

Protectant and After-Infection Activity of Fungicides Against *Botryosphaeria obtusa* on Apple

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ABSTRACT

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Reduction of radial growth of *Botryosphaeria obtusa* on fungicide-amended potato-dextrose agar medium was determined. EC₅₀ values ($\mu\text{g/ml}$) were as follows: benomyl, 0.032; bitertanol, 0.043; flusilazole, 0.045; mancozeb, 10.26; myclobutanil, 0.426; penconazole, 0.132; and tebuconazole, 0.036. Six of the fungicides were tested for their protectant and eradicator activity against *B. obtusa*. When apple (*Malus domestica*) seedlings were inoculated 7 days after fungicide application, mean disease severities (lesions per 100 cm²) were as follows: benomyl, 4.6; bitertanol, 4.6; flusilazole, 20.7; mancozeb, 0.4; penconazole, 11.6; tebuconazole, 0.9; and control (no fungicide), 101.0. Only tebuconazole gave satisfactory disease control when plants were inoculated 14 days after fungicide application. Flusilazole, penconazole, and tebuconazole reduced the severity of frog-eye leaf spot by more than 50% when applied 48 hr after inoculation or earlier. Tebuconazole and benomyl reduced the severity of black rot on detached apple fruit when applied 96 hr after inoculation or earlier. The application of 4.8 cm of simulated rain did not reduce the protectant activity of mancozeb and benomyl against *B. obtusa* but did significantly reduce the efficacy of tebuconazole and penconazole.

The fungus *Botryosphaeria obtusa* (Schwein.) Shoemaker (syn. *Physalospora obtusa* (Schwein.) Cooke) causes frog-eye leaf spot and a limb canker on apple (*Malus domestica* Borkh.) and black rot on apple fruit (5). The pathogen can cause considerable losses (6), especially in warm, humid areas. The management of these diseases is based

on elimination of inoculum sources (7) and application of fungicides on a calendar-based schedule (5).

Models have been developed (2) that use temperature and wetness duration to predict periods favorable for infection of apple fruit and foliage by *B. obtusa*. Use of these models to improve the timing of fungicide application requires the availability of fungicides with after-infection activity against *B. obtusa*, because the fungicide must be applied once infection criteria have been met. An acceptable level of protectant activity is also needed in order to reduce the need for repeated spraying when conditions remain favorable for infection. We attempted to identify fungicides with both protectant and after-infection

activity against *B. obtusa* on apple foliage and fruit.

MATERIALS AND METHODS

Preliminary screening. Eight fungicides were tested for in vitro activity against *B. obtusa*: benomyl (Benlate 50WP; E. I. du Pont de Nemours & Co., Wilmington, DE), bitertanol (Baycor 50W; Mobay Corp., Kansas City, MO), flusilazole (Nustar 10W; du Pont), mancozeb (Dithane M-45 80WP; Rohm & Haas Co., Philadelphia, PA), myclobutanil (Nova 40W; Rohm & Haas), penconazole (Topas 10W; Ciba-Geigy Corp., Greensboro, NC), pyrifenoxy (Rondo 480EC; Maag Agrichemicals, Nutley, NJ), and tebuconazole (Elite 45DF; Mobay). Fruit isolates 080 (Jackson Springs, NC), 085 (Falston, NC), and 087 (Clayton, NC) of the fungus were evaluated on potato-dextrose agar (PDA) medium amended with 0.1, 1, 10, 50, and 100 $\mu\text{g a.i./ml}$ of mancozeb or 0.01, 0.1, 0.5, 1.0, and 5.0 $\mu\text{g a.i./ml}$ of the other fungicides. Formulated fungicides were suspended in sterile distilled water and added to molten PDA. Each combination of isolate, fungicide, and concentration was replicated three times. The experiment was conducted twice.

A 5-mm-diam mycelial plug, obtained from a culture of *B. obtusa* on PDA, was placed in the center of a 9-cm-diam petri plate containing fungicide-amended PDA. The plate was kept at room temperature (approximately 24 C) under

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continuous fluorescent illumination. After 5 days, radial growth was measured and subtracted from radial growth obtained on unamended PDA.

The percentage reduction in radial growth was used as a response variable for probit analysis. The logarithm of the response was regressed on the probit of the fungicide concentration. The concentration of fungicide from the regression line that resulted in 50% reduction in radial growth (EC_{50}) was obtained for each fungicide and isolate.

