalanines. Metalaxyl (Fig. 15.8) and furalaxyl and representative of the acylalanine class of fungicides. These compounds give excellent control of various Peronosporales (Kaars Sijpesteijn 1982) but do not control fungal pathogens in the major mycological groups, such as the Ascomycetes, Basidiomycetes, and imperfect fungi. Present evidence indicates that metalaxyl specifically inhibits RNA synthesis in Pythium splendens Braun (Kerkenaar 1981) and Phytophthora megasperma Drechs. (Davidse, Gerritsma, and Hofman 1981). Nonetheless, the specific mechanism of this inhibitor has not been resolved. Metalaxyl has encountered some serious problems with fungal resistance in a relatively short period of use, which indicates action at a single target site.

Ergosterol biosynthesis inhibitors. Beginning with the introduction of triarimol in 1970 (Brown, Hall, and Miller 1970), a variety of compounds known as ergosterol biosynthesis inhibitors have been developed for agricultural use. This group includes various pyrimidines, triazoles, and imidazoles as well as miscellaneous individual compounds in the pyridine (buthiobate) morpholine (tridemorph), and

Figure 15.8. Structure of metalaxyl.
piperazine (triforine) categories (Fig. 15.9). These compounds show varying degrees of systemic activity and control a wide range of fungal diseases including powdery mildews, rusts, and smuts on a variety of commodities (Siegel 1981). They are very effective and may prove to be the most important group of fungicides ever developed.

Triarimol was the first of this group shown to have a fungitoxic mechanism based on the inhibition of the biosynthesis of ergosterol, the major fungal sterol (Ragsdale and Sisler 1972). More recent studies of other fungicides shown in Fig. 15.9 have demonstrated a primary effect of the compounds on ergosterol biosynthesis and have characterized secondary morphological and biochemical abnormalities typically produced by ergosterol biosynthesis inhibitors (Buchanauer 1977; Ragsdale 1977; Sisler and Sisler 1975; Siegel 1981). All of these fungicides except tridemorph primarily inhibit the cytochrome P-450-dependent sterol C-14 demethylation reaction, which is normally the first conversion in the pathway from lanosterol to ergosterol. There is evidence that the insertion of the C-22(23) double bond in the sterol side chain is also inhibited; however, effects on this step are obscured by the inhibition of C-14 demethylation, which occurs earlier in the pathway (Ragsdale 1977). Tridemorph inhibits ergosterol biosynthesis and produces morphological effects in fungi similar to the sterol C-14 demethylation inhibitors, but it inhibits C-14(15) double bond reduction (Kerkenaar, Barug, and Kaars Sijpesteijn 1979) or Δ8-Δ7 double bond isomerization (Kato, Shoami, and Kawase 1980) rather than C-14 demethylation.

There is evidence that the sterol C-14 demethylation inhibitors block the cytochrome P-450 mixed function oxygenase involved in the removal of the sterol C-14 methyl group (Gadher et al. 1983; Mitropoulos et al 1976). It has been hypothesized that inhibition is based on an interaction of a heterocyclic nitrogen atom of the fungicides with the protohaem iron atom of the enzyme, resulting in an exclusion of oxygen (Gadher et al. 1983).

Specificity of sterol C-14 demethylation inhibitors among organisms is probably based most often on differences in affinity of the fungicides for cytochrome P-450 enzymes. It is possible, for example, to design structures that selectively inhibit fungal sterol C-14 demethylation in preference to the same systems in mammals (Gadher et al. 1983). Nevertheless, lack of adequate specificity is sometimes encountered. A growth retardation of the host plant sometimes occurs when sterol C-14 demethylation inhibitors are applied as fungicides. This effect is due to the blocking of cytochrome P-450 enzymes involved in gibberellin biosynthesis (Coolbaugh, Hirano, and West 1978).

Although fungal strains resistant to these compounds are easily produced in the laboratory, field resistance has, thus far, not proved to be a problem. This may be due to a lack of fitness of resistant strains for survival in the field (Siegel 1981). An interesting mechanism of resistance based on an energy-dependent efflux of the fungicides has been described by Waard and Nistelrooy (1980).

**NON FUNGITOXIC PROTECTANTS**

Third-generation type of protectants characteristically show little or no fungitoxicity in vitro but decrease infection or reduce disease severity when applied to plants. Such activity implies that these compounds are converted to fungitoxicants in the host plant or that they affect pathogenicity of the fungus or resistance of the host. A compound of this type may interfere with factors critical for pathogenesis, such as the production or activity of fungal toxins and enzymes or
Figure 15.9. Structures of ergosterol biosynthesis inhibitors.
elicitors and repressors of host resistance. On the other hand, the primary action may be on the host rather than on the pathogen. A compound might increase passive resistance of the host by affecting cell-wall composition, or it might accentuate active resistance mechanisms that are triggered by a challenge of the pathogen. Various mechanisms of plant protection by the aforementioned type of chemical are discussed by Langcake (1981). In this chapter, consideration will be limited to a few examples that have proved successful in practice or for which the mechanism of protection has been studied in some detail.

