Effects of Postinfection Applications of Ergosterol Biosynthesis-Inhibiting Fungicides on Lesion Formation and Pseudothecial Development of *Venturia inaequalis*

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Appreciation is extended to J. J. Bond, E. M. Brown, and L. R. Pope for assistance during this study.

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Journal Series Paper 9830 of the of the North Carolina Agricultural Research Service, Raleigh.

Accepted for publication 10 September 1985 (submitted for electronic processing).

ABSTRACT

O'Leary, A. L., and Sutton, T. B. 1986. Effects of postinfection applications of the ergosterol biosynthesis-inhibiting fungicides on lesion formation and pseudothecial development of *Venturia inaequalis*. Phytopathology 76:119-124.

The ergosterol biosynthesis-inhibiting (EBI) fungicides, bitertanol (150.0 μg/ml), etaconazole (18.7 μg/ml), fenarimol (34.2 μg/ml), and triforine (142.0 μg/ml), applied 120 hr after inoculation of apple (*Malus domestica* Bork. 'McIntosh') foliage with conidia of *Venturia inaequalis*, resulted in the formation of chlorotic lesions 2 wk after application. Some normal sporulating lesions were observed in the bitertanol and triforine treatments after 2 wk and in all fungicide treatments 5 wk after application. Sporulation was also observed around the edges of chlorotic lesions after 5 wk. A postsymptom application of benomyl (150.0 μg/ml) was more effective than the EBI fungicides in reducing the recovery of *W. inaequalis* on acidified potato-dextrose agar from lesions 5 wk after application. All postsymptom fungicide treatments failed to prevent continued sporulation from treated lesions. Germination of conidia recovered from treated lesions was low compared to that of conidia recovered from untreated lesions. A single postharvest application of the EBI fungicides resulted in the production of fewer pseudothecia in overwintered leaves. Pseudothecia that developed in treated leaves were smaller and contained fewer ascospores compared to pseudothecia from untreated leaves. Histological studies indicated that fewer pseudothecia were initiated in treated leaves after leaf fall when EBI fungicides were applied.

Ergosterol biosynthesis-inhibiting (EBI) fungicides have shown various degrees of postinfection control of *Venturia inaequalis* (Cke.) Wint. These fungicides prevented the formation of visible lesions when applied within 3 days after inoculation (1,5,7,12,14). Applications made 3 days after infection or from the onset of a wet period suitable for infection, but before symptoms developed, resulted in the formation of chlorotic flecks (1,5,7,12,14). Schwabe et al (12) reported that conidial production was almost completely inhibited by the EBI fungicides tested when applications were made up to 5 days after inoculation. Although no sporulation was observed in chlorotic lesions resulting from treatment with fenarimol (5) and bitertanol (1), the fungus was isolated from chlorotic lesions resulting from bitertanol and etaconazole treatments (7). In all of these studies, treatments were evaluated within 3 wk after inoculation and 2 wk after the fungicide applications. Further research is needed to determine if viable conidia will be produced in chlorotic lesions more than 2 wk after fungicide application.

Although Szkolnik (14) found that a postsymptom application of several EBI fungicides did not prevent sporulation of *V. inaequalis* or reduce the number of conidia produced, Brandes and Paul (1) reported fewer conidia and little germination after bitertanol was applied to lesions. Isolation of *V. inaequalis* from chlorotic lesions (7) and continued sporulation from treated lesions (1,14) suggest that the fungus may grow saprophytically in overwintered sprayed apple leaves. Five to seven regular season applications of several EBI fungicides (9,10) or a single postharvest application of etaconazole and bitertanol (4) resulted in fewer ascospores released from overwintered leaves in the spring. These studies were not designed to determine when pseudothecial development was affected by the fungicides.

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Therefore, the purposes of our experiments were to investigate the effects of postinfection applications of several EBI fungicides on the development of *V. inaequalis* and to measure the influence of these fungicides on the saprophytic growth of the fungus in overwintered leaves.

