Resistance of *Sclerotinia homoeocarpa* to Iprodione and Benomyl

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ABSTRACT

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An iprodione/benzimidazole-resistant strain of *Sclerotinia homoeocarpa* was isolated from a creeping bentgrass putting green where dollar spot disease control with iprodione and benzimidazoles had failed. The resistant strain grew on potato-dextrose agar amended with 1,000 μg/ml of iprodione or benomyl, whereas a sensitive strain and a benzimidazole-resistant strain failed to grow on media amended with more than 1 μg/ml of iprodione. The iprodione/benzimidazole-resistant strain maintained its resistance after 4 mo away from the fungicides. Although the iprodione-resistant strain was less virulent than a sensitive and a benzimidazole-resistant strain in greenhouse trials, spray applications of iprodione and benomyl failed to control this strain under field conditions.

Iprodione is a recently developed dicarboximide fungicide that has proved efficacious on turfgrass for the control of dollar spot, caused by *Sclerotinia homoeocarpa* F. T. Bennett (8). Since the identification of benzimidazole resistance in *S. homoeocarpa* (14), iprodione has played an important role in spray programs designed to forestall benzimidazole resistance in *S. homoeocarpa* or to control dollar spot and certain other turfgrass diseases once benzimidazole resistance becomes a problem (10,13).

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In July 1981, creeping bentgrass (*Agrostis palustris* Huds.) putting greens on a southern Michigan golf course treated with iprodione and the benzimidazole fungicides benomyl and thiophanate-ethyl showed serious dollar spot disease. Research studies were conducted 1) to establish whether an iprodione- and/or benzimidazole-resistant strain of *S. homoeocarpa* was present, 2) to test the relative virulence of this strain, and 3) to identify effective fungicide controls for this disease outbreak.

MATERIALS AND METHODS

Three isolates of *S. homoeocarpa* were used for laboratory and greenhouse experiments. An iprodione/benzimidazole-sensitive (IBS) wild-type strain was isolated from creeping bentgrass in a research plot near East Lansing, MI, that had never been treated with dicarboximide or benzimidazole fungicides. A benzimidazole-resistant (BR) strain was isolated from a creeping bentgrass green near East Lansing where benzimidazole fungicides no longer controlled dollar spot; this strain had shown high levels of in vitro and in vivo resistance to benzimidazole fungicides (13; J. M. Vargas, *unpublished*). An iprodione/benzimidazole-resistant (IBR) strain was isolated from bentgrass in a putting green on a golf course in southern Michigan where iprodione and benzimidazoles had failed to control dollar spot. The three strains were isolated by placing infected leaf tissue on potato-dextrose agar (PDA). The resulting colonies were subcultured on PDA at 21 C for 5 days to provide inoculum for further studies.

In vitro fungicide bioassay. A petri plate fungicide bioassay was prepared with benomyl, iprodione, chlorothalonil, cycloheximide, and triadimefon. The fungicides were suspended in sterile distilled water at 10,000 μg a.i./ml, and aliquots were pipetted directly into autoclaved, partially cooled PDA to give concentrations of 1, 10, 100, and 1,000 μg a.i./ml of PDA.

Plugs 5 mm in diameter were cut from inoculum plates with a cork borer, inverted, then placed in the center of the treatment plates. Each of the three *S. homoeocarpa* isolates was placed on four replicate plates per fungicide concentration. The petri plates were incubated

on the laboratory bench under a 12-hr light/dark cycle at 21 ± 1 C.

Fungal colony growth was measured along the same diameter at 48-hr intervals.

This experiment was analyzed as a 5 × 4 × 3 factorial. Dosage response curves were fitted and ED_{50} (estimated dosage required to reduce colony growth of test strain to 50% of colony growth of the control strain) values were calculated.

In vivo greenhouse studies. Each *S. homoeocarpa* isolate was tested for virulence in the greenhouse. Circular (8-cm) pieces of double-layer cheesecloth were placed on PDA plates, which were then inoculated (five replicates per isolate) with the *S. homoeocarpa* isolates. After 7 days' incubation at room temperature, the cheesecloth bearing the fungal mycelium was placed on the tops of pots of creeping bentgrass (five replicates per isolate) in a greenhouse mist chamber under a 12-hr light/dark cycle at 21 ± 2 C. The cheesecloth inoculum source was removed after 5 days, and the pots were incubated on the greenhouse bench for 5 days under a 12-hr light/dark cycle at 21 ± 2 C before disease rating. Visual ratings of foliar infection were made on a 1 (no infection) to 5 (severe infection) scale.

