New Fungicides for Apple and Pear Diseases

This workshop, sponsored by the Apple and Pear Disease Workers, was held at the 72nd Annual Meeting of The American Phytopathological Society in Minneapolis, Minnesota, on 24 August 1980. One objective was to summarize basic and applied research on the control of apple and pear diseases with new fungicides. Fungicides that inhibit ergosterol biosynthesis were emphasized in the workshop because several compounds in this group should be released for commercial use on tree-fruit crops during the next few years. We hope that this workshop will help chemical manufacturers to develop and label these new fungicides more quickly in the years ahead. The workshop committee, consisting of A. L. Jones, Michigan State University, and T. B. Sutton, North Carolina State University, is grateful to the speakers and to the chairmen of the morning and afternoon sections for their participation. We extend thanks to the Program and Local Arrangements Committee of The American Phytopathological Society for help in arranging for the facilities for this workshop and to M. C. Shurtleff for his help in arranging publication of these papers.

Physical Modes of Action of Sterol-Inhibiting Fungicides Against Apple Diseases

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ABSTRACT

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Sterol-inhibiting CGA 64251, bitertanol, fenarimol, fenapanil, prochloraz, triadimefon, and triforine were evaluated on apple in the greenhouse. Against *Venturia inaequalis*, they were more effective in eradicative than in protective action. They inhibited spore production when applied before scab symptoms appeared but not when applied to existing scab lesions. Against *Podosphaera leucotricha*, CGA 64251, bitertanol, and triadimefon sprays gave excellent protection. Vapor from CGA 64251 and triadimefon gave very good protection against mildew on unsprayed trees.

During the past decade, a new family of fungicides has come into prominence. These fungicides are categorized as sterol inhibitors because of their biochemical mode of action in the inhibition of ergosterol synthesis. This paper deals with the physical mode of action of seven of these chemicals, namely, 1-[[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-yl]methyl]-*H*-1,2,4-triazole (Ciba-Geigy CGA 64251; Vangard), bitertanol (Baycor), fenarimol (Eli Lilly EL 222), fenapanil (Rohm and Haas RH 2161), prochloraz (Boots Hercules BFN 8206), triadimefon (Bayleton), and triforine (Funginex). These chemicals were evaluated rigorously in the laboratory and greenhouse on potted apple trees for their relative strengths and weaknesses in regard to protection and tenacity, after-infection activity, presymptom activity, and postsymptom activity against the apple scab pathogen *Venturia inaequalis* (Cke.) Wint. They were also evaluated for protective and vapor action against powdery mildew caused by *Podosphaera leucotricha* (Ell. & Everh.) Salm.

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MATERIALS AND METHODS

Specific experimental procedures exploring each mode of action of fungicides are governed by conditions affecting the fungal pathogen. This relationship of fungicidal activity to fungus activity is depicted in Fig. 1. Laboratory control of plant material, fungal inoculum, temperature, wetting, spraying, timing, and other variables allows the researcher to manipulate the procedure for each mode of action. The following sections on definition and procedure for each mode of action are brief because they are covered in detail elsewhere (3,4).

Apple scab. *Protection (retention).* In protection, the fungicide is applied before or during an infection period so that the chemical can kill or inactivate the spores and prevent infection of the plant (Fig. 1). The evaluative technique helps determine not only the protective mode of action of the fungicide at specific dosage rates but also its retentiveness on the leaf surface.

I made a precision application of the dilute fungicidal spray at the rate of 10 mg of dilute spray of formulated fungicide per square centimeter of leaf surface. This deposit was verified by weight on tared glass slides sprayed at the same time as the trees of each treatment. This rate did not allow for runoff from the leaf. After overnight drying, the trees received a 5-cm, simulated overhead rainfall; after 3 hr of drying, they were mass inoculated by a paint sprayer with a suspension of 70,000 *V. inaequalis* conidia per milliliter. Ideal conditions for infection were provided in the mist chamber at 20 C for 30 hr, after which the trees were returned to the greenhouse for normal maintenance. Lesion counts were made 2 wk after treatment.