Evaluation of protectant activity. Open-pollinated Delicious apple seedlings were sprayed to runoff with the following concentrations ($\mu\text{g a.i./ml}$) of

fungicides: benomyl, 112; bitertanol, 150; flusilazole, 19; mancozeb, 2,000; penconazole, 91; and tebuconazole, 101. Half of the first unfolded leaf of each seedling was removed the day fungicides were applied to identify foliage that emerged after application. Plants were inoculated with a conidial suspension of *B. obtusa* containing 10^5 conidia per milliliter 0, 1, 3, 7, and 14 days after fungicide application. Inoculum production and inoculation procedures are described elsewhere (2).

Seedlings were kept in a greenhouse at about 25–30 C. Water was provided from below to avoid washing fungicide off plant surfaces. Symptoms of frogeye

leaf spot were assessed 14 days after inoculation. The number of lesions per leaf was counted, and the area of each leaf was determined by means of a leaf area diagram. Disease severity was expressed as number of lesions per 100 cm^2 . The experimental design was a split plot with four replications. Fungicides were considered main plots, and the intervals between application and inoculation were subplots. Each experimental unit consisted of three seedlings. In addition to the fungicide treatments, an unsprayed, inoculated control and an uninoculated control were included in the trial.

The experiment was conducted twice. In the first run, plants were sprayed on different days (days 0, 7, 11, 13, and 14) and inoculated on the same day (day 14). In the second run, plants were sprayed on the same day (day 0) and inoculated on different days (days 0, 1, 3, 7, and 14); a control was included for each inoculation date to account for possible variability in inoculum concentration. Thus, in run one, as the seedlings grew, an increasing number of leaves received fungicide on each spray day, and in run two, as the seedlings grew, an increasing number of leaves were not protected by fungicide.

For each run, separate analyses of variance were conducted for disease severity on total foliage, foliage present at the time of the first fungicide application, and foliage that emerged after the first fungicide application. A Waller-Duncan *k*-ratio *t* test with *k*-ratio 100 ($P = 0.05$) was used to compare treatment means.

Evaluation of after-infection activity in foliage. Open-pollinated Delicious seedlings were inoculated with a conidial suspension containing 10^5 conidia of *B. obtusa* per milliliter. Each inoculated seedling was sealed in a polyethylene bag with a wet paper towel to provide high moisture and incubated at 20 C for 24 hr to ensure infection of the foliage (2). Plants were sprayed 24, 48, 72, and 96 hr after inoculation with the same fungicides at the same rates as described for the protectant activity test. Seedlings were transferred to a greenhouse and evaluated 2 wk after inoculation. The percentage of leaf area that was diseased was estimated as one of the following categories: 0, 0–1, 1–3, 3–6, 6–12, 12–25, 25–50, or 50–100%. The experimental design was a split plot, with fungicides as main plots and after-infection spray intervals as subplots. Unsprayed, inoculated plants and uninoculated plants were included as controls. The experimental unit consisted of three seedlings. The treatments were replicated five times on different dates.

The disease severity rating of fungicide-treated seedlings was subtracted from that of the unsprayed, inoculated control, and the percentage reduction in

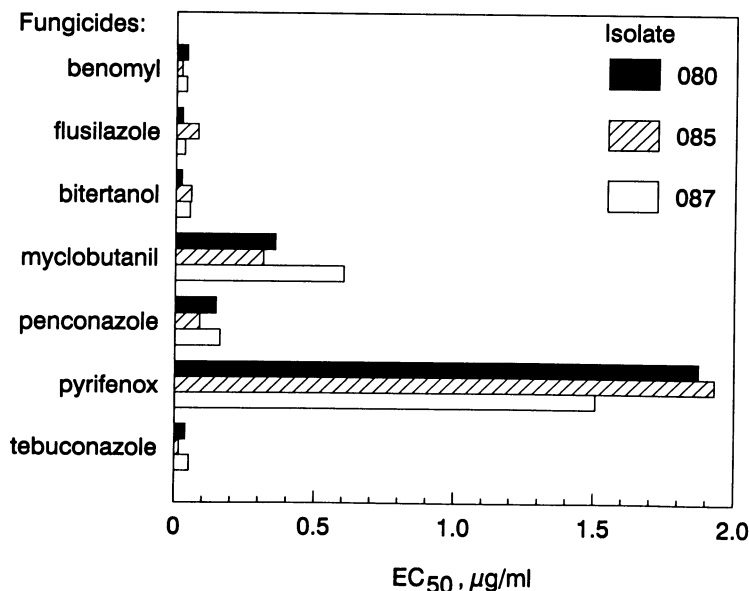


Fig. 1. EC_{50} values of seven fungicides incorporated into potato-dextrose agar medium against three isolates of *Botryosphaeria obtusa* after 5 days' growth.