MATERIALS AND METHODS

Fungicides used. The following fungicides and rates (μg a.i./ml) were used throughout this study: benomyl (Benlate 50W), 150.0, E.I. Dupont de Nemours and Co., Wilmington, DE; bitertanol (Baycor 50W), 150.0, Mobay Chemical Corp., Kansas City, MO; etaconazole (Vangard 10W), 18.7, Ciba-Geigy Corp., Greensboro, NC; fenarimol (Rubigan 12.5% EC), 32.8, Eli Lilly Co., Greenfield, IN; and triforine (Funginex 18.2% EC), 136.0, EM Industries, Hawthorne, NY.

Presymptom fungicide application study. Single shoots of McIntosh apple seedlings were inoculated with a suspension of conidia of *V. inaequalis* (5×10^4/ml). The suspension was prepared by growing the fungus on potato-dextrose agar (PDA) in 9-cm-diameter petri dishes, scraping the agar surface in distilled water, and filtering the suspension through cheesecloth. The seedlings were atomizer-sprayed with the suspension, placed in a mist chamber at 20–22 C for 48 hr, and then placed in an air-conditioned greenhouse room at 20 C and misted for 8 hr/day on an alternating 15-min schedule for 5 wk. Individual attached leaves from six seedlings were dipped into one of the fungicide suspensions 120 hr after inoculation. Control leaves were dipped in distilled water. After the fungicides had dried, the seedlings were returned to the greenhouse. The percentage of leaf area covered by sporulating and nonsporulating (chlorotic or necrotic) lesions was visually estimated 2 and 5 wk after treatment. The number of conidia produced per square millimeter of lesion (10 lesions per fungicide) was determined by scraping the surface of each sporulating lesion (normal or chlorotic) with a nylon brush into sterile distilled water and counting the resulting conidial suspension using a hemacytometer. Conidia from each lesion were placed on acidified potato-dextrose agar (APDA), incubated in the dark at 20 C for 32

hr, and the percentage of 100 conidia that germinated was recorded.

Postsymptom fungicide application study. Postsymptom activity of the fungicides was investigated in greenhouse and field studies. In the greenhouse study, McIntosh apple seedlings with one shoot were inoculated and incubated as described above. At 2 wk after inoculation, individual leaves from six seedlings with sporulating lesions were dipped into one of the fungicide suspensions or in distilled water. After the foliage dried, the seedlings were returned to the greenhouse. Five weeks after treatment, leaf pieces (4-mm square) were cut from the treated lesions, washed in running tap water for 1 hr, dipped in a 5% NaClO solution for 1 min, rinsed in sterile distilled water, and placed on APDA. Seventy-two lesions were plated for each treatment and the fungus was identified by observing the sporulating mycelium growing from the leaf pieces. Thirty lesions from each treatment were examined microscopically to determine if lesions were sporulating. Six sporulating lesions from each treatment were rubbed over the surface of APDA in petri dishes, incubated at 20 C in the dark for 32 hr, and the percentage of 100 conidia that had germinated was recorded. The experiment was repeated once and the data were combined for analysis.

In the field study, 7-yr-old McIntosh apple trees naturally infected with *V. inaequalis* were used. Leaves with sporulating lesions from four terminals per tree, two trees per fungicide, were dipped into one of the fungicide suspensions or in distilled water. Waxed paper bags were placed over the terminals to prevent subsequent natural infection. Five weeks after treatment, 48 lesions from each treatment were plated on APDA as described above, 30 lesions were observed for sporulation, and six sporulating lesions from each treatment were evaluated for germination of conidia. The experiment was repeated once and the data were combined for analysis.

Field overwintering study. Unsprayed, 5-yr-old cultivar Delicious apple trees located at the Mountain Horticultural Crops Research Station (MHCRS), Fletcher, NC, were used. Fungicide treatments were replicated three times in a completely randomized design with single-tree plots. Thirty leaves from each tree were tagged and dipped into one of the fungicide suspensions or in distilled water on 14 October 1982 and 16 October 1983. Two weeks after treatment the leaves were picked. Disks (1-cm diameter) were punched from them with a cork borer and placed in cheesecloth bags. The bags were overwintered in Saran cloth (Chicopee Mfg. Co., Cornella, GA 30531) cages placed on the ground at MHCRS in 1982. In 1983, a replicate set of disks was placed at MHCRS and Boone, NC.

