

Detection of Demethylation Inhibitor (DMI) Resistance in *Sclerotinia homoeocarpa* Populations

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ABSTRACT

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Sclerotinia homoeocarpa is an important turfgrass pathogen on golf courses in Michigan and Ohio. Isolates were collected from golf courses on which sterol demethylation inhibitor (DMI) fungicides had never been used and from courses where DMI fungicides were used but where little or no control of dollar spot was achieved. The mean relative growth value on DMI-amended media of 150 isolates from golf courses treated with DMI fungicides was two to three times higher than for 150 isolates from golf courses with no history of DMI treatments. Isolates with high relative growth values for triadimefon also had similarly high values for fenarimol and propiconazole. The difference in growth on DMI-amended media of the *S. homoeocarpa* isolates from untreated courses compared to treated courses indicated that resistance to DMI fungicides had developed. Dollar spot control on a golf course in southeast Michigan infected by DMI-resistant *S. homoeocarpa* was not obtained with triadimefon, fenarimol, or propiconazole. Chlorothalonil applied at 191 ml/100 m² was the only chemical that provided acceptable disease control for the duration of the study (1991 through 1993). A triadimefon concentration of 2 µg ml⁻¹ was an effective monitoring dose for *S. homoeocarpa* populations.

Sclerotinia homoeocarpa F.T. Bennett (8), the causal agent of dollar spot, is a widely distributed and destructive pathogen of turfgrass in the United States (13). Benzimidazole and dicarboximide fungicides have been used for dollar spot management; however, the identification of resistance in field populations of *S. homoeocarpa* to these chemicals (3,14) resulted in a need for new fungicides. The sterol demethylation inhibitor (DMI) fungicides became available in 1979 for dollar spot control in the United States. Use of DMI fungicides increased rapidly on turfgrass areas where benzimidazole- and/or dicarboximide-resistant strains of *S. homoeocarpa* were present (3,14).

DMI fungicides were used extensively and provided excellent control of dollar spot on golf courses in Michigan through the 1980s, but in 1990, a few reports of reduced control were received from golf course superintendents. In 1991, fairways on two golf courses in southern Michigan and one in northern Ohio exhibited serious outbreaks of dollar spot following repeated applications of DMI fungicides.

The objective of this study was to determine the sensitivity of populations of *S. homoeocarpa* from six golf courses to three DMI fungicides and to establish the efficacy of these DMI fungicides for control of dollar spot on a golf course where triadimefon-resistant strains had been detected previously.

MATERIALS AND METHODS

Fungal isolates. Six golf courses were selected for study based on the history of DMI fungicides used for disease management. Courses 1 through 3, located in Michigan near Warren, Webberville, and Williamston, respectively, had never been treated with DMI fungicides. Golf courses 4 through 6, located near Toledo, OH, Grosse Pointe, MI, and Southfield, MI, respectively, had been treated with DMI fungicides for several seasons including 1991. DMI fungicides had not adequately controlled dollar spot on courses 4 through 6 since 1989.

Creeping bentgrass (*Agrostis stolonifera* L.) and annual bluegrass (*Poa annua* L.) plants showing distinct dollar spot lesions were collected with a 2-cm-diameter soil probe from five fairways at each of the six golf courses during September 1991. Leaf blades were placed individually on potato-dextrose agar (PDA; Difco Laboratories, Detroit) amended with tetracycline and streptomycin sulfate at 20 µg ml⁻¹ each and incubated at 21°C for a minimum of 48 h. Single 7-mm-diameter agar plugs with mycelium of *S. homoeocarpa* were transferred from an area adjacent to each blade of grass to fresh PDA. Fifty isolates were collected from each golf course.

