Chemical Control of Alternaria Blotch of Apples Caused by *Alternaria mali*

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ABSTRACT

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Four fungicides alone or in combination were tested in the field for activity on *Alternaria mali*. No satisfactory control was achieved except with iprodione at 0.30 g a.i./L. Captan and mancozeb alone and in combination with benomyl failed to control Alternaria blotch after artificial inoculation, whereas up to 65.1% disease reduction was achieved under conditions of natural infection compared with the nontreated control. Iprodione provided 69.1–79.8% and 71.6–75.1% disease reduction for the higher (0.30 g/L) and lower rate (0.15 g/L), respectively, under conditions of natural infection. After artificial inoculation, disease reduction ranged from 53.7 to 68.9% and 34.1 to 59.8% at the higher and lower rates of iprodione, respectively. The EC_{50} of iprodione for three isolates of *A. mali* was 1.04 μg/ml. No resistance of *A. mali* to iprodione was detected in the field. The incidence and severity relationship of Alternaria blotch was described by the equation: $S^{1/2} = b + cI$, where S = disease severity, b = the intercept coefficient, c = the slope coefficient, and I = disease incidence.

Alternaria blotch, caused by *Alternaria mali* Roberts, is one of the most important diseases of apples in Japan, South Korea, China, and other countries of southeastern Asia and recently was reported in Zimbabwe (4). *A. mali* was first identified in the United States in 1924, but the fungus was not considered an important pathogen of apples (8) until a disease outbreak occurred in Japan in 1956 (11). The disease was observed in North Carolina in 1987 on strains of Delicious (2). Symptoms on the leaves resemble frogeye leafspot, caused by *Botryosphaeria obtusa* (Schwein.) Shoemaker, or captan injury. Lesions appear as circular brown spots 2–5 mm in diameter. Severe infection can result in significant defoliation. Fruit usually are not affected except in some very susceptible varieties such as Indo (11).

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Chemical control of *A. mali* has been investigated in Japan and South Korea (5,6,9). Polyoxin, captafol, and iprodione suppressed sporulation of *A. mali* and provided the best control of Alternaria blotch (5,6,9).

The objective of this study was to investigate the activity of several fungicides that are used commonly for summer disease control in apple orchards in the southeastern United States against *A. mali*. Disease incidence and severity relationships also were studied.

MATERIALS AND METHODS

Fungicide test in the field. Four fungicide treatments were tested in 1989 and six treatments in 1990. The tests were conducted at the Central Crops Research Station (CCRS) in Clayton, NC. Fungicides and fungicide combinations tested in 1989 included captan 50W at 1.2 g a.i./L; captan at 0.6 g a.i./L + benomyl (Benlate 50DF) at 0.075 g a.i./L; mancozeb (Dithane DF) at 1.8 g a.i./L; and mancozeb at 0.67 g a.i./L + benomyl at 0.075 g a.i./L. Two rates of iprodione (Rovral 4F), 0.30 g a.i./L and 0.15 g a.i./L, were included in the 1990 test.

Eighty 10-yr-old Oregon Spur Delicious trees in five-tree groups grown in two rows were used. Three groups were assigned randomly to each treatment in 1989 and two in 1990. Fungicides were applied to the drip point (1,870 L/ha) with a Swanson DA 500 speed sprayer (Durand Wayland, LaGrange, GA) driven at 4 km/hr and with 1,379-kPa manifold pressure. Fungicide treatments were applied on 25 April, 9 and 23 May, 6 and 20 June, 3 and 18 July, and 15 and 29 August 1989; and 17 April, 1, 15, and 29 May, 12 and 26 June, 10 and 24 July, and 7 August 1990. Standard insecticides and miticides were applied to all treatments.

Apple trees in the orchard were inoculated with a conidial suspension of *A. mali* to ensure disease development because Alternaria blotch had not been observed previously in the orchard. The two most pathogenic isolates in our collection (No. 1522 and 1544 from Henderson County, NC) were used. Inoculum was grown on potato-dextrose agar (PDA) and harvested after 7 days. Inoculations were conducted five times in the period from 10 May to 5 July 1989 and two times in 1990. The following spore suspension concentrations were used (conidia per milliliter): 5.0×10^4, 10 May 1989; 1.2×10^4, 23 May 1989; 9.5×10^4, 16 June 1989; 1.3×10^6, 28 June 1989; 1.3×10^6, 5 July 1989; 1.0×10^5, 12 July 1990; and 1.9×10^4, 13 July 1990. Every second and fourth tree in each five-tree group was sprayed to the drip point with a 12.4-L hand-pump backpack sprayer. Inoculations were done just before periods of rain in 1989 to ensure favorable conditions for infection. In 1990, overhead irrigation was applied late in the evening 2 hr before inoculation.

