Sclerotinia homoeocarpa Tolerance to Benzimidazole Configuration Fungicides

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ABSTRACT

Isolates of *Sclerotinia homoeocarpa* were collected during 1972 from locations in the eastern and midwestern U.S. where benomyl had failed to control Sclerotinia dollar spot on turfgrass.

Isolates from such locations were comparable in cultural morphology and disease symptom development to nontolerant isolates of *S. homoeocarpa*.

On fungicide-amended PDA, isolates from control failure locations were over 100 times as tolerant to benomyl as isolates from other areas. Tolerance to benomyl was associated with tolerance to several other benzimidazole configuration fungicides. No tolerance was detected for Actidione or chlorothalonil. Moderate tolerance to Dyrene was noted with one isolate.

In greenhouse experiments with Penncross creeping bentgrass, neither benomyl nor Bay Dam 18654 suppressed infection by tolerant isolates. Actidione and Dyrene were partially effective in controlling both tolerant and nontolerant isolates.

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Sclerotinia homoeocarpa F. T. Bennett is one of the more severe pathogens of irrigated temperate zone turfgrasses (4). For many years, cadmium and mercury compounds have been the mainstay fungicides for control of this pathogen. Recently, the fungitoxic, systemically translocated benzimidazole compounds (benomyl, thiabendazole, and the ethyl and methyl thioallophanates) have been found to be very effective against *S. homoeocarpa* and have been rapidly replacing the heavy metal fungicides for Sclerotinia dollar spot control. The benzimidazole compounds normally provide disease suppression at dosages as low as 7 g active ingredient/93 centares (7).

Recently Goldberg and Cole (6) reported a total lack of Sclerotinia dollar spot control where benomyl had been used in Ohio, Illinois, New Jersey, and Pennsylvania. Isolates of *Sclerotinia homoeocarpa* from these locations were 100 times as tolerant to benomyl in vitro as isolates from areas where no control difficulties had been experienced. The mode of action of the benzimidazole configuration in fungal cells is thought to be quite specific, involving DNA synthesis (2). This contrasts with the broad toxicological activity of many of the dithiocarbamate organo-sulfur compounds. Reports of benzimidazole control failures with other pathogens are increasingly appearing in the literature (1, 5, 9).

In light of this situation, in vitro studies were initiated to characterize the fungal isolates from control failure locations, and to assess the level of tolerance to benzimidazole fungicides among these isolates. Representative isolates were selected for studies of benzimidazole tolerance in the greenhouse. In vivo tolerance level were determined for benzimidazole fungicides alone and in combination with broad-spectrum protectant fungicides.

MATERIALS AND METHODS.—*Pathogen collection and identification.*—Samples of diseased turfgrass were collected both from areas where benomyl control failures had been reported, and from random infection centers where there were no control problems. Diseased tissue was surface sterilized in 0.5% sodium hypochlorite solution for 2 min, and leaf portions were placed on Difco potato-dextrose agar (PDA), acidified by adding 1 ml 95% lactic acid to each liter after autoclaving.

Isolates thus obtained were compared to The Pennsylvania State University *S. homoeocarpa* collection with respect to morphology, optimum pH, and growth rate-temp response in the 2-35 C range. Cultures were maintained on PDA slants. Inoculum for greenhouse studies was grown on sterile, autoclaved rye grain. Pots of 8-wk-old Penncross bentgrass were inoculated with rye-grain inoculum and incubated for 7 days in a saturated atmosphere between 24 and 30 C. Experimental design for the greenhouse bench experiments was a split plot randomized block. Fifty-four isolates with five replications were evaluated for typical disease development. Symptoms were compared among isolates. Nontolerant, as well as cadmium-tolerant strains, from The Pennsylvania State University *S. homoeocarpa* collection, were included for reference. Infection center diam, blighting severity, and amount of aerial mycelium produced were recorded.

In vitro fungicide tolerance.—Laboratory experiments were conducted with 54 isolates. The data reported in this paper are from 11 representative isolates: five benomyl-tolerant (S-46, S-47, S-49, S-50, and S-51), three benomyl-sensitive (S-7, S-8, and S-9), two previously reported cadmium-tolerant isolates (S-24 and S-25), and one isolate tolerant to both cadmium and benomyl (S-62).

