STUDIES ON POWDERY MILDEW OF APPLE IN NURSERIES

Thesis

by

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Submitted in partial fulfilment of the requirements for the degree

of

MASTER OF SCIENCE

in

MYCOLOGY AND PLANT PATHOLOGY

COLLEGE OF HORTICULTURE
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2005

ACKNOWLEDGEMENTS

With limitless modesty, I praise and thank the almighty who provided me courage and zeal to go through this project.

I take this opportunity to express my gratitudes and profound personal regards to my chairman Dr. S.K. Sharma, Sr. Scientist, Department of Mycology and Plant Pathology for his judicious guidance, prompt help and keen interest during the tenure of these investigations.

I express my heartfelt thanks to the worthy members of my advisory committee Dr. J.N. Sharma, Plant Pathologist; Dr. J.S. Chandel, Associate Professor and Dr. Rajesh Kaushal, Assistant Professor for their valuable suggestions throughout the course of investigations.

I emphatically extend my venerable thanks to Dr. T.R. Shandilya, Professor and Head, Department of Mycology and Plant Pathology for providing necessary research facilities.

I am sincerely indebted to acknowledge my senior Dr. (Mrs.) Meenu Gupta for her encouragement, cooperation and valuable suggestions.

When I see around myself speared and cheared by my jovial and loving friends Lt. Pragya, Munish Thakur, Hitendr Patiyal, Hardev Negi, Ashish Dobhal, Dinesh Negi, Ashin Chaudhary, Tara Singh, Ashutosh Bhaik, Sandeep Dharma, Arun, Pankaj and all friends. I thank Jagdish ji and Ajay Gautam ji for helping me in research work.

Every effort is motivated by an ambition and all ambition have inspirations behind. I owe this pride place to my affectionate parents for their blessings, selfless persuasion, sacrifices and reassurances, besides inspirations from my relatives. I am also grateful to my dearest sisters Pragti and Priyanka for their affection, moral support and encouragement, without whom this treatise would have remained an ambition.

Assistance received from Satyanand Stokes Library and its staff is duly acknowledged.

Finally a word of special appreciation also goes to Kanwar Jagdeep and Subhash ji (DPT Computers, Nauni) for their intense efforts and patience in typing this manuscript.

Nauni, Solan
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CERTIFICATE-I

This is to certify that the thesis entitled, **"Studies on Powdery Mildew of Apple in Nurseries"**, submitted in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE** in **MYCOLOGY AND PLANT PATHOLOGY** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.) is a record of bonafide research work carried out by **Mr. Ashwani Singh Chauhan (H-2002-25-M)** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigations have been fully acknowledged.

21/06/05

Place: Nauni, Solan
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CERTIFICATE-II

This is to certify that the thesis entitled, **"Studies on Powdery Mildew of Apple in Nurseries"**, submitted by **Mr. Ashwani Singh Chauhan (H-2002-25-M)** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), in partial fulfilment of the requirements for the award of degree of **MASTER OF SCIENCE** in **MYCOLOGY AND PLANT PATHOLOGY** has been approved by the Student's Advisory Committee after an oral examination of the same in collaboration with the external examiner.

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INTRODUCTION

Apple (*Malus domestica* Borkh.) is the most important fruit of the temperate regions of the world. In India, the major apple growing areas are Jummu & Kashmir, Himachal Pradesh and Kumaon hills of Uttar Pradesh. The efforts are also being made to extend its cultivation to north-eastern states of the country comprising Arunachal Pradesh, Assam, Manipur, Mizoram, Tripura and Sikkim. In India apple occupies an area of 2,50,000 ha with an annual production of 1.47 MT (FAO, 2004). Himachal Pradesh, "the apple state of India", has made tremendous progress in the production of high quality apple during the last few years and has become number one commercial crop in terms of quality as well as quantity. Out of 163.33 thousand ha under fruit crops, apple alone constitutes 38.46 per cent. Presently, the area under this fruit is around 84,112 ha with an annual production of about 45,900 MT (NHB, 2004)

Although area under apple cultivation is increasing every year, yet the production per unite area is decreasing despite the use of best possible plant protection measures mainly because of monoculture and cultivation at elevation providing marginal conditions for growth and development of only few cultivars.

Apple is known to suffer heavily from a variety of fungal, bacterial and viral diseases out of which fungal disease are of utmost economic importance. Amongst fungal diseases, powdery mildew caused by *Podosphaera leucotricha* is a serious disease and causes heavy losses both in orchards as well as in nurseries. The disease is prevalent in almost all the apple growing regions of the world. Symptoms of powdery mildew appear immediately after bud break and spread to young leaves and inflorescence. Affected leaves are short and narrow and are covered with whitish growth of conidia (Zherbele, 1984). In nurseries, the disease is more severe as compared to adult plants and as a result of infection, the seedlings are killed in one season.

Keeping in view the destructive nature of the pathogen and extent of losses caused due to this disease, the present investigations were undertaken with following objectives;

i) To record the prevalence and severity of powdery mildew in apple growing areas of Himachal Pradesh.

ii) To study the role of meteorological factors on the disease development

iii) To devise a suitable disease management strategy through chemical and germplasm evaluation methods.

Chapter-2

REVIEW OF LITERATURE

2.1 OCCURRENCE

Powdery mildew is one of the most important fungal disease of apple and was first noticed in 1871 on apple seedlings by Bessey in the United States. He referred the pathogen as some species of *Podosphaera,* but Ellis and Everhart (1888) named it *Sphaerotheca leucotricha.* Burrill in 1892 further changed it to *S. mali* (Dusy), but Salmon (1900) renamed it as *Podosphaera leucotricha.* This disease has been reported from almost all the apple growing areas of the world. In India, the disease was first reported from Kashmir in 1919 by Butler (Butler and Bisbey, 1960).

The economic importance of apple mildew is undoubtedly undisputed, yet no systematic survey has so far been conducted to assess the losses caused by this disease. Some reports from Europe (Nordmann, 1922; Lustner, 1923; Noack, 1928) revealed that the disease has become a serious hazard to fruit growing as the fungus attacks vegetative shoots, fruit spur, blossom and fruits from spring to autumn. In Newzealand, Cunningham (1923) described the deleterious effects of mildew infection on apple as

i) Reduction of leaf age and damage to the foliage,

ii) Distortion of fruit buds,

iii) Weakening and frequent killing of laterals and

iv) Reduction in the marketable value of the fruit by russeting, cracking and frequent deformation.

Woodward (1927) described it as a factor of great economic importance to the apple growing industry in United Kindgom. In other countries like Austria (Kock, 1927), USA (Berwith, 1936), USSR (Khonyakov *et al.*, 1977; Kalyuzhnii and Bogden, 1981) and Germany (Kolbe, 1978), the disease has been reported to be of serious nature causing huge losses. Bennet and Moore (1963) demonstrated that infection of powdery mildew resulted in small sized fruits thereby reducing the crop yield. The importance of powdery mildew under Indian conditions has also been emphasized from time-to-time by various workers (Agarwala and Thirumalachar, 1967; Bose and Sindhan, 1967; Gupta *et al.*, 1977; Putoo and Kaul, 1978; Gupta, 1981; Verma and Gupta, 1986; Sharma *et al.*, 1992 and Sandal and Surina, 1995).

2.2 MORPHOLOGICAL CHARACTERS

The conidia of *P. leucotricha* are produced in long chains and measures 22-30 x 15-20 µm (Burchill, 1978). Stevens (1954) described the perithecial stage of the pathogen. The perithecia were densely gregarious, more or less scattered and measured 78-96 µm. These have two types of appendages i.e. apical and basal. Apical appendages are 3-11 in number, widely spreading or erect, 4-7 times diameter of the perithecium with undivided apex which is blunt or rarely (once or twice) dichotomously branched or brown basaly. Basal appendages are nearly absolute or well developed, short tortuous, pale brown, simple or irregularly branched. Asci are oblong to subglobose and measure 55-70 x 44-50 µm. Spores are 22-26 x 12-14 µm. Conidia are ellipsoidal, truncate, hyaline measuring 28-30 x 12 µm under Indian conditions. Puttoo and Kaul (1978) reported that cleistothecia are globose, depressed, black, partially embedded in mycelium and measure 75 to 85 µm in diameter. These are furnished with two kinds of appendages, those which spread out from apical depressed part, are long, stiff and bristly, while the others which emerge from base, are short and tortuous.

2.3 PATHOGENICITY

The mycelium of some of the powdery mildew grow even at 5°C (Butt, 1978) but slow development at low temperatures lengthens the latent and incubation periods as in case of strawberry mildew (*Sphaerotheca macularis*). Peries (1962) obtained no sporulation after 3 weeks of mycelial growth at a temperature range of 5-13°C. According to Yarwood (1957), the germination of conidia of *Sphaerotheca pannosa* and *Podosphaera leucotricha* depended upon stimulation from the host. Infact the conidia of these two fungi are more dependent on the protective action of high humidity than any other species. These are among the shortest lived powdery mildew conidia (Longree, 1939; Stoll, 1941). Berwith (1936) reported that the infection of young apple leaves by mildew pathogen was accomplished in 45 to 48 hours at a temperature between 13 to 25°C, conidiophores being produced in 5 days. Kaspers (1967) reported that infection could occur between 4-28°C but the fructification period was lenthened at suboptimal temperature (20°C) lasting 12 days at 4°C and 4-5 days at 20°C. Soskic (1960) reported that parasite *Podosphaera leucotricha* developed best at 16°C.

2.4 EPIDEMIOLOGICAL STUDIES

There is no definite evidence available regarding the conditions which help the outbreak of apple mildew. It has been reported that the trouble in some localities is worse in hot and dry season whereas in other warm and moist summers or cool, damp sunless weather favoured the disease (Butler and Jones, 1961). Many workers have reported the occurrence and spread of powdery mildew under dry weather conditions (Nordmann, 1922; Boughey, 1949; Butikofer, 1949; Mare, 1952; Videnov, 1972).

Butikofer (1949) reported that in Switzerland, the occurrence of apple mildew was promoted by a relatively mild dry climate. Individual factors which favoured the disease were; mean April temperature exceeding 11°C, moderate atmospheric humidity (60-70%) and short period of rainfall, however, it was the combination of all these factors rather than anyone of them separately. On the other hand, Dorozliko (1974) did not detect the disease in extremely dry areas of USSR. He further stated that in dry zone, the disease appeared in spring and autumn without causing much damage. Lustner (1923) had stated that it was difficult to establish any definite correlation between meteorological conditions and incidence of mildew. The severe epidemics of the disease in 1923 in Germany was associated with abnormally cool, damp and sunless weather, where as in 1921, a similar out break coincided with an usually hot and dry period. Blumeer (1951) also observed that cool and damp spring weather was unfavourable for the spread of the disease. Videnov (1972) reported that winter periods with minimum temperature below -22°C inhibited the disease development. Zherbele and Ivanova (1984) observed that the ascospores of powdery mildew (*Podosphaera leucotricha*) are dispersed at an air temperature >5°C and RH 60-80 per cent. Grushin (1988) reported that low temperature in winter and high temperature at the end of summer of the previous year had the maximum negative effect on the development of *Podosphaera leucotricha*. Factors connected with high relative humidity in summer of the previous year favoured fungal development. The effect of weather conditions in the vegetative period of current year was insignificant. Stephan (1989) reported that in the GDR, field studies on shaded apple plants grown in pots showed the length of the incubation period of *Podosphaera leucotricha* to be determined by an effective temperature of 60°C above a daily mean temperature of 6°C. Stephans (1991) found that the primary infection on lateral buds was much lower than that of terminal buds. The first three lateral buds had a higher infection rate than subsequent one.

