Greenhouse Evaluation of the Curative and Protective Action of Sterol-Inhibiting Fungicides against Apple Scab

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ABSTRACT

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Etaconazole at 15 μg/ml, 1-[2-(2,4-dichlorophenyl)-pentyl]1*H*-1,2,4-triazole (CGA 71818) at 25 μg/ml, fenarimol at 37.5 μg/ml, and bitertanol at 125 μg/ml in combination with an adjuvant at 1 ml/L were highly effective in preventing the formation of apple scab lesions on apple leaves when applied up to 72 hr after the onset of infection periods at 15 C. Applications 120 hr after inoculation resulted in the formation of many chlorotic lesions. With bitertanol at 125 and 250 μg/ml and triforine at 234 μg/ml, chlorotic lesions developed if applications were delayed 24 hr or more after inoculation. Production of conidia was almost completely inhibited by all of the sterol-inhibiting fungicides, even when applied 120 hr after inoculation. Inadequate control was obtained with postinfection applications of dithianon. The protective activity of triforine, etaconazole, bitertanol plus adjuvant, fenarimol, and CGA-71818 was found to decrease faster between application and inoculation than that of captan and mancozeb.

Additional key words: *Malus* spp., *Venturia inaequalis*.

After the introduction of triforine in the late 1960s, other sterol-inhibiting fungicides with different chemical characteristics were released for evaluation. Several of these fungicides were highly active against the apple scab fungus *Venturia inaequalis* (Cke.) Wint. (1,2,4,6,10–13,15–17). These fungicides prevented the formation of visible lesions when applied within about 3 days after inoculation (1,6,10,11,15), and only chlorotic flecks or spots developed if they were applied later than 3 days after the onset of infection, but before symptoms were visible. The unique postinfection control activity or curative action of these fungicides was observed by several workers (1,6,10,11,15), but quantitative data on the extent to which they inhibited the production of conidia were limited. Schwabe (10) quantified the level of spore inhibition with triforine, bitertanol, and fenarimol, but these studies were conducted by using relatively high rates of the latter two fungicides. Szkolnik (15) also reported on the antisporulant activity of some sterol-inhibiting fungicides.

The efficacy of the sterol-inhibiting fungicides as protective fungicides has received less attention than their postinfection activity. Schwabe (9), Schwabe and Jones (11), and Szkolnik (15) reported that the sterol-inhibiting fungicides they studied were weak protectants, but Drandarevski and Schicke (2) and Brandes and Paul (1) reported that triforine and bitertanol, respectively, were good protective fungicides. Unfortunately, these latter two papers lacked sufficient data to support the conclusion that these fungicides were good protectants.

This report summarizes the results of greenhouse experiments with several sterol-inhibiting compounds and elaborates on their effectiveness for preventing the establishment of scab lesions, their antisporulant activity, and their protective activity. Some of these tests are with rates or fungicides (CGA-71818) not evaluated previously.

MATERIALS AND METHODS

The techniques used for evaluating the curative and protective action of the sterol-inhibiting fungicides were mentioned briefly in previous reports (9,10). They are described more fully here.

Plant material and inoculum. Ungrafted trees of MM 109 clonal rootstock with three to five actively growing shoots per tree were used throughout this investigation. Trees were grown in 175-mm-diameter pots containing 2 L of soil and fertilized every 2 wk with a balanced nutrient solution (3). Twelve single-tree replicates per treatment were used in all experiments except one, in which 18 replicates were used.

Infected leaves were obtained from potted clonal cultivar MM 109 trees which had been inoculated 2–3 wk earlier and kept in a greenhouse during incubation. Inoculum was prepared by vigorously shaking infected apple leaves in tap water and filtering the spore suspension through muslin to remove debris. The concentration of conidia was determined with a hemacytometer and the suspension was diluted to 10^5 spores per milliliter with water. Viability of spores was checked by determining the percentage germination after 24 hr on water agar.

Test fungicides and application. The following fungicides were tested: bitertanol (Baycor 25 W) from Bayer, South Africa, P.O. Box 1366, Johannesburg, South Africa 2000; captan (Orthocide 50 W) from AE and CI, P.O. Box 1122, Johannesburg, South Africa 2000; triforine (Denarin 18% EC) and dithianon (Delan 75 W) from Celamerck, P.O. Box 20096, Alkantrant, South Africa 005; etaconazole (Sonax 10 W) and 1-[2-(2,4-dichlorophenyl)-pentyl]-1*H*-1,2,4-triazole (CGA-71818, Topas 5W) from Ciba-Geigy, P.O. Box 92, Isando, South Africa 1600; fenarimol (Rubigan 12.5 EC) from Elanco, P.O. Box 98, Isando, South Africa 1600; and mancozeb (Dithane M-45 80 W) from Rohm and Haas, P.O. Box 2356, Primrose, South Africa 1416. In some experiments, the adjuvant Agri-Dex® (Bayer, South Africa) was combined with bitertanol. All fungicides were applied as dilute sprays to runoff with a hand sprayer at 28 kg/cm^2.