After-infection activity. After-infection activity is the capability of a fungicide applied at different times (hours, days) after infection to inhibit fungal development and prevent the establishment of scab lesions. This activity has also been referred to as eradication, curative action, kickback, and intervention. In

practice, each fungicide has been categorized according to the duration of after-infection activity measured from the beginning of the wet period because the start of wet conditions is much more precisely identified than is the calculated time of actual infection.

To evaluate after-infection activity, I mass inoculated the apple trees and placed them in the mist chamber for an infection period as described under protection. For fungicidal treatments made at specific times up to 30 hr after inoculation, the trees were withdrawn from the mist chamber at the desired time and immediately sprayed with the dilute fungicide. They were then returned to the greenhouse. For longer interval studies, the trees were returned to the greenhouse after the 30-hr infection period and sprayed at the desired time interval. Freshly sprayed trees were allowed to remain wet for a minimum of 15 min to allow for fungicidal effect. Scab lesion counts were made about 2 wk after inoculation.

Presymptom activity. Presymptom activity is an elaboration of after-infection activity. Some fungicides applied beyond their limit of after-infection activity have no meaningful effect on the development of typical olive-colored, sporulating scab lesions. Others so applied do not prevent lesion manifestation; however, the lesions are atypical and appear as chlorotic spots or flecks that produce few or no secondary conidia. This is defined as presymptom activity. Depending on the fungicide, dosage rate, and timing of spray, it is not unusual for presymptom activity to overlap with the actual after-infection activity.

This procedure calls for inoculation of apple trees as explained under protection. The dilute fungicidal spray was applied 5–7 days after inoculation and infection. This interval exceeds the after-infection limit of all fungicides tested in the greenhouse to date. It also precedes scab symptom appearance, which, under greenhouse conditions, occurs 8 days or more after inoculation. Two weeks after inoculation, the inoculated and sprayed leaves were collected. In the laboratory the spores were harvested with atomized water, and counts of a standardized volume of spore suspension were made with a haemocytometer. Spore counts and the actual lesion count on the same leaves provided information on production of spores per lesion and, hence, the level of presymptom activity of the fungicide.

Postsymptom activity. In postsymptom activity, the fungicide applied to sporulating scab lesions prevents or greatly inhibits further production of new conidia from the same lesions. To evaluate postsymptom activity, I inoculated the apple trees and placed them in the mist chamber for an infection period as described under protection. About 2 wk later, when abundant sporulating lesions were present on the foliage, the conidia were removed by the pressure of atomized water applied by a paint sprayer. Each fungicidal treatment was made as a dilute spray within 10 min of spore removal. After three more days the leaves were collected, the spores harvested and counted, and leaf lesion counts were made. The count of spores per lesion revealed the postsymptom activity of the fungicides tested.

Powdery mildew. McIntosh apple seedlings were used for these studies because they are readily infected with powdery mildew in the greenhouse, and the lesions can be counted; more susceptible cultivars are prone to severe leaf distortions. The seedlings were first grown in greenhouse rooms where the relative humidity and inoculum level were usually too low for significant mildew development.

Protection. About 5 days before the start of fungicidal treatments, the potted trees were transferred to a "mildew greenhouse" where warm temperature and high humidity, together with abundant inoculum from previously infected trees, encouraged mildew infection. When initial secondary mildew lesions appeared on existing foliage, the youngest leaf on every tree was marked and the spray experiment initiated. Each fungicide was applied as a thorough, dilute spray to the top and bottom surface of all leaves with a paint sprayer (6). The day after treatment, all the trees received a 7-mm overhead rainfall. This spray-rainfall procedure was repeated weekly for five consecutive weeks. At the time of the last spray, the youngest leaf was marked; after allowing at least five more days for full symptom expression, mildew lesion counts were taken on all the new leaves that developed during the course of the multiple spray program.

