Increase in Incidence and Severity of Target Turfgrass Diseases by Certain Fungicides

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ABSTRACT

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Field and laboratory studies were conducted to determine if in a situation of fungicide resistance, the use of the fungicide in question might increase the severity of the target turfgrass disease. Applications of either a mixture of cycloheximide and thiram or triphenyltin hydroxide to tall fescue (*Festuca elatior* var. *arundinacea*) colonized by a strain of *Rhizoctonia solani* resistant to these fungicides increased the incidence of Rhizoctonia blight. Applications of benomyl, thiophanate-methyl, and thiophanate-ethyl to creeping bentgrass (*Agrostis palustris*) colonized by a benzimidazole-resistant strain of *Sclerotinia homoeocarpa* resulted in significant increases in the incidence and severity of Sclerotinia dollar spot. The benzimidazole-induced increase in Sclerotinia dollar spot was detected within 7 days of the second fungicide application and continued for an additional 28 days in the absence of additional fungicide applications. Treatments of creeping bentgrass with triphenyltin hydroxide also exhibited an increase in Sclerotinia dollar spot. This increase was first detected 3 wk after the third and final fungicide application and continued for 36 days.

There has been a significant increase in recent years in the amount of fungicides used in turfgrass culture. Pesticide marketing figures for 1989 show that more fungicide was sold in the United States for use on turfgrass than any other commodity, including the various food crops (2). For United States golf courses, fungicides comprise 48% of the total pesticide budget (1).

The widespread use of turfgrass fungicides has drawn additional attention to the need to characterize the various nontarget effects they have on the suscept and its environment. Side effects that have been reported to date include modifications in the carbohydrate metabolism of the suscept (9), alterations in tissue dry weight and in the contents of nutrient elements in grass leaves and stolons (12), changes in the microbial composition of the thatch (10), and reductions in the mineralization of nitrogen in the soil (5,8).

It has also been shown that the use of certain turfgrass fungicides may bring about an increase in the incidence and severity of nontarget diseases. Jackson (6) has reported that applications of benomyl and thiabendazole increased the susceptibility of Kentucky bluegrass (*Poa pratensis* L.) to Helminthosporium leaf spot (incited by *Bipolaris sorokiniana* (Sacc.) Shoemaker). In greenhouse-based studies, Warren et al (14) found that benomyl increased the severity of Pythium blight (incited by *Pythium aphanidermatum* (Edson) Fitzp.) of creeping bentgrass (*Agrostis palustris* Huds.). Joyner and Couch (7) reported that the incidence of crown rust (incited by *Puccinia coronata* Corda) of perennial ryegrass (*Lolium perenne* L.) increased after applications of thiabendazole and thiophanate-methyl. Smith et al (11) observed the development of a patch disease, incited by an unidentified Basidiomycete, on bentgrass that had been sprayed with benomyl to control Sclerotinia dollar spot (incited by *Sclerotinia homoeocarpa* F. T. Bennett).

In addition to increasing the incidence and severity of nontarget diseases, it is also possible that under certain circumstances, the use of what would otherwise be a highly efficacious fungicide will result in an increase in the development of the target disease. This phenomenon could occur in instances of fungicide resistance. If the efficacy of the fungicide is diminished and the nature of its side effects on the development of the plant are such that its susceptibility to the pathogen is increased, applications of the material could bring about an increase in the severity of the target disease.

The purpose of the present investigation was to determine if in a situation of fungicide resistance, the use of the fungicide in question might increase the incidence and/or severity of the target disease.

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MATERIALS AND METHODS

Diminished efficacy experiments. The Sclerotinia dollar spot control experiments were conducted on a stand of Penneagle creeping bentgrass maintained at a cutting height of 5 mm and naturally infested with *S. homoeocarpa*. Previous fungicide screening trials had shown that a strain of *S. homoeocarpa* was present in this location that was susceptible to anilazine, triadimefon, propiconazole, iprodione, chlorothalonil, and cycloheximide but highly resistant to benomyl, thiophanate-methyl, thiophanate-ethyl, and triphenyltin hydroxide.