Table 1. Effect of fungicides and interval between application and inoculation on severity of frogeye leaf spot (number of lesions/100 cm^2) on apple foliage

Fungicide	Time from fungicide application to inoculation (days)				
	0	1	3	7	14
Run 1^{w,x}					
Benomyl	8.2 aAB	3.7 aA	3.8 aAB	9.0 aA	16.8 aCD
Bitertanol	15.7 aA	28.7 aA	9.9 aA	11.5 aA	34.5 aABC
Flusilazole	6.0 bAB	1.6 bA	5.0 bAB	9.5 bA	58.3 aA
Mancozeb	0.9 bB	1.0 bA	0.3 bB	2.5 bA	29.3 aBC
Penconazole	2.6 aB	1.8 aA	2.7 aB	14.6 aA	49.0 bAB
Tebuconazole	1.3 aB	0.6 aA	0.3 aB	1.5 aA	4.6 aD
Untreated control					115 ^y
Run 2^{z,x}					
Benomyl	0.0 cB	0.3 cB	0.7 cBC	4.6 bCD	13.4 aB
Bitertanol	0.3 cB	0.5 cB	0.2 cC	4.6 bCD	18.4 aB
Flusilazole	0.0 cB	1.5 cB	2.6 cB	20.7 bB	40.3 cA
Mancozeb	0.0 bB	0.0 bB	0.0 bC	0.4 bD	—
Penconazole	0.0 cB	1.6 cB	0.0 cC	11.6 bBC	32.4 aA
Tebuconazole	0.0 bB	2.6 bB	0.3 bC	0.9 bD	10.0 aB
Untreated control	46.9 A	45.6 A	45.0 A	101.0 A	

^wIn run 1, plants were sprayed on different days (days 0, 7, 11, 13, and 14) and inoculated on the same day (day 14).

^xNumbers followed by the same letter (lowercase for rows and uppercase for columns) do not differ significantly ($P = 0.05$) according to the Waller-Duncan *k*-ratio *t* test.

^yDisease severity in the control was significantly ($P = 0.05$) higher than in all other treatments.

^zIn run 2, plants were sprayed on the same day (day 0) and inoculated on different days (days 0, 1, 3, 7, and 14).

disease was used as the response variable. A Waller-Duncan *k*-ratio *t* test with *k*-ratio 100 ($P=0.05$) was used to compare fungicides. For each fungicide, the percentage reduction in disease was regressed on the interval between inoculation and fungicide application, and best-fitting lines were obtained via least-squares techniques. The interval between inoculation and fungicide application that resulted in a 50% reduction in disease was obtained by interpolation from the best-fitting lines.

Evaluation of after-infection activity on detached apple fruit. Mature Rome Beauty fruit were collected from an orchard that had not been sprayed with fungicides since petal fall. Fruit were washed with 0.05% NaClO, rinsed in tap water, allowed to dry, and inoculated with a conidial suspension (10^5 conidia/ml) of *B. obtusa*. To inoculate fruit, a piece of four-ply laboratory paper towel (Uni/Disco, Inc., Troy, MI) measuring 3×3 cm was dipped in the conidial suspension and placed on the uninjured surface on one side of the fruit.

Fruit were placed in plastic boxes ($30.5 \times 12.7 \times 6.4$ cm) and covered with a layer of aluminum foil and two layers of wet paper towels. Boxes were covered, sealed, and incubated at room temperature (about 24 C) for 21 hr. Fruit were then uncovered and allowed to dry. Drying took about 1 hr, for a total wetness period of 22 hr. A 2-hr dry period was allowed between the infection period and the first fungicide application to reduce the viability of superficial inoculum as much as possible (3) and to limit the infection process to the initial 22-hr wetness period. The same fungicides at the same application rates tested for foliage were sprayed to runoff on the fruit surface 24, 48, 72, or 96 hr after inoculation.

One week after inoculation, fruit were wiped with sterile water to reduce superficial residues of fungicides without altering cuticle permeability. Ten pieces of the inoculated area, measuring approximately 0.7×0.7 cm, along with 0.4 cm of the underlying tissue, were cut from each fruit. The 10 pieces were placed in a moist chamber consisting of a 9-cm-diam plastic petri dish with a wet paper towel (6×6 cm) covered with a sterile piece of aluminum foil (6×6 cm) in the bottom. The chambers were sealed with Parafilm (American Can Co., Greenwich, CT) and kept at room temperature under continuous fluorescent illumination. After 4 wk the proportion of pieces with pycnidia of *B. obtusa* was determined in each moist chamber.