In vivo field fungicide trial. A replicated fungicide study was established on a practice putting green with dollar spot disease on the golf course where the IBR strain had been isolated. The study included the fungicides listed above as well as anilazine. Two treatments were applied at recommended label rates to three replicate plots (0.9 × 1.5 m) on a 14-day schedule. Ratings (number of dollar spots per plot) were taken 14 days after each application.

Greenhouse and field trial data were statistically analyzed using analysis of variance.

RESULTS

In vitro fungicide bioassay. Measurements of fungal colony diameters revealed iprodione and benomyl resistance in the IBR strain after 48 hr of incubation. The BR strain also showed benomyl resistance as anticipated.

By day 6, the IBR strain was growing aggressively on media containing 1, 10, 100, and 1,000 μg/ml of iprodione, whereas the BR and IBS wild-type strains were unable to grow at any iprodione concentration above 1 μg/ml (Table 1). The IBR and BR strains were growing well on media containing 1, 10, and 100 μg/ml of benomyl, whereas the IBS strain was completely inhibited at all benomyl concentrations (Tables 1 and 2).

The IBR and BR strains showed similar growth responses to increasing concentrations of benomyl (Fig. 1). Dosage-response curves for both the IBR strain ($R^2 = 0.997$) and the BR strain ($R^2 = 0.999$) growing on benomyl-amended PDA were highly correlated. The ED_{50} values for both strains were not significantly different (Table 3). The dosage-response curve fitted to the IBR strain on iprodione-amended PDA was significant ($R^2 = 0.540$), but the dosage response was less predictable than for the IBR and BR strains on benomyl. The ED_{50} value for the IBR strain on iprodione-amended PDA was significantly lower than the ED_{50} values for the IBR or BR strain growing on benomyl-amended PDA (Table 3). A dosage-response curve could not be fitted for the BR strain on iprodione-amended PDA because of the lack of fungal growth.

Colony growth of BR and IBS strains appeared unexpectedly on 10, 100, and 1,000 μg/ml of iprodione plates between day 8 and day 14, although not in all replicates. Various replicate plates of each isolate eventually showed prolific *S. homoeocarpa* growth while other replicates within the same treatment showed no growth whatsoever. Further investigation is needed to determine the nature of the resistance shown by these spontaneously mutated *S. homoeocarpa* strains.

When the study was terminated (day 26), the IBR and BR strains were growing on media containing 1,000 μg/ml of benomyl and the IBS strain was growing on media containing 1 μg/ml of benomyl.

Responses of the three strains to cycloheximide and triadimefon at various concentrations were comparable (Table 1), but the strains did respond differently to chlorothalonil. The IBR and IBS strains showed greater growth at the 10, 100, and 1,000 μg/ml of chlorothalonil concentrations than did the BR strain.

The IBR isolate retained its resistance to both benomyl and iprodione after seven consecutive transfers on PDA over a 4-mo period.

In vivo greenhouse studies. The IBR strain was less virulent than either the BR strain or the IBS wild-type strain. On a visible foliar necrosis rating scale of 1 (no infection) to 5 (severe infection), the mean infection rating of 2 for the IBR strain was significantly less (LSD 0.05) than the mean infection rating of 3.4 for the BR strain or of 3.6 for the IBS strain. This finding was consistent with observations of field symptoms produced by the IBR strain(s), which were generally less severe than field symptoms produced by the BR strain and the IBS wild-type strain.

In vivo field fungicide trial. Iprodione and benomyl failed to give adequate control of *S. homoeocarpa* after two applications, although some disease suppression by iprodione was noted (Table 4). Benomyl showed no disease inhibition effect. The other fungicides

Table 1. Growth of three *Sclerotinia homoeocarpa* strains[a] on potato-dextrose agar amended with fungicides after 6 days at 21 ± 1 C

Fungicide	Concentration (μg a.i./ml)	Mean diameter colony growth (mm)[b]		
		IBR	IBS	BR
Benomyl	1	87.0	0.0	87.0
	10	87.0	0.0	87.0
	100	79.0	0.0	79.0
	1,000	1.8	0.0	0.0
Iprodione	1	87.0	27.8	25.0
	10	60.3	0.0	0.0
	100	30.0	0.0	0.0
	1,000	16.5	0.0	0.0
Chlorothalonil	1	67.3	72.3	44.8
	10	46.3	36.3	17.0
	100	27.0	13.0	8.0
	1,000	13.5	7.8	0.0
Cycloheximide	1	52.3	67.0	69.8
	10	8.0	9.3	14.3
	100	0.0	0.0	0.0
	1,000	0.0	0.0	0.0
Triadimefon	1	14.8	15.3	8.8
	10	0.0	3.5	0.0
	100	0.0	0.0	0.0
	1,000	0.0	0.0	0.0
Control	...	87.0	87.0	87.0
LSD (0.05)		9.3	5.3	4.9
LSD (0.01)		12.3	7.1	6.5

