# Soil Application of Benzimidazole Fungicides for the Control of Cephalosporium Stripe in the Greenhouse and Field

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### **ABSTRACT**

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Soil drenches with Benlate at rates as low as 12.0 kg a.i./ha or "in-furrow" applications of Benlate or Mertect at rates of 3.1 kg a.i./ha were effective in reducing the incidence of Cephalosporium stripe in greenhouse-grown winter wheat. Control of disease by Topsin-M was erratic or ineffective. Disease control in greenhouse studies was correlated with in vitro sensitivity of Cephalosporium gramineum to these fungicides. In-furrow application of Benlate, Mertect, or Topsin-M in field plots was not effective in consistently reducing the incidence of Cephalosporium stripe or increasing yield, even at high rates of application. Control of Cephalosporium stripe in the field with these systemic fungicides is not sufficiently consistent to be commercially feasible.

Cephalosporium stripe, caused by the fungus Cephalosporium gramineum Nisik. & Ikata (syn. Hymenula cerealis Ell. & Ev.), is an important vascular wilt disease of winter wheat (Triticum aestivum L.) in eastern Washington and northern Idaho, as well as other parts of the United States and the world (24). Plants are infected through the roots during the winter and early spring, with subsequent colonization of the stems as internodes elongate (17). Infected plants display prominent longitudinal chlorotic striping of the leaves, and often die prematurely (6). Yield loss due to Cephalosporium stripe is related to the degree of host colonization (5). Yield of infected stems may be only 20% of the yield from uninfected stems when stripes extend into the flag leaf and head (5,12).

Control of Cephalosporium stripe has relied on cultural practices such as delayed seeding, crop rotation, deep plowing, and burning of infested residue (4,13,15,19,20,25). Although some resistance to C. gramineum exists (7,14,16,18,19,25), cultivars that are highly resistant to C. gramineum and adapted to commercial production in eastern Washington are not available. Fumigation of soil with chloropicrin is effective in reducing soilborne inoculum and disease (25), but is not economically feasible. Fungicide seed treatments have been attempted, but were not successful (19).

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The potential to control vascular diseases with systemic benzimidazole fungicides has been demonstrated (9-11,22,23). For example, soil applications of Benlate, Mertect, or Topsin-M were effective in controlling Verticillium wilt of cotton, tobacco, strawberry, and potato (2,3,9-11,22), Fusarium wilt of tomato and watermelon (9-11,23), and late-wilt of maize, caused by Cephalosporium maydis Samra, Sabet, & Hingorani (21). Whether Cephalosporium stripe is amenable to control with these systemic fungicides has not been reported previously. Therefore, this study was initiated to determine the in vitro sensitivity of C. gramineum to Benlate, Mertect, and Topsin-M, and the potential for controlling Cephalosporium stripe in the field and greenhouse using soil applications of these fungicides.

## MATERIALS AND METHODS

In vitro sensitivity of C. gramineum to benzimidazoles. Three pathogenic isolates of C. gramineum (Cg84-16, Cg84-30, Cg85-4) were selected from a collection of 60 isolates because of their "typical" colony morphology and were tested for sensitivity to Benlate PNW 50W (benomyl), Mertect 340-F (thiabendazole), and Topsin-M 70W (thiophanatemethyl) to determine the concentration inhibitory to mycelial growth and conidial germination. These isolates were originally isolated from plants collected near Pullman, WA displaying symptoms of Cephalosporium stripe and maintained as stock mycelial cultures on Difco cornmeal agar (CMA) at 5 C. A stock suspension of the fungicides containing 2 mg a.i./ml in sterile deionized water was prepared and serially diluted. Two milliliters per liter of the stock suspension or each of the dilutions was added to molten (60 C) Difco potato-dextrose agar (PDA) to yield final concentrations

of 4.0, 2.0, 1.0, 0.5, and 0.25 µg a.i./ml. Inoculum blocks (8 mm in diameter) were cut with a cork borer from the edge of actively growing cultures of *C. gramineum* on CMA and transferred, mycelium down, to triplicate plates of fungicide-amended and unamended PDA. Colony diameter was measured after incubation in polyethylene bags for 14 days at 15 C in the dark.