The experimental design was a split plot with three replications. Fungicides were main plots, and application intervals were subplots. The experimental unit consisted of four fruit. Unsprayed, inoculated fruit and

uninoculated fruit were included as controls.

Fungicide retention study. Open-pollinated Delicious seedlings were sprayed to runoff with benomyl, mancozeb, penconazole, or tebuconazole at the rates previously listed. One day after fungicide application, 0, 1.2, 2.4, 3.6, or 4.8 cm of simulated rain was applied to the seedlings. Rain was provided by spraying deionized water onto plants

placed on a turntable 1.2 m in diameter positioned 1.5 m under spray nozzles set at a pressure of 68.9 kPa, with a delivery rate of 2 cm/hr.

One day after rain was applied, seedlings were inoculated with a conidial suspension of *B. obtusa* containing 10^5 conidia per milliliter and incubated for 24 hr in a greenhouse under shade (about 21 C) as previously described. Disease was assessed 14 days after inoculation.

Table 2. Effect of fungicides and interval between application and inoculation on severity of frog-eye leaf spot (number of lesions/100 cm²) on apple foliage present 14 days before inoculation (run 1) or at the time of fungicide application (run 2)

Fungicide	Time from fungicide application to inoculation (days)				
	0	1	3	7	14
Run 1^{w,x}					
Benomyl	7.0 aA	3.6 aA	2.1 aAB	3.1 aA	2.4 aB
Bitertanol	7.9 aA	29.6 aA	8.3 aA	4.0 aA	10.2 aAB
Flusilazole	6.2 bA	1.3 bA	2.7 bAB	6.4 bA	19.5 aA
Mancozeb	0.2 bA	1.0 bA	0.0 bB	0.1 bA	9.7 aAB
Penconazole	0.7 bA	0.0 bA	0.9 bB	2.8 bA	21.9 aA
Tebuconazole	0.6 aA	0.0 aA	0.2 aB	1.1 aA	0.8 aB
Untreated control					55.0 ^y
Run 2^{x,z}					
Benomyl	0.0 bB	0.6 bC	0.8 bB	2.9 aC	1.2 abCD
Bitertanol	0.6 aB	0.8 aC	0.4 aB	3.0 aC	1.3 aCD
Flusilazole	0.0 bB	0.0 bC	1.0 bB	10.6 aB	3.0 bBC
Mancozeb	0.0 aB	0.0 aC	0.0 aB	0.2 aC	1.8 aBCD
Penconazole	0.0 cB	3.2 bcB	0.0 cB	8.8 aB	4.2 bB
Tebuconazole	0.0 aB	0.1 aC	0.0 aB	0.8 aC	0.4 aD
Untreated control	93.7 A	90.0 A	84.7 A	157.0 A	123.0 A

^wIn run 1, plants were sprayed on different days (days 0, 7, 11, 13, and 14) and inoculated on the same day (day 14).

^xNumbers followed by the same letter (lowercase for rows and uppercase for columns) do not differ significantly ($P=0.05$) according to the Waller-Duncan *k*-ratio *t* test.

^yDisease severity in the control was significantly ($P=0.05$) higher than in all other treatments.

^zIn run 2, plants were sprayed on the same day (day 0) and inoculated on different days (days 0, 1, 3, 7, and 14).

Table 3. Effect of fungicides and interval between application and inoculation on severity of frog-eye leaf spot (number of lesions/100 cm²) on apple foliage that emerged within 14 days before inoculation (run 1) or after fungicide application (run 2)

Fungicide	Time from fungicide application to inoculation (days)				
	0	1	3	7	14
Run 1^{w,x}					
Benomyl	9.5 abAB	3.7 bA	5.5 bABC	4.8 bBC	32.1 aCD
Bitertanol	23.4 bA	27.7 abA	11.5 bA	19.0 bAB	59.0 aABC
Flusilazole	5.9 bAB	1.9 bA	7.3 bAB	12.6 bABC	97.9 aA
Mancozeb	1.5 bB	0.9 bA	0.6 bC	4.8 bBC	48.9 aBC
Penconazole	4.5 bAB	3.6 bA	4.5 bABC	26.3 bA	76.0 aAB
Tebuconazole	1.9 aB	1.2 aA	0.4 aC	2.0 aC	8.5 aD
Untreated control					175.0 ^y
Run 2^{x,z}					
Benomyl	0.0 bA	0.0 bA	0.7 bB	6.3 bB	25.5 aB
Bitertanol	0.0 bA	0.2 bA	0.1 bB	6.1 bB	35.4 aB
Flusilazole	0.0 cA	2.9 cA	4.1 cA	30.8 bA	77.6 aA
Mancozeb	0.0 bA	0.0 bA	0.0 bB	0.7 bB	—
Penconazole	0.0 bA	0.0 bA	0.0 bB	14.3 bB	60.6 aA
Tebuconazole	0.0 bA	5.2 bA	0.5 bB	1.0 bB	19.7 bB
Untreated control	0.0 A	1.3 A	5.3 A	46.5 A	

^wIn run 1, plants were sprayed on different days (days 0, 7, 11, 13, and 14) and inoculated on the same day (day 14).

