Application Rates and Spray Intervals for Apple Scab Control with Flusilazol and Pyrifenox

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ABSTRACT

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The triazole fungicide flusilazol and the pyridine fungicide pyrifenox, both ergosterol biosynthesis inhibitors, effectively controlled apple scab under greenhouse and orchard conditions. In greenhouse studies, single sprays of flusilazol at 8 μg/ml and pyrifenox at 38.4 μg/ml inhibited the development of visible scab lesions on apple leaves when applied 72 hr after inoculation. When sprays were applied 120 hr after inoculation, chlorotic rather than normal lesions formed. No normal lesions and fewer chlorotic lesions developed when two sprays were applied 7 days apart beginning 72 and 120 hr after inoculation. Single sprays of flusilazol or pyrifenox applied 24–72 hr before inoculation gave control of leaf scab comparable to that provided by 1,200 μg/ml captan applied at the same interval. When the interval was extended to 120 hr, captan was more effective. In field studies over a 3-yr period, flusilazol at 9.4–14 μg/ml and pyrifenox at 37.5–75 μg/ml were both highly effective in 7-day schedules for control of apple scab. Lower rates and 14-day schedules were less effective. Flusilazol at 37.5 μg/ml on a 21-day schedule was highly effective for suppressing lesions. Mixtures of flusilazol at 9 μg/ml with mancozeb at 1,440 μg/ml and of pyrifenox at 37.5 μg/ml with metiram at 1,900 μg/ml were more effective than either flusilazol or pyrifenox alone on 14-day schedules.

Additional key words: *Venturia inaequalis*

Ergosterol biosynthesis-inhibiting (EBI) fungicides have been under investigation for the control of apple scab, caused by *Venturia inaequalis* (Cke.) Wint., for more than 20 yr. Initial research with EBI fungicides for apple scab control indicated that a number of these fungicides had excellent post-infection and presymptom control activity (1,2,5,6,8,9) but less protective activity than conventional protectant fungicides (8,9). In 1984–1985, two new EBI fungicides, flusilazol (Nustar 40EC, 20DF, duPont de Nemours & Co., Wilmington, DE) and pyrifenox (Ro 15-1297, 4E, Maag Agrochemicals, Vero Beach, FL), were made available to us for study. Flusilazol is a member of the triazole group and pyrifenox is a member of the pyridine group of EBI fungicides.

The purpose of our experiments was to investigate the protective and post-infection activities of these new EBI fungicides against apple scab under greenhouse conditions and to define application rates and efficacy in the field.

MATERIALS AND METHODS

Greenhouse studies. The EBI fungicides evaluated, in addition to flusilazol and pyrifenox, were diniconazole (Spotless 25W, Chevron Chemical Co., San Francisco, CA), fenarimol (Rubigan 1E), and triflumizole (Procure 50W, Uniroyal Chemical Inc., Naugatuck, CT). Benomyl (Benlate 50W) was included as a standard fungicide treatment.

In the protectant study, single-shoot McIntosh apple (*Malus domestica* Bork.) trees in pots were sprayed to runoff at 24, 48, 72, and 120 hr before inoculation. Each fungicide was applied to three single-tree replicates at each of the four treatment times. Table 1 describes the fungicides used and rates tested. The trees were inoculated by atomizing them with a suspension of conidia of *V. inaequalis* (5×10^5 per milliliter) that was prepared by rinsing infected apple leaves with distilled water. The youngest fully unfolded leaf on each tree was tagged for later reference. Thirty minutes after inoculation, the trees were placed in a mist chamber at 20–22 C for 48 hr, then moved to an outside bench. Data were recorded 18 days after inoculation on the tagged leaf and the next two older leaves by visually estimating the percentage of leaf area covered with scab lesions. The experiment was repeated with similar results.

In the postinfection study, trees were inoculated as described for the protectant study. Six trees per fungicide were sprayed at either 72 or 120 hr after inoculation. A second spray was applied to three of the trees from each fungicide treatment 1 wk after the first spray. Table 2 describes the fungicides used and rates tested. Three trees were left unsprayed as controls. Data were recorded 19 days after inoculation on the tagged leaf and the adjacent two older leaves by estimating the percentage of leaf area covered by either sporulating or chlorotic scab lesions. The experiment was repeated once.