A suspension of conidia was prepared by washing cultures of *C. gramineum* growing on PDA with sterile distilled water. The suspension was serially diluted and 0.1-ml aliquots were spread on duplicate plates of fungicide-amended and unamended PDA at the above concentrations. Three replicates were used. Colony counts were made after incubation for 10 days at 15 C in the dark.

Fungicidal vs. fungistatic activity was determined by transferring inoculum blocks from plates where mycelial growth did not occur to unamended PDA. Growth was evaluated after incubation for 10 days at 15 C.

Sixty isolates of *C. gramineum* collected between 1983 and 1987 and

Table 1. Incidence of Cephalosporium stripe in greenhouse-grown Stephens winter wheat following soil drenches with Benlate 50WP at two concentrations before, at, or after inoculation

	Rate (mg a.i./plant)a			
Time of application	6.6	66.6		
Inoculated control	83.	6 <sup>b</sup>		
14 Days preinoculation	34.6*°	0*		
Inoculation <sup>d</sup>	46.9*	12.4*		
7 Days postinoculation	21.7*	7.2*		
15 Days postinoculation	32.2*	19.2*		
Mean	33.7	9.7		
LSD ( $P = 0.05$ )	10.0°			

<sup>&</sup>lt;sup>a</sup> Plants were grown in potting mix consisting of 55% peat, 35% pumice, and 10% sand (w/w).

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<sup>&</sup>lt;sup>b</sup>Figure represents the mean of six replicates with three pots per replicate.

<sup>&</sup>lt;sup>c</sup>Figures represent the mean of six replicates. <sup>d</sup>Plants were inoculated 2 wk after planting by cutting the roots in situ with a knife, then pouring a suspension of conidia onto the soil.

<sup>&</sup>quot;Fisher's least significant difference compares means of fungicide rate between columns. Treatment means with an asterisk (\*) are significantly different from the inoculated control according to t tests (P = 0.05). Data were arcsin square root transformed before analysis. Transformed data are presented.

maintained as stock cultures were evaluated for sensitivity to Benlate and Mertect at  $4 \mu g \, a.i./ml$ , and Topsin-M at  $10 \, \mu g \, a.i./ml$ . Three inoculum blocks (8 mm in diameter) per isolate were cut from the edge of actively growing cultures and transferred, as above, to individual plates of fungicide-amended and unamended PDA. Colony diameter was measured, and growth on amended and unamended media was compared.

Greenhouse studies. Benlate soil drenches. Two experiments were conducted with Stephens (CI 17596) winter wheat: the first using a greenhouse potting mix (55% peat:35% pumice:10% sand, w/w) and the second using Thatuna silt loam (TSL) (fine silty, mixed, mesic Xeric Argialboll) Ap horizon (collected from the Plant Pathology Farm, Pullman, WA) mixed 2:1 (v/v) with pumice (particle size up to 1 cm) to improve drainage. Four seeds per 15-cm-diameter pot were planted and maintained in a growth chamber at 15-18 C with a 12-hr light period (approximately 300  $\mu \text{E} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ ) supplied by fluorescent lights (Sylvania GRO-VHO-WS and Westinghouse CW-SHO-EW [1:2 mixture) for 4 wk. The temperature was then lowered to 5 C for 4 wk for vernalization. After vernalization, plants were moved to a greenhouse bench at 20-28 C with natural lighting until maturity.

Plants were inoculated 2 wk after planting by cutting the roots in situ with a knife and pouring a suspension of conidia (200 ml of  $5 \times 10^6$  and 300 ml of  $2 \times 10^8$  cfu/ml for experiments 1 and 2, respectively) onto the soil surface. Roots were cut by placing the knife midway between adjacent plants in a pot (about 2-3 cm from the plant base) and pushing the knife to the bottom of the pot.

Benlate was applied as a soil drench ranging from 14 days before inoculation to 15 days after inoculation at 6.6 or 66.6 mg a.i./plant (12.0 and 121.2 kg a.i./ha, respectively, based on a 72.8 kg/ha seeding rate and 25 seeds/g) in the first experiment, and 6.6, 13.3, or 132.5 mg a.i./plant (12.0, 24.2, and 241.1 kg a.i./ha, respectively) in the second experiment. Benlate was mixed with 400 ml of water immediately before drenching and then was poured on the soil surface, except at inoculation when the plants were inoculated first, then the fungicide was mixed with the balance of water needed to make 400 ml and was poured on the soil.