Before the initiation of the spray program, 10 branches were selected arbitrarily and tagged on the second and fourth tree in each group. Disease incidence and severity were assessed four times in 1989 (29 June, 20 July, and 14 and 31 August) and five times in 1990 (21 May, 8 and 29 June, 20 July, and 13 August). A modified Horsfall-Barratt scale of 0–5 was used to assess disease severity where 0 = no symptoms, 1 = 0–3, 2 = 4–6, 3 = 7–12, 4 = 13–25, and 5 = 26–50% of the leaf area covered with lesions. Disease incidence was expressed as the proportion of leaves affected. Percent defoliation was assessed in 1990 by counting the number of nodes and number of leaves present on tagged branches on 27 August.

Temperature and relative humidity were monitored by a hygrothermograph (Belfort Instrument Co., Baltimore, MD) located in a standard instrument shelter approximately 100 m north of the orchard. Rainfall data were obtained from an automatic weather system located at the same site (15). Leaf wetness was recorded with a DeWit leaf wetness meter (Valley Streams Farm, Orano, Ontario) located in the orchard.

The experiments were conducted in a completely randomized design with three and two replications for each treatment in 1989 and 1990, respectively. Data were collected from the second and fourth trees in each group, which were considered subsamples. The nontreated control had four replications. More replications of the nontreated control were used than fungicide treatments to assess the uniformity of the inoculations throughout the orchard. Data were analyzed by a general linear models procedure with the Statistical Analysis System (10). Treatment differences were determined using groups (treatments) as the error term.

Disease incidence and severity relationship. Alternaria blotch severity was expressed as the mean disease severity from a modified Horsfall-Barratt scale (described earlier) and the mean number of lesions per leaf where 1 = seven, 2 = 30, and 3 = 80 lesions per leaf, and related to the disease incidence. Number of lesions per leaf was obtained from the modified Horsfall-Barratt scale (No. 1, 2, and 3) and represent the mean number of lesions per leaf associated with each scale number. The relationship for disease severity was examined by the function: $S^{1/2} = b + cI$ where S = disease severity, b = the intercept coefficient, c = the slope coefficient, and I = disease incidence (12,13). All treatments had nine observations (sampling dates) for both incidence and severity, except iprodione which was evaluated only in 1990 (five times). Data were analyzed by regressing disease incidence on disease severity with a linear regression procedure within the Statistical Analysis System (10). Slopes for each treatment were compared by a t test (1) and differences among slopes were compared with a linear regression procedure (10).

Determination of iprodione EC_{50} for *A. mali*. PDA was amended with 0, 1.0, 2.5, 5.0, or 10 μg of iprodione per milliliter and 5-mm-diameter mycelial plugs from 7-day-old *A. mali* colonies of isolates 1522, 1540, and 1544 were placed