2.4.1 Effect of temperature

Woodward (1927) showed that on detached apple leaves, temperature between 10 and 15°C were the most favourable for the germination of conidia but on dry glass slides a temperature of 19 to 22°C under moist conditions was found to be optimum. No germination of conidia was observed at either 7°C or 28°C (Berwith, 1936). Stoll (1941) studied the germination of small conidia of *P. leucotricha* on collodion membranes, the use of which doubled the germination in comparison to dry glass slides and the optimum range was found to be 19-25°C. Yarwood *et al.* (1954) showed 10, 16 and 30°C to be minimum, optimum and maximum for conidial germination and further stated that conidia germinated poorly on glass slides. Zaracovitis (1963) found newly abstricted conidia of *Podosphaera leucotricha* to give 67$\pm$9 per cent germination on dry glass slides, incubated at 21°C in a saturated atmosphere. Coyier (1968) made a comprehensive study on the germination of conidia on detached apple leaves. At 4.3 and 10°C, there was no germination after 6 hours, which was 44 and 69 per cent respectively, after 24 hours of incubation. At 32.2°C, 10 per cent of the conidia germinated in 24 hours, but the conidia incubated at this temperature for 6 hours and transferred subsequently to 21°C germinated well. The germination of conidia incubated at 21°C was 71 per cent in 6 hours and 88 per cent in 24 hours which was maximum. The mycelial growth was also best at this temperature. Other workers have reported the poor and erratic germination of mildew fungi *in vitro* (Blumeer, 1933; 1951; Corner, 1935; Yarwood, 1951; 1957; Hirata, 1954; Koopmans, 1959). Monler (1971) studied the germination of Conidia on apple leaves, rose petal and glass slides and found minimum temperature for germination of conidia to be 4°C on apple leaves and rose petals and 10°C on glass slides, optimum 21-25°C and maximum 30°C. Suvorova (1974) reported that conidia of *Podosphaera leucotricha* to germinate between 6 to 33°C. Verma and Gupta (1988) have shown 21°C temperature to be the best for the conidial germination of *Podosphaera leucotricha.*

2.4.2 Effect of moisture

The relationship of moisture and development of disease is one of the most controversial aspect of pathogenesis in powdery mildews. Yarwood (1957) indicated favourable effect of rain, dew and sprinkler irrigation on the development of powdery mildews. On the other hand, Boughey (1949) reported that the incidence of powdery mildews decreased with the increase in rainfall. Yarwood (1936) observed that powdery mildews are normally favoured by dry weather and reasons for this observation are the mechanical damage to conidiophores by rain. Poor germination of spores in water has also been reported by Corner (1935). Different workers (Yarwood, 1936; Brodie and Neufeld, 1942; Clayton, 1942; Brodie, 1945 and Delp, 1954) showed that conidia of powdery mildew of many species could germinate even at relative humidities approaching zero (0% RH).

Most, if not all, studies on moisture relations on powdery mildews have shown that free moisture is inhibitory to powdery mildews. Not only germination is inhibited (Woodward, 1927; Yarwood, 1936; Longree, 1939; Cherewick, 1944; Brodie, 1945; Delp, 1954 and Robert, 1962) but growth of mycelium becomes abnormal (Schnathorst, 1958). Woodward (1927) reported that surplus water on the leaf surface was unfavourable for germination. He obtained successful germination of *Podosphaera leucotricha* conidia when the leaf was sprayed lightly with an automiser comparable to a light fall of dew. Once germination had taken place a vigorous growth of hyphae was induced followed by a copious development of conidiophores. On the other hand, production of mycelium and conidia did not occur on moist portions of the leaf and germination was impeded by a wet leaf surface. Moreover, a dry leaf surface favoured the rapid extension of hyphae and encouraged the production of conidial chains.

Corner (1935) observed that dipping of conidia of *Erysiphe graminis, Podosphaera leucotricha, S. pannosa* and *Oidium* spp. for 1 to 3 hours in water caused their death but floating conidia germinated readily after 24 hours and produced upright germ tubes. Berwith (1936) observed that conidia of *P. leucotricha* planted under dry conditions on glass slides at temperature 1 to 34°C failed to germinate at humidities at or below 90 per cent, but germinated only at 100 per cent RH. Similarly, Monler (1971) observed that conidia germinated on apple leaves and glass slides at 70 and 90 per cent RH, respectively.

In hanging drops of water, Berwith (1936) found less than 1 per cent germination and in raised drops of water no germination was obtained. However, Brodie (1945) reported that germination of the conidia of *P. leucotricha* might take place under drier conditions as he observed infection of apple leaves in greenhouse at RH less than 50 per cent. But Berwith (1936) had obtained no infection at temperature 1 to 10°C or at 28°C when the humidity was 90 per cent or less. Zaracovitis (1963) observed 67 per cent germination of newly abstricted conidia in a saturated atmosphere. The concentration of conidia of *P. leucotricha* in air is normally associated with wind velocity, temperature and solar radiation and is negatively associated with high RH and leaf wetness.

2.4.2.1 Germ tubes

An early caution by Brodie and Neufeld (1942) that conditions which favour germination of conidia may not be optimal for other stages of development is true as the germ tubes of these pathogens are more sensitive to moisture stress than are their parent conidia. On glass slides, the germ tube length does not increase at RH below 98 per cent though the conidia germinate. Nour (1958) in case of *E. chichoracearum* showed that an average germtube length of 32 um was produced at saturation but only 3-8 um at 85 per cent RH.

2.5 HOST RESISTANCE

Resistance of powdery mildew to plants has been normally correlated with frequency of plant hairs, high osmotic pressure of the cell sap (Homma, 1937), abundant tannin (Maranon, 1924 and Hummarlund, 1925), high acid content (Molze, 1917) and presence of leaf glands (Robertson, 1824).

In case of *P. leucotricha*, Corner (1935) found that the process of conidia germination was same both on susceptible and resistant apple cultivars but on later, conidia germinated upto the papilla stage and infection did not occur. Rarely a normal mycelium developed and produced conidia in week's time.

Csorba (1935) in Budapest had shown that the outer wall in the epidermal cell in susceptible cultivar of apple was thinner (av. 2.13 μm) in comparison to resistant ones (2.67 μm). Jeger *et al.* (1982) also observed more conidia/conidiophores on younger apple leaf in comparison to older ones. The development of the conidiophores was highest on the upper leaf surface, but the total number of conidia/ mm^2 was highest on lower surfaces, especially on younger leaves. Smykov (1987) evaluated 12 breeding lines for resistance to *Podosphaera leucotricha* by growing them among susceptible varieties for 3 years and one of these lines 120141 showed resistance to the test pathogen.

Grabowski (1987) reported Oregon Spur Delicious, Discovery and Golden Anvil Spur to be least susceptible to powdery mildew. Red Delicious, Ambred, Tydeman's Worcester, Tydeman's Late Orange, Red Baron, Surkh Tral and Dolgoe showed moderate resistance to powdery mildew (Verma and Gupta, 1988). Whereas, Wiatsma and Burger (1993) observed that Prima had a fairly high degree of mildew resistance. Pitera and Bogdanowicz (1994) reported that out of 140 cultivars of apple evaluated against powdery mildew, 17 has very light infection and were resistant to the disease.

Out of 12 varieties of apple screened against powdery mildew Sochi-1 and Sochi-2 were highly resistant (Indenko *et al.*, 1994). Pedersen *et al.* (1994) reported Belle de Boskoop, Bramleys seedling, Discovery, Filippa, Ingrid Marie and Mutsu to be moderately resistant to the disease. Vercammen *et al.* (1994) observed that NY62-306-10, NY65-707-19 and Rosana were free from powdery mildew infection.

Many cultivars like Prima, Earlidel, Red Fuji, Hi Early, Redfree, Anabela, Caricia, Liaofu, Klon U 211, Selection U211, MacExcell, Generos, Florina, Brina, Golden Mica, Red Earlibius, Perleberg 3, Co-op 6, Holsteiner Cox, Carola, Skityanka, Winter Banana, 33T9, DA 6517 and Sapipion Red had been reported to be resistant to the apple powdery mildew by various workers (Haeseldonckx, 1994; Wearing, 1995; Washington *et al.*, 1998; Hauagge and Tsuneta; 1999; Pitera *et al.*, 2000; Ognjkanov *et al.*, 2000; Yi-Kai *et al.*, 2001; Stankiewicz *et al.*, 2001; Serboiu, 2001; Tolstolik and Krasulya, 2001; Khanizadeh *et al.*, 2002; Bergamini *et al.*, 2002).

2.6 CHEMICAL CONTROL

The damage caused by powdery mildew may be manifested as a direct fall in the yield, because of suppression or distortion of plant growth and in nurseries because of sudden death of seedlings.

2.6.1 *In vitro* evaluation of fungicides

Price (1970) reported that benlate at high concentration partially inhibited germination but suppressed the production of secondary germtubes at all concentrations of 5-500 ppm. A number of commercial fungicides and dinitrophenolic compounds have been tested by Sztejnberg *et al.* (1975) for their ability to suppress further sporulation of established lesions of *P. leucotricha*.

Gupta and Sharma (1981) found that Bavistin, Benlate and Saprol did not allow the new spores to be produced on existing lesions for 26 days. Saprol was more tenacious than the other two. Butt *et al.* (1982) reported that bupirimate, fenarimol, nitrothal-isopropyl and ditalimfos all reduced conidia production by 80-85 per cent compared with undipped controls when estimated by leaf washing. Gupta and Gupta (1987) reported that Baycor (bitertanol) and Saprol (triforine) reduced sporulation of *Podosphaera leucotricha* on infected leaves and promoted the emergence of healthy new foliage. Flusilazole and bupirimate inhibited sporulation 20 days after treatment. Rathke and Jahn (1989) reported that fungicides like Afugan (pyrazophos), Sickosul (sulphur), Nimrod (bupirmite) and Rubigon (fenarimol) applied to apple leaves 6 days after inoculation with *Podosphaera leucotricha* significantly reduced sporulation 6 day after application.

Sharma and Gupta (1994) reported that carbendazim, bitertanol, triadimefon, tridemorph, triforine and thiophanate - methyl significantly inhibited spore germination of *Podosphaera leucotricha* and reduced germtube length. Maximum inhibition (94%) was obtained with triforine and the minimum (72%) with thiophanate methyl at 500 ppm. Antisporulant activity of the fungicides persisted for 14 days in all the test fungicides except carbendazim and bitertanol where the activity lasted for 21 days. Cimanowski and Bielenin (1996) while testing fungicides for their antisporulant activity in greenhouse experiments reported that all the fungicides were very active against *Podosphaera leucotricha* spores 1 day after application. The highest inhibitory and antisporulant activity was shown by flusilazole (Punch 400 EC) followed by strobilusis (BAS 4900 2F) and bupirimate (Nimrod 25 EC).

2.6.2 *In vivo* evaluation of fungicides

The first serious attempt to reduce damage caused by powdery mildew was probably made in the early nineteenth century when sulphur dusting came into use for the control of mildew on fruit trees (Forsyth, 1802). A little later Robertson in 1824 reported the use of sulphur and soap in water for the control of peach mildew. Since then several workers have reported the efficacy of various forms of sulphur in the control of powdery mildews. At present about 20 different fungicides are in use and sulphur still accounts for 25-30 per cent of the total expenditure on powdery mildew fungicides.

Vareshehagina (1981) sprayed trees with 2 per cent colloidal sulphur against *P. leucotricha* at bud burst, after flowering and later twice at 6-7 days intervals. The disease was reduced from 41 to 17 per cent on Jonathan cultivar. However, some workers have reported the injurious effects of sulphur on certain apple varieties (Woodward, 1927; Blumer and Luthi, 1949; Stachelin, 1949; Beraha *et al.*, 1955). Comai (1984) reported that the application of wettable sulphur and Bayleton Combi (triadimefon) at 200 and 150 g respectively gave same control of the disease.

Since sulphur is not particularly effective below 15°C and temperature below 15°C are common during the critical stages of powdery mildew development in the spring, it was replaced by Karathane (Coyier, 1971). Beraha *et al.* (1955) found that Karathane (3/4 lb per 100 gallon) reduced infection to 18.7 per cent compared with 55.4 for trees sprayed with water. Hammerlund (1959) found that weekly application of 0.05 per cent Karathane was significantly more effective against apple mildew than fortnightly application of 0.1 per cent Karathane. Zobrist and Bohnen (1963) reported that mildew and russeting could be controlled by 5 sprays of Karathane at 10 days intervals starting before flowering.