Vapor action. Initial tests on vapor action were conducted in closed fumigation chambers (5). The most effective mildewcide was later evaluated in larger scale greenhouse tests with a treated cheesecloth canopy over a large group of trees.

The circular fumigation chamber was constructed of a plywood top and floor ring, approximately 80 cm in diameter, with a vertical separation of 60 cm maintained by threaded rods (Fig. 2). A flexible, translucent, fiberglass sheet of 60 cm width encircled the unit, and the overlapped ends were kept in place with several spring-hook catches. The open base of the chamber was placed on crushed stone on the greenhouse bench. Water seeping from a bleed irrigation hose (dew hose) embedded in the crushed stones kept the stones wet, thus maintaining 100% relative humidity within the enclosed chamber throughout the test.

At the start of the test, several pots of trees that were essentially free from mildew received a dilute spray of the test fungicide and were placed immediately in the fumigation chamber. Other mildew-free trees without treatment (including water) were placed in the chambers but not in direct contact with the sprayed trees. Mildewed trees were also placed in the chamber to provide inoculum. Just before closing the fumigation chamber, all trees received a liberal inoculation by having spores blown onto them from dry seedlings with abundant new infections. The trees were kept in the closed chamber 2–4 days and then returned to a mildew-free house where lesion counts were made about 1 wk later on the youngest susceptible leaves that were exposed to the fungicide vapor.

In studies with the fumigation canopy, the fungicide with the most consistent record of effective mildew control in the fumigation chambers—CGA 64251—was used. In the two experiments reported here, McIntosh apple seedlings relatively free of powdery mildew were transferred to the "mildew greenhouse" on the day of

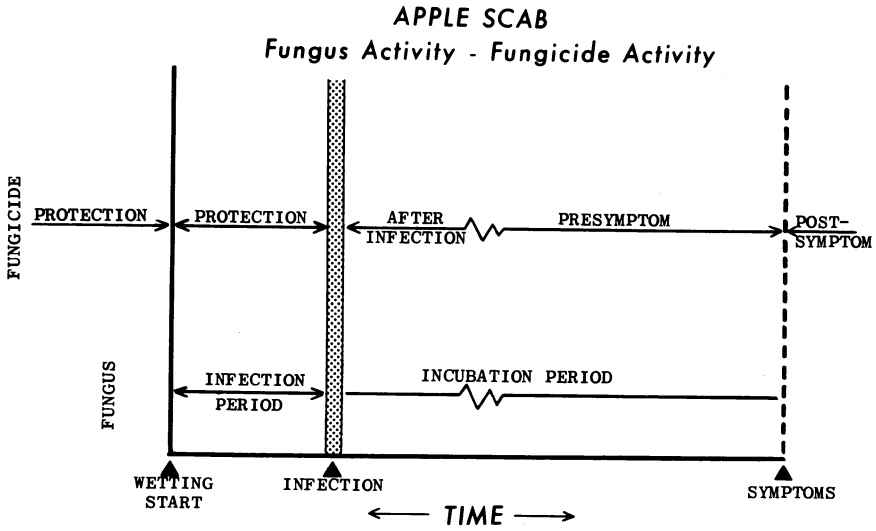

Fig. 1. Relationship of fungicidal modes of action to apple scab fungus activity.

treatment. A wooden framework over the trees made it possible to stretch a pretreated cheesecloth to form a canopy over the top and two sides of a group of trees. Two sides were left open (Fig. 2). About fifty 15-cm pots of three seedlings each, occupying a greenhouse bench area of 140 × 180 cm, formed the treatment group; a similar number was in the control group.