Disease incidence was evaluated at maturity by cutting stems at the base and separating them into healthy and diseased classes (based on the presence of stripes in leaves) from which the percentage of infected stems was calculated. In both experiments, treatments were arranged in a randomized complete block factorial design, with time of fungicide application and rate as the main factors. There were six

replicates in the first experiment and four replicates in the second experiment. Two or three untreated controls per replicate were included. Disease incidence data were arcsin square root transformed before analysis; the transformed data are presented. Analysis of variance was conducted to determine significance among the main factors. Treatment means were compared with the mean of the untreated controls using t tests to determine significance.

"In-furrow" application. Benlate, Mertect, or Topsin-M were applied in a liquid or dry-powdered formulation to the bottom of the seed hole immediately before planting to simulate an "infurrow" application in the field.

A single experiment was conducted to determine the effect of fungicide formulation on control of Cephalosporium stripe. Seeds were sown in potting mix, as previously described, containing oat kernels (1% w/w) colonized by C. gramineum (8). Benlate or Mertect (DF) was applied at rates of 1.7, 3.4, 6.8, or 13.6 mg a.i./plant (3.1, 6.2, 12.4, and 24.8 kg a.i./ha, respectively, based on a 72.8 kg/ha seeding rate and 25 seeds/g). Dry treatments were applied as a 3.75% a.i. formulation in talc, and the liquid treatments were applied in 3 ml of water. Roots of plants were cut with a knife in situ, as previously described, 15 days after seeding to facilitate infection from inoculum produced on the colonized oat kernels. Disease incidence was evaluated as before. Treatments were arranged in a randomized complete block factorial design with fungicide, formulation, and rate as the main factors. Analysis of data was conducted as previously described.

Two experiments were conducted with Stephens wheat grown in the TSL:pumice mix, with Benlate, Mertect, and Topsin-M applied at rates of 1.7, 3.4, 6.8, and 13.6 mg a.i./plant. Plants were grown for 4 or 8 wk in a growth chamber at 15–18 C, as described previously, then inoculated by cutting the roots in situ and pouring a suspension of conidia (100 ml of  $9 \times 10^5$ 

or 300 ml of  $9 \times 10^7 \text{ cfu/ml}$ , respectively) onto the soil surface. Plants were vernalized, then allowed to grow to maturity in the greenhouse before evaluating disease incidence. Treatments were arranged in a randomized complete block factorial design with fungicides and rates as the main factors. One and three untreated, inoculated controls were included in each of the six replicates in the first and second experiments, respectively.

Field experiments. Field plot experiments were conducted between 1984 and 1987 at the Plant Pathology Farm, Pullman, WA. These plots will be referred to as the 1985, 1986, and 1987 plots, respectively. The plot area was in a winter wheat-summer fallow rotation. Four-row plots (4.3 m  $\times$  1.8 m) were seeded with a deep-furrow, split-packerwheel drill with John Deere HZ hoeopeners spaced 40 cm apart. Fertility and weed management were consistent with local commercial practices. Fungicides were applied as granular formulations on silica sand (16 mesh) at seeding with a Barber Spreader. Granular fungicides were prepared by moistening silica sand in a widemouth bottle, adding drypowdered fungicide (Benlate PNW 50W, Mertect DF, or Topsin-M 70W), then mixing the sand and fungicide until all of the fungicide adhered to the sand. The fungicide-coated sand was then spread on paper and allowed to dry.

Nugaines (CI 13968) and Hyslop (CI 14564) winter wheat were seeded on 17 September 1984 at the rate of 72.8 kg/ha. Benlate and Mertect were applied in the furrow with the seed at the rate of 11.1 kg a.i./ha as 10.4% a.i. granular formulations. Half of each plot was not treated with fungicide and served as an untreated control for that fungicide-cultivar combination. Treatments were arranged in a randomized complete block factorial design with cultivar and fungicide application as the main factors; there were four replicates. Naturally infested wheat straw (approximately 800 kg/ha) was spread over the plot site and

Table 2. Incidence of Cephalosporium stripe in greenhouse-grown Stephens winter wheat following soil drenches with Benlate 50WP at three concentrations before, at, or after inoculation

		R	ate (mg a.i./plant	t) <sup>a</sup>
Time of application	_	6.6	13.3	132.5
Inoculated control	71.4 <sup>b</sup>			
14 Days preinoculation		49.8*°	14.4*	0*
Inoculation <sup>d</sup>		17.9*	9.4*	7.6*
14 Days postinoculation		6.2*	0*	0*
LSĎ		16.3 <sup>e</sup>		

<sup>&</sup>lt;sup>a</sup> Plants were grown in Thatuna silt loam + pumice (2:1).