Moore *et al.* (1964) found that fortnightly applications of DNOC (Dinocap) at 0.025 per cent were as effective against *P. leucotricha* as 1 per cent lime sulphur. Spray with less concentration of fungicide at 7 days interval gave the best control and were better than equivalent low volume applications. Srivastava and Roy (1984) found that the disease was successfully controlled (73.1%) by 2 foliar sprays of Karathane EC (0.05 per cent) given just after appearance of disease at an interval of one month. Karathane has also been reported to control powdery mildew (Burchill and Cook, 1975 and Yoder and Hickey, 1983), however, Karathane in some cases has also been proved to be less effective in comparison to other fungicides (Bourdin *et al.*, 1973; Al-Shabbi, 1976 and Sharma and Gupta, 1980). Sharma *et al.* (1992) reported that *Podosphaera leucotricha* was well controlled in apple nurseries by 4 sprays of Dinocap or bitertanol from bud burst to fruit development.

A major breakthrough in the control of powdery mildew has been achieved after the development of systemic fungicides which not only protect the new leaves, but also reduce the level of existing disease. Bose and Sindhan (1967) have reported the control of apple mildew with 0.05 per cent benlate. Foschi *et al.* (1972) reported benomyl and thiophanate methyl to be effective against *Podosphaera leucotricha*. Cimanowski *et al.* (1974) found benlate, Topsin M and fundazol to be effective protectant. Dijke and Alink (1974) found that weekly application of Topsin at 35 g/ha gave good control of apple mildew and was more effective than fortnightly application at 100 g/ha. Saprol and Topsin M gave 95-97 per cent control of *P. leucotricha* (Ketskhoveli *et al.*, 1975, Lokaj, 1975).

Gupta *et al.* (1977) showed that infection by *P. leucotricha* on variety Red June was reduced in descending order by carbendazim (0.05%), binapacryl (0.1%) and benomyl (0.05%) while on Baldwin, carbendazim was best followed by benomyl and binapacryl. Benomyl and carbendazim were equally effective on Laxten Fortune and Laxten Superb cultivars, but carbendazim was better on Laxten Equisite. Putoo and Kaul (1978) found bavistin to be the best in controlling the disease. Bayleton at 0.05 per cent was highly effective and also prevented secondary infection in terminal buds, reduced the risk of infection in the following year. According to Verheyden and Soenen (1979) bayleton was consistently better than many fungicides in reducing the incidence of powdery mildew in many apple cultivars including Golden Delicious.

Sanchez and Rios (1979) in field trails found that Bayleton 25 WP was superior to Baycor 25 WP after 8 applications at 10-15 days interval starting from petal fall. Yurut (1978) reported thiophanate methyl to reduce apple mildew by 79.14 per cent.

Sharma and Gupta (1980) reported that out of 5 fungicides tested against *P. leucotricha* on the susceptible cultivar, Jonathan, the best results were obtained with Bavistin followed by Morocide and Morestan. Four sprays (at dormant, bud swell, petal fall and fruit development stages) of either fungicide than 2 or 3 sprays.

Gupta (1981) further found binapacryl (0.1%) and carbendazim (0.05%) to reduce disease equally on Granny Smith and Red June cultivars. Borovinova (1984) reported that Baycor 25 was effective when combined with 0.01 per cent bayleton and when used alone proved ineffective. Srivastava and Roy (1984) successfully controlled the disease with Topsin M 0.1 per cent (64.4%), Saprol EC 0.05% (69.69) and Bavistin 0.1 per cent (68.7%).

In the field trials against primary and secondary infection, Matijevic (1985) observed fungicides based on triadimefon, fenarimol and ditelimfos + benomyl were most effective, whereas, Nowacka *et al.* (1985) reported that Topaz C 50 WP (captan + penconazole) used throughout the season effectively controlled powdery mildew. Ramirezlegarreta (1985) reported that three applications of Bayleton (triadimefon) immediately after flowering reduced the infection of the disease.

Mandoza *et al.* (1991) observed that five sprays of tetraconazole (1.0 lt/ha), triadimenol (0.18 lt/ha), hexaconazole (0.28 and 0.34 lt/ha) sprayed against powdery mildew controlled the disease when sprayed at 15 days interval effectively. Berrie (1997) showed that the application of DMI fungicides (triadimefon, triadimenol, penconazole, fenarimol and mycobutanil) were better to control the powdery mildew of apple. Sholberg and Haag (1994) reported that McIntosh apple seedlings, inoculated with *Podosphaera leucotricha* developed significantly fewer powdery mildew colonies when sprayed with the demethylation inhibiting (DMI) fungicides like myclobutanil, flusilazole, triademefon and propiconazole than control.

Score (difenoconazole) and Topaz (penconazole) were also found to be effective to control powdery mildew on apple (Fedulova, 1994). Sharma and Gupta

(1994) found bitertanol to be the best fungicide against powdery mildew followed by carbendazim and triforine in the field.

Yoder (2000) observed that Five myclobutanil applciations followed by three applications of triadimefon (MS-T3) reduced primary infection to 19 per cent as compared to trees not receiving any fungicide. Cardei (2001) reported that in Golden Delicious, Starkrimson, Jonathan, Idared and Mutsu, the bromuconazole and dodine combination was most effective in controlling powdery mildew. Sun-RuiHong *et al.* (2001) also found that penconazole to be the most effective fungicide against apple powdery mildew.

Akhtar *et al.* (2002) reported the efficacy of 3 fungicides, viz., Shincer (carbendazim) 50 EC 100 g /100 l of water (LW), Bavistin (carbendazim) 50 DF 100g/100 LW and Topaz (penconazole) 100 EC 50 ml/100 LW in controlling powdery mildew infection of apple. Several other workers have reported the effectiveness of various other systemic fungicides like triadimefon (Kundart, 1977; Borovinova, 1978; Gjaerum *et al.*, 1981; Gupta and Gupta, 1991 and Jacobocuellar and Ramirezlegarreta, 1992), bavistin (Peresypkin and Khalil, 1977; Gupta, 1988; Sharma and Sharma, 1991) and thiophanate methyl (Burchill and Williamson, 1971; Cole *et al.*, 1971; Foschi *et al.*, 1972 and Sholberg and Haag, 1994) as antisporulent as well as for control of the disease.

Chapter-3

MATERIALS AND METHODS

The present studies were conducted in the laboratories of Department of Mycology and Plant Pathology at College of Horticulture, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan (H.P.). The field trails were laid out in the fields of HRS, Kandaghat, Solan.

3.1 SURVEY, COLLECTION AND MAINTENANCE OF MATERIAL

Apple nurseries in different localities of Solan, Shimla and Kullu Districts of Himachal Pradesh were surveyed periodically during the year 2004 from April to June to record the incidence and severity of powdery mildew under field conditions. The infected plant parts mainly leaves and shoots showing characteristic symptoms were collected in polythene bags, brought to the laboratory and preserved for further studies.

3.1.1 Disease incidence and severity

In order to record the incidence and severity of powdery mildew; nurseries were visited during April to June and the per cent disease incidence was calculated as follows:

$$\text{Disease incidence (\%)} = \frac{\text{Number of diseased plant}}{\text{Total number of plants}} \times 100$$

To record disease severity, 10 plants per locality were selected at random and 10 leaves from top to bottom were observed and rating was done as per Sharma (1985).

Rating	Description
0	No infection
1	1 to 10% leaf area mildewed
2	11 to 25% leaf area mildewed
3	26 to 50% leaf area mildewed
4	51 to 75% leaf area mildewed
5	76% and above leaf area mildewed

The disease severity (%) was calculated (McKinney, 1923) as under

$$\text{Severity (\%)} = \frac{\text{Sum of all disease ratings}}{\text{Total number of ratings x Maximum disease grade}} \times 100$$

3.2 IDENTIFICATION

The samples of diseased plant were brought to laboratory and thoroughly examined under microscope to detect the presence of anamorph and teleomorph of powdery mildew pathogen. Morphological characters of the powdery mildew fungus were studied microscopically. The following anamorphic characters were studied by the method given by Khan and Sharma (1995).

a) Mode of parasitism in relation to ectophytic and endophytic nature of mycelium
b) Morphology of conidiophores in relation to branching
c) Arrangement of conidia on conidiophores
d) Shape of conidia
e) Dimension of conidia (Length and Breadth)
f) Length / Breadth (L/B) index
g) Presence and absence of fibrosin bodies in conidia
h) Morphology of germ tube
i) Development of appressoria

3.2.1 Mode of parasitism

To observe mode of parasitism in relation to ectophytic/endophytic nature of mycelium, infected apple leaves were observed under a stereo binocular microscope.

3.2.2 Conidial dimensions

For conidial dimensions, 300 conidia selected at random from the slides prepared from the samples were measured with the help of an ocular micrometer. L/B index was determined by dividing length of a conidium with its breadth and mean values were calculated.

3.2.3 Occurrence of fibrosin bodies

For determining presence of fibrosin bodies, conidia were gently dusted on clean glass slides. A few drops of aqueous KOH solutions (3%) were placed on each slide to pronounce the fibrosin bodies. The treated conidia were examined microscopically for,

a) Presence or absence of well developed discrete fibrosin bodies
b) Number of fibrosin bodies per conidium

In all, 100 conidia were counted from each slide (replicated thrice) selected at random in different microscopic fields.

3.2.4 Conidial germination and germ tube morphology

Conidia from freshly sporulating mildew colonies were dusted on the clean glass slides placed on a glass rod triangle, kept in a petriplate (90 mm) containing sterilized distilled water at the bottom and moistened cotton wool lining the inner surface of the upper lid. The petriplates were incubated at $25 \pm 1^{\circ}C$. After 24 h of incubation, slides were examined microscopically to record.

a) Morphology of germ tube i.e. simple or forked
b) Per cent germination of conidia
c) Per cent forked germ tubes
d) Per cent simple germ tubes
e) Appressorial development

For each of the aforesaid observation, 100 conidia from each slide were randomly selected in different microscopic fields totalling 300 for each observation.

To record the development of appressoria, one set of slides were inoculated and incubated as described above for 36 h prior to recording observations.

3.3 PATHOGENICITY

In order to prove the pathogenicity of the fungus associated with the powdery mildew of apple seedlings, the surface of leaves (detached leaves or seedlings of apple) was first sterilized with 70 per cent ethanol and then thoroughly washed with sterilized distilled water. The conidia were dusted on healthy detached leaves and leaves of apple seedlings. After inoculation, drops (5 μl) of sterilized water were placed on corners of the inoculated leaves with the help of micropipette to provide high humidity for the germination of conidia and incubated at room temperature i.e. 20-25°C. Suitable control without any conidial dusting were also kept. The incubation period on both detached leaves and leaves of apple seedlings was calculated as follows.

$$i = ts - ti$$

Where,

i	-	Incubation period in hours
ti	-	Time of inoculation of leaves
ts	-	Time of appearance of disease symptoms

3.4 EPIDEMIOLOGICAL STUDIES

3.4.1 Effect of temperature on conidial germination under *in vitro* conditions

With a view to find out the optimum temperature for conidial germination, conidia from freshly sporulating colonies were uniformly dusted over the sterilized dry and clean glass slides kept on glass rod triangles placed in Petriplates (90 mm) containing cotton wool moistened with sterilized distilled water. Two such slides were kept in each Petridish. Petridishes containing slides were subsequently transferred to incubator maintained at 5,10, 15, 20, 25, 30 and 35°C. All the treatments were replicated four times and experiment was repeated twice. After 48 hrs of incubation, slides were examined microscopically to record conidial germination and germ tube length. About 300 conidia selected randomly from different microscopic fields were to calculate per cent germination and germ tube length.

3.4.2 Effect of relative humidity on conidial germination under *in vitro* conditions

In order to find out the effect of relative humidity on conidial germination, eight humidity levels viz., 100.0,99.10,98.20,96.90,95.60,89.90,85.70, and 80.50 were maintained using sulphuric acid (H_2SO_4) of known specific gravity as recommended by Stevens (1916). (Appendix 1).