Evaluating curative action. Experimental trees were placed in an inoculation chamber at 15 C, inoculated with a conidial suspension, and exposed to wet periods of various durations with an overhead irrigation system programmed to operate 20 sec every 30 min. This system delivered about 8 mm of water every 24 hr. Fungicides were applied 24, 48, 72, and 120 hr after inoculation to groups of trees exposed to 24, 48, 72, and 72 hr of wetting,

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respectively. Trees sprayed 120 hr after inoculation were exposed to a 72-hr wet period followed by a 48-hr dry period at 15 C. Nonsprayed control trees were maintained at all time intervals.

Evaluating preventive action. To determine the preventive action of the sterol-inhibiting fungicides, treatments were applied 2–4, 24, 48, 72, 96, and 120 hr before inoculation. During the preinoculation period, trees were placed outside the greenhouse. Records were kept on the number of leaves that unfolded between the longest time of spraying and inoculation. Following inoculation, all trees were kept continuously wet for 48 hr at 15 C. Scab development was assessed after the trees were incubated in a greenhouse at approximately 20 C for 2–3 wk.

Disease assessment and data analysis. Scab development was determined by counting the number of lesions on seven leaves per shoot and calculating the percentage scab per tree using the method of Kremer and Unterstenhöfer (7). To determine conidial production, seven leaves were picked from each shoot, leaves from three trees were pooled, weighed, suspended in 250 ml of water, and shaken for 1 min to suspend the conidia. Spore concentrations were determined with a hemacytometer and the results were expressed as the numbers of conidia per milliliter from 10 g of leaf tissue.

Percent disease control was based on the difference between disease incidence in the control and each fungicide treatment. Differences were divided by the disease incidence in the control and multiplied by 100. Percent reduction in number of conidia produced was computed similarly.

RESULTS

Curative action. Three experiments were conducted to evaluate the curative action of the sterol-inhibiting fungicides 24–120 hr after inoculation (Figs. 1–3). In the first experiment, fenarimol at 25 and 37.5 μg/ml were similarly effective at most intervals, but the lower rate was less effective when applied 48 hr after inoculation (Fig. 1). Etaconazole at 15 μg/ml reduced the scab incidence 96% when applied 24–72 hr after inoculation and 84% when applied 120 hr after inoculation. Etaconazole was more effective at 15 μg/ml than at 7.5 μg/ml and equally as effective as fenarimol at 37.5 μg/ml. Triforine at 234 μg/ml provided less disease control than fenarimol and etaconazole at all delay intervals. Disease development in trees treated with dithianon was high, even at the 24-hr delay interval. Despite differences in disease control among treatments, all treatments except dithianon reduced conidial