Disks were fixed in a mixture of 2-propanol, water, propionic acid, and formaldehyde (45:45:5:5, v/v)(FPP) when pseudothecia in the leaf disks had matured (21 April 1983 and 9 April 1984). Sixty disks per treatment were evaluated for each location. The number of pseudothecia in each disk and the diameter and number of ascospores in 10 randomly chosen disks from each treatment were recorded.

To determine when pseudothecial development was inhibited, samples of leaf disks (10 disks per treatment) were collected from MHCRS for microscopic examination on 11 November, 19 December, 17 February, and 9 April. The leaf disks were fixed in FPP, dehydrated, embedded in Paraplast+ (MP = 56 C, Sherwood Medical Industries, St. Louis, MO), sectioned at 12 μm and stained according to a modified Conant's staining schedule (6).

Laboratory overwintering study. Disks (1-cm diam) cut from unsprayed, healthy McIntosh apple leaves were sterilized in propylene oxide. Cellulose sponges (15.2 × 10.2 × 2.5 cm) were moistened and autoclaved for 1 hr at 137.9 kPa; molded plastic boxes (16.5 × 11.4 × 6.4 cm) with lids were sterilized with propylene oxide for 24 hr. Under aseptic conditions, one sponge was placed in each box and 30 disks, abaxial side up, were placed on each sponge. The disks were inoculated with 0.1 ml of a conidial suspension of *V. inaequalis*. The inoculum was prepared by growing two isolates, one of each mating type, on PDA in 9-cm-diameter petri dishes for 3 wk at 20 C under fluorescent lights. Conidia were collected by placing 8 ml of sterile distilled water in each dish, scraping the agar surface with a sterile spatula, and pouring the suspension through sterile cheesecloth. The conidial suspensions of each isolate were combined and the final concentration was adjusted to 6.0×10^4 conidia per milliliter. Five drops of Tween-20 were added to each 100 ml of conidial suspension. After inoculation, the boxes were placed at 20 C for 2 wk to encourage mycelial growth over the leaf disks. Disks were then dipped for 5 sec in one of the fungicide suspensions or in sterile distilled water (60 disks per treatment). The boxes remained uncovered in a laminar flow hood for 15 min to allow evaporation of surface moisture. The boxes were placed at 20 C for 1 wk and then at 8 C for 15 wk. Ten disks per treatment were cleared in a solution of 1 N potassium hydroxide solution, autoclaved for 8 min at 137.9 kPa, and observed with a compound microscope at 5 wk. The remaining disks were cleared and observed at 15 wk.

RESULTS

Presymptom fungicide application study. Sporulating lesions were observed on seedlings treated with benomyl, bitertanol, and triforine 2 wk after the fungicide applications (Table 1). Only chlorotic lesions were observed in the etaconazole and fenarimol treatments. Sporulating lesions were evident in all treatments 5 wk after application (Table 1). Sporulation was observed only in lesions or around chlorotic flecks which were visible at 2 wk; no secondary lesions developed. In the triforine, etaconazole, bitertanol, and fenarimol treatments, sporulation was observed around the edges of the chlorotic lesions after 5 wk (Fig. 1). Some chlorotic lesions in the bitertanol, etaconazole, and fenarimol treatments became necrotic. Fewer conidia were produced from

TABLE 1. The formation and sporulation of *Venturia inaequalis* lesions on McIntosh apple seedlings at 2 and 5 wk after a 120-hr postinoculation application of EBI[v] fungicides and benomyl

Treatment	Dose (μg a.i./ml)	2 wk		5 wk	
		Chlorotic and necrotic lesions[w] (%)	Normal lesions[x] (%)	Chlorotic and necrotic lesions (%)	Normal lesions (%)
Control[y]		0[z]	30	0	36
Triforine 18.2% EC	142.0	7	9	8	12
Etaconazole 10W	18.7	8	0	10	11
Benomyl 50W	150.0	5	5	3	12
Bitertanol 50W	150.0	6	2	6	8
Fenarimol 12.5% EC	34.2	8	0	4	6