Commercial fungicides were suspended in sterile distilled water and added in appropriate quantities to partially cooled, sterile PDA. All concns were calculated on a w/v active ingredient basis. The supplemented medium was then poured into sterile, plastic petri plates, allowed to cool and used immediately. Dosages used were 0, 1, 10, 100, and 1,000 μg fungicide active ingredient per

ml of agar. Fungicides tested were 2-(4-thiazolyl) benzimidazole, (thiabendazole); methyl 1-(butylcarbamoyl) -2-benzimidazolecarbamate, (benomyl); α-(2, 4-dichlorophenyl)-α-phenyl-5-pyrimidinemethanol, (triarimol); methyl 1-(5 cyanopentyl) amino carbonyl-1, 1H-benzimidazole, (Bay Dam 18654); dimethyl 4,4-*O*-phenylenebis [3-thioallophanate], (thioallophanate-methyl); diethyl 4,4-*O*-phenylenebis [3-thioallophanate], (thioallophanate-ethyl); cadmium succinate, (cadminate); 2,4-dichloro-6-(*o*-chloroanilino)-s-triazine, (Dyrene); cycloheximide 3-[2-(3,5-dimethyl-2-oxycyclohexyl)-2-hydroxyethyl] glutarimide, (Actidione); and tetrachloroisophthalonitrile (chlorothalonil). The thioallophanates have been shown to undergo biotransformation to alkyl 2-benzimidazolecarbamate within plants (10), and in this paper will be considered benzimidazole fungicides.

Under natural conditions, the pathogen spreads from leaf to leaf within infection centers by mycelial proliferation; hence, inverted 5-mm diam agar plugs of mycelium were taken from the periphery of fungal colonies and transferred to the center of fungicide-amended PDA plates. Each chemical treatment was replicated three times. All cultures were incubated in the dark at 24±1 C. After the initial lag period, which was less than 48 h, radial growth rates were linear when plotted arithmetically against time. Hence, beginning at 48 h, colony diam were recorded at 24-h intervals. The diam measurements were converted to an "r" value for the linear growth phase, which is equivalent to radial growth in mm per 24 h (3).

Greenhouse experiments.—Two isolates were selected which exhibited similar virulence and symptom patterns in greenhouse pathogenicity tests, but differed in benomyl tolerance in vitro. Isolate S-50 tolerated benomyl dosages over 100-fold greater than those tolerated by S-9 in agar plate experiments. Inoculum was prepared by growing the two isolates on autoclaved rye grain. Penncross creeping bentgrass, *Agrostis palustris* Huds., was grown in 10.16 cm (4-inch) diam plastic pots and inoculated 8 wk after seedling emergence. Fungicides were applied at dosages equivalent to the standard 93 centare (1,000 ft^2)

TABLE 1. Mycelial growth of *Sclerotinia homoeocarpa* isolates on potato-dextrose agar amended with various fungicides

Fungicide	Formulation (% AI)	Dosage (μg/g agar)	Radial growth (mm/day)			
			S-9	S-24	S-50	S-62
benomyl	50% wp	0	15.1	16.4	14.0	8.5
		1	0.0	0.0	14.0	7.5
		10	0.0	0.0	13.4	6.2
		100	0.0	0.0	8.8	4.5
Thiabendazole	43% fl	0	15.7	16.1	15.8	11.3
		1	0.0	2.5	14.0	10.8
		10	0.0	0.0	13.8	7.8
		100	0.0	0.0	3.8	0.0
Bay Dam 18654	50% wp	0	13.3	13.8	12.2	9.6
		1	0.0	0.0	11.9	9.1
		10	0.0	0.0	11.5	8.4
		100	0.0	0.0	8.4	4.1
thioallophanate methyl	50% wp	0	13.3	13.8	12.4	7.7
		1	0.0	0.0	12.0	7.4
		10	0.0	0.0	10.6	6.8
		100	0.0	0.0	9.9	6.3
thioallophanate ethyl	50% wp	0	14.4	16.8	13.3	9.5
		1	0.0	0.0	12.5	9.1
		10	0.0	0.0	11.2	7.4
		100	0.0	0.0	11.5	6.9
Cadminate	60% wp	0	12.5	15.3	13.5	9.6
		1	11.8	14.9	12.3	9.3
		10	6.0	14.0	8.8	9.3
		100	0.0	12.7	0.0	7.6
triarimol	25% wp	0	14.5	14.1	13.1	8.7
		1	3.8	3.8	2.8	3.1
		10	0.0	0.0	0.0	0.0
Dyrene	50% wp	0	12.3	15.9	12.2	8.5
		1	8.5	12.6	6.8	8.5
		10	0.0	4.1	0.0	0.0
Actidione	2% wp	0	12.8	14.8	12.8	7.8
		1	5.9	4.7	4.8	3.9
		10	0.0	0.0	0.0	0.0
chlorothalonil	75% wp	0	12.1	14.5	12.3	8.3
		1	0.0	0.0	0.0	0.0
		10	0.0	0.0	0.0	0.0