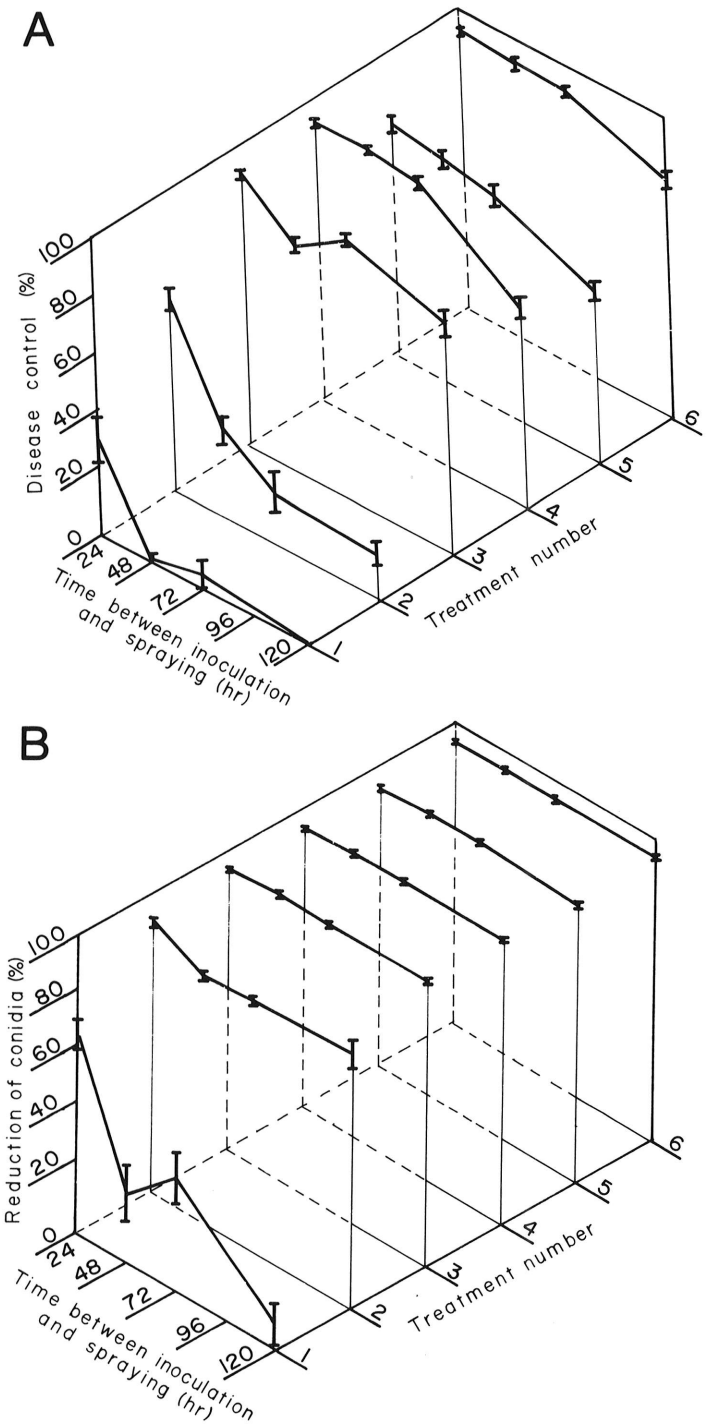

Fig. 1. Curative control of scab on leaves of potted cultivar MM 109 rootstock apple trees in the greenhouse with various fungicide treatments applied at various times after inoculation. Treatments were: 1 = dithianon 562.5 μg/ml; 2 = triforine 234 μg/ml; 3 = fenarimol 25 μg/ml; 4 = fenarimol 37.5 μg/ml; 5 = etaconazole 7.5 μg/ml; and 6 = etaconazole 15 μg/ml. **A,** Disease reduction. **B,** Reduction in numbers of conidia produced. I = standard error of the mean. (Experiment 1)

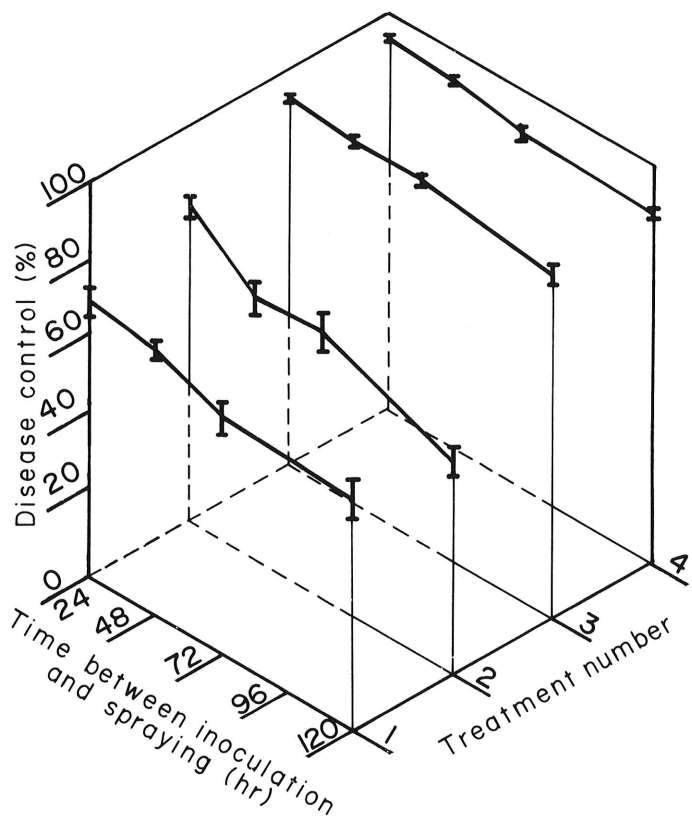

Fig. 2. Curative control of scab on leaves of potted cultivar MM 109 rootstock apple trees in the greenhouse with various fungicide treatments applied at various times after inoculation. Treatments were: 1 = triforine 234 μg/ml; 2 = bitertanol 250 μg/ml; 3 = etaconazole 15 μg/ml; and 4 = CGA-71818 25 μg/ml. I = standard error of the mean. (Experiment 2)

production 85–99% at all time intervals compared to the control (Fig. 1B).