Fungicide screening. The following DMI fungicides were used in this study: triadimefon (Bayleton 25% DF, Miles Inc., Kansas City, MO), fenarimol (Rubigan 1 AS, Dow Elanco Products Co., Indianapolis, IN), and propiconazole (Banner 1.1 EC, Ciba-Geigy Co., Greensboro, NC). To determine the sensitivity of the *S. homoeocarpa* isolates to the DMI fungicides, a 7-

mm-diameter agar plug was cut from 1-week-old colonies and transferred to plates containing 20 ml of PDA amended with triadimefon or fenarimol at 0, 0.01, 0.05, 0.1, and 0.5 µg ml⁻¹ or propiconazole at 0, 0.01, 0.05, 0.1, and 0.2 µg ml⁻¹.

Only one isolate was tested per petri dish. The fungicides were dissolved in acetone and added to molten PDA (60°C) after sterilization. The final acetone concentration was 0.1% (vol/vol) in all treatment and control media. The colony diameter (minus the diameter of the inoculation plug) was determined after incubation for 4 days at 21°C. The results were expressed as the relative growth (colony diameter on DMI-amended medium divided by the diameter on unamended medium multiplied by 100) for each isolate. ED₅₀ values were calculated by regressing the relative growth against the log of the fungicide concentration. The experiment was replicated three times. Chi-square analysis was used to analyze the frequency distributions based on relative growth for isolates of *S. homoeocarpa* from the six golf courses.

Field trials. The efficacy of triadimefon, fenarimol, and propiconazole for control of dollar spot on a fairway composed of a mixture of annual bluegrass and creeping bentgrass was evaluated at golf course 5 from 1991 through 1993. In addition, iprodione (Chipco 26019 2 FL, Rhone-Poulenc Ag Co., Research Triangle Park, NC) and chlorothalonil (Daconil 2787 4.17 FL, ISK Biotech Co., Mentor, OH) were tested alone and in combination with triadimefon. The fairway was irrigated daily, fertilized with 0.12 kg of N per 100 m² every 14 days, and mowed to a height of 1.0 cm.

Fungicides were applied to 1.8- × 1.8-m plots, replicated three times in a randomized block design, with a CO₂ plot sprayer. Treatments were initiated on 28 August 1991, 21 July 1992, and 22 July 1993, with subsequent applications made at 10- or 21-day intervals. Plots treated at 10-day intervals were sprayed four, seven, and six times, and those treated at 21-day intervals were sprayed two, four, and three times, during 1991, 1992, and 1993, respectively.

Visual dollar spot disease assessments were made on 16 September 1991, 1 October 1992, and 19 September 1993 when disease severity in the unsprayed control treatment was greatest. A 0 to 9 scale, where 0 = 0 to 9% and 9 = 90 to 100% of the plot area with symptoms of dollar spot,

Table 1. Sensitivity to three sterol demethylation inhibiting (DMI) fungicides of *Sclerotinia homoeocarpa* isolates from three golf courses (1 through 3) where DMI fungicides were never used and three courses (4 through 6) where DMI fungicides were used extensively

Fungicide concentration in amended medium	Golf course	Relative growth ^y	
		Mean ^z	Range
Triadimefon, 0.5 µg ml ⁻¹	1-3	29	17-40
	4-6	61	27-85
Fenarimol, 0.5 µg ml ⁻¹	1-3	17	7-38
	4-6	42	20-57
Propiconazole, 0.5 µg ml ⁻¹	1-3	11	3-36
	4-6	38	10-64

^y Colony diameter on DMI-amended medium/the corresponding diameter on unamended medium × 100.

^z Each value is the mean of 50 isolates from each of the three golf courses (150 isolates total).