The cheesecloth was first soaked in a suspension of the fungicide, with the excess squeezed out to prevent any drip of the fungicide directly onto the trees. This cheesecloth was immediately stretched and stapled onto the wooden framework to form the canopy. The top was approximately 15 cm above the top of the tallest seedlings. Similarly, cheesecloth was soaked in distilled water, squeezed out, and stretched onto a frame over control trees on the bench adjacent to the chemical treatment group. Just before placement of the canopy, all trees of both groups were inoculated liberally by having spores blown onto them from dry, heavily mildewed trees. Many infected trees in the greenhouse room provided additional inoculum via air currents. The greenhouse room was misted overnight the day of treatment and 2 days later. After 2 wk, the cheesecloth canopies were removed and powdery mildew leaf lesions were counted.

RESULTS AND DISCUSSION

Apple scab. I used a whole-number control rating, ranging from 0 to 10 with 0 representing complete control, to compare fungicidal performance in controlling apple scab (Table 1). This rating, developed from $\log_{10}$ paper and an arbitrary zero, provides a weighted comparison of fungicidal efficacy. A control rating is calculated from the raw lesion counts for each treatment in each experiment, based on the severity of scab on the water-sprayed control trees in that experiment. The control rating for each fungicide in the tables is the average for a minimum of three experiments in each mode of action category. The minimum percentage of control for control ratings 0, 1, 2, etc., is 100, 98, 94, 89, 83, 76, 68, 59, 47, 29, and 0, respectively.

Protection (retention). Sterol-inhibiting fungicides in general were weak to ineffective protectants against apple scab (Table 1). With control ratings of 4–10, their protective action was decidedly inferior to that of mancozeb, captan, dodine, dichlone, and glyodin, which had ratings of 1–3. One of the sterol inhibitors —CGA 64251—stood uniquely apart from the others in protection. At 30 μg/ml, it gave a fairly good measure of protection (control rating of 4) compared with fenarimol, bitertanol, triadimefon, fenapanil, triforine, and prochloraz, which gave very little or no meaningful protection against scab at active rates of 167–300 μg/ml. The fungicide retention technique depends highly on the kill or inactivation of spores. Most of the sterol inhibitors tested did not effectively prevent conidial germination and, therefore, did not prevent infection.

After-infection activity. The after-infection activity of sterol inhibitors exceeded that of protection (Table 1). In this mode of action, they outperformed standard fungicides like mancozeb, captan, and glyodin, whose strength lies largely in protection against apple scab. CGA 64251 and fenarimol showed a potency equivalent to that of phenylmercuric acetate, the unique eradicative fungicide used in apple orchards for two decades preceding discontinuance by regulatory action about 1971. In fact, these two sterol inhibitors exceeded the performance of phenylmercuric acetate at 30 μg/ml in applications 2 and 3 days after inoculation (Table 2). The eradicative potency of the organic mercurials has not been equalled before now by any approved fungicide. Other sterol inhibitors, although less effective than CGA 64251 and fenarimol, had an after-infection potency equivalent to that of dichlone. Dichlone is the most effective after-infection scab fungicide among all fungicides currently approved for use on apple.

Presymptom activity. All seven sterol inhibitors tested shared with dodine and benomyl a high level of presymptom activity (Table 1). This activity was lacking in captan, mancozeb, dichlone, and glyodin. The sterol inhibitors