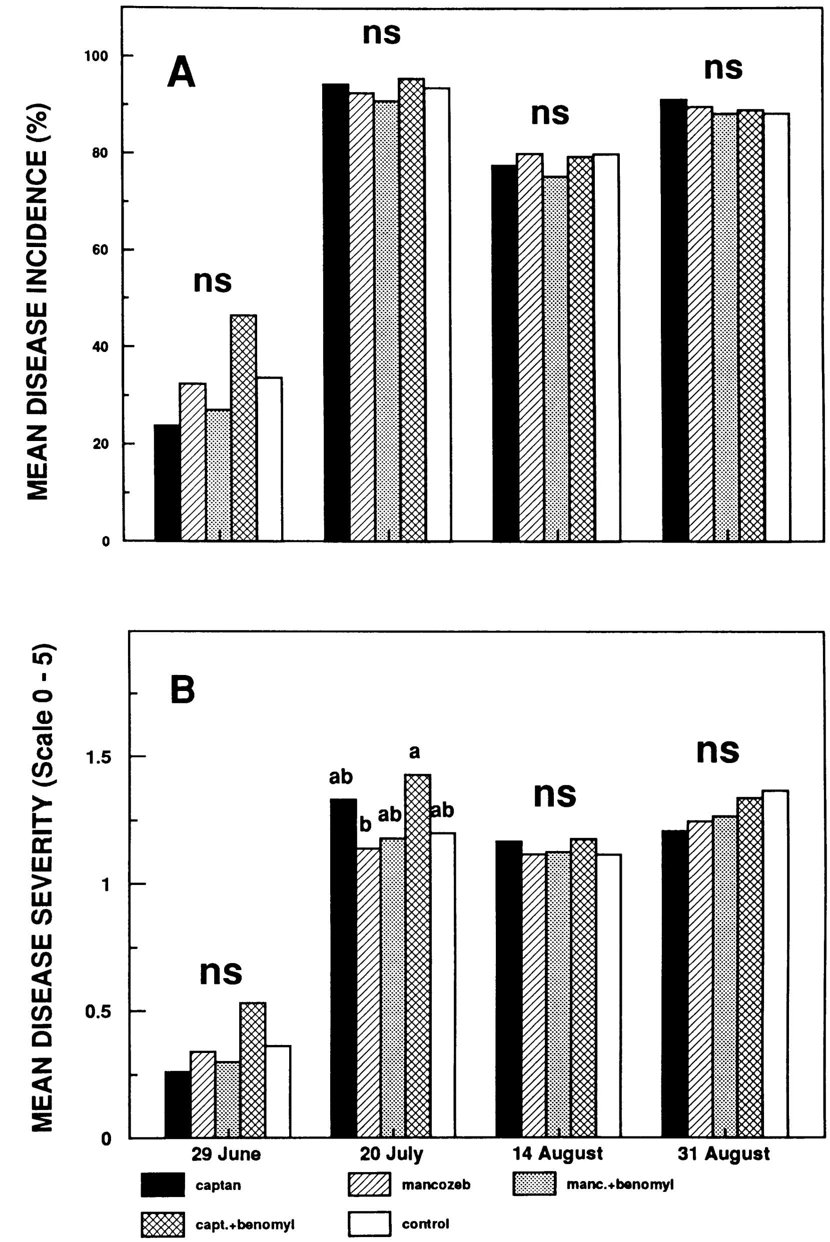

Fig. 1. Fungicide treatments and mean disease **(A)** incidence and **(B)** severity of Alternaria leaf blotch of apple at the Central Crops Research Station, 1989.

onto the centers of the plates. The fungus was allowed to grow for 4 days at 24 C in continuous dark. Colony diameters were measured and compared to growth on the unamended control. EC_{50}s were determined by regressing the $\log_{10}$ of the iprodione concentration on the probit of the percent inhibition. Data were analyzed by a probit procedure (10). Each fungicide combination-isolate number was replicated three times and the experiment was repeated three times.

Monitoring resistance of *A. mali* to iprodione in the field. One hundred lesions were selected arbitrarily from leaves collected from the trees treated with the two rates of iprodione, captan, and the nontreated control. Tissue with lesions was surface-sterilized in 0.5% NaOCl for 10 s, cut from the leaf with a 5-mm-diameter sterilized cork borer, and placed onto PDA plates amended with 5 μg of iprodione per milliliter. This concentration was used based on the EC_{50} of resistant sectors of cultures of *A. mali* that arose in our preliminary laboratory experiment and the MIC reported for isolates from Japan (6.25 μg of ipridione per milliliter) (9). Any growth of *A. mali* from the lesions was recorded after 3 days. Monitoring was conducted on 10 and 25 July and 16 August 1990.

RESULTS

Disease development in the field. No disease was observed in the orchard after the first two inoculations in 1989. Although both inoculations were done in the evening prior to rain, the subsequent weather was dry. Alternaria blotch first was observed after the third inoculation on 16 June. The inoculation was followed by a 12-hr wetting period, and rainfall occurred on three of the following 5 days. Disease severity increased most between 29 June and 20 July, after the fourth and fifth inoculations. During that period, average daily temperature was 26.2 C and 10 wetting periods were recorded. Disease severity did not increase from 21 July to 31 August, although mean daily temperature (27 C) and rainfall (16 wetting periods) were favorable for disease development (N. Filajdić, *unpublished*).