[v] Ergosterol biosynthesis-inhibiting.
[w] Percent leaf area covered with chlorotic and necrotic lesions.
[x] Percent leaf area covered with normal sporulating lesions.
[y] Control leaves dipped in tap water.
[z] Data are the means of four replicates with four leaves per replicate.

normal lesions as well as from lesions which were initially chlorotic in all of the fungicide treatments; conidial production from treated lesions was reduced 77–90% compared to that in the control treatment (Table 2). Conidia recovered from treated lesions were similar in size and shape to those produced in the controls. Germination of conidia from the fungicide treatments was reduced compared to that of conidia from the control treatment (Table 2). The morphology of germ tubes produced by conidia from treated lesions was similar to the morphology of germ tubes produced by conidia from untreated lesions.

Postsymptom fungicide application study. Benomyl was more effective than the EBI fungicides in reducing the recovery of the fungus from lesions 5 wk after treatment (Table 3). Etaconazole and fenarimol were the most effective EBI compounds tested. All of the fungicides failed to prevent continued sporulation from treated lesions; however, the fungicides significantly reduced the percentage of lesions sporulating. Benomyl was the most effective and triforine the least effective (Table 3). Sporulating lesions did not increase in size but the center of some lesions in all fungicide treatments became necrotic and sporulation was observed around the edges of the necrotic areas. Germination of conidia recovered from the lesions was reduced in all treatments (Table 3). Although triforine was the least effective in preventing sporulation from treated lesions, it was the most effective in reducing germination of conidia.

Field overwintering study. Few pseudothecia were produced in leaves treated with bitertanol, fenarimol, and etaconazole (Table 4). Triforine performed similarly to benomyl. The diameters of pseudothecia in all fungicide treatments except benomyl in 1982 were significantly smaller than the control pseudothecia (Table 4). The differences among fungicides were not entirely consistent between locations and years; in general, however, treatment with the EBI compounds resulted in smaller pseudothecia. Fewer ascospores were produced in pseudothecia from fungicide-treated leaves compared to untreated leaves (Table 5). Etaconazole and fenarimol were the most effective, and all of the EBI compounds were more effective than benomyl.

Histological examinations of leaf disks from untreated lesions collected on 11 November showed extensive mycelial growth of *V. inaequalis* in the inner leaf tissues and numerous ascogonial initials. Normal mycelial growth was evident in the inner leaf tissues of the fungicide-treated leaf disks, but in the bitertanol, etaconazole, and fenarimol treatments some dark-pigmented, thick-walled hyphae

TABLE 2. Production and germination of *Venturia inaequalis* conidia 5 wk after a 120-hr postinoculation application of EBI[u] fungicides and benomyl on McIntosh apple seedlings

Treatment	Dose (μg a.i./ml)	Conidia/mm² lesion (no.)[v]	Reduction (%)[w]	Germination (%)[x]
Control[y]		6,407 a[z]	0	87 a
Triforine 18.2% EC	142.0	1,464 b	77	62 d
Benomyl 50W	150.0	1,453 b	84	72 c
Etaconazole 10W	18.7	1,002 bc	84	72 c
Bitertanol 50W	150.0	768 c	88	78 b
Fenarimol 12.5% EC	34.2	638 c	90	69 c

[u] Ergosterol biosynthesis-inhibiting.
[v] Data are the means of 10 lesions per treatment.
[w] Reduction in conidial production compared to the control.
[x] Data are the percentage of 100 conidia observed.
[y] Control leaves dipped in tap water.
[z] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to the Waller-Duncan k-ratio t-test.