Isolates of *S. homoeocarpa* recovered from benomyl-control-failure locations have been found to grow well on potato-dextrose agar amended with benomyl and thiophanate-methyl at concentrations of 100 μg a.i./ml, whereas the growth of isolates from other areas was impeded completely at 1 μg a.i./ml (4,13). In the present investigation, it was determined that the strain of *S. homoeocarpa* colonizing the foliage of the plants in the plot area in question was able to grow well on potato-dextrose agar amended with benomyl at a concentration of 100 μg a.i./ml.

The Rhizoctonia blight study was conducted on a stand of K-31 tall fescue (*Festuca elatior* L. var. *arundinacea* (Schreb.) Wimm.) cut at 6.5 cm and naturally infested with *Rhizoctonia solani* Kühn. Earlier fungicide screening trials at this location had shown that a strain of *R. solani* was present that was susceptible to anilazine, triadimefon, fenarimol, thiophanate-methyl, vinclozolin, mancozeb, iprodione, propiconazole, and chlorothalonil but highly resistant to triphenyltin hydroxide and a mixture of cycloheximide and thiram labeled for control of Rhizoctonia blight.

Sclerotinia dollar spot tests. Two separate field experiments were conducted in the Sclerotinia dollar spot series. One experiment assessed the disease enhancement potential of benzimidazole fungicides labeled for the control of Sclerotinia dollar spot and the other measured the extent to which triphenyltin hydroxide heightens the development of the disease.

The first benzimidazole test involved benomyl applied at 14.2 g a.i./93 m^2 and five other fungicides commonly used for Sclerotinia dollar spot control. These materials and their rates (g a.i./93 m^2) were as follows: anilazine (113.1), chlorothalonil (118.3), cycloheximide (0.64), iprodione (14.2), and triadimefon (7.1). The second benzimidazole experiment was conducted concurrently in the same plot location. It involved benomyl and thiophanate-methyl and thiophanate-ethyl applied at 14.2 and 56.7 g a.i./93 m^2, respectively. Triphenyltin hydroxide was tested at 7.1 and 68.0 g a.i./93 m^2. Seven fungicides commonly used for Sclerotinia dollar spot control were also included in this experiment. These materials and their rates (g a.i./93 m^2) were as follows: anilazine (113.4), propiconazole (3.9), fenarimol (5.7), chlorothalonil (118.3), triadimefon (7.1), iprodione (14.2), and vinclozolin (28.4).

All fungicides were applied at a dilution rate of 19 L/93 m^2 with a carbon dioxide-pressurized sprayer equipped with a UniJet 8002 flat-fan nozzle (Spraying Systems Co., Wheaton, IL) delivering 276 kPa at the nozzle. The individual plots were 1.8 m^2, and each treatment was repeated through four randomized blocks.

In the experiment involving benzimidazoles and other fungicides labeled for Sclerotinia dollar spot, applications were made on 18 and 25 August, respectively. Three disease ratings were performed, beginning at the time of the second application and continuing at 7-day intervals for two successive weeks. In the experiment involving only benzimidazoles, applications were made on 7, 14, and 21 July. Five disease ratings were performed, beginning at the time of the third application and continuing at 7-day intervals for four successive weeks.

For the triphenyltin hydroxide test, applications were made on 29 June and 6 and 12 July. Seven ratings were performed, beginning at the time of the third application and continuing at 7-day intervals for six successive weeks.

The magnitude of disease in each experiment was visually rated using a scale of 0–10, where 0 = no disease, 1 = 10%, and 10 = 100% of the foliage in the plot area blighted. The data from each rating were subjected to analysis of variance and means compared by Duncan's multiple range test.

Rhizoctonia blight experiments. Two separate field experiments were involved in the Rhizoctonia blight series on tall fescue. One experiment assessed the Rhizoctonia blight enhancement potential of a mixture of cycloheximide and thiram, and the other measured the extent to which triphenyltin hydroxide heightened the development of the disease.