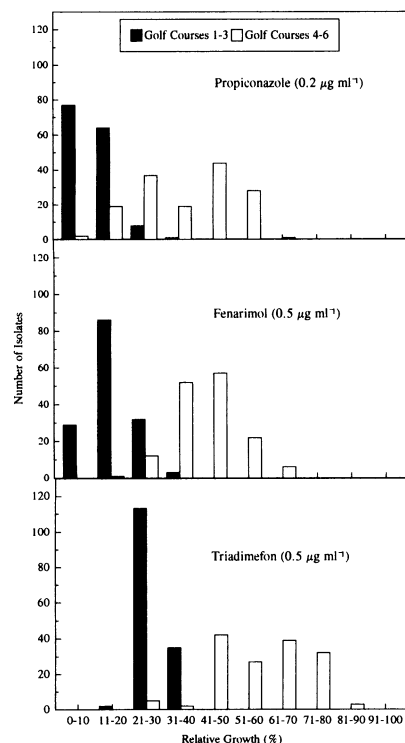


Fig. 1. Frequency distributions of relative growth of *Sclerotinia homoeocarpa* isolates sampled from six golf courses on media amended with three sterol demethylation inhibitor fungicides.

was used to rate each plot. The ratings were subjected to a square root transformation prior to analysis of variance (11). Treatment means were separated using Tukey's honestly significant difference test.

RESULTS

Fungicide screening. Mean ED₅₀ values for isolates from golf courses 1 through 3 and 4 through 6 were 0.12 and >0.5 µg ml⁻¹ for triadimefon, 0.03 and 0.26 µg ml⁻¹ for fenarimol, and 0.002 and 0.103 µg ml⁻¹ for propiconazole, respectively. At the highest concentration tested, the relative growth of isolates from golf courses 1 through 3 was two to three times lower, depending on the DMI fungicide, compared to the relative growth of isolates from golf courses 4 through 6 (Table 1).

Isolates that grew well on PDA amended with triadimefon also grew well on PDA amended with fenarimol and propicon-

azole. Chi-square analysis of the frequency distribution data for each fungicide indicated that isolates from courses 1 through 3 had significantly ($P < 0.001$) less growth on fungicide-amended media than isolates from courses 4 through 6 (Fig. 1). When representative isolates from golf courses 1 through 3 and 4 through 6 were tested on PDA amended with triadimefon at 1 and 2 µg ml⁻¹, growth of isolates from courses 4 through 6 was 4.7- and 7.8-fold greater, respectively, than the growth of isolates from golf courses 1 through 3 (Table 2).

Field trials. Disease incidence was higher in 1991 than in 1992 and 1993. The severity of dollar spot in plots treated with triadimefon, fenarimol, and propiconazole was reduced compared to untreated plots in 1992 but not in 1991 or 1993 (Table 3). The severity of dollar spot in plots treated with iprodione or with iprodione plus triadimefon was not statistically ($P = 0.05$) different in 1991 or 1992 compared to the severity of dollar spot in plots treated with triadimefon. The severity of dollar spot in plots treated only with iprodione was reduced significantly ($P = 0.05$) compared to the severity in the untreated control in 1992 but not in 1991 or 1993. Chlorothalonil applied alone on a 10-day schedule provided complete control in each of the three years, whereas chlorothalonil plus triadimefon on a 21-day schedule provided complete control in 1992 and 1993 but not in 1991.

DISCUSSION

The significant difference in the sensitivity of isolates from golf courses 1 through 3 compared to isolates from courses 4 through 6 to three DMI fungicides indicates that the *S. homoeocarpa* populations, where DMI fungicides have been used, have shifted toward resistance to this group of fungicides. This is the first documented case of DMI resistance in a turfgrass pathogen population. There have been previous reports of DMI resistance in *Sphaerotheca fuliginea* (cucumber powdery mildew) (7), *Guignardia bidwellii* (grape black rot) (12), and *Venturia inaequalis* (apple scab) (10).

All triadimefon-resistant isolates of *S. homoeocarpa* were cross-resistant to fenarimol and propiconazole, based on both

Table 2. Comparison of two monitoring doses of the sterol demethylation inhibiting (DMI) fungicide triadimefon on the growth of *Sclerotinia homoeocarpa* isolates from three golf courses (1 through 3) where DMI fungicides were never used and three courses (4 through 6) where DMI fungicides were used extensively

Golf courses	Relative growth on triadimefon-amended medium ^z	
	1 µg ml ⁻¹	2 µg ml ⁻¹
1-3	8.0	3.7
4-6	38.0	29.0

^z Relative growth (colony diameter on triadimefon-amended medium/the diameter on unamended medium × 100) was calculated for each isolate. Each value is the mean of 15 isolates, five from each golf course.