Equal values of required solutions were poured in Petriplates (90 mm) which were used as humidity chambers. Conidia from freshly sporulating colonies were uniformly dusted over the sterilized, clean dry glass slides kept in these humidity chambers. Two such slides were kept in each Petriplate. The sides of each Petriplate were sealed with parafilm and subsequently transferred to an incubator maintained at 25$\pm$1°C. All the treatments were replicated four times and the experiment was repeated twice. After 24 h of incubation, slides were microscopically examined to record conidial germination and germ tube length. A total of 300 conidia selected randomly from different microscopic fields were examined to calculate per cent conidial germination and germ tube lengths.

3.4.3 Role of meteorological factors on disease development under field conditions

In order to find out the role of meteorological factors on disease development; severity of powdery mildew of apple was recorded in the nursery maintained by Department of Pomology, Dr.Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan (H.P.) during the crop season 2004 starting from the first week of May. Simultaneously, meteorological data on temperature, relative humidity (RH) and cumulative rainfall were also recorded. Simple, partial and multiple correlations and regression equations between disease severity and above three independent factors were worked out.

3.5 DISEASE MANAGEMENT

3.5.1 Germplasm screening

In all 113 apple cvs/lines available with the Regional Horticulture Research Station, Mashobra of Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni-Solan were screened for their reaction to powdery mildew under natural epiphytotic conditions during the 2004 crop season. The severity of powdery mildew was recorded at regular intervals by adopting the earlier mentioned scale given by McKinney (1923). Severity was further graded from 0 to 4 by using the following criteria:

Grade	Description	Infection type
Highly resistant	Plant completely free from the disease	0
Moderately resistant	Mycelium developed in small patches and covering 1 to 10 per cent leaf area	1
Moderately susceptible	Mycelium development slow and 11-25 per cent leaf area covered	2
Susceptible	Many small colonies appearing, later coalescing and covering 26-75 per cent area	3
Highly susceptible	Entire leaf (76-100%) covered by mildew uniformly	4

3.5.2 Management through fungicides

Various systemic and non-systemic fungicides (given below) were tested under laboratory and field conditions against powdery mildew pathogen.

Trade name	Common name	Formulation	Chemical name
Karathane	Dinocap	48 EC	Mixture of 2, 4-dinitro-6-octylphenyl crotonate and 2, 6-dinitro-4-octylphenyl crotonate
Sulfex	Wettable sulpher	80 WP	Wettable sulpher
Bavistin	Carbendazim	50 WP	2 (metroxycarbamoyl) benzimidazol
Bright	Carbendazim (12%)	50 WP	2 (metroxycarbamoyl) benzimidazol
	Mancozeb (63%)	50 WP	Methyl 1-(butycarbomyl)-2-benzimidozole carbamate)
Contaf	Hexaconazole	5 EC	2-(2, 4 dichlorophenyl)-1-(1H-1, 2,4-triazol-1-yl) hexane-2-ol
Score	Difenconazole	25 EC	α-(22chlorophenyl-α-(4 chlorophenyl) -5-pyrimidine methanol
Glow	Hexaconazole	5 EC	2-(2, 4 dichlorophenyl)-1-(1H-1, 2,4-triazol-1-yl) hexane-2-ol

3.5.2.1 *In vitro* evaluation of fungicides

The efficacy of seven different fungicides viz., Bright, Sulfex, Score, Karathane, Contaf, Bavistin and Glow at different concentrations (50, 100, 250ppm) were tested against the test pathogen by slide germination method given by the American Phytopathological Society (Anonymous, 1943).

One ml each of spore suspension of *P. leucotricha* and fungicides suspension were placed in sterilized cavity slides. The cavity slides were placed in humid chamber on sterilized glass rods by lining the wet blotting paper to maintain high (more than 90 per cent) humidity. The Petriplates were sealed with plastic film and incubated at 25$\pm$1°C for 48 hrs. Four replications were kept for each treatment. Observations on conidial germination and per cent inhibition was calculated by using the formula given by Vincent (1947).

$$R = \frac{C - T}{C} \times 100$$

Where,

R	-	Per cent reduction
C	-	Number of conidia produced in control
T	-	Number of conidia produced in treatment

3.5.2.2 Post symptoms anti-sporulent activity of fungicides

To determine the post symptom anti-sporulent activity, an experiment was laid out in completely randomized block design during 2004 in the polyhouse of Department of Mycology and Plant Pathology, Nauni, Solan (H.P.). Apple seedlings were raised in the pots (6" dia) filled with sterilized soil.

Leaves of established apple plants were inoculated with conidia of *P. leucotricha* taken from infected apple leaves with the help of camel hairbrush. A small drop of water (5 µl) was placed on the corner of each inoculated leaf with the help of a micropipette to provide humidity for conidial germination. After the appearance of the symptoms, twenty leaves (5 leaves per plant) each having 5 to 10 mildew pustules (one treatment) were tagged and subjected to seven fungicides spray treatment viz., Bright (0.25%), Contaf (0.05%), Karathane (0.04%), Bavistin (0.05%), Sulphur (0.25%), Score (0.15%), Glow (0.05%) and a check (only water). After 3, 5, 7 and 10 days of single spray, five tagged leaves were removed from each treatment and one lesion (5 mm^2) per leaf was cut with the help of a cork borer and washed into 5 ml of distilled water with the help of a camel hair brush (Gupta and Gupta, 1987). One drop (0.1 ml) of conidial suspension was placed on a haemocytometer to record the number of conidia and reduction (%) in number of conidia over control was calculated by the method given by Vincent (1947).

$$R = \frac{C - T}{C} \times 100$$

Where,

R	-	Per cent reduction
C	-	Number of conidia produced in control
T	-	Number of conidia produced in treatment

3.5.2.3 Evaluation of fungicides against powdery mildew under field conditions

A field trail was laid out at the experimental farm of HRS, Kandaghat with seven fungicides viz., Contaf (0.05%), Score (0.05%), Bavistin (0.05%), Karathane (0.04%), Sulfex (0.025%), Bright (0.25%) and Glow (0.05%) against powdery mildew of apple in randomized block design during April to August 2004 and each treatment was replicated thrice.. Spray application of fungicides was started with the initiation of disease i.e. in the first weak of April. In all, five sprays were applied at fortnightly intervals and observations on disease incidence and disease severity were recorded ten days after the last spray.

3.5.2.4 Effect of number of sprays on the management of disease

A field trial was laid out at the experimental farm of HRS, Kandaghat with seven fungicides viz., Contaf (0.05%), Score (0.05%), Bavistin (0.05%), Karathane (0.04%), Sulfex (0.025%), Bright (0.25%) and Glow (0.05%) against powdery mildew in randomized block design during April to August 2004 to find out the optimum number of sprays required for the management of powdery mildew of apple. The fungicides were applied in three sets comprising two, three and four sprays of fungicides, respectively. Each treatment was replicated thrice. The application of fungicides was started with the initiation of disease in the month of April. Observations on disease incidence and severity were recorded ten days after last spray application.

Chapter -4

EXPERIMENTAL RESULTS

4.1 SURVEY

A survey was conducted to determine the incidence and severity of powdery mildew in apple nurseries in different localities of Solan, Shimla and Kullu districts of Himachal Pradesh during the months of April to June in 2004 and the data have been presented in Table 1.

Table 1. Incidence and severity of powdery mildew in apple nurseries in Himachal Pradesh

Location	Disease incidence (%)	Disease severity (%)
Solan		
Nauni	65.00	45.00
Pandah	42.41	33.92
Dedghrat	32.67	26.76
Jardhari	27.47	23.52
Mean	41.89	32.30
Shimla		
Kotkhai	62.30	51.25
Nihari	60.00	47.25
Sarahan	58.50	50.56
Rohru	35.00	27.77
Mean	53.95	44.20
Kullu		
Seobagh	75.00	60.00
Bajaura	40.33	43.25
Mohal	45.00	33.00
Shirar	30.67	22.77
Basegran	55.00	42.00
Mean	49.20	40.20
Overall mean	48.79	38.90

The Data (Table 1) showed that the disease was present in all the areas surveyed and incidence varied from 27.47 per cent in Jardhari (Solan) to 75.00 per cent at Seobagh (Kullu) in nurseries. Similarly, the disease severity was maximum at Seobagh (60.00%) followed by Kotkhai (51.25%) and Sarahan (50.56%) and was minimum in Shirar (22.77%) in Kullu district, Seobagh in Kullu district, Nauni in Solan district and Kotkhai in Shimla district recorded maximum disease incidence and disease severity during the year under report.

The data further showed that out of various districts surveyed, Shimla district had the highest incidence (53.95%) and severity (44.20%) followed by Kullu district with 49.20 per cent and 40.20 per cent and minimum 41.89% and 32.30 per cent disease incidence and severity in Solan district, respectively.

4.2 SYMPTOMATOLOGY

Powdery mildew damaged both the nursery and the mature trees, reduced the vegetative growth, destroyed flower buds and produced dieback symptoms under severe conditions. The symptoms appeared on the leaves, stems and fruits with small whitish felt-like patches of fungal structure. In nature, early infections were usually caused by windborne conidia which germinated within few hours, formed appressaria, penetrated epidermal cells, developing haustoria and secondary hyphae and thus produced lesions. In nurseries, the fungus was more destructive and all developing leaves were covered with powdery mass leading to severe defoliation (Plate 1).

4.3 MORPHOLOGICAL CHARACTERS AND IDENTIFICATION

Infected apple leaves showing characteristic symptoms of the disease were collected, brought to the laboratory and examined microscopically to detect the presence of anamorph and teleomorph of the powdery mildew pathogen. All the samples collected from localities in Solan and Shimla districts were found to contain only anamorphs of the powdery mildew fungus, while those from Kinnaur district also contained teleomorph (perithecia) of the fungus. Observations pertaining to anamorphic and teleomorphic characters of the fungus were recorded (Table 2) and subsequently used to identify the fungus.

a) Leaves

b) Twigs

c) Seedlings

Plate 1. Symptoms of powdery mildew

Microscopic observations revealed that the mycelium was ectophytic. The condiophores were unbranched and septate with a cylindrical foot cell. Conidia were ellipsoidal and truncate in shape and arranged in long chains in basipetal succession. The size of the conidia was about 28-30 x 12-14 μm. When observed under high power (450 x), L/B index value was less than three (mostly 2.23). About 95.67 per cent conidia exhibited the presence of fibrosin bodies and an average number of fibrosin bodies per conidium was 8.33. Germination of conidia was observed to be 49.94 per cent (Plate 2). Observations on germtube morphology revealed that germinating conidia produced simple germtubes which emerged laterally from the side wall of the conidium. All the conidia produced simple germtubes and no forked germtube was observed in all the samples examined. For appressorial formation, it was found that one appressoria is obtained from one germ tube. Perithecia are subglobose, dark brown and have apical and basal appendages. One ascus is present in each perithecium. Ascospores are hyaline, subglobose or oblong, single celled, measuring 22-26 x 12-14 μm.

Table 2. Morphological characters of the pathogen causing powdery mildew of apple

1. Anamorphic characters	
Mycelium	Ectophytic
Conidiophores	Unbranched septate with cylindrical foot cell
Shape of conidia	Ellipsoidal, truncate
Conidial dimensions	
Dimension (μm)	28-30 x 12-14
Length/Breadth Index	2.23
Conidial germination (%)	49.94
Germtube morphology	
Simple (%)	100.00
Forked (%)	Nil
Conidia with fibrosin bodies (%)	95.67
Number of fibrosin bodies per conidium	8.33
Appressorial development	One appresoria from one germtube
2. Teleomorphic characters	
Perithecial dimensions	
Size (μm)	78-96
Shape	Subglobose
Appendages	Basal and Apical
Number of asci per perithecia	One
Ascospores (μm)	22-28 x 11-15

On the basis of above mentioned anamorphic and teleomorphic characters, the fungus was identified as *Podosphaera leucotricha* (Ell. and Ev.) Salmon.