<sup>&</sup>lt;sup>b</sup>Figure represents the mean of four replicates with two pots per replicate.

<sup>&</sup>lt;sup>c</sup> Figures represent the mean of four replicates.

<sup>&</sup>lt;sup>d</sup>Plants were inoculated 2 wk after planting by cutting the roots in situ with a knife, then pouring a suspension of conidia onto the soil.

<sup>&</sup>lt;sup>e</sup> Fisher's least significant difference (P = 0.05) compares treatment means within or between columns. Treatment means with an asterisk (\*) are significantly different from the inoculated control according to t tests (P = 0.05). Data were arcsin square root transformed before analysis. Transformed data are presented.

incorporated to a depth of 7–10 cm with a disk before planting.

Daws winter wheat (CI 17419) was seeded on 12 September 1985 and 11 September 1986 at the rate of 72.8 kg/ha. Benlate and Topsin-M were applied in the furrow at rates of 1.1, 2.2, 4.5, and 6.7 kg a.i./ha as 4.5 and 5.0% a.i. granular formulations, respectively. Treatments were arranged in a randomized complete block factorial design with fungicide and rate as the main factors; one untreated control was included in each of the four replicates. The plots received 134 and 202 kg/ha colonized oat kernels broadcast

after planting in 1985 and 1986, respectively.

Disease incidence was evaluated when wheat was in the watery-ripe to soft-dough stage of development (Feeke's scale 10.5.4–11.2). A 0.5-m section of row was removed from one of the outside rows of each plot by cutting the stems at the base, dividing them into healthy or diseased classes, and calculating the percentage of infected stems. Yield was determined by harvesting the two center rows from each plot. Stems were cut by hand, bundled, and threshed with a Vogel stationary bundle thresher.

**Table 3.** Incidence of Cephalosporium stripe in greenhouse-grown Stephens winter wheat following "in-furrow" application of liquid or dry formulations of Benlate PNW 50W or Mertect DF at four rates at seeding

		Rate (mg a.i./plant) <sup>a</sup>					
Treatment		1.7	3.4	6.8	13.6		
Inoculated control <sup>b</sup>	82.4°						
In-furrow: dry							
Benlate		74.9 <sup>d</sup>	66.5*	60.4*	58.7*		
Mertect		61.5*	43.2*	36.9*	7.7*		
In-furrow: liquid							
Benlate		76.4	67.6*	50.6*	10.0*		
Mertect		58.8*	40.8*	4.2*	8.9*		
LSD(P = 0.05)		16.2°					

<sup>&</sup>lt;sup>a</sup> Plants were grown in potting mix consisting of 55% peat, 35% pumice, 10% sand (w/w). Fungicides were applied to the bottom of the seed hole immediately before seeding. Dry treatments were applied as a 3.75% a.i. formulation in talc; liquid treatments were applied in 3 ml of water.

**Table 4.** Incidence of Cephalosporium stripe in greenhouse-grown Stephens winter wheat following "in-furrow" application of Benlate PNW 50W, Mertect 340-F, or Topsin-M 70W at four rates

Fungicide	_	Rate (mg a.i./plant) <sup>a</sup>				
	_	1.7	3.4	6.8	13.6	Mean
Experiment 1						
Inoculated control <sup>b</sup>	80.2°					
Belate		72.8	56.3*	53.1*	29.8*	53.0*
Mertect		77.4	55.3*	50.0*	34.5*	54.3*
Topsin-M		79.3	81.7	75.8	74.7	77.9
Mean		76.5	64.5	59.7	46.3*	
LSD		12.1 <sup>d</sup>				10.5°
Experiment 2						
Inoculated control	37.0 <sup>f</sup>					
Benlate		16.3 <sup>g</sup>	8.0*	9.2*	22.0	13.9*
Mertect		17.8*	20.6	19.9	3.5*	15.4*
Topsin-M		18.7	7.1*	20.2	7.1*	13.3*
Mean		17.6	11.9	16.4	10.9	
LSD		NS				NS

<sup>&</sup>lt;sup>a</sup> Plants were grown in Thatuna silt loam + pumice (2:1, v/v). Fungicides were applied in 3 ml of water to the bottom of the seed hole immediately before seeding.