**Antipenetrant compounds.** Fungi often must penetrate cutanized epidermal walls of plant cells in order to establish infection of the underlying tissue. Penetration of the epidermis involves special enzymic activity, mechanical forces, or a combination of both. The penetration process, therefore, is a potential target for disease control by chemicals that may be nonfungitoxic in vitro.

The most successful group of nonfungitoxic protectants developed thus far are fungal melanin biosynthesis inhibitors (Fig. 15.10). These compounds specifically block the capacity of appressoria to penetrate cutanized plant epidermal walls (Inoue et al. 1982; Woloshuk and Sisler 1982). Tricyclazole, pyroquilon, chlorbenthiazole, and fthalide have all proved effective for practical control of rice blast disease caused by *Pyricularia oryzae* Cav. These melanin biosynthesis inhibitors have a peculiarly narrow disease control spectrum. Their practical use at present is limited to the control of rice blast disease. However, tricyclazole, pyroquilon, and PP-389 protect bean tissue from infection by *Colletotrichum lindemuthianum* (Saac. & Magn.) Scribner in experimental test systems (Wolkow, Sisler, and Vigil 1983). Tricyclazole acts as an antipenetrant against both *C. lindemuthianum* (Wolkow et al. 1983) and *Colletotrichum lagenarium* (Pass.) Ell. & Halst. (Kubo et al. 1982) and presumably displays some degree of antipenetrant activity against *Colletotrichum* species in general.

Tricyclazole, the first of these compounds to be recognized as a melanin biosynthesis inhibitor (Chrysasyi Tokousbalides and Sisler 1978), blocks the conversion of 1,3,8-trihydroxynaphthalene (1,3,8-THN) to vermelone and the conversion of 1,3,6,8-tetrahydroxynaphthalene (1,3,6,8-THN) to scytalone in the polyketide pathway to melanin (Chrysasyi Tokousbalides and Sisler 1979). Other compounds shown in Fig. 15.10 act in a similar or identical manner to tricyclazole (Woloshuk and Sisler 1982; Yamaguchi, Sekido, and Misato 1982). Wheeler (1982) studied the pathway from 1,3,6,8,-THN to 1,8-dihydroxynaphthalene in cell-free systems from *Verticillium dahliae* Kleb. and found that the conversion of
1,3,6,8-THN to scytalone and the conversion of 1,3,8-THN to vermelone are both tricyclazole-sensitive, NADPH-dependent reactions.

The antipenetrant action of melanin biosynthesis inhibitors is based on an interference with melanization of appressorial walls in P. oryzae (Woloshuk and Sisler 1982), C. lindenuthianum (Wolkow et al. 1983), and C. lagenarium (Kubo et al. 1982). Melanization seems to confer the wall rigidity and architecture needed to focus the turgor forces involved in the penetration process (Kubo et al. 1982; Wolkow et al. 1983; Woloshuk, Sisler, and Vigil 1983). There remains a question of whether a simple deposition of melanin between wall microfibrils or a cross-linking of wall polymers by an oxidation product (quinone) of the immediate melanin precursor is more important in contributing to wall rigidity (Wolkow et al. 1983).

Although the spectrum of activity of melanin biosynthesis inhibitors is very narrow in regard to disease control, they provide examples of nonfungitoxic compounds that have proved extraordinarily successful in practice.

There is good evidence that cutinase plays a critical role in the penetration of plant epidermal walls by some pathogens. Maiti and Kolattukudy (1979) showed that either specific cutinase antiserum or nonfungitoxic concentrations of the potent cutinase inhibitor diisopropylfluorophosphate protect pea epicotyls from infection by Fusarium solani Mart. Similar protection of papaya fruit from infection by Colletotrichum gloeosporioides Penz. is also afforded by these agents (Dickman, Patil, and Kolattukudy 1982). More recently Koller, Allan, and Kolattukudy (1982) showed that nonfungitoxic concentrations of several organic phosphorus insecticides and fungicides protect pea epicotyl segments from infection by F. solani. In view of the fact that the inhibitors do not prevent infection of wounded stem sections, and since the effectiveness of the compounds as protectants generally corresponds to their effectiveness as cutinase inhibitors, it is likely that protection is based on inhibition of cutinase.