Field study with flusilazol in 1984 and 1985. Fungicides were applied dilute with a handgun sprayer at 2,760 kPa to runoff. In 1984, flusilazol was applied on 14-day schedules at 4.7, 9.4, and 14.0 μg/ml and on a 21-day schedule at 37.5 μg/ml. Captan (Captan 50W) was sprayed on a 14-day schedule as a standard fungicide control. Treatments were applied to three-tree plots replicated three times in a block of 9-yr-old McIntosh apple trees on M26 rootstock. The first spray was applied on 2 May (tight cluster). Primary infection periods (and severity) were

Table 1. Incidence of apple scab 18 days after McIntosh trees were inoculated with conidia of *Venturia inaequalis* in the greenhouse (fungicides applied 24, 48, 72, and 120 hr before inoculation)

Treatment	Dosage (μg a.i./ml)	Leaf area infected (%)			
		24 hr[x]	48 hr	72 hr	120 hr
Pyrifenox	38.4	0.3 b[y]	1.0 b	1.9 b	22.6 b
Fenarimol	14.4	0.0 b	2.5 b	3.7 b	19.2 b
Triflumizole	115.0	0.0 b	0.8 b	3.6 b	10.8 c
Flusilazol[z]	8.0	0.0 b	0.6 b	1.6 b	11.7 c
Captan	1,200.0	0.0 b	0.0 b	0.0 b	1.1 d
Control	···	30.4 a	34.6 a	29.3 a	32.1 a

[x] Time refers to the interval between application of fungicides and inoculation.

[y] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

[z] Flusilazol 40EC was used.

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identified by an electronic disease predictor (4) (Reuter-Stokes, Inc., Twinsburg, OH) on 22 April (moderate), 4 May (low), 13 May (low), 18 May (low), 22 May (high), 25 May (high), 28 May (moderate), and 8 June (low).

In 1985, treatments were applied to two-tree plots, replicated three times, in a block of 8-yr-old McIntosh apple trees on M26 rootstock. The fungicide treatments tested in 1984 were repeated, and 7-day schedules of flusilazol at 4.7, 9.4, and 14.0 μg/ml were added to the experiment. The first sprays were applied on 22 April (tight cluster). Primary infection periods (and severity) were identified by a disease predictor on 24 April (low), 5 May (low), 15 May (low), 20 May (low), 26 May (moderate), 11 June (low), and 15 June (moderate).

The effects of flusilazol on the development and morphology of conidia was determined by examining scab lesions from selected treatments in 1985 with a Jeol JSM-35C scanning electron microscope (Jeol Ltd., Peabody, MS). Leaf disks (3.5 mm diameter) with lesions were cut from randomly collected leaves with a cork borer and fixed in phosphate-buffered 4% glutaraldehyde for 2 hr. Samples were washed once in 0.1 M phosphate buffer (pH 7.4), dehydrated in an ethanol series, and critical-point-dried. Dried samples were mounted on stubs and sputter-coated with 20 nm of gold.

Field studies with pyrifenox in 1985. Pyrifenox was tested in a block of 9-yr-old McIntosh apple trees on M7 and M26 rootstocks. The fungicide was applied alone at 25, 37.5, and 75 μg/ml and at 37.5 μg/ml in combination with captan at 450 μg/ml. Mancozeb (Dithane M-45 80W) and triadimefon (Bayleton 50W) were tank-mixed and applied as a standard fungicide treatment. Applications were made on 16 April (one-quarter-inch green tip), 23 April (pink), 30 April (bloom), and 7 May (petal fall), and cover sprays were made on 15, 22, and 29 May, 10 and 21 June, 10 July, and 1 August. Sprays were applied to single-tree plots replicated three times. Primary infection periods were as reported for the flusilazol test in 1985.

Field studies in 1986. Both fungicides were applied to a 10-yr-old block of McIntosh apple trees on M7 and M26 rootstocks. Sprays were applied to single-tree plots replicated three times. Flusilazol at 9.4 μg/ml was sprayed alone on 7- and 14-day schedules and in combination with mancozeb (Manzate 200 80W) at 1,440 μg/ml on a 14-day schedule. Pyrifenox at 37.5 μg/ml was applied alone on 7- and 14-day schedules and in combination with metiram (Polyram 80W) at 1,900 μg/ml on a 14-day schedule. Primary infection periods (and severity) were predicted on 14 April (moderate), 20 April (low), 28 April (low), 6 May (low), 15 May (high), 17 May (moderate), 27 May (moderate), 1 June (low), 5 June (low), 7 June (low), 10 June (high), 14 June (moderate), and 15 June (low). The 7-day treatments were applied on 15 April (one-half-inch green tip), 23 April (tight cluster), 3 May (full bloom), 10 May (petal fall), and cover sprays were made on 17, 23, and 29 May, 5 and 18 June, and 1, 14, and 29 July. The 14-day treatments were applied on 15 April, 3, 17, and 23 May, 5 and 18 June, and 1, 14, and 29 July.