In the second experiment, etaconazole at 15 μg/ml and CGA-71818 at 25 μg/ml were equally effective when applied 24–120 hr after inoculation (Fig. 2). Both fungicides reduced disease development more at all time intervals than bitertanol at 250 μg/ml and triforine at 234 μg/ml. At 24 hr after inoculation, bitertanol provided 84% disease control compared to 73% for triforine. At 48–120 hr after inoculation, bitertanol and triforine were equally effective. Conidial production was reduced 99–100% by all treatments at all time intervals.

In the third experiment, bitertanol plus the adjuvant and etaconazole at 15 μg/ml were equally effective and reduced disease development more than bitertanol alone (Fig. 3). Disease control with CGA-71818 was sporadic and disease development was higher at the 24-hr delay interval than at the 48- and 72-hr intervals. Disease development in trees treated with triforine was high at all time intervals. Again, conidial production was reduced 95–100% by all treatments at all time intervals.

Protective action. This experiment consisted of two trials. In trial A, mancozeb gave 98–100% control of scab when applied 3–72 hr before inoculation, 86–92% control when applied 96 hr before inoculation, and 68% control when applied 120 hr before inoculation (Fig. 4). Triforine at 234 μg/ml provided less disease control at each application interval before inoculation. In trial B, etaconazole at 15 μg/ml gave 93–100% control of scab when applied 3–48 hr before inoculation and was equally as effective as mancozeb. However, mancozeb prevented disease more effectively than etaconazole when applied 72–120 hr before inoculation. In trials A and B, an average of 2.3 and 3.5 leaves unfolded per shoot during the 96 and 120 hr preinoculation periods, respectively.

In the last experiment, captan and mancozeb were equally effective at each time period between spraying and inoculation (Fig. 5). Fenarimol applied 3 and 24 hr before inoculaton was equally as effective as captan and mancozeb and more effective than fenarimol applied 48–120 hr before inoculation. More disease developed in trees treated with CGA-71818 24 hr before inoculation than in trees treated 3 and 48 hr before inoculation. More disease developed in trees treated with CGA-71818 72–120 hr before inoculation than in trees treated with captan or mancozeb. Disease control with bitertanol at 62.5 μg/ml in combination with

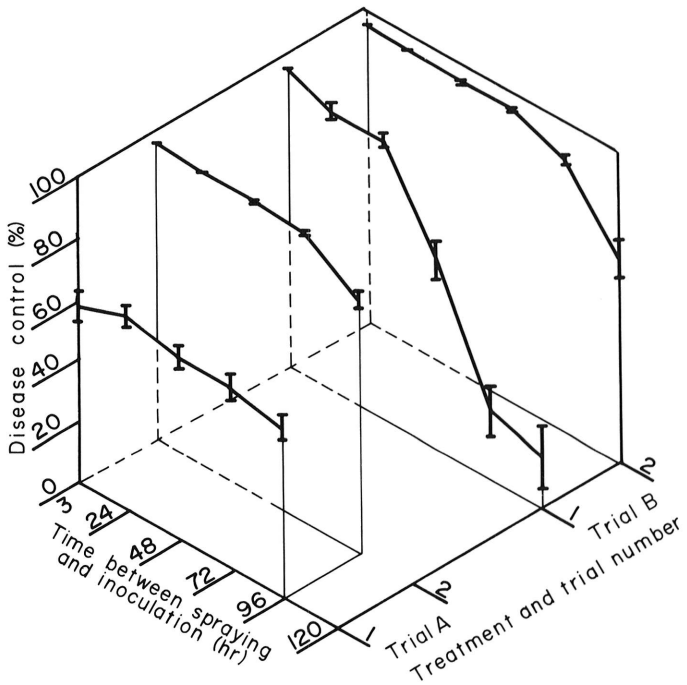

Fig. 4. Protective control of scab on leaves of potted cultivar MM 109 rootstock apple trees in the greenhouse with fungicides applied at various times before inoculation with conidia of *Venturia inaequalis*. Treatments 1 and 2 were triforine 234 μg/ml and mancozeb 1,200 μg/ml, respectively, in trial A and etaconazole 15 μg/ml and mancozeb 1,200 μg/ml, respectively, in trial B. I = standard error of the mean.