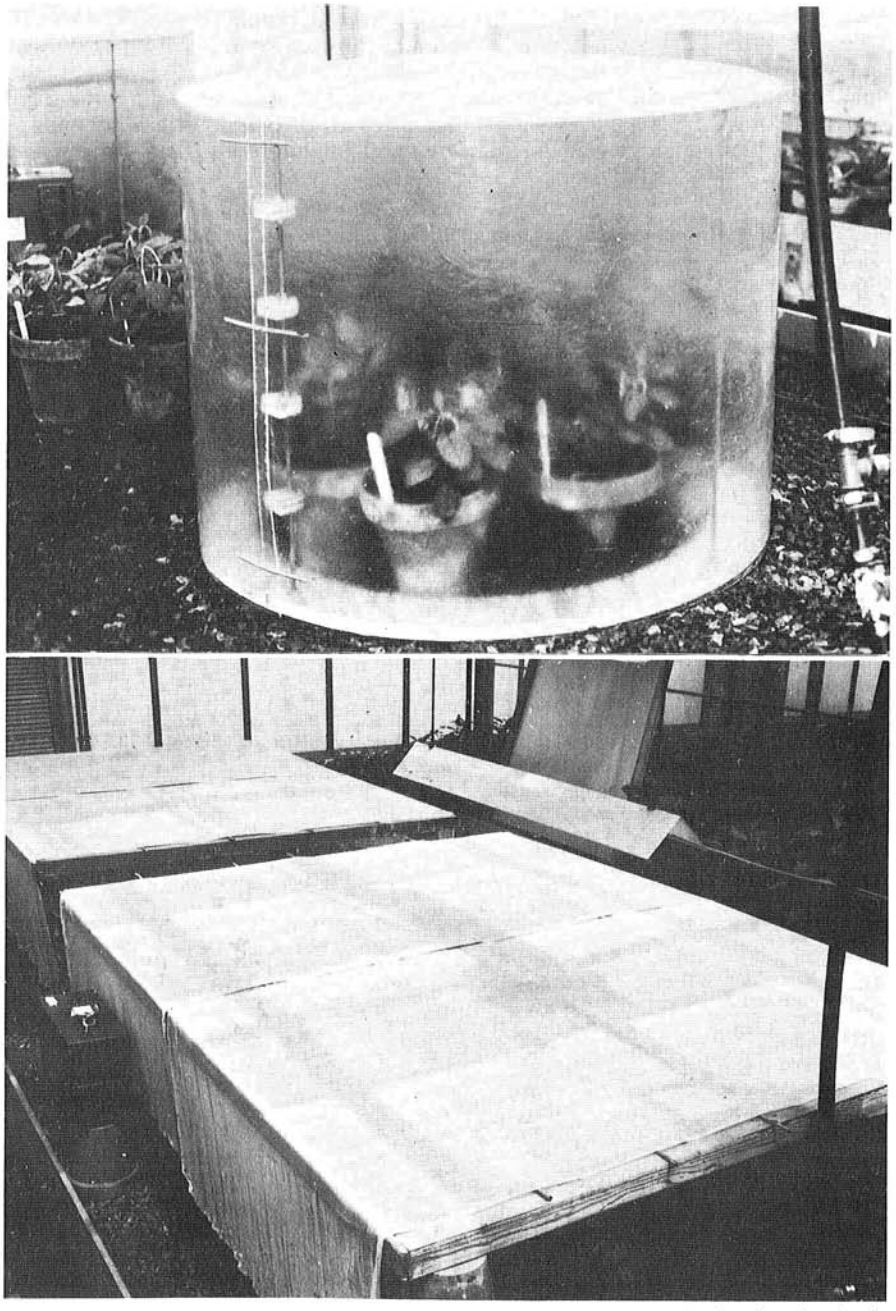

Fig. 2. Control of apple powdery mildew by fungicidal vapor action. **(Top)** Fumigation chamber in which untreated trees are protected by vapor from those treated. **(Bottom)** Fungicide-treated cheesecloth canopy supplying vapors that protect untreated trees beneath it.

evidently have a potent and adverse action against the established scab fungal mycelium during incubation. Different rates of the sterol inhibitors have not yet been fully evaluated for presymptom activity, but this level of activity is probably proportionate to the active rate of the toxicant, as has been demonstrated for dodine and benomyl.

The specific benefit derived from presymptom activity comes from a great reduction in secondary scab inoculum, which then reduces the pressure on fungicidal sprays applied later. Presymptom activity has been recognized in the orchard performance of fungicides, but further studies need to be made to define more clearly the value of this activity as influenced by such factors as dosage rates, timing, moisture requirements, duration of incubation periods, redistribution of fungicides, and effective threshold levels.