Data collection for field trials. The incidence of primary scab was estimated by counting the total lesions per replicate that we could find in a 3-min period in 1985 and in a 4-min period in 1986. Terminal leaf scab was evaluated by rating 20 terminals per replicate. Fruit scab was evaluated by examining all fruit in each replicate.

RESULTS

Greenhouse studies. Trees sprayed with captan had a low incidence of scab even when sprayed 120 hr before inoculation (Table 1). The incidence of scab on trees sprayed with EBI fungicides at 24, 48, and 72 hr before inoculation was similar to that on trees sprayed with captan. On trees sprayed 120 hr before inoculation, trees sprayed with EBI fungicides had a significantly higher incidence of scab than trees sprayed with captan. Among the treatments containing EBI fungicides, trees sprayed with triflumizole at 115 μg/ml and flusilazol at 8 μg/ml had significantly less scab than trees sprayed with pyrifenox at 38.4 μg/ml and fenarimol at 14.4 μg/ml. Only sporulating lesions were observed in all treatments where symptoms were present.

In the postinfection studies, a single spray of flusilazol at 8 μg/ml or of pyrifenox at 38.4 μg/ml applied 72 hr after inoculation prevented the development of normal lesions more effectively than benomyl or diniconazole (Table 2). A second spray 7 days later reduced the percentage of leaf area covered with lesions, especially in the triflumizole and fenarimol treatments. Chlorotic rather than normal lesions were observed in all but the diniconazole and benomyl treatments.

More leaf area was covered with scab lesions when the first fungicide treatments were applied 120 rather than 72 hr after inoculation (Table 2). When fungicides were applied 120 hr after inoculation, nearly all lesions in the pyrifenox and flusilazol single-spray treatments were chlorotic. When a second treatment was applied 7 days after the first treatment, the percentage of leaf area with scab lesions was lower than with a single-spray treatment and all lesions were chlorotic.

Field studies with flusilazol in 1984 and 1985. In 1984, flusilazol was more effective at 9.4 and 14 than at 4.7 μg/ml in controlling infection of the leaves and fruit (Table 3). Flusilazol at 37.5 μg/ml was very effective on a 21-day calendar schedule. When the application dates for the 21-day schedule were compared with the dates of actual infection periods, the spray dates fell just before or after these infection periods.

In 1985, flusilazol was more effective when applied on a 7-day than on a 14-day schedule (Table 3). Regardless of the dosage, only a few chlorotic lesions

Table 2. Leaf area infected with apple scab 14 days after McIntosh trees in the greenhouse were inoculated with conidia of *Venturia inaequalis* (fungicides applied 72 and 120 hr after inoculation and repeated 7 days after the first spray on half of the trees in each treatment)

		72 hr After inoculation				120 hr After inoculation			
		One spray		Two sprays		One spray		Two sprays	
Treatment	Dosage (μg a.i./ml)	Total[w] (%)	Normal[x] (%)	Total (%)	Normal (%)	Total (%)	Normal (%)	Total (%)	Normal (%)
Benomyl	300.0	37.3 a[y]	35.3 ab	16.7 b	10.3 b	31.3 a	22.0 b	15.7 b	11.3 b
Diniconazole	12.5	23.0 ab	21.0 bc	15.0 b	7.0 b	17.7 bc	4.3 cd	16.7 b	4.0 c
Fenarimol	14.4	12.3 bc	3.7 cd	3.3 b	0.0 b	28.3 ab	11.7 c	12.0 b	2.0 c
Triflumizole	115.0	11.3 bc	7.3 cd	3.3 b	0.0 b	28.0 ab	4.7 cd	16.3 b	0.0 c
Pyrifenox	38.4	5.3 bc	2.7 d	3.3 b	0.0 b	11.0 c	0.0 d	9.7 b	0.0 c
Flusilazol[z]	8.0	2.3 c	0.0 d	2.0 b	0.0 b	15.3 bc	0.7 d	8.7 b	0.0 c
Control	···	39.7 a	39.7 a	40.7 a	40.7 a	33.3 a	33.3 a	37.0 a	37.0 a