With both series of experiments, each fungicide was applied in the equivalent of 19 L of water per 93 m^2 with the same equipment and nozzle pressure described earlier for the Sclerotinia dollar spot trials. The individual plots were 0.6 m^2, and each treatment was repeated through five randomized blocks in the cycloheximide-thiram series and four randomized blocks in the triphenyltin hydroxide tests.

The materials included in the cycloheximide-thiram experiments and their dosage levels (a.i./93 m^2) were as follows: cycloheximide (0.64 g) + thiram (63.8 g), anilazine (85.1 g), triadimefon (7.1 g), fenarimol (5.7 g), thiophanate-methyl (28.4 g), vinclozolin (28.4 g), mancozeb (28.4 g), iprodione (28.4 g), propiconazole (15.6 ml), and chlorothalonil (88.7 g). Two separate trials were conducted. In both tests, the treatments were made on 26 June and 5 July. Seven days after the second application, 300 leaves were collected at random from each plot and stored at −10 C until the disease incidence ratings were performed.

In the triphenyltin hydroxide test, the dosage levels (g a.i./93 m^2) were as follows: triphenyltin hydroxide (7.1 and 68.0), mancozeb (90.7), anilazine (113.4), iprodione (14.2), and chlorothalonil (118.3). Three hundred leaves were collected at random from each plot 16 days after treatment and stored at −10 C until the disease incidence ratings were per-