Disease incidence data were transformed using the arcsin square root function; means of transformed data are presented. Analysis of variance was used to determine significance of main effects and interactions, and Fisher's least significant difference was used to compare treatment means when appropriate. T tests were used to compare treatment means with those of the untreated controls during 1985–1986 and 1986–1987.

#### **RESULTS**

In vitro sensitivity of C. gramineum to benzimidazoles. The concentrations of Benlate, Mertect, and Topsin-M that reduced mycelial growth by 50% were between 0.25 and 0.5  $\mu$ g/ml, 0 and 0.25  $\mu$ g/ml, and 1.0 and 2.0  $\mu$ g/ml, respectively. Complete inhibition of mycelial growth was apparent at 1.0  $\mu$ g/ml for both Benlate and Mertect, but growth still occurred at 4.0  $\mu$ g/ml with Topsin-M. Germination of conidia was completely inhibited by  $0.25 \mu g/ml$  of Benlate or Mertect, the lowest concentration tested. With Topsin-M, however, germination of conidia occurred at concentrations up to 1.0  $\mu$ g/ml, but was completely inhibited at  $2.0 \mu g/ml$ . There was only a slight reduction in the percent germination of conidia at 1.0  $\mu$ g/ml of Topsin-M compared with the control. The three isolates of C. gramineum used to determine the inhibitory concentrations of Benlate, Mertect, and Topsin-M behaved similarly.

Mycelial growth from inoculum blocks placed initially on fungicide-amended PDA resumed when the blocks were transferred to unamended PDA. These results suggest that the effect of Benlate, Mertect, and Topsin-M is fungistatic with respect to mycelial growth.

None of the 60 isolates of *C. gramineum* was tolerant of Benlate, Mertect, or Topsin-M, as indicated by growth from all three inoculum blocks placed on fungicide-amended PDA. However, two of the 60 isolates grew from one of the inoculum blocks, (one each on Benlate- and Mertect-amended PDA). Tolerant isolates remained tolerant after being maintained for 3 wk on unamended CMA then transferred back to Benlate- or Mertect-amended CMA.

Effect of soil drenches with Benlate on Cephalosporium stripe. Benlate soil drenches significantly reduced the incidence of Cephalosporium stripe at all application times and rates compared with the untreated control (Tables I and 2). The magnitude of reduction in disease incidence was greater with an increasing rate of fungicide application in both rooting media. Time of fungicide application did not have a significant effect on disease incidence when plants were grown in the high-organic matter

<sup>&</sup>lt;sup>b</sup>Oat kernels colonized by *Cephalosporium gramineum* were mixed into the rooting medium (1%, w/w) before planting. Roots were cut with a knife in situ 15 days after planting.

Figure represents the mean of six replicates with two pots per replicate.

<sup>&</sup>lt;sup>d</sup>Figures represent the mean of six replicates.

<sup>&</sup>lt;sup>e</sup> Fisher's least significant difference compares means within or between columns. Treatments with asterisks (\*) are significantly different from the inoculated control based on t tests (P = 0.05). Data were arcsin square root transformed before analysis. Transformed data are presented.

<sup>&</sup>lt;sup>b</sup>Plants were inoculated by cutting the roots in situ with a knife, then pouring a suspension of conidia onto the soil surface.

<sup>&</sup>lt;sup>c</sup> Figures represent the mean of six replicates.

<sup>&</sup>lt;sup>d</sup>Fisher's least significant difference (P = 0.05) compares means of application rates; NS = not significant. Treatments with asterisks (\*) are significantly different from the inoculated control based on t tests (P = 0.05). Data were arcsin square root transformed before analysis. Transformed data are presented.

Fisher's least significant difference (P = 0.05) compares means of fungicides.

Figure represents the mean of six replicates with three pots per replicate.