While these studies provide evidence that some plant diseases can be controlled solely by inhibiting cutinase activity of the pathogen, the practical value of this protective mechanism remains to be proved.

Compounds affecting host-parasite interaction. The compound 2, 2-dichloro-3,3-dimethyl cyclopropane carboxylic acid (DDCC) (Fig. 15.11) is specific for control of P. oryzae on rice (Langcake and Wickins 1973). DDCC does not prevent epidermal penetration of rice plants by P. oryzae, but markedly accentuates host cellular responses once penetration has occurred (Cartwright, Langcake, and Ride 1980). Cells of rice plants treated with DDCC respond in a rapid, hypersensitive fashion to penetration by P. oryzae, which is in contrast to a delayed, mild reaction by cells of untreated plants. Rapid development of intracellular hyphae occurs in cells of the untreated plants, but hyphal development is halted soon after penetration in cells of treated plants. Suppression of hyphal development apparently results from the accumulation of the phytoalexins, momilactones A
and B, in the tissue surrounding the infection centers. The accumulation of these fungitoxic substances is more rapid and far greater in magnitude in DDCC-treated leaves than in leaves of untreated plants (Cartwright et al. 1980).

While it is evident that protection of rice plants by DDCC is based on a marked enhancement of the host resistance system, the mechanism by which this is triggered remains obscure.

Probenazole (Oryzemate), a relative of saccharin, controls rice blast disease caused by *P. oryzae* and bacterial leaf blight caused by *Xanthomonas oryzae* (Uyeda & Ishiyama) Dowson. Probenazole (Fig. 15.11) does not show appreciable toxicity to hyphal growth of *P. oryzae* on agar media or to conidial germination on glass slides. However, conidial germination on rice leaf sheaths is quite sensitive to the compound (Watanabe 1977). While this effect suggests that probenazole may act prior to penetration, other tests show that the compound suppresses lesion spread after the fungus has penetrated. Analyses of probenazole-treated plants inoculated with *P. oryzae* reveal an accumulation of α-linoleic acid and three other fungitoxic material with similar properties. These are considered to form a chemical barrier against the pathogen. Moreover, there is an augmentation of peroxidase, phenylalanine ammonia lyase, and catechol-O-methyltransferase in inoculated plants treated with probenazole. These increased enzyme activities are believed to reflect an enhanced lignoid barrier formation around the invading pathogen (Sekizawa and Mase 1981). While enhancement of the resistance reaction of the rice plant to *P. oryzae* is regarded as the major mechanism of action of probenazole (Sekizawa and Mase 1981), the specific target of this compound in the host or pathogen has not been identified.

Aluminum tris-O-ethyl phosphonate (fosetyl-Al) (Fig. 15.11) controls a variety of fungal diseases, particularly those caused by certain species of *Phytophthora*. Moreover, concentrations that control *Phytophthora* diseases show little toxicity to mycelial growth of the pathogens in vitro (Bompieri et al. 1980; Farhi, Tsao, and Menge 1981). On the other hand, fosetyl-Al at 5 μg/mL strongly inhibits sporangial formation in vitro by *Phytophthora parasitica* Dast. and *Phytophthora citrophthora* (Smith & Smith) Leonian and inhibits zoospore release in the latter species (Farhi et al. 1981).

The compound is degraded in buffer or plant tissue to phosphorous acid, a product on which protective activity is apparently based (Bompieri et al. 1980). Fosetyl-Al triggers a necrotic blocking (defense) reaction, with increased phenolic accumulation in plant tissue around infection sites, and as a consequence further advance of the pathogen into the tissue is halted. The blocking reaction is triggered in tissue of tomato, pepper, and French bean, but not in tissue of potato. Induction of the defense reactions is suppressed by phosphate ions (Bompieri et al. 1980). While substantial evidence indicates that protection by fosetyl-Al is mediated through host defense systems, the primary target that triggers induction or accentuation of these systems has not been identified.

**CONCLUSIONS**

Chemicals in current use for control of plant fungal diseases are almost exclusively fungitoxic compounds. However, nonfungitoxic chemicals that specifically interfere with fungal parasitism or accentuate host—plant defenses are now emerging. Continued advances can be expected with conventional fungitoxic protectants, but new and unusual developments are likely to involve chemicals that regulate fungal parasitic systems or host defense mechanisms. Experience
thus far indicates that the principal limitation of specific compounds in the latter category will be a narrow-disease control spectrum. This limitation and the fungal resistance problems encountered with certain systemic fungicides will necessitate the continued use of the older, less specific surface protectants.

ACKNOWLEDGMENT

Contribution No. A-3489, Publication No. 6562 of the Maryland Agricultural Experiment Station.

LITERATURE CITED


Mode of Action and Selectivity of Fungicides