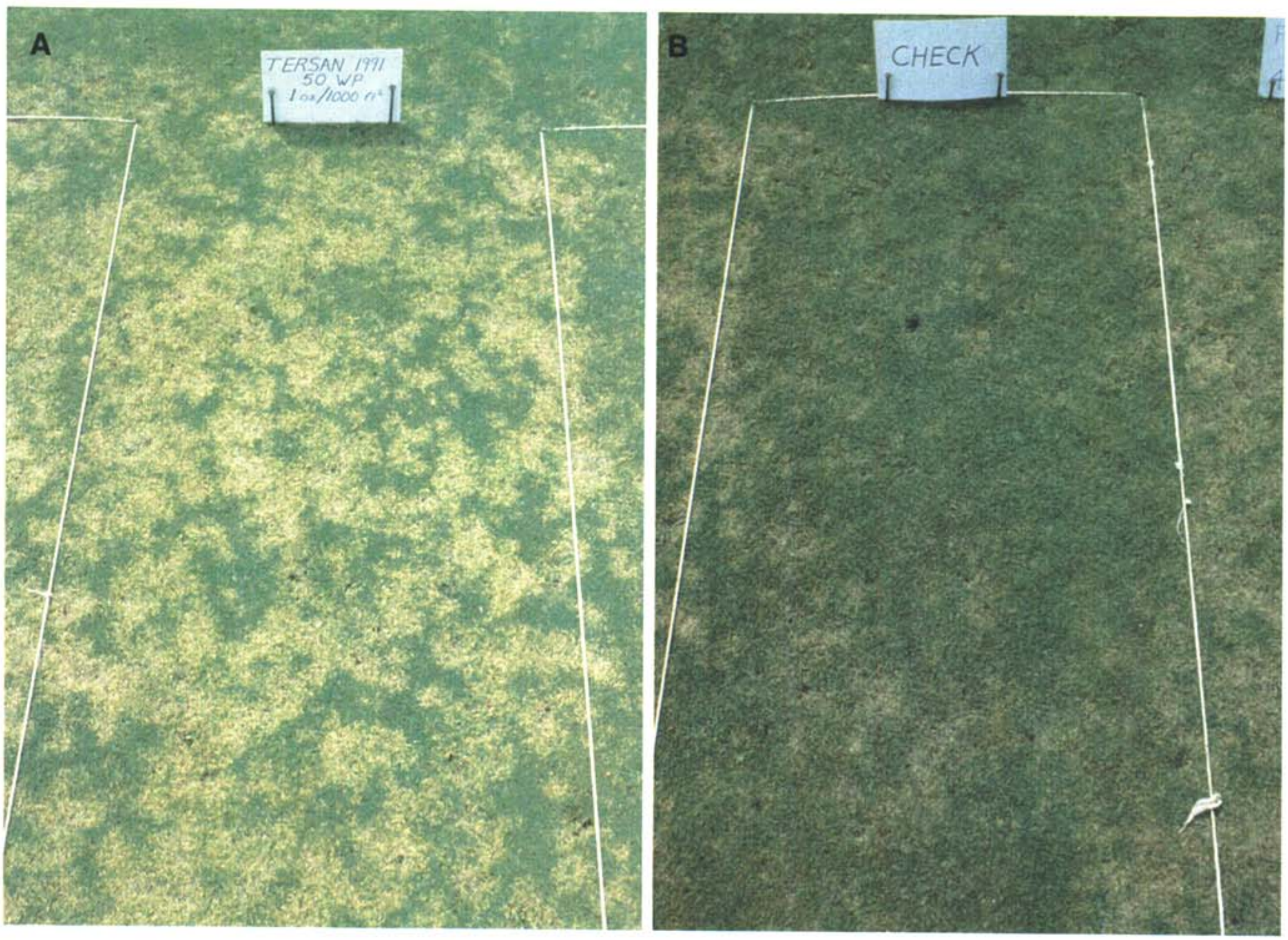

Fig. 1. Illustration of **(A)** benomyl-induced increase in the incidence and severity of Sclerotinia dollar spot of Penneagle creeping bentgrass incited by a benzimidazole-configuration resistant strain of *Sclerotinia homoeocarpa* over **(B)** a nontreated check plot.

Table 1. Benomyl-induced increase in the mean disease incidence of Sclerotinia dollar spot of Penneagle creeping bentgrass incited by a benzimidazole-resistant strain of *Sclerotinia homoeocarpa*

Fungicide	Rate (g a.i./93 m²)	Mean disease incidence[y]		
		1 September	9 September	16 September
Benomyl	14.2	70.0 a[z]	90.0 a	87.5 a
Check		62.5 a	72.5 b	45.0 b
Anilazine	113.1	22.5 b	20.0 c–e	20.0 c
Chlorothalonil	118.3	20.0 b	25.0 cd	22.5 c
Cycloheximide	0.64	12.5 bc	35.0 c	32.5 bc
Iprodione	14.2	0.0 c	12.5 de	0.0 d
Triadimefon	7.1	0.0 c	0.0 e	0.0 d

[y] Percent blighted foliage per plot. Last fungicide application date, 25 August.
[z] Means in a column followed by the same letter are not significantly different ($P = 0.05$) from each other according to Duncan's multiple range test.

Table 2. Increase in mean disease incidence of Sclerotinia dollar spot of Penneagle creeping bentgrass incited by a benzimidazole-resistant strain of *Sclerotinia homoeocarpa* induced by benomyl, thiophanate-ethyl, and thiophanate-methyl

Fungicide	Rate (g a.i./93 m²)	Mean disease incidence[y]			
		28 July	4 August	10 August	17 August
Thiophanate-methyl	14.2	82.5 a[z]	82.5 a	75.0 a	55.0 a
Thiophanate-ethyl	14.2	80.0 a	82.5 a	75.0 a	57.5 a
Benomyl	14.2	75.0 a	72.5 ab	60.0 a	55.0 a
Thiophanate-ethyl	56.7	75.0 a	80.0 a	72.5 a	60.5 a
Thiophanate-methyl	56.7	70.0 a	77.5 ab	65.0 a	55.0 a
Benomyl	56.7	55.0 b	57.5 bc	60.0 a	55.0 a
Check		45.0 b	42.5 c	27.5 b	25.0 b

[y] Percent blighted foliage per plot. Last fungicide application date, 21 July.
[z] Means in a column followed by the same letter are not significantly different ($P = 0.05$) from each other according to Duncan's multiple range test.