4.4 PATHOGENICITY

In order to test the pathogenicity of *Podosphaera leucotricha*, apple seedlings were grown in the pots (10 cm dia) in polyhouse of Department of Mycology and Plant Pathology. These apple seedlings in pots as well as detached leaves of apple were brought to the laboratory, inoculated by dusting conidia from infected leaves with camel hair brush and incubated at 25°C under moist conditions. The observations on incubation period on both detached leaves and leaves of apple seedlings were recorded and presented in Table 3.

Table 3. Pathogenicity of *Podosphaera leucotricha* fungus

Plant part	Incubation period (days)
i) Detached leaves	8
ii) Leaves of potted seedlings	12

It is evident from the data (Table 3) that the incubation period were shorter i.e. 8 days on detached leaves as compared to 12 days on the leaves of apple.

4.5 EPIDEMIOLOGICAL STUDIES

4.5.1 Effect of temperature on conidial germination under *in vitro* conditions

To ascertain the most suitable temperature for the conidial germination of *Podosphaera leucotricha*, temperature regimes ranging from 5-35°C were maintained and conidial germination was seen by slide germination test. Observations on per cent germination as well as length of germtubes were recorded after 48 hrs of incubation.

Data (Table 4) revealed that conidia of *Podosphaera leucotricha* could germinate over a wide range of temperature (5-35°C) however, the increase in temperature there was an increase in conidial germination, maximum germination (49.94%) was obtained at 25°C, closely followed by 30°C (47.05%) and 20°C (46.19%), though both were statistically at par with each other. Minimum germination was recorded at 5°C followed by 35°C giving 28.36 and 33.59 per cent, respectively. 10 and 15°C was statistically at par with each other giving 36.09 and 37.58 per cent, respectively.

Table 4. Effect of temperature on conidial germination and germtube length under *in vitro* conditions

Temperature (°C)	Conidial germination (%)	Germtube length (μm)
5	28.36 (24.06)	8.75
10	36.09 (32.76)	14.17
15	37.58 (33.62)	15.25
20	46.19 (42.82)	15.33
25	49.94 (44.97)	18.33
30	47.05 (43.31)	17.75
35	33.59 (31.52)	9.33
$CD_{0.05}$	(1.54)	2.93

Figures in parentheses are arc sine transformed values

Data further showed that maximum germtube length of conidia of *Podosphaera leucotricha* was recorded at 25°C (18.33 μm) closely followed by 20°C (17.75μm)), though statistically at par with each other. Minimum germtube length was recorded at 5°C (8.75 μm) and 35°C (9.33 μm). Germtube length at 10, 15 and 20°C was statistically at par with each other.

4.5.2 Effect of relative humidity on conidial germination

In order to study the effect of different relative humidity levels on conidial germination and germ tube length of *Podosphaera leucotricha,* eight humidity levels (100.0, 99.10, 98.20, 96.90, 95.60, 89.90, 85.70 and 80.50%) were evaluated by slide germination technique under *in vitro* conditions. Observations recorded on conidial germination and germ tube length are presented in Table 5.

It is evident from the data (Table 5) that conidia germinated at all the humidity levels tested ranging from 80.50 to 100.00 per cent. Conidial germination was highest at 95.60 per cent RH (84.71%) followed by 89.90 per cent (79.49%) and 96.90 per cent (79.00%), however, all were statistically at par with each other. Conidial germination was minimum at 100 per cent (45.08%) relative humidity.

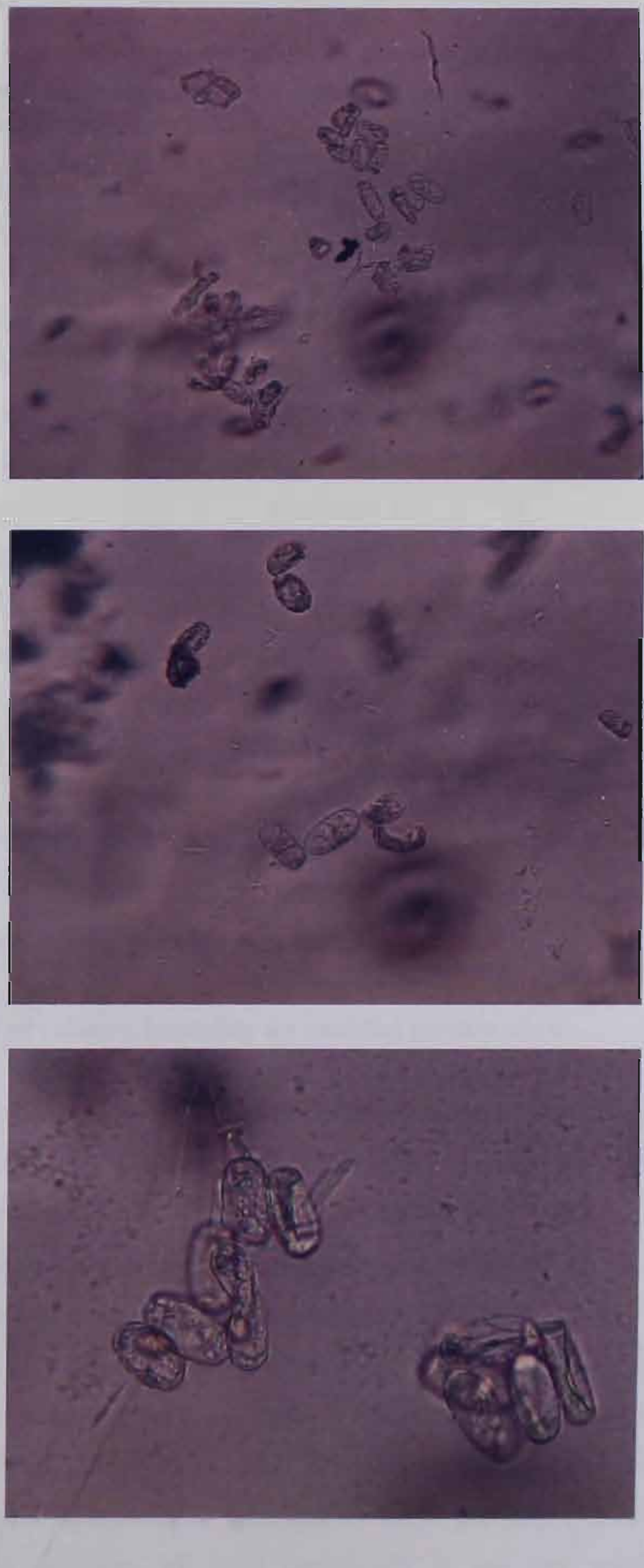

Plate 2. Conidias of *Podosphaera leucotricha*

Table 5. Effect of relative humidity on conidial germination and germtube length under *in vitro* conditions

Relative humidity levels (%)	Conidial germination (%)	Germtube length (μm)
100.00	45.08 (42.16)	7.85
99.10	53.01 (46.95)	8.37
98.20	66.63 (54.32)	9.67
96.90	79.00 (62.74)	9.95
95.60	84.71 (67.07)	13.03
89.90	79.49 (63.22)	12.60
85.70	73.21 (59.68)	11.24
80.50	71.41 (58.15)	10.83
$CD_{0.05}$	(5.59)	1.45

Figures in parentheses are arc sine transformed values

With regard to germ tube length at different humidity levels, maximum germ tube length (13.03 μm) was observed at 95.60 per cent RH followed by 89.90 per cent and 85.70 per cent RH giving 12.60 μm and 11.24 μm long germ tubes, respectively. Minimum germ tube length was observed at 100 per cent RH (7.85 μm). Free moisture was found to be least supportive to the elongation of germ tubes.

3. ROLE OF METEOROLOGICAL FACTORS ON DISEASE DEVELOPMENT UNDER FIELD CONDITIONS

In order to study the effect of meteorological factors on disease development under field conditions, experiments were conducted at the experiment farm of the Department of Pomology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan. The severity of the disease was recorded at weekly intervals commencing from 7th May and continued upto 23rd July, 2004. The information of weather parameters and disease development (Table 6) revealed that the disease appeared in the first week of May and severity of the disease ranged from 0.02 to 12.33 per cent. It assumed serious proportions during the months of June and July. Since the disease progress is dependent on meteorological parameters such as mean air temperature, relative humidity and cumulative rainfall, therefore simple, partial and multiple correlations were worked out to establish the relative contribution of these factors in the spread of the disease.

Table 6. Effect of meteorological factors on development of powdery mildew of apple

Date of observation	Temperature (°C)	Rainfall (mm)	Relative humidity (%)	Increase in disease severity (%)
07.05.2004	19.55	24.00	53.50	0.02
14.05.2004	25.05	0.00	38.00	3.85
21.05.2004	28.15	0.00	34.00	5.44
28.05.2004	24.40	39.00	56.00	6.88
04.06.2004	24.85	8.80	53.00	12.33
11.06.2004	23.35	30.60	62.50	10.43
18.06.2004	23.95	35.40	77.00	9.87
25.06.2004	24.55	16.60	76.00	7.46
02.07.2004	24.75	2.40	59.00	6.03
09.07.2004	26.20	30.40	74.00	5.65
16.07.2004	19.50	39.80	75.00	2.00
23.07.2004	24.45	2.00	56.50	0.07

4.5.3.1 Simple correlation

Simple correlation coefficients worked out between disease severity and meteorological factors are presented in Table 7.

Table 7. Simple correlation coefficients between disease severity and meteorological factors

Meteorological factor	Simple correlation coefficient
Temperature	-0.5149*
Relative humidity	0.6742*
Rainfall	0.1751

* Significant at 5 per cent

The data (Table 7) revealed that value of simple correlation coefficient was significant between disease severity and temperature, and relative humidity, while it was non-significant with rainfall indicating their dependence on disease development.

The correlation between disease severity and relative humidity was highly significant and positive (0.6742) showing consistent effect of relative humidity on disease development. Similarly, correlation coefficient between mean air temperature and disease severity was found significant and negative (-0.5149). However, the correlation with disease severity and rainfall (0.1751) was positive but non-significant.

4.5.3.2 Partial correlation

Change in one parameter is often influenced by simultaneous changes in several other parameter of a disease epidemic, therefore, valid conclusions could not be drawn from simple correlation coefficients study. Hence the partial correlation coefficient was worked out among various combinations of disease severity with mean air temperature, relative humidity and cumulative rainfall. The data after analysis is presented in Table 8.

Table 8. Partial correlation coefficients between disease severity and meteorological factors

Meteorological factor	Partial correlation coefficient
Temperature	0.3449
Relative humidity	-0.6642*
Rainfall	0.2350

* Significant at 5 per cent

The data presented in Table 8 indicated that partial correlation between disease severity and relative humidity (-0.6642) was highly significant and negative, whereas with temperature and rainfall, it was positive but non-significant.

4.5.3.3 Multiple correlation

The coefficient of multiple correlation (R^2) was calculated to measure the contribution of linear function of independent variables such as mean air temperature (X_1), Relative humidity (X_2), and rainfall (X_3) on dependent variable, disease severity (Y_1) and is presented in Table 9.

Table 9. Multiple correlation and regression equation between disease severity and meteorological factors

R^2	Multiple coefficient determination (%)	Regression equation		
0.6194	61.94	X_1	X_2	X_3
Y_1=	-122.76 + (115.19)	1.5600 x 1 + (92.5470)	2.0870 x 2* + (0.1055)	0.04614 x 3 (0.1083)

* Significant at 5 per cent

Where: Y_1= Disease severity
X_1= Mean air temperature
X_2= Relative humidity
X_3= Rainfall

The multiple correlation coefficient (Table 9) between disease severity and group of independent variable was found to be 0.6194, which indicates that 61.94 per cent change in disease severity was caused by mean air temperature, relative humidity and cumulative rainfall collectively, whereas the rest of the variation was due to unexplained factors (error variation) and the factor not included in the investigations.

4.5.3.4 Regression equation

The multiple regression equation (Table 9) showed that a unit change in relative humidity could influence the disease severity up to an extent of 2.0870 units, followed by mean air temperature (115600 units) and cumulative rainfall (0.04614 units) in the positive direction.