[w] Percentage of leaf area covered with both normal sporulating lesions and chlorotic lesions was determined by rating three leaves per replicate.
[x] Percentage of leaf area covered with normal sporulating lesions.
[y] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] Flusilazol 40EC was used.

developed in 7-day schedules. Both normal and chlorotic lesions were observed in 14-day schedules, especially at the lower rates. The effectiveness of both schedules improved with higher rates of flusilazol. Numerous non-sporulating, chlorotic and necrotic lesions developed on trees treated with 37.5 μg/ml on the 21-day schedule. These lesions were probably initiated in the infection period on 5 May. This infection period occurred 13 days after the first spray on 22 April and 8 days before the second application on 13 May. Control of fruit scab was good with all treatments except flusilazol at 4.7 μg/ml on the 14-day schedule.

The absence of mature conidia in chlorotic lesions on leaves treated every 21 days with flusilazol was verified by examining the lesions with scanning electron microscopy. The density and morphology of conidia in lesions from unsprayed trees and in sporulating lesions from leaves of trees sprayed with flusilazol at 4.7 μg/ml on a 14-day schedule were similar in appearance (Fig. 1A,B). Only subcuticular fungal growth and a few immature conidia were observed in chlorotic lesions from leaves sprayed on the 21-day schedule with flusilazol at 37.5 μg/ml (Fig. 1C).

Field studies with pyrifenox in 1985. The effectiveness of pyrifenox improved as the rates were increased from 25 to 75 μg/ml (Table 4); only chlorotic primary lesions formed at 75 μg/ml. The combination of pyrifenox at 37.5 μg/ml and captan at 450 μg/ml was slightly more effective than pyrifenox alone at 37.5 μg/ml. The combination of mancozeb and triadimefon had a higher number of lesions than the other treatments. Control of fruit scab was better with pyrifenox at 37.5 and 75 than at 25 μg/ml.

Field studies in 1986. From 14 to 23 May, frequent and long wet periods followed earlier infection periods. Under these severe conditions, flusilazol at 9.4 μg/ml and pyrifenox at 37.5 μg/ml were more effective in preventing infection of apple leaves when applied on 7-day rather than 14-day schedules (Table 5). On 14-day schedules, flusilazol and pyrifenox were more effective when combined with mancozeb and metiram, respectively, than when applied alone. Control of fruit scab was good except when flusilazol and pyrifenox were applied alone on 14-day schedules.

Table 3. Control of apple scab on McIntosh trees with several rates of flusilazol applied in 7-, 14-, and 21-day schedules in the orchard

Treatment and rate (μg a.i./ml)	Interval (days)	Infection (%) in 1984: Terminal leaves[v]	Infection (%) in 1984: Fruit[w]	1985: Types of lesions on 30 May: Total[x] (no.)	1985: Types of lesions on 30 May: Chlorotic (%)	1985: Terminal leaves infected[w] (%)	1985: Fruit infected[x] (%)
Flusilazol (4.7)[y]	7	···	···	30.3 d[z]	100.0	0.0 c	1.4 c
	14	18.2 bc	62.8 b	78.5 c	68.2	4.3 b	7.3 b
Flusilazol (9.4)	7	···	···	8.2 d	100.0	0.0 c	2.2 c
	14	9.7 abc	9.0 a	20.5 d	66.1	0.8 bc	2.0 c
Flusilazol (14.0)	7	···	···	1.8 d	100.0	0.0 c	0.6 c
	14	5.1 ab	4.5 a	14.7 d	92.8	0.2 c	1.1 c
Flusilazol (37.5)	21	1.3 a	0.6 a	257.7 b	100.0	0.3 c	1.4 c
Captan (1,200)	14	23.7 c	11.0 a	94.3 c	17.3	0.5 c	1.4 c
Control	···	94.3 d	84.7 b	315.5 a	35.2	83.0 a	100.0 a

[v] Terminal leaf scab was evaluated on 7 August 1984 and 17 June 1985 by rating 20 terminals per replicate.
[w] Fruit scab was evaluated on 18 June 1984 and 19 August 1985 by examining all fruit in each replicate.
[x] Number of lesions per tree counted in 3 min.
[y] Flusilazol 40EC was used in 1984, and flusilazol 20DF was used in 1985.
[z] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