Table 3. Increase in mean disease incidence of Sclerotinia dollar spot of Penneagle creeping bentgrass induced by triphenyltin hydroxide

Fungicide	Rate (g a.i./93 m²)	Mean disease incidence[y]		
		31 July	9 August	29 August
Triphenyltin hydroxide	68.0	77.6 a[z]	83.0 a	88.0 a
Triphenyltin hydroxide	7.1	75.0 a	83.0 a	44.3 b
Check		55.0 b	60.0 b	38.0 bc
Anilazine	113.4	28.0 c	48.0 bc	38.0 bc
Propiconazole	3.9	23.0 cd	38.0 cd	38.0 bc
Fenarimol	5.7	18.0 c–e	18.0 ef	15.0 cd
Iprodione	14.2	20.0 c–e	25.0 de	35.0 bc
Triadimefon	7.1	8.0 de	8.0 fg	8.0 d
Vinclozolin	28.4	5.0 de	0.0 g	3.0 d
Chlorothalonil	118.3	0.0 e	23.0 de	13.3 cd

[y] Percent blighted foliage per plot. Last fungicide application date, 12 July.
[z] Means followed by the same letter are not significantly different ($P = 0.05$) from each other according to Duncan's multiple range test.

Table 4. Increase in mean disease incidence of Rhizoctonia blight of K-31 tall fescue induced by cycloheximide-thiram

Fungicide	Rate (g a.i./93 m²)	Mean disease incidence[y]	
		Trial A	Trial B
Cycloheximide-thiram	0.64 + 63.8	26.2 a[z]	24.0 a
Check		16.6 b	11.2 b
Anilazine	85.1	8.0 c	5.0 cd
Triadimefon	7.1	6.8 c	4.4 cd
Fenarimol	5.7	6.4 c	7.2 c
Thiophanate	28.4	6.0 c	4.4 cd
Vinclozolin	28.4	5.8 c	6.6 c
Mancozeb	90.7	4.6 c	3.6 de
Iprodione	28.4	4.6 c	3.4 de
Propiconazole	15.6	4.2 c	2.8 e
Chlorothalonil	88.7	3.8 c	3.4 de

[y] Percent diseased leaves per plot.
[z] Means in a column followed by the same letter are not significantly different ($P = 0.05$) from each other according to Duncan's multiple range test.

formed.

Evaluation of level of disease development in each trial was based on the percentage of diseased leaves per plot. A total of 100 randomly selected leaves were examined from the samples collected from each plot of the individual experiments and rated for the presence or absence of leaf lesions incited by *R. solani*. The data from each rating were subjected to analysis of variance and means compared by Duncan's multiple range test.

The Rhizoctonia blight enhancement potential of triphenyltin hydroxide was also evaluated in laboratory-based experiments. K-31 tall fescue plants were grown in 7.5 × 8.5 cm polystyrene containers with Weblite (a granular, heat-expanded shale manufactured by the Webster Brick Co., Salem, VA) serving as the support medium. Before seed germination, watering was accomplished with tap water. After seed germination, irrigation was performed once every 7 days with a 20-20-20 water soluble fertilizer at a rate of 273 ppm of nitrogen. The intervening daily irrigations were accomplished with tap water.

Three weeks from the time of seed germination, the containers were divided into three groups. Triphenyltin hydroxide was applied at 7.1 and 68.0 g a.i., respectively, in the equivalent of 19 L/93 m². One application of each dosage level was made. The individual treatments were replicated nine times and the experiment was performed twice.

The isolate of *R. solani* used in this experiment was an anastomosis group 1 isolate that was resistant to triphenyltin hydroxide and had been recovered from the foliage of K-31 tall fescue. Two hours after the fungicide applications, foliage of each treatment group was sprayed with an aqueous suspension of mycelial fragments of *R. solani* that had been grown in potato-dextrose broth and chopped in a blender. Immediately after inoculation, plants were stored in a Percival dew chamber (Percival Manufacturing Co., Boone, IA) in a moisture-saturated atmosphere at 24 C for 72 hr.