[a] IBR = iprodione/benzimidazole-resistant strain, IBS = iprodione/benzimidazole-sensitive strain, BR = benzimidazole-resistant strain.
[b] Mean of four replicate plates.

Table 2. Analysis of variance of effect of various fungicides on growth of test strains of *Sclerotinia homoeocarpa*

Source of variation	df	Mean squares
Fungicide (F)	4	9,917.5*[a]
Rate (R)	3	22,114.2*
Strain (S)	2	9,291.8*
F × R	12	2,381.7*
R × S	6	664.2*
F × R × S	24	634.8*
Error	180	23.0
Total	239	...

[a] * = Significant at the 0.01 level.

gave adequate control of *S. homoeocarpa* after two applications. The iprodione/benzimidazole-resistant strain was easily controlled in the field once appropriate fungicides were identified and applied.

DISCUSSION

The primary fungicides used for dollar spot control during the past 5 yr at the golf course where the IBR strain was identified were the benzimidazole fungicides benomyl, thiophanate-ethyl, and thiophanate-methyl. Iprodione was introduced into the spray program 3 yr ago, along with infrequent chlorothalonil applications, when the benzimidazoles no longer gave adequate control of *S. homoeocarpa*. Use of the benzimidazoles, however, was not discontinued.

The IBR strain was resistant to iprodione and benomyl but sensitive to chlorothalonil, cycloheximide, triadimefon, and, in the field, anilazine. Multiple resistance to both iprodione and benomyl has been reported previously in *Monilinia fructicola* (Wint.) Honey (12) and *Botrytis cinerea* Pers. ex Fr. (7), where Pappas et al found the multiple-resistant *B. cinerea* strain to be sensitive to chlorothalonil and other fungicides with multisite modes of action.

The literature includes reports of the spontaneously acquired in vitro resistance phenomenon observed in the IBS and BR strains when both iprodione-sensitive isolates mutated spontaneously and began growing on iprodione-amended PDA. Leroux et al (5) and Dennis and Davis (3) reported similar findings in in vitro studies with iprodione-sensitive *B. cinerea* strains on iprodione-amended media. The apparent ease with which some iprodione-sensitive fungi mutate to resistant types in vitro suggests the possibility of a similar process in the field.

The occurrence of spontaneously iprodione-resistant phytopathogenic fungi within wild populations is well documented (1,3,6,12). The literature describes iprodione-resistant strains that are both as aggressive as and less aggressive than sensitive strains. Working with dicarboximide-resistant *B. cinerea*, Hisada et al (4) and Spengler et al (11) identified strains that were less aggressive than sensitive strains, whereas Pappas et al (7) and Schuepp and Kung (9) identified strains that were as aggressive as sensitive strains. Dennis and Davis (3) documented the 2-yr existence of iprodione-resistant *B. cinerea* strains in field plots, although at last report (2) these strains were no longer detectable. Such reports have led some workers to doubt that disease control failures with iprodione and the other dicarboximides will ever become widespread because organisms resistant to this group of fungicides generally lack field fitness. Work with the IBR strain described here and with iprodione-resistant *Fusarium nivale* (Fr.) Ces. (1) shows that 1) rapid population shifts toward iprodione-resistant strains can occur in the field, 2) multiple resistance to dicarboximides and benzimidazoles can occur in the field, and 3) this can result in disease control failures with iprodione.

