Control of apple powdery mildew *(Podosphaera leucotricha)* in British Columbia by demethylation-inhibiting fungicides

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In greenhouse studies inoculated McIntosh apple seedlings sprayed with the demethylation-inhibiting (DMI) fungicides myclobutanil, flusilazole, triadimefon and propiconazole developed significantly fewer powdery mildew colonies than control plants. Fewer colonies were observed on myclobutanil-treated plants than on plants treated with the other fungicides. An average of only 0.60 mildew colonies developed on the leaves of myclobutanil-treated plants within 10 days after inoculation compared with an average of 48.2 colonies on the leaves of the control plants. The DMI fungicides were more effective than thiophanate-methyl in field trials conducted in 1989 and 1990 on infected Jonagold apple foliage. The DMI fungicides flusilazole and myclobutanil effectively controlled foliar powdery mildew under heavy inoculum pressure on Jonathan apple trees in 1987 and 1988, respectively. The cultivars used herein showed no detectable phytotoxic effects of applying DMI fungicides, neither injury to fruit or foliage nor altered shape or weight.

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Une étude effectuée en serre sur des jeunes plants inoculés de pommiers McIntosh, ayant été traités à l'aide de fongicides inhibant la déméthylation, soit le myclobutanil, le flusilazole, le triadimefon et le propiconazole, a permis de démontrer que ces plants ont form6 beaucoup moins de colonies de blanc que les plants témoins. Les plants traites au myclobutanil ont present6 moins de colonies que les plants traites avec les autres fongicides. Dix jours après l'inoculation, une moyenne de 0,60 colonie de blanc seulement est apparue sur les feuilles des plants ayant reçu du myclobutanil, alors que dans le cas des plants témoins une moyenne de 48,2 colonies se sont développées. Les fongicides inhibant la déméthylation se sont révélés plus efficaces que le thiophanate-methyl dans les essais au champ qui ont été effectues en 1989 et en 1990 sur le feuillage infect6 des pommes Jonagold. En 1987 et en 1988, du flusilazole et du myclobutanil appliques sur des pommiers Jonathan, en presence d'une forte action de l'inoculum, ont permis de lutter efficacement contre le blanc sur le feuillage. Par suite de l'application des fongicides, aucun effet phytotoxique (dommages aux fruits ou aux feuillages, modification de la forme ou du poids du fruit) n'a été détecté sur les cultivars dont il est question ici.

Introduction

Powdery mildew (PM), caused by *Podosphaera leucofricha* (Ell. & Ev.) Salm., is an important disease of apple in the interior of British Columbia. Disease severity and need for control measures are related to host susceptibility and to the intended market for the cultivar (Yoder and Hickey 1983). The pathogen may cause death of vegetative shoots or flower buds, and russetting of fruit (Jones and Sutton 1984). The grower's primary concern with mildew is the russet symptoms that markedly reduce fruit quality (Spotts *et al.* 1981). Infected young trees of susceptible cultivars may be seriously damaged or become poorly shaped because of retarded vegetative growth or loss of terminal buds. In British Columbia the very susceptible apple cultivars, such as McIntosh and Golden Delicious, are treated regularly with fungicides for control of fruit russet. The fungicides most commonly used for powdery mildew are sulfur and thiophanate-methyl. Several new cultivars have been recently introduced, which also will require regular fungicide treatments (Anon. 1992).

P. leucofricha is an obligate ,parasite that overwinters on apple as mycelium in dormant buds infected during the previous growing season (Hickey and Yoder 1990). The "primary infection phase" of the disease is initiated by conidia produced on overwintering mycelium at bud break, which infect young leaves, flowers and shoots. Newly formed conidia from these sources are inoculum for the "secondary infection phase", which is the infection of healthy leaves during the growing season (Burchill 1960). The

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reduction of primary inoculum and the protection of leaves, fruit and buds from secondary infections are two areas of concern for effective disease control measures. Timely application of fungicides is widely used to prevent new infections and to reduce the number of spores produced on new lesions.