dosage commonly used in turfgrass research, and are reported as g/93 centare equivalent. Fungicides and dosages tested were as follows: benomyl, 28.2 and 56.4 g, 50% WP formulation; Bay Dam 18654, 28.2 and 56.4 g, 50% WP; Dyrene, 112.8 and 225.6 g, 50% WP; and Actidione, 56.4 and 112.8 g, 2% WP. The fungicides were applied as an aqueous spray to the pots. After the pots were sprayed with fungicides and allowed to dry, each pot of grass was inoculated by placing a few kernels of rye-grain inoculum on the foliage in the center of the grass area. The pots were then placed beneath individual plastic covers and incubated for 3 days in a partially shaded section of the greenhouse. Temp beneath the covers was 24-28 C. Pots were arranged in a split plot randomized block design with three replications. Each pot was considered a replicate.

Disease severity was evaluated 7 days after inoculation on the basis of infection center diam. A visual 0-5 rating scale was employed: 0 = no disease, 1 = infection center < 2 cm in diam, 2 = 2.1 - 4.0 cm, 3 = 4.1 - 6.0 cm, 4 = 6.1 - 8.0 cm, 5 = 8.1 - 10.0 cm or essentially the complete foliage surface of the pot.

Data obtained from greenhouse experiments were subjected to analyses of variance and Duncan's modified least significant difference tests.

RESULTS.—*Pathogen collection and identification.*—The isolates from the control failure locations (designated benomyl-tolerant) were similar to the nontolerant isolates in terms of cultural appearance and morphology. No isolate produced conidia or any other spore forms. Both sensitive and tolerant isolates produced black flake-like pseudosclerotia after several weeks on PDA. Tolerant and nontolerant isolates had similar pH and growth-temp responses when grown on PDA agar. Optimum temp was 25-30 C for all isolates, with a large decrease in growth at 35 C.

When inoculated on mature Penncross bentgrass, typical Sclerotinia dollar spot symptoms developed. Pathogenicity and virulence results were similar for both tolerant and nontolerant isolates on 8-wk-old Penncross. The tolerant isolates (benomyl, cadmium, and benomyl/cadmium) were well within the range exhibited by nontolerant isolates for infection center diam, severity of foliar blighting, and aerial mycelium production on the diseased foliage.

In vitro fungicide tolerance.—All isolates from benomyl control failure sites grew well on PDA containing 100 μg/ml benomyl. Isolates from areas where no control problems had been reported were uniformly sensitive to benomyl, with complete growth inhibition at 1 μg/ml of agar. Isolates which were tolerant to benomyl were also tolerant to thiabendazole, Bay Dam 18654, thioallophanate-methyl, and thioallophanate-ethyl. Benzimidazole tolerance was not necessarily associated with cadmium tolerance (Table 1).

Isolates were found which were tolerant to cadmium alone, to benzimidazoles alone, and to both cadmium and benzimidazoles. In general, isolates tolerant to both cadmium and benzimidazoles were not as tolerant to the benzimidazoles as those isolates that were only tolerant to benzimidazoles. Tolerance reponse to thiabendazole was less uniform among isolates than tolerance response to the other benzimidazole compounds.

Isolates tolerant to benzimidazole compounds were not tolerant to triarimol, Dyrene, Actidione, or chlorothalonil. However, one cadmium tolerant isolate, S-24, exhibited a 10-fold increase in tolerance to Dyrene. Triarimol is a full systemic, xylem-translocated fungicide; Actidione is locally systemic; and Dyrene and chlorothalonil are protectant materials (Table 1).