In 1990, the disease was observed first on 19 May; however, disease progress was slow until 12 July when trees were inoculated. The period from 19 May to 12 July was characterized by two hot dry periods in early June and again in late June and early July. After inoculation, disease severity increased rapidly until the last disease severity record was taken on 13 August. Mean daily temperature during that period was 25.8 C and eight wetting periods were recorded.

Fungicide tests in the field. In 1989, none of the fungicide treatments reduced disease incidence compared with the nontreated control on any of the four sampling dates (Fig. 1A). Disease incidence in the captan + benomyl treatment was greater, although not significantly so, on 29 June than in the nontreated control. Disease severity was similar to the nontreated control in all treatments on all sample dates except 20 July when disease severity was greater in the captan + benomyl treatment than the mancozeb treatment (Fig. 1B). Only 11.8 and 9.1% disease reduction was achieved by captan and mancozeb treatments, respectively.

In 1990, disease incidence on 21 May was less in all treatments than in the control (Fig. 2A). On 20 July, disease incidence was less in treatments with either rate of iprodione than all other treatments. Only the iprodione (0.30 g/L) treatment provided a significant disease reduction on 13 August (Fig. 2A). During the period of natural infection (21 May–29 June), disease severity generally was less than the nonsprayed control in all treatments except for the captan treatment on 29 June (although treatment means were not always significantly different from the nonsprayed control). Iprodione at 0.30 and 0.15 g/L and mancozeb treatments provided the best control during the period. During the period of rapid disease increase after inoculation, only the iprodione treatments provided commercially acceptable control. Iprodione at 0.30 g/L provided 68.9 and 53.7% disease reduction on 20 July and 13 August, respectively, when compared with the nontreated control, whereas 59.8 and 34.1% disease reduc-