The most promising new fungicides for control of powdery mildew are the broad-spectrum, sterol-inhibiting compounds (Ogawa and English 1991). With the exception of the morpholines, all sterol inhibitors have a common site of action within the biosynthesis pathway and are grouped together as demethylation inhibitors or DMIs (Scheinpflug 1988). Although myclobutanil was registered for the control of powdery mildew on apples and grapes in Canada, it is unclear whether the fungicidal properties of myclobutanil are comparable to those of thiophanate-methyl or to other DMI fungicides such as triadimefon, which are used to control powdery mildew on apples in the United States.

This study compares the activities of DMI fungicides and thiophanate-methyl on mildew of apple plants under controlled conditions in the greenhouse and in an orchard. Results of tests to evaluate phytotoxicity to apple also are presented.

Materials and methods

Fungicides

The fungicides used in these experiments [flusilazole (Nustar 20 DF, Dupont Canada Inc.), myclobutanil (Nova 40 W, Rohm and Haas Canada Inc.), propiconazole (Orbit 40 W, Ciba-Geigy Canada Ltd.), thiophanate-methyl (Easout 70 W, Ciba-Geigy Canada Ltd.) and triadimefon (Bayleton 50 W, Chemagro Ltd.)] were commercial formulations provided by the manufacturers.

Greenhouse studies

McIntosh apple seeds were stratified for 6 weeks at 10°C and planted in 10-cm dia. pots in a soil mixture containing equal volumes of loam, sand and vermiculite. The pots were placed in the greenhouse (22°C day, 18°C night, 77-84% RH) for germination and subsequent growth for approximately three weeks without pesticides for disease or insect control.

The inoculum source was infected apple shoots from an eight year old Jonagold tree in the Summerland Research Station orchard. The fungus was identified as *Podosphaera leucotricha* (Ell. & Ev.) Salm. on the basis of symptom development and a comparison of the morphological characters of the conidia and fruiting bodies with those described for *P. leucotricha* by Ogawa and English (1991). The infected shoots were placed in a 1°C cold storage room for approximately 4 hrs while the fungicide suspensions were being prepared. McIntosh seedlings were sprayed to runoff using a hand operated mister (Table 1). The leaves were allowed to dry for 30-min before inoculation with *P. leucotricha* conidia. Each treatment consisted of 10 seedlings. A conidial suspension was prepared by brushing conidia from diseased shoots into sterile water containing 20 µl/mL of Triton X 100. The concentration was adjusted to 8.0×10^{11} conidia/mL with a haemacytometer. Within 15-min of preparation the suspension was sprayed on the leaves. The seedlings were inoculated using the method Dekker (1982) developed to evaluate powdery mildew on cucumber leaves.

Mildew development was estimated by counting colonies on leaves 6, 8 and 10 days after inoculation. Each small white spot at least 3-mm in diameter was counted as a colony. Mildew colonies were counted on both surfaces of six leaves at positions -1 to +4, where leaf 0 was the youngest leaf behind the shoot apex at the time of inoculation and -1 was the next unrolled leaf and youngest leaf at the shoot tip when the colonies were counted (Jeger *et al.* 1986). One plant was removed from each treatment because it had on average fifteen times more colonies than the mean, and therefore had been apparently infected with powdery mildew before the start of the experiment. Conidial production per cm^2 of leaf surface was estimated 10 days after inoculation for samples containing 10 leaves from each of the six positions. The 10 leaf samples were rinsed with 25 mL of sterile water to remove conidia and 10 aliquots were counted in a haemacytometer chamber. The average of these 10 counts was used as the concentration of conidia in the 25 mL suspensions. Leaf area was determined by tracing each leaf on drawing paper and measuring the area with a digitizing tablet (Jandel Scientific, Corte Madera, CA).

Orchard studies

For the field test uniform trees of apple cv. Jonagold were selected in the orchard at the Summerland Research Station. Apple trees on M.106 or M.26 rootstocks, approximately 4m and 3m high, respectively, were 9 years old when the first test was conducted in 1989. Thirty-five selected trees in two rows were grouped into five randomized blocks with seven random single trees separated by an unsprayed control tree. Treatments (Table 3) were applied until runoff with a handgun operated at 690 kPa on May 4 (pink stage of blossom development), May 16 (petal fall), June 2 (first foliage cover spray), and June 15 (second cover). The active ingredient (a.i.) dosages applied for the DMI materials were those recommended by the manufacturer while for thiophanate-methyl the dosage was that recommended for British Columbia (Anon. 1992).
Secondary powdery mildew development was evaluated on

June 26 by selecting 10 shoots at random on each single tree replicate and determining the incidence and the number of leaves with mildew. Mildew severity was based on visual estimates of the percentage of bottom leaf surface area showing symptoms using 10 increments between 10 and 100%. Severity was the average percent for all of the infected leaves on each tree.