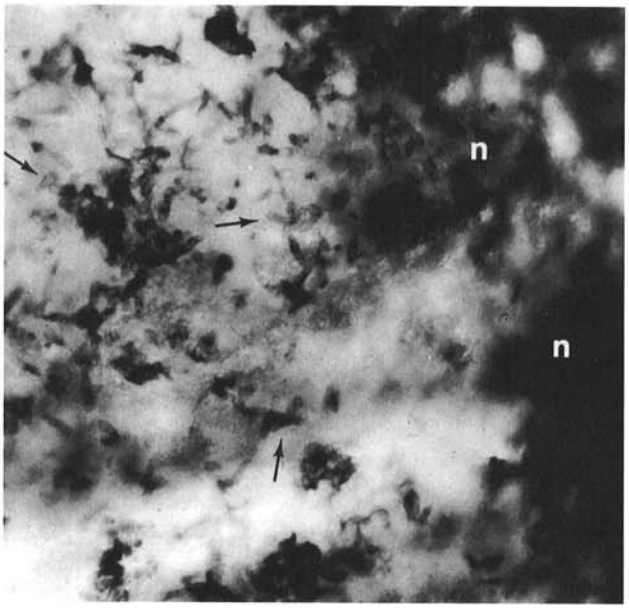

Fig. 1. Conidia of *Venturia inaequalis* (arrows) produced around the edge of a necrotic apple leaf lesion (n) 5 wk after a 120-hr postinfection application of etaconazole (×100).

TABLE 3. Isolation and sporulation of *Venturia inaequalis* from lesions and germination of conidia recovered 5 wk after a postsymptom application of EBI[r] fungicides on inoculated McIntosh apple seedlings in the greenhouse and on naturally-infected McIntosh apple trees located at the Central Crops Research Station, Clayton, NC

Treatment	Dose μg a.i./ml)	Recovery from lesions (%)		Lesions sporulating (%)		Conidia germinated[s] (%)	
		Inoc.[t]	Naturally Infected[u]	Inoc.[v]	Naturally infected[w]	Inoc.[x]	Naturally infected
Control[y]		76.4 a[z]	71.9 a	100 a	98 a	94.8 a	89.7 a
Triforine 18.2% EC	142.0	70.1 ab	67.7 ab	85 b	83 b	52.1 e	47.1 f
Bitertanol 50W	150.0	67.4 b	65.6 ab	72 c	70 c	79.2 b	75.3 c
Fenarimol 12.5% EC	34.2	51.4 c	60.4 bc	68 c	65 c	67.2 c	65.4 e
Etaconazole 10W	18.7	50.0 c	53.1 cd	65 d	55 d	62.9 d	69.3 d
Benomyl 50W	150.0	38.2 d	48.9 d	35 d	30 e	78.7 b	76.7 b

[r] Ergosterol biosynthesis-inhibiting.
[s] Data are the percentage of 100 conidia observed.
[t] Data are the means of six replicates with 12 lesions per replicate.
[u] Data are the means of eight replicates with six lesions per replicate.
[v] Data are the means of six replicates with 10 lesions per replicate.
[w] Data are the means of three replicates with 10 lesions per replicate.
[x] Data are the means of six replicates with 100 conidia per replicate.
[y] Control leaves dipped in tap water.
[z] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to the Waller-Duncan k-ratio t-test.

were observed growing from the subcuticular stroma into the palisade region. Few ascogonial initials were present and those that had formed were smaller (25 μm in diameter) compared to control ascogonial initials (30–35 μm in diameter). On 19 December, control pseudothecia had increased in diameter (65–70 μm) and were filled with pseudoparaphyses. In the benomyl, bitertanol, fenarimol, and triforine treatments, most of the pseudothecia were smaller (50–60 μm in diameter) but similar to the controls in their development. Some pseudothecia in the etaconazole treatment were abnormal with lightly pigmented, loosely arranged cells comprising the peridium. Asci had formed by 17 February in the control pseudothecia and were approximately one-half mature size. Development of pseudothecia in the fungicide treatments was similar to the controls, but they were smaller in size. Control pseudothecia contained numerous asci filled with mature ascospores on 9 April. Pseudothecia were filled with undifferentiated asci in the fungicide treatments; however a few asci in each pseudothecium typically contained four or eight mature ascospores. Abnormal, aseptate ascospores were observed in the fenarimol and etaconazole treatments (Fig. 2).

