Sensitivity of *Sclerotinia homoeocarpa* Isolates to Propiconazole and Impact on Control of Dollar Spot

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ABSTRACT

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In response to reports of reduced efficacy of propiconazole for control of dollar spot, isolates of *Sclerotinia homoeocarpa* were collected from several locations in Georgia and tested for sensitivity to propiconazole and other demethylation-inhibiting (DMI) fungicides. Two discriminatory concentrations of propiconazole (0.02 and 0.2 $\mu\text{g ml}^{-1}$) were used to detect lower in vitro sensitivity in two populations that had been exposed repeatedly to propiconazole than in four nonexposed populations. Mean 50% effective concentration (EC_{50}) values for a nonexposed population (baseline) and a DMI-exposed population were 0.0049 and 0.0283 $\mu\text{g ml}^{-1}$, respectively. Positive correlations were significant among $\log_{10} \text{EC}_{50}$ values for propiconazole, fenarimol, and myclobutanil but not between triadimefon and any of the other three fungicides, indicating cross-resistance relationships in this pathogen may not be universal among the DMIs. In greenhouse experiments, propiconazole-treated bentgrass was inoculated with seven isolates of *S. homoeocarpa* differing in sensitivity to propiconazole. Incubation period decreased and relative area under the disease progress curve and disease severity 28 days after inoculation increased linearly with increasing $\log_{10} \text{EC}_{50}$ value of the isolate. Results of this study confirm a significant relationship between in vitro sensitivity of *S. homoeocarpa* and in planta control efficacy of propiconazole and provide evidence of field resistance to propiconazole in *S. homoeocarpa* in Georgia.

Additional keywords: *Agrostis palustris*, disease management, fungicide resistance, sterol inhibitors

Dollar spot, caused by *Sclerotinia homoeocarpa* F. T. Bennett, is the most common turfgrass disease in North America (7), causing considerable reduction in quality of amenity turfgrasses. In Georgia, the disease is most severe and economically damaging on creeping bentgrass (*Agrostis palustris* Huds.) managed for golf putting greens, with most epidemics occurring in late spring, early summer, and fall. Cultural practices alone are not sufficient to control the disease on bentgrass (24), and disease thresholds on golf courses are particularly low; therefore, fungicides are applied preventatively, often on a 2-week schedule, when conditions are favorable for the disease (23). This intensive fungicide use can result in selection for resistant strains in pathogen populations (12,24) and a decrease in fungicide efficacy over time.

The sterol demethylation-inhibiting (DMI) fungicides, available for dollar spot control in the United States since 1979

(12), offer several advantages over other classes of fungicides. These fungicides provide a broad spectrum of activity, controlling many important turfgrass diseases with a single application. In addition, the DMIs can be used at low application rates and at long application intervals because of their systemic activity (15). Equally important is the ability of the DMIs to control strains of *S. homoeocarpa* resistant to the benzimidazole (25) and dicarboximide (8) fungicides.

Evidence of field resistance to a fungicide is based on an observable decrease in disease control under field conditions that is associated with decreased sensitivity of the pathogen to the fungicide. In documented cases of field resistance to DMI fungicides, in vitro mycelial growth assays often have been used to detect reduced pathogen sensitivity. In vitro mycelial growth assays were used to detect resistance to the DMI fungicides in field populations of *S. homoeocarpa* in Illinois, Kentucky, and Michigan, at sites where control failure had been observed (9,12,24). Similar assays were used to detect reduced sensitivity to the DMIs in a population of *S. homoeocarpa* in Ontario, Canada, at a site where field resistance to the DMIs had not been observed (14). Reports such as these indicate the need to authenticate in

vitro test results with in planta loss of fungicide efficacy prior to confirming a fungicide resistance problem.

Little is known about the sensitivity of *S. homoeocarpa* populations in Georgia to the DMI fungicides. DMI use on turf in the southeastern United States generally is limited to golf course greens where susceptible creeping bentgrass has been established. Recently, reduced efficacy of the DMI fungicides on bentgrass greens has been observed at golf courses with a history of DMI usage. The reduced efficacy is manifested as shorter intervals of dollar spot control on golf greens treated with propiconazole than the intervals listed on the fungicide label. The research reported here was initiated to investigate these reports. The objectives of this research were to (i) establish a baseline sensitivity for monitoring propiconazole sensitivity of *S. homoeocarpa* populations in Georgia, (ii) compare propiconazole sensitivity in six populations of *S. homoeocarpa* in Georgia with differing histories of exposure to the fungicide, (iii) evaluate potential for cross-resistance in *S. homoeocarpa* to other DMI fungicides, and (iv) determine the relationship between fungicide sensitivity of *S. homoeocarpa* in vitro and control of dollar spot with fungicide in inoculated bentgrass.