Postsymptom activity. All seven sterol inhibitors tested failed in postsymptom activity against apple scab (Table 1). Continued production of conidia from existing active scab lesions was not abated by these fungicides, which is in sharp contrast to their presymptom activity. They differed greatly from dodine, benomyl, phenylmercuric acetate, dichlone, and glyodin, which performed effectively in the postsymptom mode of action. Of the fungicides evaluated, only dodine, benomyl, and phenylmercuric acetate were very active in both the presymptom and postsymptom modes of action. Unfortunately, dodine and benomyl are prone to pathogen resistance. As noted above, the benefit from postsymptom activity lies in the reduction of pathogen inoculum pressure and improvement of chances for better scab control with succeeding sprays.

Powdery mildew. Bitertanol, triadimefon, and CGA 64251 applied weekly as a dilute spray were highly effective in protecting apple foliage (Table 3). Control of mildew was greater than that obtained with the standard mildewcides sulfur, benomyl, and dinocap. That differences in efficacy within the family of sterol inhibitors do occur was evident from the lower activity of triforine, which provided control equivalent to that of dinocap rather than to that of the three sterol inhibitors noted above.

A growth regulator response was evident on seedlings receiving repeated applications of CGA 64251. The plants were shorter than those receiving other effective mildewcides. The leaves were also smaller, somewhat cupped, less flexible, and darker green than those of other treatments. These responses were not observed on apple trees in the apple scab experiments involving just a single application of the sterol inhibitors.

Within the enclosed fumigation chambers, the chemical vapor emission from trees sprayed with either CGA 64251 or triadimefon gave substantial protection against powdery mildew to untreated trees in the same chamber (Table 4). These mildewcides also produced marked differences in vapor action among sterol inhibitors, as evident by the occurrence of only 3 or 4 mildew lesions per leaf when compared with 17 lesions for fenarimol and 41 for triforine. The fenarimol activity was about the same as sulfur, and the triforine was about as inactive in this mode of action as was dinocap. No growth regulator response was observed on sprayed trees or on untreated trees exposed to the chemical vapor inside the chamber. Although it is common knowledge that sulfur vapor controls powdery mildew, it appears that temperatures higher than

Table 1. Fungicidal modes of action against apple scab

Treatment	Dose (μg a.i./ml)	Control rating[a]			
		Protection[b]	After infection[b]	Pre-symptom[c]	Post-symptom[c]
Water	...	10	10	10	10
Mancozeb	1,440	1	10	10	9
Captan	1,200	2	10	10	10
Dodine	293	2	7	tr	1
Dichlone	150	3	5	9	1
Glyodin	720	3	10	10	1
Phenylmercuric acetate	30	4	tr	0	1
CGA 64251	30	4	tr	0	10
Benomyl	225	7	7	0	1
Fenarimol	40	9	tr	0	10
Bitertanol	300	9	6	tr	10
Triadimefon	300	9	6	tr	10
Fenapanil	300	9	5	1	10
Triforine	167	10	5	2	10
Prochloraz	300	10	8	0	10

[a] Ratings of 0, 1, 2, etc., are equivalent to a minimum percentage of disease control of 100, 98, 94, 89, 83, 76, 68, 59, 47, 29, and 0, respectively; tr = trace, or >99.5%.
[b] Based on control of scab lesion development.
[c] Based on inhibition of spore production from scab lesions.