<sup>&</sup>lt;sup>8</sup> Figures represent the mean of six replicates.

potting mix (Table 1). There was a significant fungicide rate × application time interaction for plants grown in the TSL-pumice mix, however, that resulted from a significantly higher incidence of disease only at the lowest rate of application 14 days before inoculation (Table 2).

Effect of "in-furrow" applications of benzimidazoles on Cephalosporium stripe. Applications of Benlate and Mertect in both liquid and dry form resulted in significantly lower incidences of Cephalosporium stripe compared with the inoculated control at all application rates, except the lowest rate of Benlate (Table 3). There was a significant rate × formulation × fungicide interaction that resulted from lower disease incidence following application of Mertect rather than Benlate at all rates, except the lowest and highest rates of the dry and liquid formulations, respectively.

Mertect and Benlate were more effective than Topsin-M in reducing the incidence of Cephalosporium stripe in one of two subsequent experiments using "in-furrow" applications of liquid fungicides (Table 4). Mertect and Benlate were not significantly different in the first experiment, and both reduced disease more than Topsin-M when averaged across rate of application. However, in the second experiment, where disease incidence was much lower, disease control was more erratic, although

Table 5. Incidence of Cephalosporium stripe and yield of Hyslop and Nugaines winter wheat in field plots following in-furrow application of Benlate or Mertect at seeding in Pullman, WA, during 1984–1985

Cultivar/ treatment <sup>a</sup>	Disease incidence <sup>b</sup>	Yield (kg/ha)	
Nugaines			
Benlate	$30.8^{\circ}$	98.0*	
Benlate			
untreated control	40.5	71.3	
Mertect	26.2	86.0	
Mertect			
untreated control	24.4	77.7	
Hyslop			
Benlate	47.8	69.0	
Benlate			
untreated control	52.8	55.2	
Mertect	37.5*	77.8	
Mertect			
untreated control	52.7	64.6	
$LSD^d$	11.4	19.2	

<sup>&</sup>lt;sup>a</sup>Silica sand (16 mesh) coated with Benlate PNW or Mertect DF (10.4% a.i.) was applied with the seed in the seed row at the rate of 11.1 kg a.i./ha.

fungicide-treated and untreated control.

Asterisks (\*) indicate a significant difference

with the untreated control.

several treatments were significantly better than the control, and the three fungicides were not significantly different when averaged across rate of application. It is not known why disease incidence was lower in the second experiment.

Control of Cephalosporium in field plots. Cephalosporium stripe incidence was moderate in 1985 and 1987, but too low for meaningful comparisons in 1986. Therefore, 1986 data are not presented.

In 1985, disease incidence averaged about 7% less and yield averaged 15.5 kg/ha higher in the fungicide-treated plots than in the untreated controls (Table 5). Although there was a trend toward reduced incidence of disease and increased yield with fungicide application, only the Mertect-treated Hyslop wheat had a significantly lower disease incidence, and only the Benlate-treated Nugaines had a significantly greater yield.

In 1987, only Topsin-M provided a significant reduction in disease incidence when averaged across all rates, compared with the untreated control (Table 6). Topsin-M, however, was not significantly different than Benlate. There were no differences among rates within the fungicides according to F tests, although Benlate at 4.5 kg/ha and Topsin-M at 2.2 kg/ha had disease incidences significantly less than the untreated control. Yield was not significantly increased by any treatment in 1987.

#### DISCUSSION

Cephalosporium stripe was reduced by soil drenches with Benlate at rates as low as 12.0 kg a.i./ha or "in-furrow" applications of Benlate or Mertect under greenhouse conditions (Tables 1-4) at rates of 3.1 kg a.i./ha. Topsin-M exhibited erratic control when disease

incidence was low, and no control when disease incidence was high, at similar rates. Under field conditions, control of Cephalosporium stripe with these three fungicides was erratic even at application rates (11 kg a.i./ha) that would not be economically feasible (Tables 5 and 6).

Others have reported similar results with Verticillium wilt of cotton and latewilt of maize. Relatively low concentrations of Benlate controlled both diseases in the greenhouse, but much higher application rates were necessary in the field; even then control was erratic (9-11,21). The greater control obtained in the greenhouse was attributed to confinement of the root system within a pot, and the complete uptake of the chemical applied to a pot (10). In the field, roots only partially contact a soilapplied fungicide, unless the fungicide is thoroughly mixed throughout the area of root distribution (10,11).