We conclude that failure of iprodione and the benzimidazoles to control dollar spot on a golf course in Michigan during 1981 was preceded by the selection of benzimidazole-resistant *S. homoeocarpa*

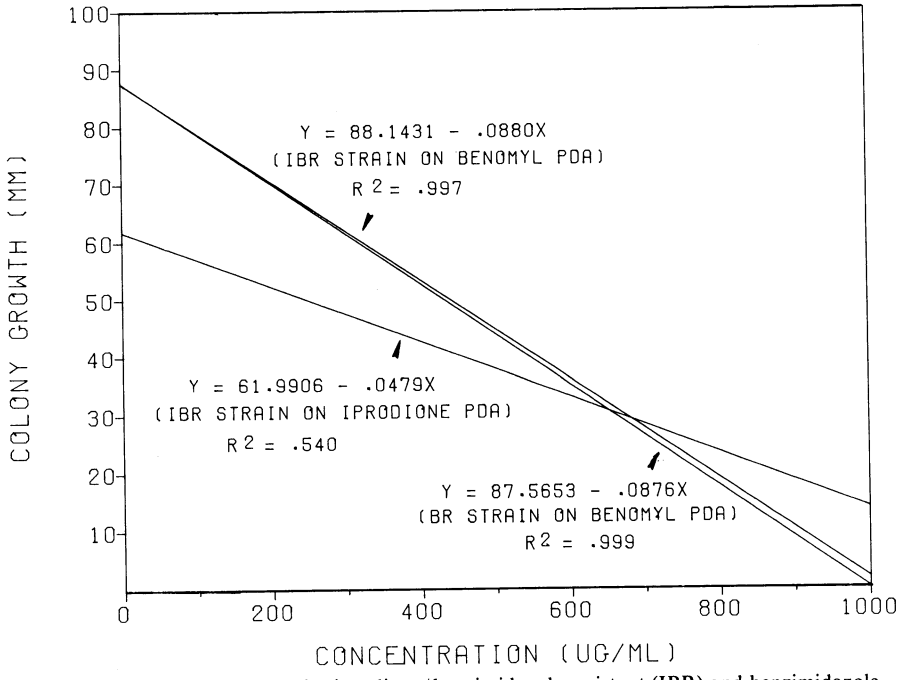

Fig. 1. Dosage-response curves for iprodione/benzimidazole-resistant (IBR) and benzimidazole-resistant (BR) strains of *Sclerotinia homoeocarpa*.

Table 3. Analysis of benzimidazole- and iprodione-resistant strains of *Sclerotinia homoeocarpa* grown for 6 days on benomyl- and iprodione-amended PDA

Treatment[a]	Coefficient of determination (R^2)	Regression equation slope	ED_{50}[b] (μg a.i./ml)
IBR strain on benomyl-amended PDA	0.997	0.0880	507.5
BR strain on benomyl-amended PDA	0.999	0.0876	503.2
IBR strain on iprodione-amended PDA	0.540	0.0479	380.5
BR strain on iprodione-amended PDA	0.125	ns[c]	...
IBS strain (control)	...	0.0000	...
LSD (0.05)		0.0020	55.7
LSD (0.01)		0.0029	80.1

[a] IBR = iprodione/benzimidazole-resistant, BR = benzimidazole-resistant, IBS = iprodione/benzimidazole-sensitive.
[b] ED_{50} = Estimated dosage at which the diameter of the colony growth of the resistant strain equals 50% of the diameter of the colony growth of the control strain.
[c] ns = Not significant ($P = 0.05$); growth occurred only at 1 μg/ml.

Table 4. Control of iprodione/benzimidazole-resistant (IBR) *Sclerotinia homoeocarpa* strain(s) on a creeping bentgrass putting green

Fungicide	Concentration (g a.i./92.9 m^2)	No. of dollar spots[x] Application on 17 July 1981[y]	No. of dollar spots[x] Applications on 17 and 31 July 1981[z]
Triadimefon	7.1	1.3 a	0.0 a
	14.2	1.7 a	0.0 a
Chlorothalonil	71.5	2.7 a	0.0 a
Anilazine	85.1	2.0 a	0.0 a
Cycloheximide	0.6	7.3 a	0.3 a
Iprodione	28.4	16.7 ab	10.7 b
Control	...	28.3 b	24.7 c
Benomyl	14.2	28.7 b	21.3 c

[x] Mean of three replicate field plots (0.9 × 1.5 m). Means followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).
[y] Ratings taken 31 July 1981.
[z] Ratings taken 14 August 1981.

strains through long-term use of benzimidazole fungicides and, more recently, by further selection of iprodione-resistant strains through long-term iprodione use. In light of the numerous reports of iprodione/benzimidazole multiple-resistant phytopathogenic fungi and the recently documented cases of field disease control failures with iprodione, researchers may need to reassess the role of iprodione and other dicarboximides in the prevention and control of benzimidazole resistance and in field disease control in general. It would seem wise to anticipate future iprodione resistance problems and to adopt defensive usage patterns to avoid the buildup of resistant organisms.

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