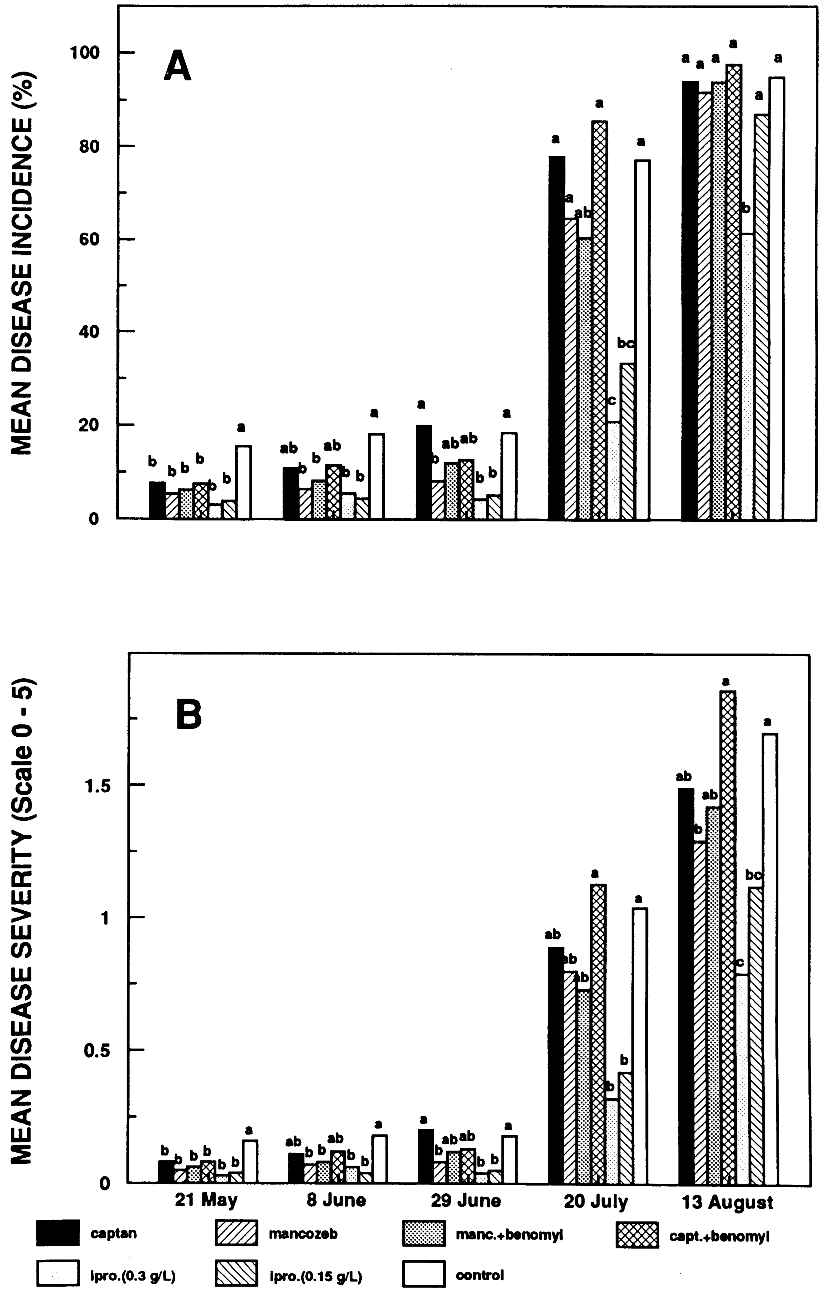

Fig. 2. Fungicide treatments and mean disease (**A**) incidence and (**B**) severity of Alternaria leaf blotch of apple at the Central Crops Research Station, 1990.

tion was provided by the lower rate of iprodione. Disease severity was generally less, though not always significantly so, in treatments containing mancozeb as opposed to captan.

Percent defoliation was 7.5 and 25.0 for the higher (0.30 g/L) and lower (0.15 g/L) rates of iprodione, respectively. Captan + benomyl, captan, and the control treatments had 56.8, 52.4, and 38.6% defoliation, respectively, whereas the mancozeb and mancozeb + benomyl treatments had 38.5 and 29.9% defoliation, respectively.

Disease incidence and severity relationship. There was no significant difference ($P = 0.05$) between slopes of the regression lines for any treatments applied in 1989 and 1990, and data from both years were combined to compare individual treatments. There were no significant differences among treatments for either measure of disease severity when iprodione treatments were excluded from the overall analysis. The general relationship between incidence and severity (as expressed by the modified Horsfall-Barratt scale) was similar for all fungicide treatments and the nontreated control when iprodione was included in the analysis (Table 1 and Fig. 3). When the number of lesions per leaf was used as a measure of disease severity, regression coefficients generally were similar for all fungicide treatments and the nontreated control except for iprodione at 0.30 g/L, which had a slope significantly steeper than most other treatments (Table 1 and Fig. 4). When disease severity was expressed as a mean derived from the modified Horsfall-Barratt scale and all data were used, $b = -19.0$ and $c = 94.02$ ($R^2 = 98.2\%$). When disease severity was expressed as the (mean number of lesions per leaf)$^{0.5}$, $b = -1.72$ and $c = 18.86$ ($R^2 = 95.3\%$).

Determination of iprodione EC_{50} for *A. mali*. The mean EC_{50}s for isolates 1522, 1540, and 1544 of *A. mali* were 0.78, 2.68, and 1.03 μg/ml, respectively.

Monitoring for resistance of *A. mali* to iprodione in the field. *A. mali* failed to grow from any lesion from any treatment plated on PDA amended with 5 μg of iprodione per milliliter. *A. mali* grew from all lesions plated on nonamended PDA.

DISCUSSION

Fungicides commonly used for summer disease control (captan, mancozeb, and mixtures with benomyl) resulted in poor control of Alternaria blotch under conditions of natural infection. Our observations in commercial orchards in North Carolina over the past 2 yr corroborate these findings. Only iprodione resulted in reduced disease incidence and defoliation when the inoculum level was high (after artificial inoculation). The use of iprodione to control Alternaria blotch is dependent on its registration on apples in the United States. If iprodione is registered, application schedules need to be developed to provide satisfactory control while minimizing the likelihood of resistance developing. McPhee reported that strains of *A. alternata* (Fr.:Fr.) Keissl. resistant to iprodione arose frequently in Canada (7). We failed to find any resistant isolates in our field samples; however, iprodione was used for only one season.