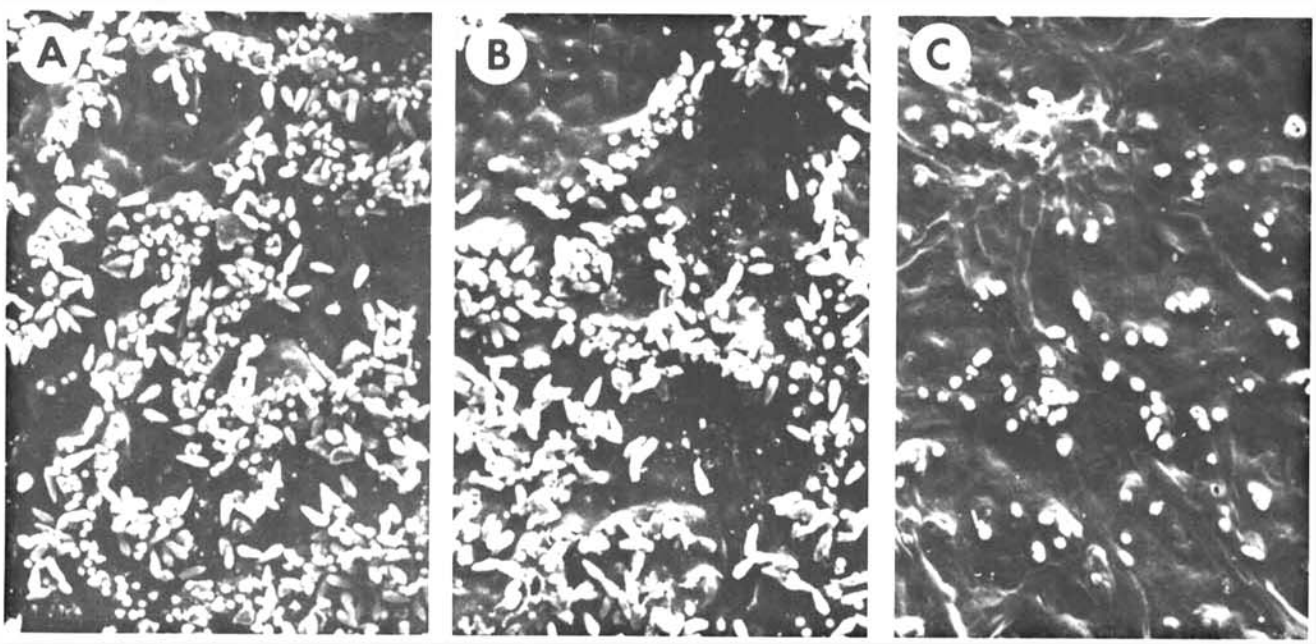

Fig. 1. Scanning electron micrographs of lesions caused by *Venturia inaequalis* on apple leaves either unsprayed or sprayed with flusilazol. **(A)** Unsprayed lesion with many normal conidia (×200). **(B)** Lesion sprayed at 4.7 μg/ml on a 14-day schedule showing density and morphology of conidia similar to unsprayed lesions (×220). **(C)** Lesion sprayed at 37.5 μg/ml on a 21-day schedule with few immature conidia (×220).

DISCUSSION

Our results in the greenhouse indicate that flusilazol at 8 μg/ml and pyrifenox at 37.5 μg/ml have excellent postinfection control activity when applied as a single spray 72 hr after inoculation. When single postinfection treatments were applied 120 hr after inoculation, chlorotic lesions formed in place of normal lesions, suggesting that we had exceeded the time limit for postinfection control with these fungicides at the rates tested. Postinfection control with all the EBI fungicides tested was improved by making a second application 7 days after the first application. Improved control from back-to-back applications was particularly evident with fenarimol at 14.4 μg/ml and triflumizole at 115 μg/ml. Previous studies indicated that 25 μg/ml was the marginal rate for fenarimol as a single postinfection spray (8). Our results suggest that when EBI fungicides are used at low rates or later than 72 hr after the beginning of an infection period, repeated applications will improve control of apple scab. The poor postinfection control activity of benomyl in the greenhouse study may be because inoculum had been collected from an orchard where benomyl-resistant strains were previously detected.