Laboratory overwintering study. Extensive mycelial growth, hyphal coiling, and numerous pseudothecial initials were observed in the control disks. Disks treated with the fungicides had much less mycelial growth and no hyphal coiling or initials. An average of eight pseudothecia per disk was observed in the control disks at the end of 15 wk.

TABLE 4. Numbers and diameters of pseudothecia from leaf disks infected by *Venturia inaequalis* and treated with EBI[v] fungicides on 14 and 16 October 1982 and 1983 and overwintered at the Mountain Horticultural Crops Research Station (MHCRS) in 1982 and 1983 and at Boone (BOONE), NC, in 1983

		Pseudothecia/disk[w]			Diameters of pseudothecia[x] (μm)		
		MHCRS		BOONE	MHCRS		BOONE
Treatment	Dose (μg a.i./ml)	1982	1983	1983	1982	1983	1983
Control[y]		13.7 a[z]	18.2 a	20.0 a	153.9 a	160.5 a	157.2 a
Benomyl 50W	150.0	12.5 a	16.7 b	10.8 b	133.0 a	149.0 b	128.5 b
Triforine 18.2% EC	136.0	10.5 bc	14.6 b	11.5 b	128.3 bc	136.5 c	125.4 b
Bitertanol 50W	150.0	8.4 c	6.1 c	8.9 c	123.8 bc	127.9 d	126.2 b
Fenarimol 12.5% EC	32.8	5.6 d	6.6 d	5.5 d	128.1 bc	130.1 cd	125.3 b
Etaconazole	18.7	5.2 d	2.9 d	4.8 d	119.1 c	128.1 cd	121.3 b

[v] Ergosterol biosynthesis-inhibiting.
[w] Data are the means of three replicates with 20 disks per replicate.
[x] Data are the means of three replicates with 10 pseudothecia per replicate.
[y] Control leaves dipped in tap water.
[z] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to the Waller-Duncan k-ratio t-test.