Table 2. After-infection mode of action against apple scab of fungicides applied 1–3 days after inoculation

Treatment	Dose (μg a.i./ml)	Scab control rating[a] for spray applied at		
		1 day	2 days	3 days
Water	...	10	10	10
CGA 64251	30	tr	1	tr
Fenarimol	40	1	tr	tr
Phenylmercuric acetate	30	tr	2	7
Fenapanil	300	5	4	6
Dichlone	150	5	8	10
Triforine	167	5	8	9
Bitertanol	300	6	7	8
Triadimefon	300	6	8	10
Dodine	293	7	8	10
Benomyl	225	7	8	9
Prochloraz	300	8	7	10
Captan	1,200	10	9	10

[a] Ratings of 0, 1, 2, etc., are equivalent to a minimum percentage of disease control of 100, 98, 94, 89, 83, 76, 68, 59, 47, 29, and 0, respectively; tr = trace, or >99.5%.

Table 3. Fungicidal protective action in the control of apple powdery mildew

Treatment	Dose (μg a.i./ml)	Leaves with powdery mildew (%)
Water	...	100
Bitertanol	150	1
Triadimefon	150	1
CGA 64251	30	2
Sulfur	900	6
Dichlone	300	18
Benomyl	225	23
Triforine	167	49
Dinocap	75	50
Captan	1,200	99

Table 4. Vapor action of fungicides in a closed fumigation chamber in the protection of apple trees against powdery mildew[a]

Treatment	Dose (μg a.i./ml)	Powdery mildew	
		Leaves infected (%)	Lesions per leaf (no.)
Water	...	99	50
CGA 64251	7.5	56	3
Triadimefon	18.8	59	4
Sulfur	900.0	87	13
Fenarimol	9.4	92	17
Dinocap	75.0	90	27
Triforine	83.7	98	41

[a] Data from untreated trees in the same chamber, but not in contact, with those treated.

Table 5. Vapor action of CGA 64251 from a pretreated cheesecloth canopy in the control of powdery mildew on untreated apple trees in the greenhouse

Treatment of canopy	Powdery mildew	
	Leaves infected (%)	Lesions per leaf (no.)
Test I		
Water	88	29
CGA 64251, 30 μg a.i./ml	64	12
Test II		
Water	75	19
CGA 64251, 30 μg a.i./ml	40	3

the 23 C in these fumigation chambers would be needed for greater activity by sulfur.

Although the cheesecloth canopy impregnated with CGA 64251 did not form a closed chamber, it allowed an ample vapor environment among the trees below to provide substantial mildew control. In contrast, trees beneath the adjacent canopy without chemical treatment had a high level of mildew (Table 5). Not only were fewer leaves infected with CGA 64251, but the number of lesions per infected leaf was greatly reduced under the canopy treated with the sterol inhibitor. No growth regulator effect from the chemical vapor was evident. The favorable results suggest realistic benefits from vapor activity of sterol inhibitors in the control of powdery mildew diseases in the greenhouse and under certain circumstances in the field.

CONCLUSION

The sterol synthesis inhibitors are broad-spectrum fungicides. In addition to the activity reported here against apple scab and powdery mildew, several of these chemicals provide good to excellent protective, after-infection, or presymptom activity against cedar-apple rust incited by *Gymnosporangium juniperi-virginianae* and cherry leaf spot incited by *Coccomyces hiemalis* (1,2). They also provide excellent control of brown rots of stone fruits incited by *Monilinia fructicola* through both protective and after-infection activity (1). Such activity against a number of unrelated fungal pathogens stresses the potency of the sterol-inhibiting action. The broad spectrum also suggests that other biochemical modes of action may be involved.

From the standpoint of continued agricultural production, it is encouraging to have promising new fungicides that may be added to the depleted arsenal of fruit disease control materials. The depletion of the arsenal has stemmed from discontinuance of registered fungicides because of unfavorable efficacy, termination of patents, actions of regulatory agencies, and development of fungal resistance. Two major apple scab fungicides, dodine and benomyl, can no longer be readily recommended in certain apple-growing areas because of resistance. Sterol-inhibiting fungicides appear to hold much promise, particularly through their after-infection and pre-symptom modes of action. They can be successfully integrated into effective orchard disease management programs through current high-concentrate, low-volume spray application programs.

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