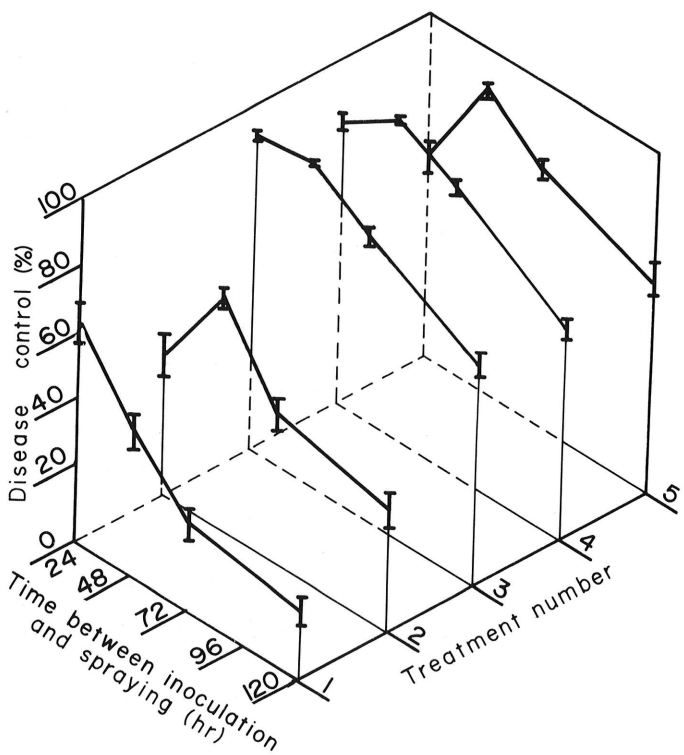

Fig. 3. Curative control of scab on leaves of potted cultivar MM 109 rootstock apple trees in the greenhouse with various fungicide treatments applied after inoculation. Treatments were: 1 = triforine 234 μg/ml; 2 = bitertanol 125 μg/ml; 3 = bitertanol 125 μg/ml plus Agri-Dex® 1 ml/L; 4 = etaconazole 15 μg/ml; and 5 = CGA-71818 25 μg/ml. I = standard error of the mean. (Experiment 3)

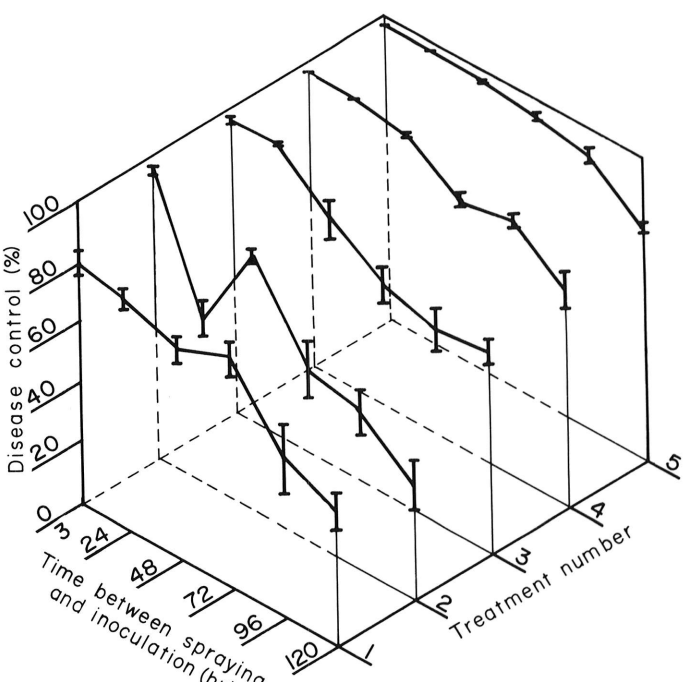

Fig. 5. Protective control of scab on leaves of potted cultivar MM 109 rootstock apple trees in the greenhouse with fungicides applied at various times before inoculation with conidia of *Venturia inaequalis*. Treatments were: 1 = bitertanol 62.5 μg/ml plus Agri-Dex® 250 μl/L; 2 = CGA-71818 25 μg/ml; 3 = fenarimol 31.25 μg/ml; 4 = captan 1,000 μg/ml; and 5 = mancozeb 1,200 μg/ml. I = standard error of the mean.

the adjuvant at 250 μl/L did not equal or exceed that obtained with captan or mancozeb. In this experiment, an average of 3.3 leaves unfolded during the 120-hr preinoculation period. All trees in this experiment received 5.1 mm of rain the night before the last set of fungicide applications 3 hr before inoculation.