Disease incidence and severity relationships have been studied in other pathosystems, such as powdery mildew on apple (3,12,13) and wheat (3). The transformation used by Seem et al (12,13) provided a good fit for disease incidence and severity relationships for all treatments. The slope of the regression equation for iprodione at 0.30 g/L was not as steep as those of other treatments (though not significantly less) when disease severity was expressed as a mean derived from the modified Horsfall-Barratt scale. Conversely, when the number of lesions per leaf was used as an indicator of disease severity, the slope of the iprodione at 0.30 g/L was steeper than all others. Both results were expected as iprodione at 0.30 g/L significantly reduced disease severity compared with the other treatments. A similar trend was observed by Seem et al (12,13) in treatments that were most effective on apple powdery mildew. Although there were slight differences in the slopes of the incidence-severity relationships among treatments, we believe that they are similar enough so that incidence can be used as a reliable indicator of severity

Table 1. Comparison of coefficients from regression lines of disease incidence on disease severity for Alternaria blotch of apple

	Regression coefficients				Coefficient of determination	
	Intercept		Slope			
Treatment	A^x	B^y	A	B	A	B
Captan	−22.5	0.8	99.1	18.7 bc^z	98.5	90.1
Mancozeb	−19.5	−1.7	97.7	19.6 bc	99.0	95.3
Mancozeb + benomyl	−20.1	−1.3	95.5	18.8 bc	98.9	95.3
Captan + benomyl	−19.0	−1.4	91.9	18.1 bc	98.7	97.4
Iprodione (0.30 g/L)	−13.2	−13.2	78.0	29.5 a	94.3	94.3
Iprodione (0.15 g/L)	−16.4	−5.9	92.7	20.7 b	97.2	99.6
Control	−21.7	−0.1	95.5	17.9 c	98.1	98.3

[x] Mean disease severity expressed as (average rating + 0.5)$^{0.5}$ using a modified Horsfall-Barratt scale of 0–5 where 0 = no symptoms, 1 = 0–3, 2 = 4–6, 3 = 7–12, 4 = 13–25, and 5 = 26–50% of the leaf area covered with lesions.

[y] Mean disease severity expressed as (number of lesions per leaf)$^{0.5}$.

[z] Means within column followed by different letters are significantly different ($P = 0.05$), according to the Waller-Duncan k-ratio t test.

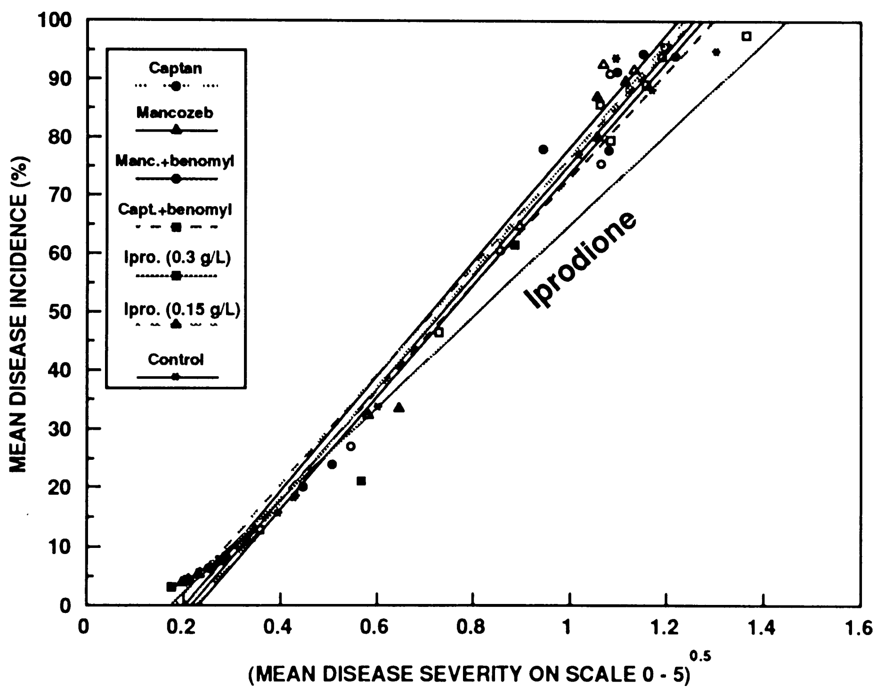

Fig. 3. Regression of disease incidence on disease severity for various fungicide treatments at the Central Crops Research Station, 1989 and 1990. Disease severity was expressed as the mean from a modified Horsfall-Barratt scale of 0–5 where 0 = no symptoms, 1 = 1–3, 2 = 4–6, 3 = 7–12, 4 = 13–25, and 5 = 26–50% of the leaf area covered with lesions.