^xNumbers followed by the same letter (lowercase for rows and uppercase for columns) do not differ significantly ($P=0.05$) according to the Waller-Duncan *k*-ratio *t* test.

^yDisease severity in the control was significantly ($P=0.05$) higher than in all other treatments.

^zIn run 2, plants were sprayed on the same day (day 0) and inoculated on different days (days 0, 1, 3, 7, and 14).

The percentage of diseased leaf area was estimated using the scale described previously.

The experimental design was a split plot with three replications, with rain levels as whole plots and fungicides as subplots. An analysis of variance was conducted, and a Waller-Duncan *k*-ratio *t* test ($P = 0.05$) was used to compare treatment means.

RESULTS

In vitro study. Benomyl, flusilazole, bitertanol, penconazole, and tebuconazole strongly inhibited radial growth of *B. obtusa* on PDA, as indicated by their low EC_{50} values (Fig. 1). As a result, these

fungicides were selected for testing on plants. Myclobutanil was moderately active, and pyrifenoxy exhibited very little activity in vitro against *B. obtusa*. EC_{50} values for mancozeb were 10.1, 5.4, and 15.2 $\mu\text{g/ml}$ for isolates 080, 085, and 087, respectively. Mancozeb was also selected for further testing because of its widespread use as a standard protectant against apple diseases. The three isolates of *B. obtusa* exhibited similar degrees of sensitivity to each fungicide.

All fungicides tested reduced the severity of frogeye leaf spot on total foliage by about 90% when applied 7 days or less before inoculation (Table 1). Only tebuconazole reduced disease by more

than 90% when plants were inoculated 14 days after fungicide application.

When data only from foliage present at the time of the first fungicide application (day 0) were analyzed (Table 2), a significant reduction ($P < 0.05$) in protectant activity at 14 days was observed only for flusilazole and penconazole in both experimental runs. Protectant activity of mancozeb decreased significantly at 14 days in run 2.

Disease severity on foliage that emerged after day 0 (date of first fungicide spray) increased as the interval between fungicide application and inoculation increased (Table 3). The increase was not significant ($P < 0.05$) for tebuconazole. In run one, all fungicide treatments at all application dates lowered disease severity compared with the untreated control. In run two, flusilazole did not differ from the control when applied 3 or 7 days before inoculation.

In the second run of the experiment, a severe attack of powdery mildew (caused by *Podosphaera leucotricha* (Ellis & Everh.) Salm.) on young foliage hampered the inoculation of seedlings in the 14-day-interval replicates for the control and mancozeb treatments. As a consequence, disease severities on total foliage and young foliage for the 14-day interval were compared only among the remaining treatments in this run.

After-infection activity in foliage. Tebuconazole, penconazole, and flusilazole reduced disease more than benomyl ($P < 0.05$) (Fig. 2). Treatment with bitertanol and mancozeb resulted in more disease than treatment with benomyl.

Benomyl reduced frogeye leaf spot by more than 50% when applied 24 hr after inoculation (Fig. 3). Tebuconazole, penconazole, and flusilazole reduced disease severity more than 50% even when applied 48 hr after inoculation. Bitertanol and mancozeb did not exhibit any useful after-infection activity against *B. obtusa* in apple foliage.

After-infection activity in detached apple fruit. Tebuconazole and benomyl reduced disease severity on fruit ($P < 0.05$) compared with an untreated control and with the other fungicides (Fig. 4). The time interval between inoculation and fungicide application had no significant effect on disease severity. The fungicide \times time interaction was also not significant. However, a significant decrease in disease severity with increasing time interval was found in the case of tebuconazole (Fig. 5).