DISCUSSION

Our results confirm the excellent curative activity of the sterol-inhibiting fungicides against apple scab. When applied up to 72 hr after inoculation, fenarimol, etaconazole, and CGA-71818 prevented symptom expression more effectively than triforine and bitertanol. When postinfection treatments were applied 120 hr after inoculation, nonsporulating chlorotic spots were formed in place of normal lesions. Etaconazole at 15 μg/ml provided 96% disease control when applied 3 days postinfection while 7.5 μg/ml provided 75% disease control, suggesting we had reached the lowest effective rate for this fungicide. Even at 7.5 μg/ml, etaconazole inhibited conidium production very effectively. Previous tests with etaconazole were conducted at 18.5 μg/ml (6) and at 30 μg/ml (15). By using etaconazole at the lower rates tested in this study, it may be possible to avoid the phytotoxicity problems noted with higher rates (6).

Results of previous tests with 40 μg/ml fenarimol (15) were similar to the results reported here with 37.5 μg/ml fenarimol. It appears 25 μg/ml is the marginal rate for fenarimol. Also, 234 μg/ml appears to be a marginal rate for triforine. Schwabe (10) obtained nearly 100% disease control with triforine at 468 μg/ml, even when applied 120 hr after inoculation. In this study, triforine at 234 μg/ml was less effective than all other sterol-inhibitors for preventing chlorotic lesions and in two of the three experiments it was also less effective for inhibiting sporulation.

Chlorotic spots were common when bitertanol was used alone, but (as noted previously [11]) adding the adjuvant to bitertanol increased its activity as indicated by the development of fewer chlorotic flecks. The fact that all materials were applied at the same time and only bitertanol treatments developed chlorotic flecks suggests bitertanol was slower acting than fenarimol, etaconazole, and CGA-71818.

Further research is needed to determine whether, under conditions favorable for scab development, chlorotic lesions will produce conidia after the use of sterol-inhibiting fungicides is discontinued. There are indications with fenarimol that chlorotic lesions will remain sterile (4), but Kelly and Jones (6) were able to isolate the scab fungus from chlorotic spots in leaves from trees sprayed with etaconazole and bitertanol. Under field conditions, chlorotic lesions may also be invaded by leaf spotting fungi which increase damage to leaf tissue (8). Until more is known about the potential problems with leaf spotting fungi and the viability of scab in chlorotic lesions, it is advisable to spray within 72 hr after infection and prevent symptom expression altogether. However, if sprays must be delayed, application up to 120 hr postinoculation and probably later will prevent sporulation.

Although some sterol-inhibitors provided better protection than others, none were as effective, particularly more than 48 hr after spraying, as the conventional fungicides mancozeb and captan. However, these conclusions conflict with reports of good protective activity with the sterol-inhibiting fungicides (1,2). A difference in the procedures used to evaluate protective action may account for these differing results. In all experiments where protective action was weak, fungicide-treated trees either were exposed to simulated rain (14,15) or had received overhead irrigation in the inoculation chamber (10). In experiments where protective action was good, inoculated trees were incubated in high humidity chambers (1). Because sterol-inhibiting fungicides do not inhibit spore germination (1), we suspect the simulated rain or irrigation as well as some systemic movement reduce the level of fungicide on the leaf to marginal levels. By the time germinated spores were susceptible to the fungicide, adequate deposits of fungicide were no longer present on the leaves. Conventional fungicides were effective because they act much earlier in the development of the fungus and may have better retention qualities than sterol-inhibiting fungicides. Our results also suggest that the generally excellent scab control obtained with the sterol-inhibiting fungicides applied on 7- to 10-day schedules in orchard trials, may be due more to the curative action of these fungicides than their protective action.

Finally, our results suggest that the sterol-inhibiting fungicides could be used very effectively in predictive programs for scab control. By using the information reported here together with the new instrumentation available for the determination of scab infection periods (5), the outstanding curative action of these fungicides can be exploited fully. Also, there are indications that by adding an older type protective fungicide (11), the overall effectiveness of these compounds can be improved.

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