Previous studies have shown that EBI fungicides provide less protection against apple scab than the conventional fungicides mancozeb and captan (8,9). In our trials, neither flusilazol nor pyrifenox provided as much protection as captan. The protective action of the EBI fungicides lasted about 3 days.

Based on our field results, rates of 9.4–14 μg/ml for flusilazol and 37.5–75 μg/ml for pyrifenox, which were highly effective in this investigation, should be adequate to control apple scab when applied on 7-day schedules. These rates are not adequate when applied on 14-day schedules. When applied on 7-day schedules, these fungicides controlled scab by combining up to 3 days of protective action with their postinfection activity. The postinfection activity prevented development of visible lesions when sprays were applied up to 72 hr after inoculation. Chlorotic rather than normal lesions were formed when sprays were applied later than 72 hr but before the onset of visible infections. Repeated sprays with EBI materials improved the postinfection efficiency of previous sprays when used in regular schedules.

Although many lesions developed on leaves of trees treated with flusilazol at 37.5 μg/ml on the 21-day schedule, all of the lesions were chlorotic. Only a few immature conidia were produced in these lesions. This high rate of flusilazol may have value as an emergency treatment late in the incubation period.

The possibility of extending application intervals with mixtures was proposed by Schwabe et al (7) based on greenhouse trials with bitertanol and metiram. In field trials, mixtures of flusilazol at 9.4 μg/ml or pyrifenox at 37.5 μg/ml with a conventional protectant fungicide, particularly where the conventional fungicide was at or near full recommended rates, gave good control on 14-day schedules. Mixtures applied on a 10-day interval should be tested because 14 days may be the maximum interval. An additional advantage of mixtures may be to reduce the possible selection of strains with reduced sensitivity to EBI fungicides (3).

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Table 4. Control of apple scab on McIntosh apple with several rates of pyrifenox applied on 14-day schedules in the orchard (1985)

Treatment and rate (μg a.i./ml)	Scab lesions[w] Total (no.)	Scab lesions[w] Chlorotic (%)	Leaves infected[x] (%)	Fruit infected[y] (%)
Pyrifenox (25)	78.0 bc[z]	97.0 a	6.1 b	6.0 b
Pyrifenox (37.5)	69.7 bc	96.6 a	2.6 cd	2.6 bc
Pyrifenox (75)	10.3 c	100.0 a	7.7 b	1.1 c
Pyrifenox (38) + captan (450)	19.3 c	85.5 a	0.6 d	0.0 c
Mancozeb (1,920) + triadimefon 38; mancozeb (1,440) starting 29 May	100.0 b	37.9 b	6.8 bc	0.1 c
Check	239.0 a	2.2 c	70.2 a	97.6 a

[w] Number of lesions per tree counted in 3 min on 30 May 1985.
[x] Leaf infection was evaluated on 26 June by rating 20 terminals per replicate.
[y] Fruit infection was evaluated on 16 August by examining all fruit in each replicate.
[z] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.

Table 5. Control of apple scab on McIntosh trees with flusilazol 20DF and pyrifenox applied alone on 7- and 14-day schedules and in combination with protectant fungicides on 14-day schedules in the orchard (1986)

Treatment and rate (μg a.i./ml)	Interval (days)	Lesions[w] (no.)	Terminal leaves infected[x] (%)	10 June Lesions (no.)	10 June Lesions chlorotic (%)	10 June Fruit infected[y] (%)
Flusilazol (9.4)	7	0.0 b[z]	2.4 c	21.3	95.8 a	2.5 c
	14	62.3 a	17.7 b	222.3	84.0 b	32.8 b
Flusilazol (9.4) + mancozeb (1,440)	14	1.0 b	15.7 b	199.0	90.5 ab	7.0 c
Pyrifenox (37.5)	7	0.7 b	6.8 bc	75.0	94.2 ab	5.2 c
	14	14.3 b	18.2 b	302.7	91.8 ab	30.4 b
Pyrifenox (37.5) + metiram (1,900)	14	1.3 b	7.5 bc	60.0	92.8 ab	3.8 c
Control	...	85.3 a	91.0 a	...	...	100.0 a

[w] Number of lesions per tree counted 4 min on 13 May.
[x] Terminal leaf scab was evaluated on 20 terminals per replicate.
[y] Fruit infection was evaluated on all fruit in each replicate.
[z] Means within a column followed by the same leter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.