Fungicide retention study. The mean severity of frogeye leaf spot on unsprayed plants was 6.2% of the leaf area, which was significantly greater than that observed in any of the fungicide-rain combinations. Application of mancozeb provided the best leaf spot control regardless of the rain levels considered (Fig. 6). Demethylation-inhibiting (DMI) fungicides resulted in significantly

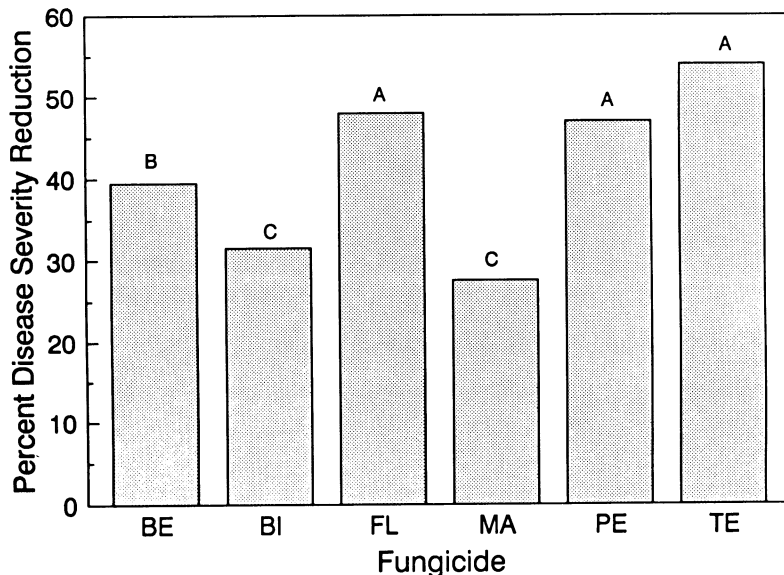


Fig. 2. Main effect of fungicides applied after infection of apple foliage by *Botryosphaeria obtusa* on reduction in severity of frogeye leaf spot, compared to an untreated control. Data are means of five replications and four application times. Bars labeled with the same letter do not differ significantly ($P < 0.05$) according to the Waller-Duncan *k*-ratio *t* test). BE = benomyl, BI = bitertanol, FL = flusilazole, MA = mancozeb, PE = penconazole, TE = tebuconazole.

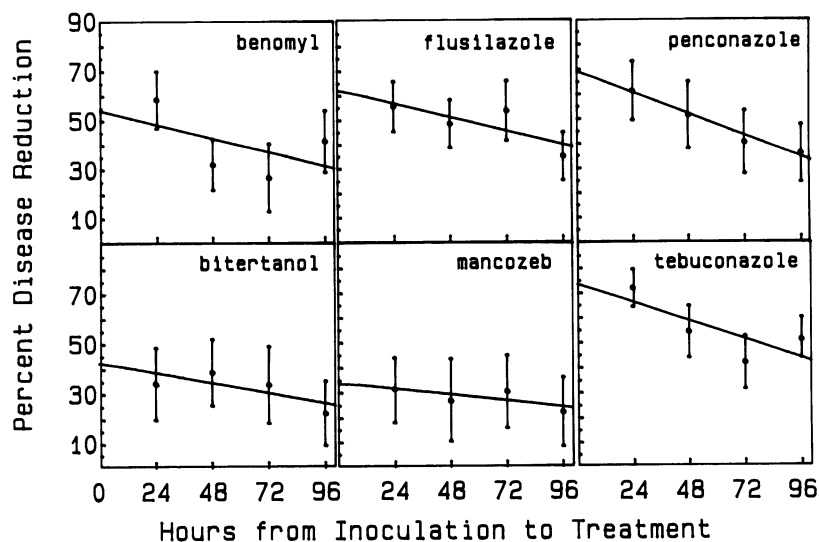


Fig. 3. Effect of fungicides and application times after inoculation of apple foliage with *Botryosphaeria obtusa* on reduction in severity of frogeye leaf spot relative to an untreated, inoculated control. Data points are means of five replications at different times. Sloped lines are best-fitting lines obtained by linear regression. Vertical lines are standard errors of the means.

higher disease severities than mancozeb when 1.2 and 4.8 cm of rain was applied. Benomyl resulted in intermediate levels of disease at those rain levels. No significant differences among fungicides were observed with 2.4 or 3.6 cm of rain.

DISCUSSION

In this study, several DMI fungicides and benomyl were shown to have strong inhibitory activity against *B. obtusa* in vitro at rates much lower than the recommended field rates. In vitro activity of some DMI fungicides against other apple pathogens has been demonstrated (11).

All fungicides tested exhibited good protectant activity against *B. obtusa* in apple foliage for up to 7 days. DMI fungicides gave levels of protection similar to those obtained with mancozeb and benomyl, which have been the standard preventive fungicides used for frog-eye leaf spot and black rot in the orchard. Tebuconazole provided adequate foliage protection (>90% reduction) for periods as long as 14 days, because it provided some protection of foliage that unfolded after fungicide application (Table 3).