Greenhouse experiments.—Infection center development and disease severity for nontolerant isolate

TABLE 2. Influence of various fungicide sprays on infection and symptom development by a tolerant and nontolerant isolate of *Sclerotinia homoeocarpa* on Penncross bentgrass in the greenhouse

Fungicide	Formulation	Dosage (g/93 centares)	Control Efficacy[a]	
			Isol. S-9	Isol. S-50
benomyl	50% wp	28.2	0.0 G[b]	5.0 A
		56.4	0.0 G	5.0 A
Bay Dam 18654	50% wp	28.2	0.3 G	4.7 AB
		56.4	0.3 G	4.7 AB
Dyrene	50% wp	112.8	3.3 CD	3.0 DE
		225.6	3.0 DE	2.3 E
Actidione	2% wp	56.4	2.3 E	3.0 DE
		112.8	3.0 DE	2.3 E
benomyl + Dyrene		28.2 + 112.8	0.0 G	3.0 DE
		56.4 + 225.6	0.0 G	2.7 DE
Bay Dam 18654 + Dyrene		28.2 + 112.8	1.3 F	2.7 DE
		56.4 + 225.6	0.0 G	3.0 DE
benomyl + Actidione		28.2 + 56.4	0.0 G	3.0 DE
		56.4 + 112.8	0.0 G	3.3 CD
Bay Dam 18654 + Actidione		28.2 + 56.4	0.0 G	2.3 E
		56.4 + 112.8	0.3 G	2.7 DE
No fungicide check		...	4.3 AB	4.0 BC

[a]Mean of three replications. Control efficacy based on infection center diam. 0 = no disease; 1 = 2 cm; 2 = 2.1 - 4.0 cm; 3 = 4.1 - 6.0 cm; 4 = 6.1 - 8.0 cm; 5 = 8.1 - 10.0 cm or essentially the entire pot foliage surface.

[b]Means not followed by the same letter are significantly different (Duncan's modified least significant difference test, $P = 0.01$). Comparisons may be made between, as well as within, columns.

S-9 and tolerant isolate S-50 were similar, with mean disease ratings of 4.3 and 4.0, respectively, for the unsprayed check pots. Sensitive isolate S-9 was controlled completely with benomyl and Bay Dam 18654, either singly or in combination with Actidione or Dyrene. Actidione and Dyrene alone gave partial disease control. Neither benomyl nor Bay Dam 18654 suppressed the disease caused by tolerant isolate S-50. Infection centers produced by the tolerant isolate in benomyl-sprayed pots were significantly larger than in the unsprayed pots. Both the 28.2 and 56.4 g/93 centare dosages received mean ratings of 5.0; i.e., complete death of all foliage. A similar trend was noted with Bay Dam 18654. Actidione and Dyrene, either alone or in combination with the benzimidazoles, partially suppressed disease severity (Table 2).

DISCUSSION.—The data obtained in the present experiments indicate that on the basis of cultural morphology, pH and temp requirements, and disease symptom development, benzimidazole-tolerant isolates of *Sclerotinia homoeocarpa* are indistinguishable from nontolerant isolates.

Field reports from benomyl control failure locations have noted increased mycelial proliferation in infection sites. In our greenhouse experiments, tolerant isolates showed increased disease severity on sprayed bentgrass when compared to the unsprayed checks. We believe that these field reports of increased mycelial proliferation may be related to a severity phenomenon rather than indicating a morphological or cultural variant. Our data to date would tend to support the severity hypothesis.

Laboratory tolerance to benomyl was clearly correlated with tolerance to other benzimidazole configurations, including thiabendazole, Bay Dam 18654, thioallophanate-ethyl, and thioallophanate-methyl. However, benomyl-tolerant isolates varied considerably in their response to thiabendazole. These results are in agreement with those of Bollen and Scholten (1) on tolerance by *Botrytis*. It is recognized that the pH (5.6) of the medium may differentially affect the fungitoxic characteristics of the various benzimidazole fungicides and this may have been a factor in the present investigations.

The absence of strong tolerance in agar culture to the local systemic and protectant fungicides chlorothalonil, Actidione, and Dyrene would suggest that these materials could be used as replacements for benzimidazoles in areas where control failures occur. However, Nicholson et al. (8) reported from Illinois the presence of *S. homoeocarpa* isolates tolerant to Dyrene. The experimental systemic triarimol may also offer a possible replacement.

The occasional occurrence of both benzimidazole and cadmium tolerance in the same isolate would seem to raise many questions of practical consideration about the maximum extent to which fungicide tolerance may develop. Multifungicide-tolerant strains could provide a monumental problem in commercial disease control, if such strains are able to survive and compete over the long term in natural situations.

The epidemiological significance of tolerance by *S. homoeocarpa* (6) and other pathogens (1, 5, 9) to the benzimidazole fungicides will not be determined until combinations of propagules of tolerant and sensitive isolates are introduced in known numbers into field plot situations, where survival under various fungicide regimens can be measured. Such work with *S. homoeocarpa* is presently underway.

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