Control of vascular diseases requires a continual supply of fungicide at an inhibitory or toxic concentration in the xylem (1-3,22). In field experiments, the fungicide was limited to the soil in the seed zone. Although the chemical was absorbed by the wheat plants, as evidenced by partial control of Cephalosporium stripe, the duration of chemical concentration high enough to inhibit the pathogen was probably too short. The infection period of winter wheat by C. gramineum extends over a long period that begins in November, 2 to 3 mo after planting. Infection probably occurred long after the concentration of fungicide dropped below the critical concentration necessary to inhibit the pathogen.

Control of Cephalosporium stripe in the greenhouse was correlated with in vitro sensitivity of *C. gramineum* to Benlate, Mertect, and Topsin-M. Benlate

Table 6. Incidence of Cephalosporium stripe and yield of Daws winter wheat in field plots following in-furrow application of Benlate PNW or Topsin-M at four rates at seeding in Pullman, WA, during 1986–1987

	Rate (kg a.i./ha) <sup>a</sup>				
•	1.1	2.2	4.5	6.7	Mean
		Disease inc	cidence <sup>b</sup>		
23.5°					
	25.3	22.6	14.1*	12.3	18.6
	15.6	9.5*	15.1	19.3	14.9*
	20.4	16.1	14.6*	15.8	
		Vield (k	o/ha)		
149.0		Tield (A	B/ 1141)		
	140.2	137.4	135.9	147.1	140.1
		140.9	155.3	144.3	147.4
	144.6	139.2	145.6	145.7	
	23.5°	23.5° 25.3 15.6 20.4  149.0  140.2 149.0	23.5°  25.3 22.6 15.6 20.4 16.1  Yield (k) 149.0  140.2 137.4 149.0 140.9	1.1 2.2 4.5  Disease incidence <sup>b</sup> 23.5°  25.3 22.6 14.1* 15.6 9.5* 15.1 20.4 16.1 14.6*  Yield (kg/ha)  149.0  140.2 137.4 135.9 149.0 140.9 155.3	1.1 2.2 4.5 6.7  Disease incidence <sup>b</sup> 23.5°  25.3 22.6 14.1* 12.3 15.6 9.5* 15.1 19.3 20.4 16.1 14.6* 15.8   Yield (kg/ha)  149.0  140.2 137.4 135.9 147.1 149.0 140.9 155.3 144.3

<sup>&</sup>lt;sup>a</sup> Silica sand (16 mesh) coated with Benlate PNW or Topsin-M (4.5 and 5.0% a.i., respectively) was applied with the seed in the seed row.

bDisease incidence is the percentage of stems displaying symptoms at the watery ripe to soft-dough stage (Feeke's stage 10.5.4-11.2). Figures represent the mean of four replicates. drisher's least significant difference (P=0.05) for comparing means within columns is intended for comparisons between the

<sup>&</sup>lt;sup>b</sup>Disease incidence is the percentage of stems displaying symptoms at the watery ripe to soft-dough stage (Feeke's stage 10.5.4-11.2).

Figures represent the mean of four replicates.

<sup>&</sup>lt;sup>d</sup>Fisher's least significant difference (P = 0.05) compares treatment means within or between columns; NS = not significant. Means with an asterisk (\*) are significantly different from the untreated control based on t tests (P = 0.05).

and Mertect were inhibitory to growth of mycelia and germination of conidia at lower concentrations than Topsin-M and provided greater disease control. Previous work has shown reduced efficacy of the thiophanates compared with benomyl and thiabendazole (9-11).

Tolerance to these fungicides was not detected among the 60 isolates of C. gramineum tested, as indicated by growth from all of the inoculum blocks placed on fungicide-amended media. Two isolates, however, were able to adapt to these benzimidazole fungicides. The fact that growth occurred from only a single inoculum block with each tolerant isolate suggests that either selection of a tolerant individual from within a genetically heterogeneous isolate or a mutation and selection for tolerance occurred with these isolates. The relative ease with which C. gramineum can adapt to the benzimidazoles and the erratic control of Cephalosporium stripe in field plots would preclude the use of these systemic fungicides for control of Cephalosporium stripe.

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