Flusilazole, penconazole, and tebuconazole exhibited levels of after-infection activity against *B. obtusa* in apple foliage that should be commercially acceptable. The time that elapsed between inoculation and fungicide application determined the degree of disease reduction achieved with these fungicides. When they were applied later than 48 hr after inoculation, disease control was inconsistent. After-infection activity of a fungicide is related to the amount of material absorbed by the plant and its distribution in the leaf. As the fungus grows within the leaf, more fungicide is required to inhibit the pathogen in the tissues, and control can be achieved only at points where fungicide accumulates in sufficient amount to exert fungicidal or fungistatic activity. Thus, at some time before the level of eradicant activity becomes insignificant, some variation in performance is expected.

The degree of after-infection activity of DMI fungicides against frog-eye leaf spot was less than that of DMI fungicides against other apple diseases, such as scab (caused by *Venturia inaequalis* (Cooke) Wint.), in which disease reduction of 90% or more can be achieved when fungicides are applied earlier than 72 hr after inoculation (10). Frog-eye leaf spot lesions can become visible to the unaided eye in less than 48 hr (2). Thus, it is possible that lesions develop too rapidly for the fungicide to prevent tissue damage.

Only benomyl and tebuconazole reduced colonization of fruit by *B. obtusa*. The interval between inoculation and fungicide application, within the range considered in this study, did not seem to have any effect on the degree

of disease reduction achieved. The incubation period of *B. obtusa* in apple fruit is longer than that on foliage; initial lesions are visible with the unaided eye about 1 wk after inoculation (2). Therefore, applications as late as 96 hr after inoculation could conceivably prevent fungal growth and lesion development.

The degree of control achieved with different fungicides may be related to their ability to penetrate the fruit cuticle. Fruit treated with mancozeb or flusilazole had more disease than the untreated control. The reasons for this result are not clear. These fungicides may have altered intraspecific and interspecific competition on and/or beneath the cuticle of the fruit, thus resulting in more

disease than was observed in the absence of the fungicides.

In general, mancozeb and benomyl exhibited better retention properties during rainfall than the DMI fungicides penconazole and tebuconazole. Similar experiments involving mancozeb, benomyl, and the DMI fungicide fenarimol exposed to a 5-cm artificial rain resulted in a ranking of apple scab severity (12) similar to that obtained here for frog-eye leaf spot with mancozeb, benomyl, and the DMI fungicides exposed to 4.8 cm of rain.

The DMI fungicides have shown some protectant and excellent after-infection activity against apple diseases such as scab (9,10,13), powdery mildew (4), and

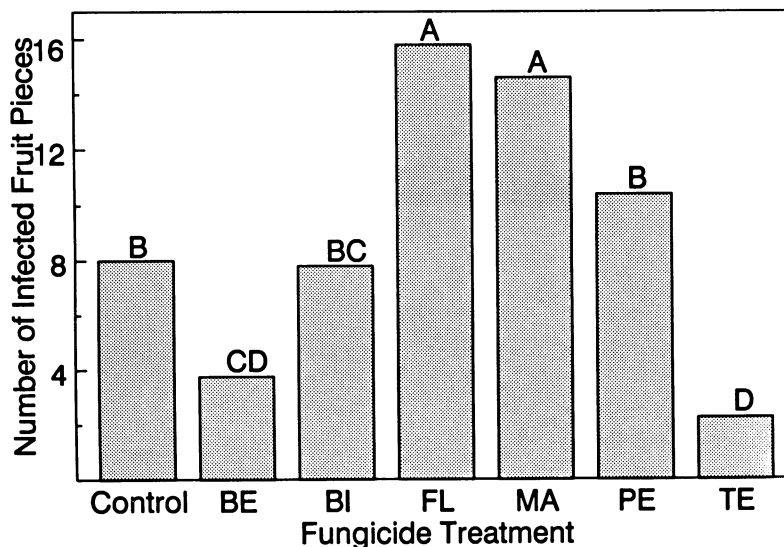


Fig. 4. Main effects of fungicides applied after infection of apple fruit by *Botryosphaeria obtusa* on development of black rot. Fruit pieces were cut from the inoculated area of the fruit and kept in moist chambers for 1 mo. Bars labeled with the same letter do not differ significantly ($P < 0.05$) according to the Waller-Duncan k -ratio t test. BE = benomyl, BI = bitertanol, FL = flusilazole, MA = mancozeb, PE = penconazole, TE = tebuconazole.