4.6 DISEASE MANAGEMENT

4.6.1 Germplasm screening

Different apple cultivars growing at RHRS, Mashobra, were screened against powdery mildew during the year 2004 under natural epiphytotic conditions. In all, 113 cultivars were screened and the disease index was calculated by using the formula givên earlier. The data have been presented in Table 10.

Table 10. Reaction of apple cultivars/germplasm lines to powdery mildew (*Podosphaera leucotricha*) under natural epiphytotic conditions

Sr. No.	Cultivars	Disease severity (%)	Disease reaction
1	Ambred	44.12	MS
2	American Mother	58.02	S
3	Bagzanka Kaschenco	6.22	HR
4	Baldwin	78.01	HS
5	Beauty of Bath	70.28	S
6	Benoni	79.25	HS
7	Boskoop	68.00	S
8	Buckingham	60.08	S
9	Cherry Cox	42.00	MS
10	Chinese Cinnamon	44.02	MS
11	Co-Pippin	46.00	MS
12	Coop-12	40.20	MS
13	Coop-13	43.22	MS
14	Cortland	80.28	HS
15	Cox's Orange Pippin	62.29	S
16	Crimes Golden Double Life	40.02	MS
17	Democrat	64.01	S
18	Desert of Isaev	82.00	HS

Sr. No.	Cultivars	Disease severity (%)	Disease reaction
19	Director Van De Plassche	66.20	S
20	Dummellow's Seedlings	45.02	MS
21	Early Baldwin	82.09	HS
22	Early Golden	38.55	MS
23	Early Red Bird	40.32	MS
24	Early Shanbury	68.44	S
25	Ellison's Orange	36.66	MS
26	English King	39.86	MS
27	Fanny	84.95	HS
28	Golden Autumn	80.02	HS
29	Goldene Chinies	35.70	MS
30	Golden Delicious	37.58	MS
31	Golden Hornett	8.02	HR
32	Golden Spur Delicious	42.44	MS
33	Grand Alexander	54.56	S
34	Granny Smith	78.02	HS
35	Hardeman	72.86	S
36	Ingrid Marie	25.75	MS
37	James Grieve	64.00	S
38	Jonathan	83.09	HS
39	Kashmira	40.44	MS
40	Kesri	52.66	S
41	King David	28.26	MS
42	King of Tompkins County	34.82	HS
43	Lady Sudley	36.38	MS
44	Laxton's Superb	70.12	MS
46	Lord Lambourne	68.28	S
47	Lobo	74.72	S
48	Lodi Barly Golden	66.70	S
49	Lungwort	64.61	S
50	McFree	86.00	HS
51	Mantet	62.78	S
52	Margaret	66.86	S
53	McIntosh	80.05	HS
54	McIntosh Double Red	82.21	HS
55	Melrose	42.41	MS
56	Memory of Michurin	78.02	HS
57	Merton Worcestor	28.72	MS
58	Miller's Seedling	82.24	HS
59	Mutsu	22.18	MR
60	Northern Sinap	6.22	HR
61	Northern Spy	60.32	S
62	Orange	68.38	S
63	Park Dale	24.72	S
64	Pearson's Late	68.36	MS
65	Peas Good Non Such	58.28	S
66	Pippin Litosky	22.72	S
67	Pomon Chinese	26.89	MS
68	Prince	70.00	S
69	Priscella	79.10	HS
70	Red Astrachan	56.58	S
71	Red Baldwin	82.94	HS
72	Red Delicious	36.72	MS

Sr. No.	Cultivars	Disease severity (%)	Disease reaction
73	Red Gold	72.00	S
74	Red Stachon	22.22	MR
75	Ribiston Pippin	52.68	S
76	Richared	46.48	MS
77	Rose Marine	38.48	MS
78	Royal Delicious	26.76	MS
79	Senator	40.68	MS
80	Sir Prize	78.21	HS
81	Sharp's Early	30.86	MS
82	Stark Earliest	32.78	MS
83	Stark Early Blaze	82.12	HS
84	Stark John Grieve	40.62	MS
85	Starkrimson Delicious (Spur Type)	44.65	MS
86	Starkrimson Delicious	84.05	HS
87	Stark Staymared	46.52	MS
88	Stark Spur Golden	66.18	S
89	Summer Champion	80.12	HS
90	Surkh Trel	20.82	MR
91	Surrovets	44.58	MS
92	Top Red (Spur Type)	32.84	MS
93	Turley Winesap	60.44	S
94	Tydeman's Early Worcester	40.50	MS
95	Tydeman's Worcestor	36.62	MS
96	Tydeman's Worcestor (Spur Type)	32.58	MS
97	Vance Delicious	34.66	MS
98	Vance Delicious (Spur Type)	28.52	MS
99	Versifield	18.78	MR
100	Victory	74.00	S
101	Wagener	28.68	MS
102	Wealthy Double Red	40.62	MS
103	White Dotted Red	65.12	S
104	Williams Favourite	44.18	MS
105	Wilson Red June	42.62	MS
106	Winter Banana	60.32	S
107	Winter Superior	30.68	MS
108	Winston	72.18	S
109	Worcestor Pearmain	34.45	MS
110	Yantaska Altaiskaya	5.78	HR
111	Yellow Newton	42.52	MS
112	York-A-Red	44.64	MS
113	Zilleton Crimon	70.23	S

1-10%	HR
11-25%	MR
26-50%	MS
51-75%	S
76& and above	HS

From the data (Table 10), it is evident that only four cvs. / lines viz., Bagzanka Kaschenco, Golden Hornett, Northan Sinap, Yantaska Altaiskaya were observed highly resistant against the pathogen whereas four cvs./lines viz., Red Stachon, Mutsu, Surkh Trel and Versifield showed moderately resistant reaction. Ambred, Cherry Cox, Chinies Cinnamon, Coop-12, Coop-13, Co-Pippin, Crime Golden Double life, Dummellow's seedlings, Early Golden, Early Red Bird, Ellison's Orange, English King, Golden Chinies, Golden Delicious, Golden Spur Delicious, Ingrid, Marie, Kashmira, King David, Lady Sudley, Laxton's Superb, Melrose, Merton, Worcstor, Pearson's Late, Pomon Chinese, Red Delicious, Richared, Rose Marine, Royal Delicious, Sentor, Sharp's Early, Stark Earliest, Stark John Grieve, Starkrimson Delicious (Spur Type), Tydenmen's Early Worcestor, Tydemans' Worcestor, Tydeman's Worcestor (Spur Type), Wagener, Wealthy Double Red, Williams Favourite, Wilson Red June, Winter Superior, Worcestor Pearmain, York-A-Red were found to be moderately susceptible. All other varieties screened against the pathogen exhibited susceptible to highly susceptible reaction under field conditions.

4.6.2 *In vitro* evaluation of fungicides

Seven systemic and non-systemic fungicides namely, Bright, Contaf, Sulphur, Glow, Karathane, Score and Bavistin were evaluated to see their inhibitory effect on conidial germination and germ tube length of *P. leucotricha* by slide germination technique under *in vitro* conditions. Observations recorded on conidial inhibition and germ tube length are presented in Table 11.

It is evident from the data (Table 11) that all the fungicides at all the concentrations inhibited conidial germination, however, maximum inhibition was observed in Karathane (62.14%) followed by Score (47.90%) and Bavistin (41.49%) which were statistically at par with each other. Bright (19.54%) and Contaf (26.22%), on the other hand, gave minimum conidial inhibition. Of the three concentrations, maximum inhibition (37.81%) was recorded at 250 ppm followed by 100 ppm (32.42%) and was minimum (29.89%) at 50 ppm. Interaction studies revealed that Karathane at all concentrations gave maximum inhibition of conidia of *P. leucotricha* being maximum at 250 ppm followed by 100 ppm, though both were statistically at par with each other.

Data also showed that minimum germ tube length of *P. leucotricha* was recorded with Karathane (5.84 μm) followed by Score (9.92 μm), Bavistin (10.29 μm) and was maximum in Bright (10.96 μm). In all the three concentrations, maximum germ tube length was recorded at 50 ppm (12.24 μm) and was minimum in 250 ppm (10.17 μm). With the increase in concentration of fungicides, the germ tube length decreased, showing thereby a negative correlation between the concentration and germ tube length.

4.6.3 Post symptom anti-sporulant activity of fungicides

In order to study the post symptoms anti-sporulant activity of fungicides, an experiment was laid out on apple seedlings raised in pots maintained in polyhouse of Department of Mycology and Plant Pathology, Nauni. Plants were inoculated with conidia of *P. leucotricha.* After the appearance of symptoms, the plants were subjected to one spray of fungicide and after 3, 5, 7 and 10 days of spray, five tagged leaves were removed from each treatment. One lesion (5 mm^2) was removed and percent reduction and total number of conidia was calculated and presented in Table 12.

It is evident from the data (Table 12) that Bavistin 3 days after first spray caused significant reduction (37.78%) in the number of conidia followed by Score (35.56%), Karathane (35.49%) and Contaf (31.11%), however, all were statistically at par with each other while sulphur (21.11%) was least effective. By 5th day all fungicides continued to reduce the conidial number, Bavistin being the best giving 33.33 per cent reduction. On 7th day, reduction level further came down and a similar trend was observed on 10th day.

On the basis of overall reduction in conidia production, Bavistin was best giving (27.96%) followed by Karathane (25.19%) and Glow (23.50%) while Score (16.57) was least effective. The overall mean reductions of conidial formation at 4 durations indicated that there was significant decrease on 3rd day which further decreased and was minimum on 10th day of spray. It also showed that with the increase in number of days, there was corresponding decrease in conidia production giving thereby a negative correlation between number of days on conidia production.

4.6.4 Effect of number of sprays on the management of powdery mildew of apple

In order to find out optimum number of sprays required to control powdery mildew effectively, seven fungicides namely Bright, Bavistin, Sulphur, Glow, Karathane, Score and Contaf were sprayed or one year old apple seedlings at experimental farm of HRS, Kandaghat. Four, three and two sprays of each fungicide were given at 15 days interval starting from bud burst. Data on incidence and severity of powdery mildew were recorded 10 days after the last spray.

The data (Table 13) show that all the fungicides in general, were effective in reducing the incidence and severity of powdery mildew irrespective of number of sprays four sprays of any fungicides were better than three sprays followed by two sprays. Four sprays of Karathane were highly effective in giving 13.85 per cent disease incidence closely followed by Contaf and Sulphur giving 15.26 and 16.46 per cent disease incidence, respectively, however, all the three were statistically at par with each other. This was closely followed by Sore (17.70%), Glow (20.53%) and Bavistin (23.51%). Maximum disease incidence (33.66%) was recorded in case of Bright. Three sprays of any fungicide were further found to be effective than two sprays. Three sprays of Score (17.04%) were equally effective as compare to four sprays of the same fungicides (17.70%). Similarly 3 sprays of Karathane (17.92%) were comparable with 4 sprays of either Sulphur, Score and Contaf giving 16.39, 17.70 and 15.26 per cent disease incidence, respectively.

Similarly disease severity was also found to be minimum in case of 4 sprays of Karathane, Score, Contaf and Sulphur giving 12.55, 13.82, 14.78 and 14.90 per cent severity, respectively as compared to three and two sprays of any fungicide.

4.6.4 Evaluation of fungicides against powdery mildew under field conditions

All the fungicides which were tested under *in vitro* conditions were also evaluated under field conditions during April to August 2004. In all, 3 sprays of each fungicide were given at 15 days interval. Data on disease incidence and severity were recorded 10 days after the last spray and is presented in Table 14.