The magnitude of disease was determined by means of a visual rating scale of 0–10, where 0 = no disease, 1 = 10%, and 10 = 100% of the foliage in each container was blighted. The data were subjected to analysis of variance and means compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

The use of benzimidazoles on bentgrass colonized by a strain of *S. homoeocarpa* resistant to this group of fungicides resulted in a significant increase in the incidence and severity of Sclerotinia dollar spot. The level of disease in the plots treated with benzimidazole was significantly greater than that in the nontreated controls (Fig. 1). This increase

Table 5. Increase in mean disease incidence of Rizoctonia blight of K-31 tall fescue induced by triphenyltin hydroxide (field test)

Fungicide	Rate (g a.i./93 m^2)	Mean disease incidence[y]
Triphenyltin hydroxide	68.0	63.8 a[z]
Triphenyltin hydroxide	7.1	27.0 b
Check		7.0 c
Mancozeb	90.7	3.3 c
Anilazine	113.4	3.0 c
Iprodione	14.2	3.0 c
Chlorothalonil	118.3	2.5 c

[y] Percent diseased leaves per plot.
[z] Means folowed by the same letter are not significantly different ($P = 0.05$) from each other according to Duncan's multiple range test.

Table 6. Increase in mean disease incidence of Rhizoctonia blight of K-31 tall fescue induced by triphenyltin hydroxide (laboratory test)

Fungicide	Rate (g a.i./93 m^2)	Mean disease incidence[y]	
		Test A	Test B
Triphenyltin hydroxide	68.0	71.1 a[z]	56.7 a
Triphenyltin hydroxide	7.1	36.7 b	36.7 b
Check		34.4 b	32.2 b

[y] Percent diseased leaves per container.
[z] Means in a column followed by the same letter are not significantly different ($P = 0.05$) from each other according to Duncan's multiple range test.

was detected in the first trial 14 days after the second fungicide application and had not diminished in intensity at the time of the second and final rating 7 days later (Table 1). In the second trial, the benzimidazole-induced increase was first detected 7 days after the third fungicide application and had not diminished in intensity at the time of the final rating 21 days later (Fig. 1 and Table 2).

Applications of triphenyltin hydroxide at both the high and low dosage levels also resulted in a significant increase in the incidence and severity of Sclerotinia dollar spot. This increase was first detected 3 wk after the third fungicide application. It continued for 2 wk in the plots treated with 7.1 g a.i./93 m^2, but in the plots treated with 68.1 g a.i./93 m^2, it still had not diminished in intensity at the time of the final rating 4 wk later (Table 3).

In the Rhizoctonia blight field experiments on tall fescue, fenarimol, anilazine, triadimefon, thiophanate-methyl, mancozeb, chlorothalonil, vinclozolin, iprodione, and propiconazole all gave adequate disease control. However, the plots treated with the cycloheximide-thiram mixture (Table 4) and those treated with triphenyltin hydroxide showed significant increases in disease incidence (Table 5). In the triphenyltin hydroxide laboratory-based experiment, the incidence of Rhizoctonia blight was significantly higher in plants treated at the 68.0 g a.i./93 m^2 rate (Table 6).

The mixture of cycloheximide + thiram used in this study was marketed in the United States for more than 20 yr for control of Rhizoctonia blight of tall fescue, and the benzimidazole-configuration fungicides benomyl, thiophanate-methyl, and thiophanate-ethyl are used extensively for control of Sclerotinia dollar spot of creeping bentgrass (3).

This is the first report that the application of these fungicides to stands of turfgrass being colonized by a resistant strain of the pathogen in question can result in a significant increase in the incidence and severity of the target disease. The findings of this study also provide additional insight where the development of protocols for the field testing of turfgrass fungicides is concerned. For example, in cases of fungicide resistance, studies on the impact of the fungicide on the situation at hand should include tests to determine if it brings about an increase in the incidence and severity of the target disease.

Also, the triphenyltin hydroxide-induced increase in incidence and severity of both Sclerotinia dollar spot of creeping bentgrass and Rhizoctonia blight of tall fescue documented by this study links with the earlier reports of fungicide-enhanced increases of such nontarget diseases as Helminthosporium leaf spot of Kentucky bluegrass (6), Pythium blight of creeping bentgrass (14), and crown rust of perennial ryegrass (7) to present a strong case for the need to determine the impact of candidate turfgrass fungicides on the development of nontarget diseases. The nontarget diseases included in a specific testing program should be those that commonly occur in conjunction with outbreaks of the target disease. This information would be particularly valuable to the turfgrass management specialist in planning multiple component fungicide application programs.

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