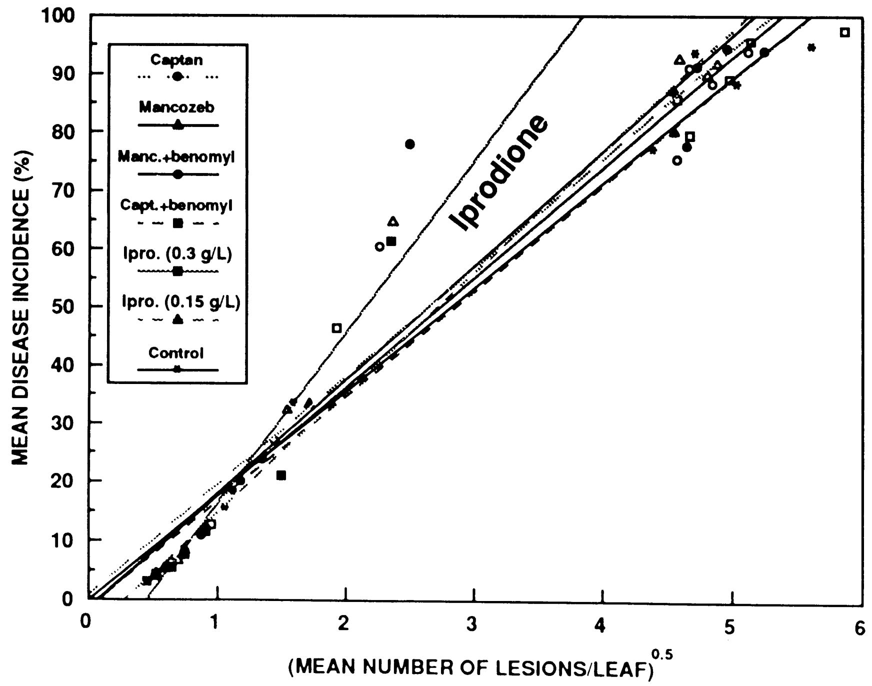

Fig. 4. Regression of disease incidence on disease severity for various fungicide treatments at the Central Crops Research Station, 1989 and 1990. Disease severity was expressed as the mean number of lesions per leaf where 1 = seven, 2 = 30, and 3 = 80 lesions per leaf.

in fungicide tests for control of Alternaria blotch. However, this relationship needs to be evaluated in tests that include additional efficacious fungicides.

Factors favoring the increase of Alternaria blotch in the orchard are not completely understood. Infection can occur in as few as 6 hr at favorable temperatures (12–28 C), (N. Filajdić and T. B. Sutton, *unpublished*). This suggests that the potential for disease development is great because *Alternaria* spp. are polycyclic pathogens. Our observations in commercial orchards suggest that if disease incidence and severity is low, standard fungicide programs used by growers will control the disease. Reduced disease incidence and severity in treatments with mancozeb during the period of 21 May to 29 June 1990 may be a reflection of this phenomenon. However, once the inoculum density reaches a certain threshold, none of the currently registered fungicides used at the rates tested are effective. This was observed in our plots in 1990 after the inoculation on 12 and 13 July. Thus, growers with light crops because of poor pollination or freeze injury will need to maintain a standard spray program on susceptible cultivars instead of a reduced one to keep inoculum levels low and prevent buildup of *A. mali* in their orchards.

It is unclear how the loss of EBDC fungicides will affect the incidence and severity of Alternaria blotch. EBDC fungicides traditionally have been used for control of Alternaria diseases in other crops (14) and provided acceptable control in our plots under light disease pressure. Based on the poor control obtained with captan and the captan + benomyl mixture in our tests, we hypothesize that the disease is likely to increase on strains of Delicious throughout much of the southeastern and eastern apple-growing regions of the United States.

ACKNOWLEDGMENT

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