Twenty-five fruit per replicate were harvested on September 11 and each fruit was examined for russet due to mildew. Possible phytotoxic effects that might alter fruit size were assessed using measurements of fruit shape, the ratio of length to diameter, and of fruit weight.

In 1990 the DMI fungicides were tested on the untreated control trees, which served as border trees the previous year. Treatments (Table 4) were applied on April 24 (pink), May 4 (full bloom), May 11 (petal fall), May 23 (first cover) and May 30 (second cover). Secondary mildew development on foliage was evaluated on June 15 and russet and fruit shape and weight were evaluated on September 13.

Trees of cv. Jonathan also were used in this study because this cultivar was reported to be extremely susceptible to powdery mildew (Koepsell and Pscheidt 1990). Six trees were sprayed with flusilazole in 1987, and six alternating control trees were sprayed with myclobutanil in 1988. Flusilazole was applied on April 9 (tight-cluster), April 21 (pink), May 8 (petal-fall), May 22 (first cover) and June 5 (second cover). Foliar powdery mildew (PM) was evaluated on June 15 and fruit PM on September 24. Myclobutanil was applied on April 29 (pink), May 10 (petal fall), May 20 (first cover) and June 1 (second cover). Foliar PM was evaluated on June 17 and fruit PM on September 24.

Results

Greenhouse trials

Powdery mildew colonies developed on every leaf of the unsprayed control plants except leaf -1, which was small and tightly rolled at the time of inoculation (Table 1). Control and treated leaves at positions 2 *to* 4 had many more colonies than younger leaves. For control plants, leaves at position 2 had about 15 colonies while those at 3 or 4 had about 13 colonies. Leaves 1 to 4 treated with fungicide had significantly fewer colonies than comparable control leaves. PM colonies were observed on leaves at position 4 when the plants were sprayed with myclobutanil at 4.50 µg/mL. The six fungicides evaluated in this experiment were equally effective in controlling the formation of colonies at each leaf position.

Conidia per cm^2 of leaf area on the control plants were increasingly more abundant as the age of the leaves increased (Table 2). Each of the five fungicide treatments significantly reduced conidial numbers in leaves at positions 0 to 4. The total number of conidia, averaged per cm^2 of leaf area for the 6 leaves per plant was 88.4, 148.1, 170.4, 354.4 and 518.9 conidia for myclobutanil, triadimefon, propiconazole, flusilazole and thiophanate-methyl, respectively, compared with 1827.1 for the control.

There were 4.0±3.1 visible powdery mildew colonies on control plants 6 days after inoculation and the number increased to 48.2±27.2 by the tenth day (Fig. 1). Thiophanate-methyl or myclobutanil reduced the number of colonies that developed during the 10 days of incubation (Fig. 1). Leaves on plants sprayed with myclobutanil were free of mildew colonies during the first 8 days of inoculation and less colonies were visible after 10 days of incubation (Fig. 1).

Field trials

The effectiveness of the DMI fungicides was shown in field trials on cv. Jonagold in 1989 and 1990 (Tables 3 and 4). In 1989, trees treated with four sprays of myclobutanil (45.0 µg/mL) or triadimefon (37.5 µg/mL) had lower incidences of mildew 7.0 and 8.6%, respectively, compared with the other fungicides and with the 68.6% incidence in the control (Table 3). Myclobutanil and triadimefon also reduced foliar mildew severity to 1.0 and 1.4%, respectively, compared with 18.6% in the control. Fruit weight and shape were not affected by application of these fungicides. Trees sprayed with thiophanate-methyl (350 µg/ml) had a higher incidence of mildew, 37.8%, and disease severity, 7.1%, than plants sprayed with myclobutanil and triadimefon. In 1990, myclobutanil was applied five times at 30.0 µg/mL dosages and gave complete control of foliar mildew. Thiophanate-methyl (350 µg/mL) was less effective than myclobutanil, but reduced mildew incidence to 11.8%, which was lower than that observed in 1989. As in 1989 fruit weight and shape were not affected by fungicide application.