Table 11. Effect of fungicides on conidial inhibition and germ tube length of *Podosphaera leucotricha* under *in vitro* conditions

Fungicides	Conidial inhibition (%)				Germtube length (µm)			
	Concentration (ppm)			Mean	Concentration (ppm)			Mean
	50	100	250		50	100	250	
Bright	17.43 (24.52)	19.20 (25.60)	21.99 (26.77)	19.54 (25.63)	11.44	11.11	10.33	10.96
Contaf	22.56 (27.45)	26.46 (30.18)	29.65 (32.16)	26.22 (29.93)	11.11	10.33	10.33	10.59
Sulphur	34.91 (35.06)	40.10 (39.20)	44.48 (41.52)	39.83 (38.59)	12.44	10.55	8.44	10.48
Glow	23.93 (28.28)	24.23 (27.81)	41.51 (39.86)	29.89 (31.98)	11.22	10.11	9.99	10.44
Karathane	53.84 (47.21)	60.31 (50.98)	72.28 (58.25)	62.14 (52.15)	6.86	6.33	4.34	5.84
Score	45.95 (42.66)	48.32 (44.03)	49.44 (44.68)	47.90 (43.79)	11.66	9.22	8.89	9.92
Bavistin	40.54 (39.30)	40.74 (39.53)	43.20 (41.06)	41.49 (38.63)	11.66	10.00	9.22	10.29
Control	-	-	-	-	21.54	20.22	19.78	20.51
Overall mean	29.89 (30.56)	32.42 (32.17)	37.81 (35.54)	-	12.24	10.98	10.17	-

$CD_{0.05}$

	Conidial inhibition		Germtube length
Fungicides	(8.31)	Fungicides	(2.56)
Conc.	(5.09)	Conc.	(0.91)
Interaction	(4.21)	Interaction	(1.27)

Table 12. Effect of fungicides on formation of Conidia of *P. Leucolricha* on apple leaves

Fungicides	Conc. (%)	Number of days								Overall mean	
		3		5		7		10			
		Total number of conidia	Reduction (%)	Total number of conidia	Reduction (%)	Total number of conidia	Reduction (%)	Total number of conidia	Reduction (%)	Total number of conidia	Reduction (%)
Bright	0.25	63.33	29.63 (32.92)	71.33	20.74 (26.95)	72.67	19.26 (24.94)	76.33	15.20 (22.68)	70.91	21.21 (27.12)
Bavistin	0.025	56.00	37.78 (37.91)	60.00	33.33 (35.26)	68.00	24.44 (29.53)	75.33	16.30 (23.66)	64.83	27.96 (31.59)
Sulphur	0.05	62.00	31.11 (33.89)	66.00	26.67 (31.08)	72.67	19.26 (25.97)	72.67	19.26 (25.97)	68.33	24.07 (29.22)
Glow	0.04	58.00	35.49 (36.51)	67.33	25.19 (29.98)	73.33	18.52 (25.37)	76.67	14.82 (22.55)	68.83	23.50 (28.60)
Karathene	0.05	57.33	35.56 (36.60)	66.00	26.67 (31.08)	69.33	22.96 (28.33)	76.00	15.56 (23.09)	67.17	25.19 (29.78)
Score	0.05	71.00	21.11 (27.29)	74.00	17.78 (24.88)	74.67	14.81 (22.59)	75.33	12.59 (20.67)	73.75	16.57 (23.86)
Contaf	0.05	62.00	31.11 (33.90)	64.00	28.83 (32.40)	72.00	20.00 (26.54)	78.00	13.33 (21.37)	69.00	23.32 (28.55)
Control		90.00	-	90.00	-	90.00	-	90.00	-		
Overall mean		64.96	31.68 (29.86)	69.83	25.60 (30.23)	70.08	19.89 (26.18)	77.54	15.29 (22.85)		

$CD_{0.05}$

Fungicides	(2.75)	Fungicides	(2.09)
No. of days	(1.94)	No. of days	(1.47)
Fungicides x No. of days	(5.47)	Fungicides x No of days	(4.16)

Table 13. Effect of number of sprays on the management of powdery mildew of apple

Fungicides	Conc. (%)	Number of sprays: 2		3		4		Overall mean	
		Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)	Disease incidence (%)	Disease severity (%)
Bright	0.25	51.00 (45.58)	40.22 (39.36)	41.00 (39.79)	30.22 (33.34)	33.66 (35.45)	25.00 (29.97)	41.89 (40.27)	31.81 (34.22)
Glow	0.05	24.66 (29.76)	18.78 (25.67)	22.66 (24.98)	21.03 (27.28)	20.53 (26.92)	16.67 (24.08)	22.62 (28.36)	18.82 (25.68)
Bavistin	0.025	33.75 (35.49)	28.66 (32.35)	28.58 (32.28)	24.22 (29.46)	23.51 (28.94)	19.21 (25.96)	28.61 (32.24)	24.03 (29.26)
Sulphur	0.05	26.41 (30.84)	19.78 (26.24)	21.59 (27.53)	14.46 (22.09)	16.39 (23.68)	10.47 (18.51)	21.46 (27.35)	14.90 (22.28)
Karathane	0.04	19.59 (26.26)	13.33 (21.21)	17.92 (24.98)	14.55 (22.40)	13.85 (21.75)	9.75 (17.94)	17.12 (24.33)	12.55 (20.51)
Score	0.05	20.37 (26.45)	16.43 (23.42)	17.04 (24.00)	12.40 (19.84)	17.70 (24.84)	12.83 (20.93)	18.37 (25.09)	13.82 (21.40)
Contaf	0.05	25.59 (29.99)	20.00 (26.17)	18.90 (25.31)	14.25 (21.73)	15.26 (22.92)	10.09 (18.43)	19.92 (26.07)	14.78 (22.11)
Control	-	68.69 (56.08)	72.54 (59.02)	69.77 (57.38)	73.34 (58.54)	70.17 (56.70)	78.53 (62.61)	69.54 (56.72)	74.80 (60.05)
Overall mean	-	33.76 (35.06)	28.59 (31.68)	29.68 (32.03)	25.55 (29.34)	26.38 (30.15)	22.82 (27.30)		

$CD_{0.05}$

Fungicide x Disease incidence	(3.29)	Fungicide x Disease Severity	(3.56)
No. of sprays x Disease incidence	(2.26)	No. of sprays x Disease severity	(2.18)
Interaction	(4.51)	Interaction	(4.39)

Table 14. Evaluation of fungicides against powdery mildew under field conditions

Treatment	Conc. (%)	Disease incidence (%)	Disease severity (%)
Bright	0.25	47.82 (43.75)	38.33 (38.240
Bavistin	0.025	30.09 (33.23)	24.00 (29.30)
Sulphur	0.05	18.99 (25.82)	13.78 (21.78)
Glow	0.04	24.41 (29.51)	17.78 (24.74)
Karathane	0.05	12.92 (21.03)	9.33 (17.41)
Score	0.05	17.59 (24.69)	12.00 (20.26)
Contaf	0.05	18.37 (24.91)	14.23 (21.73)
Control	-	69.77 (56.87)	73.34 (59.31)
$CD_{0.05}$		(7.25)	(7.77)

Figures in parenthesis are arc sine transformed values

It is evident from the data (Table 14) that all the fungicides were effective in reducing incidence and severity of the disease. Minimum incidence was recorded in Karathane (13.92%) followed by Contaf (18.37%), Sulphur (18.99%) and was maximum in Glow (24.41%), however, all were statistically at par with each other. Maximum disease incidence of 47.82 and 30.09 per cent was recorded in case of Bright and Bavistin, respectively.

Data further showed that minimum disease severity was registered in plants sprayed with Karathane followed by Sulphur and Glow giving 9.33, 13.77 and 17.48 per cent disease severity, respectively. Bright and Bavistin, on the other hand, recorded highest disease severity of 38.33 and 24.33 per cent, respectively.

Chapter-5

DISCUSSION

Apple is one of the most important fruit crops and is reported to have originated in the temperate region of western Asia. It is commercially grown in USA, China, France, Italy, Turkey, Argentina, West Germany, Spain and Japan. In India, the commercial cultivation of the crop is largely confined to the Himalayan states of J&K, Himachal Pradesh and Uttranchal whereas, north eastern states comprising Sikkim, Andhra Pradesh, Moghalaya, Manipur, Nagaland and Assam have a meagre share of less than one per cent in the total apple production. Apple, being the principal fruit crop of Himachal Pradesh has bean receiving special attention in the state. It has rightly become the golden crop of the hills and Himachal Pradesh alone produces about 25 per cent of total apple produced in the country. Though there are several diseases inflicting injury partly or wholly to an apple tree, one of most serious foliage disease is powdery mildew ranging next to scab in its prevalence.

Keeping in view the economic importance of the disease, research gaps and meagre information available in literature, it was thought worthwhile to investigate the prevalence of the disease in different apple growing areas, to ascertain exact identification of the pathogen involved, role of meteorological factors on disease development, sources of resistance and management of the disease by recently available fungicides and the results obtained are discussed herein in the light of available literature as follows:

A survey conducted to determine incidence and severity of powdery mildew in apple nurseries revealed that the disease occurred in moderate to severe form in various parts of Solan, Shimla and Kullu districts of Himachal Pradesh. The disease incidence ranged from 27.47 to 75.00 per cent and severity from 22.77 to 60.00 per cent. The data also suggested that Nauni in Solan district and Kotkhai in Shimla district recorded maximum disease incidence and disease severity. This may be attributed to the prevalence of high humidity in these areas which may have helped in more germination of conidia and subsequent disease development.

Powdery mildew damaged both the nursery and the mature trees, reduced the vegetative growth, destroyed flower buds and produced dieback symptoms under severe conditions. The symptoms appeared on the leaves, stems and fruits with small whitish felt-like patches of fungal structure. In nature, early infections were usually caused by windborne conidia which germinated within few hours, formed appressoria, penetrated epidermal cells, developing haustoria and secondary hyphae and thus produced lesions. In nurseries, the fungus was more destructive and all developing leaves were covered with powdery mass leading to severe defoliation. Similar type of symptoms of powdery mildew on apple have been observed by Ellis *et al.* (1981).

In the present investigations, microscopic examination of diseased samples collected from different localities in Solan and Shimla districts were found to contain only anamorph of the pathogen while those from Kinnaur districts also contained teleomorph which have been used successfully for establishing the identity of the causal agent of powdery mildew on apple (Burchill, 1978, Stevens, 1954 and Puttoo and Kaul, 1978).

In the present studies, anamorphic characters such as ectophytic mycelium, unbranched conidiophores with cylindrical foot cell and ellipsoidal and truacate conidia arranged in basipetal succession, conidial size, presence of fibrosin bodies in conidium, simple germtubes and one appressorial formation from one germtube and colour of pertithecia, apical and basal appendages, presence of one ascus in each perithecium clearly indicated the involvement of *Podosphaera leucotricha* as the causal agent of this disease in Himachal Pradesh and the fungus was identified as *Podosphaera leucotricha* (Ell & Ever) Salm.

In pathogenicity tests, detached leaves were found more susceptible to the disease with an incubation period of 8 days as compared to leaves of potted seedlings which had an incupation period of 12 days . Kaspers (1967) also reported a 12 days incubation period of *Podosphaera leucotricha* at 4°C and 4-5 days at 20°C.

Results pertaining to the effect of various temperature regimes on conidial germination revealed that maximum (49.94%) conidia germinated at 25°C under moist conditions followed by 30°C (47.05%) and 20°C (46.19%) as compared to 28.36 and 33.59 per cent at 5 and 35°C, respectively, which further suggested that too high or too low temperatures did not favour conidial germination. These results are in conformity with the observations of Coyier (1968). These results also lends support to the findings of Monler (1971) who reported, 4, 21-25 and 30°C as average minimum, optimum and maximum temperatures, respectively, for conidial germination of *Podosphaera leucotricha*.

Germtubes showed nearly similar growth at all temperatures although maximum germtube length was recorded at 25°C . Several workers have reported a temperature range of 20-26°C to be optimum for germ tube growth for most powdery mildews (Berwith, 1936, Manners and Hossain, 1963, Monlar, 1971, Hewitt, 1974).

Among the different humidity levels evaluated for conidial germination, maximum conidial germination occurred at relative humidity 95.60 per cent followed by RH levels 89.90 and 96.90 per cent while minimum germination occurred at 99.10 and 100 per cent relative humidity. These results are in agreement with the observations of Yarwood (1936) who suggested that powdery mildews are normally favoured by dry weather and reasons for this observations are the mechanical damage to conidiophores by rain. Poor germination of spores in water has also been reported by various workers (Corner, 1935, Brodie and Neufeld, 1942, Clayton, 1942, Brodie, 1945 and Delp, 1954).