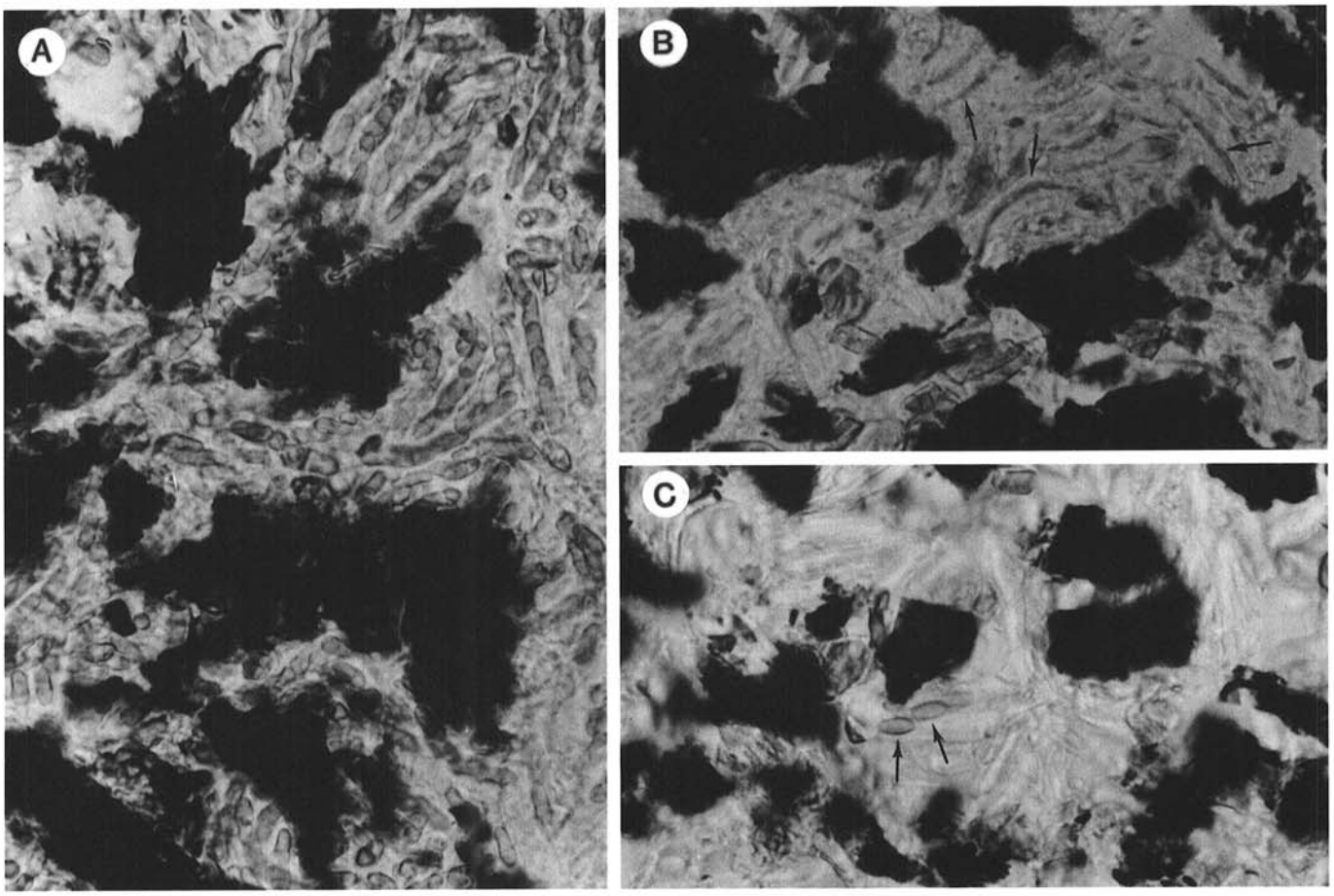

Fig. 2. Squash mounts of mature pseudothecia of *Venturia inaequalis* recovered from overwintered apple leaves which were dipped 2 wk prior to leaf fall in **A,** distilled water, **B,** etaconazole (arrows indicate empty asci), or **C,** fenarimol (arrows indicate aseptate ascospores). All ×400.

DISCUSSION

Chlorotic lesions, resulting from a postinfection, presymptom application of the EBI fungicides, produced conidia when maintained under conditions favorable for sporulation. Sporulation typically occurred around the edges of the chlorotic tissue. Kelley and Jones (7) observed sporulation on leaves treated with bitertanol and etaconazole 120 hr after inoculation, but not on leaves treated with fenarimol. Using electron microscopy, Hoch and Szkolnik (5) observed that after a postinfection application of fenarimol, the subcuticular stroma in chlorotic lesions was necrotic, whereas in the dodine and benomyl treatments, some portions of the stroma were necrotic while other portions appeared normal. In this study, some of the chlorotic lesions in the fenarimol, etaconazole, and bitertanol treatments were necrotic after 5 wk, but sporulation was observed in leaf tissue surrounding the necrotic as well as the chlorotic areas. Under the conditions of this experiment, neither benomyl nor the EBI compounds completely eradicated the fungus or prevented sporulation when applied 120 hr after infection.

Previous experiments have shown that postinfection applications of EBI fungicides prevented sporulation, but evaluations were usually made 1–2 wk after the fungicides were applied (1,3,7,12,14). Brandes and Paul (1) observed conidiophores growing from subcuticular stroma 2 wk after postinfection application of bitertanol, but no conidia had formed. Our results indicate that evaluation of sporulation inhibition should be made 5 wk or more after fungicide applications are discontinued.