Field trials in 1987 with flusilazole (32.0 µg/mL) and in 1988 with myclobutanil (40.0 µg/mL) on apple cv. Jonathan showed that both these fungicides effectively controlled powdery mildew (Table 5). The incidence of powdery mildew on unsprayed trees was extremely high in 1988 with 87% of the leaves infected and a severity rating of 39%. Myclobutanil reduced the number of infected leaves to 21.7% and the severity to 4.6%. Furthermore, russet due to mildew was reduced from 7.0 to 1.7%. Neither DMI fungicide significantly affected fruit weight and shape and injury was not observed on leaves or fruit.

Discussion

The most effective DMI fungicide in these studies was myclobutanil. In greenhouse tests at a rate of 4.5 µg/mL, myclobutanil allowed only an average of 0.56 mildew colonies per plant compared to **47.1**1 for the control. It also was very effective in reducing sporulation and in delaying the development of powdery mildew colonies. In the 1989 and 1990 field trials with cv. Jonagold, myclobutanil was more effective in reducing mildew development than the other fungicides tested, however, the other DMI fungicides also showed good control of mildew. In greenhouse tests flusilazole, triadimefon and propiconizole were as effective as myclobutanil even at the lower rates that were applied to the plants. In the Jonagold apple field trial of 1989 flusilazole and propiconazole were not as effective as triadimefon or myclobutanil, probably because they were used at considerably lower rates. The manufacturers of flusilazole and propiconazole recommended these rates because there is concern that DMI fungicides will cause phytotoxicity. Roper *et al.* (1985) found, in greenhouse and field tests on powdery mildew with the DMI, etaconazole, that it had growth-inhibiting effects, however, the rate was 260 µg/mL compared to 45 µg/mL for myclobutanil which was the highest rate used in our trials.

Thiophanate-methyl, a benzimidazole fungicide, was not as effective as the DMI compounds. In greenhouse tests thiophanate-methyl at 350 µg/mL provided slightly less effective disease control than the DMIs at less than 5 µg/mL. At those dosages, conidial production (per cm^2 of leaf area) was significantly higher on leaf 3 with thiophanate-methyl than for the three DMIs except flusilazole. Thiophanate-methyl also was not as effective as myclobutanil in reducing colony incidence (Fig. 1). In the 1989 and 1990 field trials thiophanate-methyl at 350 µg/mL was less effective than 45 µg/mL or less of the DMIs. Roper *et a/.* (1985) reported that another benzimidazole, benomyl, did not control powdery mildew as well as the DMI, etaconazole.

Field trials with apple cultivars Jonagold and Jonathan, and greenhouse tests on the highly susceptible McIntosh apple seedlings, have shown that DMI fungicides are very effective for the control of secondary powdery mildew. They provide better control at lower rates than thiophanate-methyl, which has long been the standard fungicide for powdery mildew control in British Columbia. The fact that myclobutanil was the most effective mildewcide in these tests is important and helped to register it for control of apple powdery mildew in Canada. The other DMIs are in development and may be registered in the future. The DMIs have been used to control apple scab (Wilcox *et a/.* 1992) and can be used in spray programs where control of both mildew and scab are required. Thiophanate-methyl is very limited in its control of apple scab due to widespread resistance to benzimidazoles in British Columbia (Sholberg *et al.* 1989).

Phytotoxic effects were not detected on foliage or fruit in any of these trials. Spotts and Cervantes (1986) found that the DMIs tradimefon and etaconazole did not significantly affect fruit set, percent floral buds, or fruit weight and shape. However, they noted that if the application rate was high enough there could be effects on fruit shape, as they found in an experiment when triadimefon was used at twice the rate, 0.28 kg a.i./ha and applied ten times during the season. The myclobutanil label states a rate of 0.14 kg a.i./ha, which provides a wide safety margin before any phytotoxic effects might occur.

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Table 1. Number of colonies of *Podosphaeraleucotricha* on inoculated McIntosh apple seedling leaves after 10 days of incubation in a 22°C greenhouse.