ED₅₀ and mean relative growth determinations. Baseline sensitivity to DMI fungicides has been reported for *V. inaequalis* (4,9,12). In addition, cross-resistance was observed among the DMI fungicides tested; however, baseline sensitivities varied depending on the intrinsic activity of the fungicide (5). Köller et al. (5) reported that routine monitoring based on one DMI fungicide should be indicative for the whole group because of cross-resistance among DMI fungicides.

Mycelial growth is the developmental stage most effectively inhibited by DMI fungicides under normal conditions (6). The sensitivity to DMI fungicides of *S. homoeocarpa* populations was based on an agar plug assay, while resistance was established based on ED₅₀ values and confirmed with relative growth values. These methods have been effective previously in determining DMI sensitivity distribution (1,4,9,12).

The sensitivity of individual isolates should be determined with a range of fungicide concentrations as a prerequisite for resistance monitoring (2). Smith et al. (9) demonstrated the need for precise sensitivity data and sufficiently large sample sizes for monitoring quantitative fungal population shifts. Smith et al. (9) proposed using a discriminatory dose for monitoring, based on relative growth, to follow shifts in populations toward resistance; the objective was to test the response of isolates to only two fungicide concentrations, rather than five to eight and still maintain a high level of precision and accuracy. Our work has shown that this method is an effective tool for eliminating the cumbersome task of using multiple concentrations for determining and comparing the sensitivity of fungal populations.

The development of resistance to DMI fungicides in *S. homoeocarpa* populations poses a significant challenge to the management of dollar spot because populations resistant to the benzimidazole and dicarboximide fungicides already exist (3,14). We have shown that *S. homoeocarpa* strains now exist that show multiple resistance to three major classes of fungicides. The only available treatment for

Table 3. Control of dollar spot on a golf course fairway with fungicides applied every 10 or 21 days

Fungicide	Product/ 100 m ²	Interval (days)	Disease ratings ^{y,z}			
			1991	1992	1993	Avg.
Triadimefon 25% DF	30.5 g	21	9.0 c	3.3 b	4.3 c	5.5 bc
Fenarimol 1 AS	55.7 ml	21	9.0 c	3.0 b	5.0 c	5.7 bc
Propiconazole 1.1 EC	31.8 ml	21	9.0 c	2.3 ab	6.3 c	5.9 bc
Iprodione 2 FL	63.7 ml	21	9.0 c	2.3 ab	3.0 bc	4.8 bc
Iprodione 2 FL + triadimefon 25% DF	63.7 ml + 30.5 g	21	8.7 c	1.7 ab	0.7 ab	3.7 a-c
Chlorothalonil 4.17 FL	191.0 ml	10	0.0 a	0.0 a	0.0 a	0.0 a
Triadimefon 25% DF + chlorothalonil 4.17 FL	30.5 g + 191.0 ml	21	6.0 b	0.0 a	0.0 a	2.0 ab
Control	9.0 c	8.0 c	4.0 c	7.0 c

^y 0 = no disease; 9 = severe disease; based on three replications per fungicide treatment.

^z Statistical analysis performed on square root transformation data using Tukey's honestly significant difference test at $P = 0.05$.

dollar spot management on golf courses where resistance to benzimidazole, dicarboximide, and DMI fungicides exists is the contact fungicide chlorothalonil.

Fungicide spray programs must be developed to delay the selection of *S. homoeocarpa* populations with high levels of resistance to DMI fungicides. It may be possible to minimize the selection pressure on *S. homoeocarpa* populations by limiting the use of DMIs during the time of year when dollar spot is most severe. This may allow the wild-type DMI-sensitive strains of *S. homoeocarpa* to increase in the populations.

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