Our studies confirm the poor postsymptom activity of one application of the EBI fungicides against *V. inaequalis*. All of these compounds were less effective than benomyl in reducing recovery of the fungus from lesions and preventing sporulation. Szkolnik (14) also found benomyl to be more effective than the EBI fungicides in a postsymptom application. Kelley and Jones (7) observed that when two postsymptom or two presymptom applications of etaconazole were made within 7 days of each other throughout the season, the incidence of scab lesions was significantly reduced compared to that following a single presymptom or postsymptom application. Further research is needed to determine if two-spray sequences of these fungicides would be more effective than single-spray applications in presymptom and postsymptom spray schedules.

Previous studies have shown that EBI fungicides do not inhibit spore germination (13,14). Drandarevski and Schicke (3), however, observed that conidia produced in lesions following a postsymptom application of triforine had lower germination rates, especially when two to four times the normal dose was applied. Our results indicate that conidia produced from lesions after a presymptom or a postsymptom application of the EBI fungicides had lower germination rates than the control conidia. Although these fungicides do not inhibit germination when they are applied directly to conidia, the germination of mature conidia produced from treated mycelium is reduced.

Studies have shown that seasonal and postseasonal applications of EBI fungicides and benomyl resulted in the production of fewer pseudothecia (2,8,9,10) and the release of fewer ascospores from overwintered leaves (2-4,8-11). Our studies indicate that a single postseason application of these fungicides results in the production of fewer and smaller pseudothecia that contain fewer ascospores. The poor performance of benomyl in this experiment may be due to resistant strains of *V. inaequalis* which have been previously detected in the orchard used in this study.

Histological observations of the naturally infected leaf disks indicated that pseudothecia were initiated following EBI fungicide treatments, while no pseudothecial initials were observed in any of the inoculated leaf disks in the laboratory. With the inoculated leaf disk technique, the fungal mycelium grew over the leaf surface and did not form a subcuticular stroma. Consequently, a greater surface area of the mycelium was exposed for uptake of the fungicides. In naturally infected leaves, such factors as the thick-walled, multilayered stroma and the uptake of the compounds by other fungal saprophytes, may limit the amount of the fungicides that comes in contact with the pathogen. More studies investigating the effects of various fungicide rates and multiple fungicide applications on pseudothecial formation are needed.

TABLE 5. Numbers of ascospores in pseudothecia from leaf disks infected by *Venturia inaequalis* and treated with EBI[w] fungicides on 14 and 16 October 1982 and 1983 and overwintered at the Mountain Horticultural Crops Research Station (MHCRS) in 1982 and 1983 and at Boone (BOONE), NC, in 1983

Treatment	Dose (μg a.i./ml)	Ascospores/pseudothecium (no.)[x] MHCRS 1982	MHCRS 1983	BOONE 1983
Control[y]		638 a[z]	622 a	636 a
Benomyl 50W	150.0	415 b	460 b	407 b
Triforine 18.2% EC	136.0	173 c	92 c	181 c
Bitertanol 50W	150.0	64 c	103 bc	135 c
Fenarimol 12.5% EC	32.8	144 c	16 d	67 d
Etaconazole 10W	18.7	19 d	17 d	17 d

[w] Ergosterol biosynthesis-inhibiting.
[x] Data are the means of three replicates with 10 pseudothecia per replicate.
[y] Control leaves dipped in tap water.
[z] Means within a column followed by the same letter are not significantly different ($P = 0.05$) according to the Waller-Duncan k-ratio t-test.

The EBI fungicides appeared to produce a permanent change in mycelium treated at leaf fall. Pseudothecia that were initiated in the treated disks remained smaller than the control pseudothecia throughout their development. Pseudoparaphyses and asci developed normally in all treatments, but at maturity, most of the asci in the EBI fungicide treatments were empty. A few asci in each pseudothecium contained mature ascospores, but the contents of most of the asci were undifferentiated. In the fenarimol and etaconazole treatments, several asci contained only one or two ascospores and they were aseptate. Siegel (13) pointed out that the levels of ergosterol in mycelium treated with EBI fungicides does not decline rapidly, indicating that the rate of ergosterol use is slower than its biosynthesis. Mycelium treated prior to leaf-fall may contain enough reserve ergosterol to initiate pseudothecial formation, but not enough for the synthesis of cell walls necessary for the formation of ascospores.

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