Observations of germ tube lengths showed similar growth at all moisture levels and maximum germ tube length was recorded at 35.60 per cent relative humidity. Free moisture was found least supportive to the elongation of germ tubes and minimum germtube length was recorded at 95.60 per cent relative humidity. Free moisture was found least supportive to the elongation of germ tubes and minimum germ tube length was observed at 100 per cent relative humidity (7.85 μm). An early caution by Brodie and Neufeld (1942) that conditions which favour germination of conidia may not be optimal for other stages of development is true as the germ tubes of these pathogens are more sensitive to moisture stress than are their parent conidia.

The results on the influence of environmental factors on the disease development under field conditions revealed that the disease was initiated in the first week of May indicating a influence of temperature, rainfall and relative humidity on disease development. Significant and positive correlation (0.6742) observed between disease severity and RH suggested the prominent role of RH in disease development. These results to a greater extent also corroborate the findings of various workers (Yarwood, 1957, Berwith, 1936, Monlar, 1971 and Grushin, 1988) who reported high incidence of the disease under high humidity conditions. In the present studies, a significant and negative correlation (-0.5149) have been found between disease severity and mean air temperature. Similarly, many workers have reported that occurrence of apple mildew was favoured by a relatively mild, dry climate (Butikofer, 1949, Blumeer, 1951, Videnov, 1972). Moreover, partial correlation and regression equations between disease severity and environmental factors have further elaborated the role of temperature, relative humidity for disease development. These results corroborate the findings of Yarwood (1957) who attributed higher incidence of powdery mildews to be due to higher humidity besides, low temperatures and reduced light intensity. Similarly, Khan (1989) also observed moderate temperatures and reduced light intensity to favour disease development.

Among the 113 cultivars/lines screened for resistance against powdery mildew, four cultivars/lines viz, Bagzanka Kaschenco, Golden Hornett, Northern Spy and Yantanska Attainspaya were observed highly resistant against the pathogen whereas Red Stachon, Mutsu, Surkh Trel and Versifield showed moderately resistant reaction while the remaining exhibited moderately susceptible to highly susceptible reactions. Many cultivars / lines viz., Prima, Earlidel, Red Fuzi, Redfree, Liaofu, MacExcell, Co-op 6 and Winter Banana have been reported to possess resistance against *Podosphaera leucotricha* (Serboiu, 2001, Khanizadeh *et al.*, 2002, Bergemini *et al.*, 2002). However, inconsistencies in disease reactions of various cultivars/lines to powdery mildew have been reported (Angelov, 1979). Corbaz and Taillens (1994) attributed these inconsistencies in cucumber lines against powdery mildew to multiplicity of the causal agents involved and the prevalence of different race spectra in various parts of the world.

Among the systemic and non-systemic fungicides evaluated for their fungitoxicity against *Podosphaera leucotricha* by slide germination method, Karathane, Score and Bavistin were found to be most effective at all the concentrations evaluated. Efficacy of score, bavistin have also been reported by

Cimanowski and Goszczynski (1990) and Sharma and Gupta (1994) in inhibiting spore germination of *Podosphaera leucotricha* and reducing germ tube length under *in vitro* conditions.

The post symptom anti-sporulent activity of fungicides against *Podosphaera leucotricha* revealed that the residues of Bavistin, Karathane, Glow and Contaf showed higher degree of anti-sporulant activity for a longer period (10 d) which thereby checked build up of secondary inoculum. The reduction in conidial formation by Bavistin consonance the findings of Gupta and Gupta (1991) and Sharma and Gupta (1994) who observed that Bavistin is effective in controlling appie powdery mildew and its antisporulant activity persisted for 21 days. Thus, long persistent and anti-sporulant activity of Bavistin, Karathane and hexaconazole can be used in planning effective spray schedules for the management of this disease.

For the management of powdery mildew of apple, a field trial was laid out at research farm of HRS, Kandaghat under natural epiphytotic conditions. The results showed that Karathane, followed by Score, Contaf, Sulphur and Glow were effective in reducing the incidence as well as severity of the disease. Moore *et al.* (1964) found that fortnightly applications of Dinocap at 0.025 per cent a.i. were as effective against *Podosphaera leucotricha* as 1 per cent lime sulphur Srivastava and Roy (1984) found that the disease was successfully controlled (73.1%) by 2 foliar sprays of Karathane (0.05 per cent) given just after appearance of disease at an interval of one month. Sharma *et al.* (1992) reported that *Podosphaera leucotricha* was well controlled in apple nurseries by 4 sprays of Dinocap or bittertanol from bud burst to fruit development. Mandoza *et al.* (1991) found that five sprays at 15 days interval of tetraconazole (1.0 lt.ha), triadimenol (0.18 lt/ha), hexaconazole (0.28 and 0.34 lt/ha) and bitertanol (0.4 lt/ha) controlled the apple powdery mildew.

Field trials conducted to find out the optimum number of sprays required to control the powdery mildew effectively revealed that 4 sprays of Karatane were highly effective in reducing disease incidence (13.85%) closely followed by Contaf and Sulphur giving 15.26 and 16.39 per cent disease incidence, respectively. Similarly, disease severity was also found to be minimum in case of 4 sprays of Karathane, Score, Contaf and Sulphur giving 12.55m 13.82, 14.78 and 14.90 per cent diseases severity, respectively. Zobrist and Bohnen (1963) reported that mildew attack and russetting could be controlled by 5 Karathane sprays at 10 days intervals before flowering, 2 foliar sprays of Karathane EC (0.05 per cent) given just after appearance of disease at an interval of one month have also been reported effective in controlling powdery mildew of apple by Srivastava and Roy (1984). Sharma *et al.* (1992) found that 4 sprays of Dinocap or bitertanol from bud burst to fruit development effectively managed *Podosphaera leucotricha* under field conditions.

Chapter-6

SUMMARY

The present investigations on powdery mildew of apple were undertaken with regard to its occurrence, symptomatology, identity of the pathogen involved, pathogenicity, relationship of environmental factors with disease development, screening of germplasm and to evolve an effective disease management strategy using chemical methods. The results obtained are summarized as under:

The disease was found prevalent in moderate to severe form in Solan, Shimla and Kullu districts of Himachal Pradesh. The incidence of disease ranged from 27.47 to 75 per cent and severity from 22.77 to 60.00 per cent during 2004 crop season in apple nursery.

The characteristic symptoms consisted of small whitish felt like patches of fungal structures on the leaves and stems. These patches soon joined together to form larger area. Severe foliar colonization lead to accelerated senescence with leaves becoming brown and finally dries up leading to severe defoliation.

Microscopic examination of diseased samples showed the presence of typical anamorphs of the pathogen like ectophytic mycelium, unbranched conidiophores with cylindrical foot cells, ellipsoidal and truncate conidia arranged in long chains, in basipetal succession, presence of fibrosin bodies in conidium, simple germtubes and an appressorial formation from one germtube and teleomorph of the pathogen like subglobose and dark brown perithecia having apical and basal appendages and presence of one ascus in each perithecium and single celled, hyaline subglobose ascospores clearly establish the involvement of *Podophaesa leucotricha* (Ell. & Ev.) Salmon. as causal agent of this disease in Himachal Pradesh.

In pathogenicity test, detached leaves were found more susceptible to the disease with incubation period of 8 days as compared to leaves of potted seedlings.

Temperature (25°C) and relative humidity (95.60%) showed maximum conidial germination and germtube length of *Podosphaera leucotricha.*

The environmental factors i.e. mean air temperature, relative humidity and quantitative rainfall influence the powdery mildew development.

Simple correlation coefficient between disease severity and relative humidity was found to be highly significant and positive (0.6742) while temperature showed significant but negative correlation (-0.5149). Rainfall also showed a lower degree of positive correlation (0.1751). The partial correlation coefficients between disease severity and relative humidity was highly significant and negative (-0.6642). Regression equation between disease severity and environmental factors explained 61.94 per cent of disease of which relative humidity was significant.

Out of 113 cultivars / lines screened against *Podosphaera leucotricha,* 4 cultivars /lines viz., Bagzanka, Kaschenco. Golden Hornett, Northern Sinap, Yantaska Attaiskaya were observed to be highly resistant against the pathogen while Red Starchan, Mutsu, Surleh Trel and Versifield showed moderately resistant reaction. The rest of the cultivars/lines exhibited moderately susceptible to highly susceptible reactions.

In vitro evaluation of fungitoxicants against *Podosphaera leucotricha* showed Karathane, Score and Bavistin to be the best and inhibited the conidial germination and germ tube length effectively, giving 62.14, 47.90, 41.49 per cent conidial germination, respectively.

Post symptom antisporulant activity of fungicides against *Podosphaera leucotricha* revealed that residues of Karathane, Glow and Contaf exhibited a higher degree of antisporulant activity for a longer period (upto 10 days) which clearly reduced build up of secondary inoculum and can be used in planning spray programmes for the management of this disease. Field trails conducted for the management of powdery mildew of apple revealed Karathane, Score, Contaf, Sulphur and Glow to be most effective in reducing the disease incidence as well as severity of the disease.

Field studies carried out to find out the optimum number of sprays required to control the powdery mildew effectively showed that four sprays of Karathane were highly effective in reducing disease incidence (13.85%) closely followed by Contaf and Sulphur giving 15.26 and 16.39 per cent disease incidence, respectively. Similarly, disease severity was also found to be minimum in case of four sprays of Karathane, Score, Contaf and Sulphur giving 12.55, 13.82, 14.78 and 14.90 per cent disease severity, respectively

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Year of Award of Degree	:	2005
No. of pages in thesis	:	62+1
No. of words in Abstract	:	299

ABSTRACT

Powdery mildew of apple was found prevalent in moderate to severe form in Solan, Shimla and Kullu districts of Himachal Pradesh. Incidence of disease ranged from 27.47 to 75 per cent and severity from 22.77 to 60.00 per cent. The characteristic symptoms consisted of small whitish felt like patches of fungal structures on the leaves and stems. Severe infection on leaves lead to accelerated senescence with leaves becoming brown and finally dries up leading to severe defoliation. Microscopic examination of diseased samples showed presence of typical anamorphs of the pathogen unbranched conidiophores, truncate conidia arranged in long chains, in basipetal succession, simple germtubes and presence of one ascus in each perithecium and single celled, hyaline subglobose ascospores establish the involvement of *Podophaesa leucotricha*) as causal agent of the disease. Detached leaves were found more susceptible to the disease with incubation period of 8 days as compared to leaves of potted seedlings. Temperature (25°C) and relative humidity (95.60%) showed maximum conidial germination and germtube length of the fungus. Environmental factors i.e. mean air temperature, relative humidity and quantitative rainfall influence the powdery mildew development. Out of 113 cultivars, four showed highly resistant reaction while rest of the cultivars/lines exhibited moderately susceptible to highly susceptible reactions. *In vitro* evaluation of fungicides showed Karathane, Score and Bavistin to be the best and inhibited the conidial germination and germ tube length. Post symptom antisporulant activity of fungicides against the pathogen revealed that Karathane, Glow and Contaf had higher degree of antisporulant activity for a longer period (upto 10 days). In the field, Karathane, Score, Contaf, Sulphur and Glow found to be most effective in reducing the disease incidence as well as severity of the disease. Four sprays of either fungicide were proved to be better than three and two sprays of the fungicides.

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Whether sponsored by some state/ Central Govt./Univ./SAARC : NA

Scholarship/ Stipend/ Fellowship, any other financial assistance received during the study period : NA

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Appendix-I

Relative humidity levels using sulphuric acid

Per cent sulphuric acid	Per cent relative humidity at 25°C
Water	100.0
3.03	99.1
5.96	98.2
8.77	96.9
11.60	95.6
19.61	89.9
23.47	85.7
27.32	80.5

Source: Stevens, 1916

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