Treatment	Rate a.i. (µg/ml)	Leaf Position*					
		-1	0	1	2	3	4
Control	—	0.0 a**	0.22 a	4.33 a	15.67 a	13.67 b	13.22 a
Myclobutanil	2.52	0.0 a	0.00 a	0.00 b	0.00 b	0.56 b	3.56 b
Myclobutanil	4.50	0.0 a	0.00 a	0.00 b	0.00 b	0.00 b	0.56 b
Flusilazole	1.34	0.0 a	0.00 a	0.00 b	0.22 b	1.00 b	1.44 b
Triadimefon	3.75	0.0 a	0.11 a	0.11 b	0.67 b	3.44 b	3.78 b
Propiconazole	1.16	0.0 a	0.00 a	0.00 b	0.00 b	0.56 b	1.89 b
Thiophanate- methyl	350.0	0.0 a	0.33 a	0.00 b	1.44 b	2.11 b	3.11 b

* Powdery mildew development was monitored on both surfaces of the six leaves at positions -1 to 4 where leaf 0 was the youngest expanded leaf at the time of inoculation and -1 was the adjacent unrolled leaf.

** Treatment means in the same column followed by the same letter are not significantly different at $P < 0.05$ according to the Waller-Duncan K-ratio T test.

Table 2. Number of conidia of *Podosphaera leucotricha* per cm² of leaf area washed from McIntosh apple leaves at different stages of development 10 days after inoculation.

Treatment	Rate a.i. (µg/mL)	Leaf Position*						
		-1	0	1	2	3	4	Total
Control	—	54.5 ab**	137.0	155.3 a	242.0 a	563.9 a	674.4 a	1827.1
Myclobutanil	2.52	7.5 bc	18.1 b	0.0 c	7.1 d	23.2 c	32.5 b	88.4
Propiconazole	1.16	20.8 abc	15.0 b	17.2 bc	20.3 cd	30.7 c	66.4 b	170.4
Flusilazole	1.34	43.5 abc	16.4 b	65.0 b	106.2 b	49.8 bc	73.5 b	354.4
Triadimefon	3.75	0.0 d	13.2 b	14.0 bc	24.7 cd	35.7 c	60.5 b	148.1
Thiophanate-methyl	350.0	67.1 a	63.5 b	61.5 bc	83.6 bc	120.0 b	123.2 b	518.9

* Powdery mildew development was monitored on both surfaces of the six leaves at positions -1 to 4 where leaf 0 was the youngest leaf at the time of inoculation and -1 was the adjacent unrolled leaf.

** Treatment means in the same column followed by the same letter are not significantly different at $P < 0.05$ according to the Waller-Duncan K-ratio T test.

Table 3. Effects of DMI fungicides and thiophanate-methyl on powdery mildew incidence and severity, and on fruit weight and shape of Jonagold apple in field trials in 1989.

Treatment	Rate a.i. (µg/ml)	% Foliage Mildew		Fruit	
		Incidence	Severity	Weight (g)	Shape*
Control	—	68.6 a**	18.6 a	202 a	0.865 a
Myclobutanil	45.0	7.0 f	1.0 d	204 a	0.875 a
Triadimefon	37.5	8.6 ef	1.4 d	214 a	0.861 a
Myclobutanil	25.0	16.8 de	2.6 cd	202 a	0.882 a
Propiconazole	12.0	23.2 cd	3.6 cd	202 a	0.877 a
Flusilazole	13.0	27.6 c	4.3 c	206 a	0.878 a
Thiophanate- methyl	350.0	37.8 b	7.1 b	210 a	0.862 a

* Ratio of length to diameter.

** Numbers followed by the same letter within the columns are not significantly different at P= 0.05 according to Duncan's multiple range test.

Table 4. Effects of DMI fungicides and thiophanate methyl on powdery mildew incidence and severity on foliage, fruit mildew, and on fruit weight and shape of Jonagold apple in field trials in 1990.