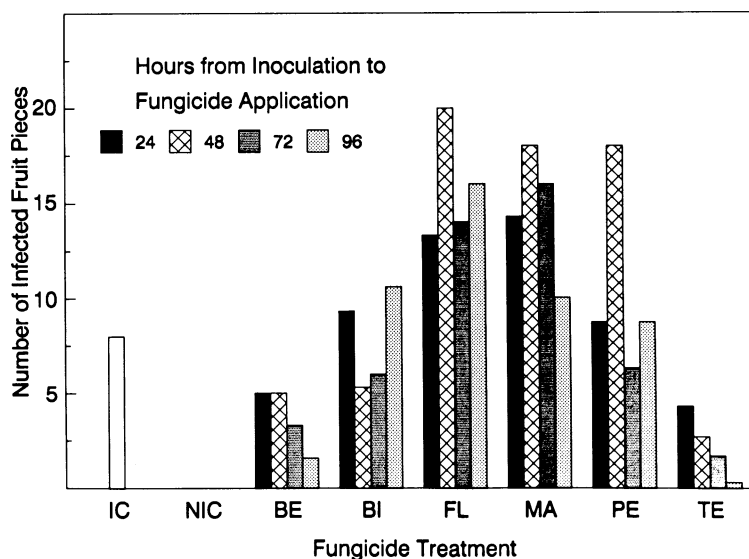


Fig. 5. Effect of fungicides and application times after inoculation of apple fruit with *Botryosphaeria obtusa* on development of black rot. Fruit pieces were cut from the inoculated area of the fruit and kept in moist chambers for 1 mo. Application times within fungicides did not differ significantly ($P < 0.05$) according to the Waller-Duncan k -ratio t test) except for tebuconazole. IC = inoculated control, NIC = noninoculated control, BE = benomyl, BI = bitertanol, FL = flusilazole, MA = mancozeb, PE = penconazole, TE = tebuconazole.

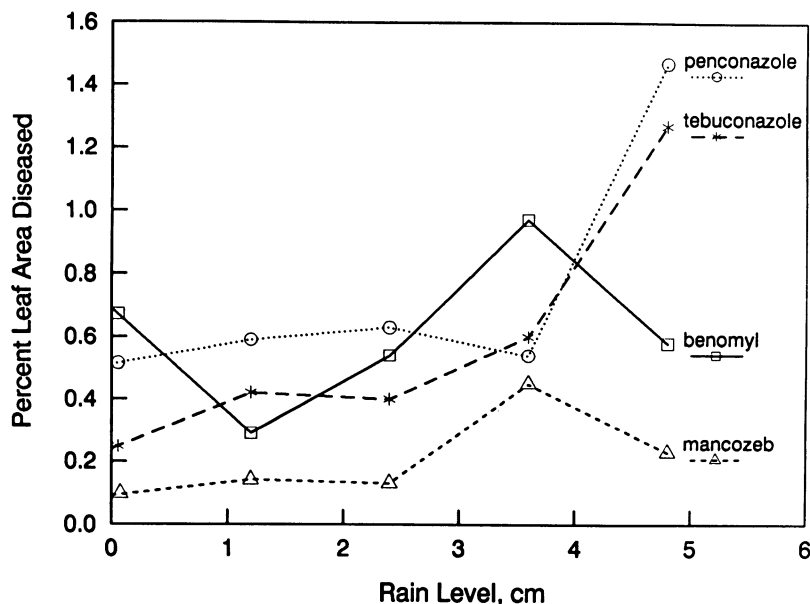


Fig. 6. Effect of simulated rain on the protectant activity of four fungicides against *Botryosphaeria obtusa* on apple foliage. Least significant differences (LSDs) (Waller-Duncan *k*-ratio *t* test) among fungicide means within each rain level (RL) were as follows: RL 0, 0.53%; RL 1.2, 0.29%; RL 4.8, 0.98%. LSD values were not calculated for RL 2.4 and 3.6 because the *F* test from analysis of variance for these RLs indicated no significant differences among fungicides.

cedar-apple rust (13). In our study, tebuconazole was the only DMI fungicide that provided useful levels of protection against and eradication of *B. obtusa*, particularly in apple foliage. Thus, it could be a useful tool in integrated management of these four apple diseases, especially in combination with materials with better retention properties, such as mancozeb or benomyl. Models that predict infection periods for apple scab, cedar-apple rust, and frog-eye leaf spot and black rot have been

developed (1,2,8). The use of a fungicide such as tebuconazole in combination with these models may significantly reduce fungicide use in some instances.

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