Treatment	Rate a.i. (µg/ml)	% Foliage Mildew		Fruit		
		Incidence	Severity	% Mildew	Wt (g)	Shape*
Control	—	49.0 a**	13.18 a	14.0 a	183.0 a	0.883 a
Myclobutanil	30.0	0.0 c	0.00 b	0.0 b	188.8 a	0.887 a
Propiconazole	12.0	2.6 bc	0.40 b	0.0 b	183.4 a	0.891 a
Flusilazole	13.0	5.6 bc	0.80 b	0.0 b	220.0 a	0.893 a
Thiophanate- methyl	350.0	11.8 b	1.44 b	3.0 b	197.4 a	0.896 a

* Ratio of length to diameter.

** Numbers followed by the same letter within the columns are not significantly different at P= 0.05 according to Duncan's multiple range test.

Note: An additional application of propiconazole was applied 30 days before harvest on August 15 and flusilazole was applied four times with the last application on May 23, 1990.

Table 5. Effects of flusilazole and myclobutanil on powdery mildew incidence and severity on leaves, fruit mildew and on fruit weight and shape of Jonathan apple in field trials in 1987 and 1988.

Year	Treatment	Rate a.i. (µg/ml)	Incidence	Severity	Fruit		
					% Mildew	Wt (g)	Shape*
1987	Control	—	68.3 b**	22.5 b	0.0 a	141.1 a	0.838 a
	Flusilazole	32.0	27.4 a	6.2 a	0.0 a	142.5 a	0.831 a
1988	Control	—	87.0 b	39.0 b	7.0 b	124.5 a	0.889 a
	Myclobutanil	40.0	21.7 a	4.6 a	1.7 a	131.1 a	0.873 a

* Ratio of length to diameter.

** Numbers followed by the same letter within the columns are not significantly different at P= 0.05 according to the analysis of variance and **F** test.

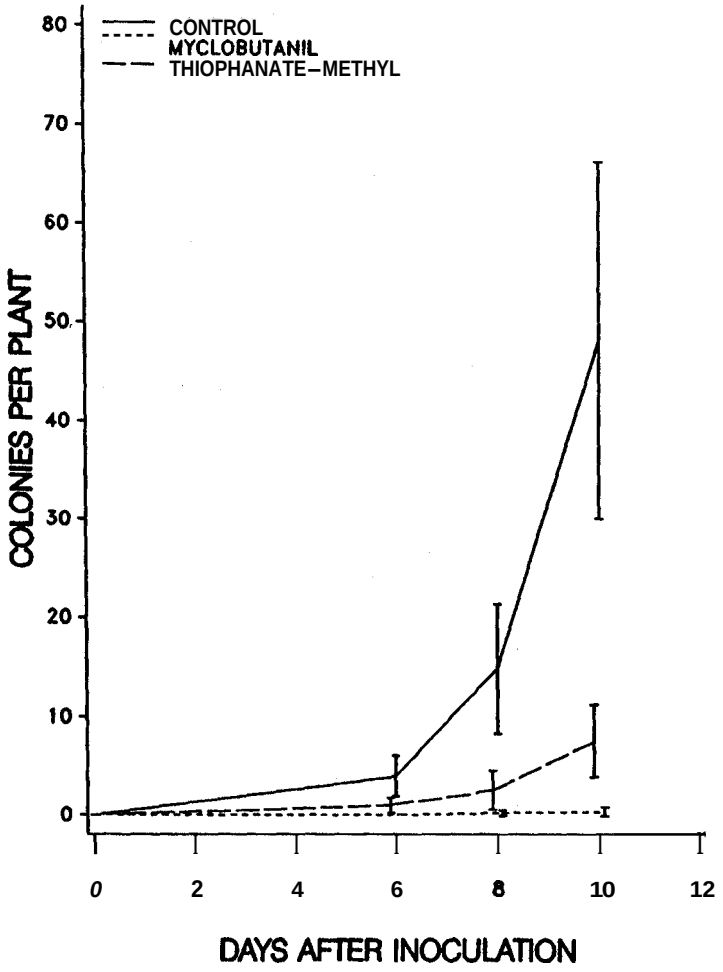

Figure 1. Powdery mildew colonies on McIntosh apple seedlings inoculated in the greenhouse and treated with the registered fungicides myclobutanil (4.5 µg/mL) and thiophanate-methyl (350.0 µg/mL). Myclobutanil is a DMI fungicide and thiophanate-methyl is a benzimidazole.