MATERIALS AND METHODS

Sampling locations and fungal isolations. Over 400 isolates of *S. homoeocarpa* were collected from creeping bentgrass putting greens exhibiting dollar spot symptoms at six golf courses across northern Georgia in the fall of 1999 and spring and fall of 2000. A representative sample of at least 50 isolates was collected from each location. DMI fungicide use varied among the sampling locations, with four of the sites having no documented history of exposure to DMIs and two of the sites having a history of DMI exposure. Population AAC, located in Atlanta, GA, had been exposed to propiconazole (Banner Maxx EC; Syngenta Crop Protection, Greensboro, NC) and triadimefon (Bayleton 50; Bayer Corp., Kansas City, MO) at 0.025 kg a.i. ha^{-1} twice per year for a minimum of 6 years prior to sampling. Population CCC, located in Roswell, GA, had been exposed to the same rate of propiconazole at least twice per year for a minimum of 4 years prior to sampling.

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Each isolate was obtained from a single dollar spot infection center and, in heavy infestations (>5 spots/m²), the minimum linear distance between sampled infection centers was 30 cm. Individual leaf blades were removed from the margins of an infection center, surface sterilized for 30 s in a 70% ethanol solution and a 0.5% sodium hypochlorite solution, rinsed once in sterile water, and placed in petri dishes (9-cm-diameter) containing potato dextrose agar (PDA; Difco Laboratories, Detroit). The petri dishes were sealed with Parafilm and cultures were incubated at room temperature (25°C) for 3 days. The pathogen was identified by visual comparison of colony growth with known cultures of *S. homoeocarpa* (6,14). Mycelial plugs from each isolate were placed on sterilized rye grains, allowed to grow at 25°C for 21 days, and frozen at -20°C until needed. One sensitive isolate, S084, and one resistant isolate, S088, with known in vitro sensitivities and field-tested levels of resistance to the DMI and benzimidazole fungicides (3), were used in each of the experiments as standards for comparison with collected isolates (Table 1).

Baseline sensitivity determination. The sensitivities of 59 isolates of *S. homoeocarpa* from a population (HMGC) not exposed to propiconazole and 69 isolates from a population (CCC) exposed to propiconazole were determined using a modified mycelial growth assay (21). Hyphal plugs (6 mm in diameter) from the edge of colonies actively growing on PDA were homogenized in 5 ml of sterile potato dextrose broth (PDB) to provide a uniform suspension of mycelial fragments. Petri dishes (9 cm in diameter) of PDA amended with propiconazole at a concentration of 0, 0.0002, 0.0006, 0.002, 0.006, 0.02, 0.06, 0.2, 0.6, 2, 6, or 20 µg a.i. ml⁻¹ were prepared. Technical grade propiconazole (97.2% a.i.; Syngenta Crop Protection, Greensboro, NC) was dissolved in acetone to make fungicide concentrations. Fungicide solutions were added to autoclaved PDA cooled to 60°C, such that the final concentration of acetone was 0.1% (vol/vol) in all treatments, including the nonamended control. Acetone at this concentration does not inhibit growth of *S. homoeocarpa* (14). A 5-mm-diameter plug of agar was removed from the center of each dish to form a well in the solidified medium. Fifty microliters of the mycelial suspension of each isolate was transferred to the well in each of two replicate plates per fungicide concentration.

Cultures were incubated at room temperature (25°C), and radial growth of colonies was measured when colony diameters of controls reached at least 50 mm (3 to 4 days of incubation). The diameter of each colony was measured in two perpendicular directions and the mean diameter was adjusted by subtracting the diameter of the well. Relative growth (RG = [the mean

adjusted colony diameter on propiconazole-amended medium/the mean adjusted colony diameter on nonamended medium] × 100%) was determined for each isolate and fungicide concentration.

The 50% effective concentration (EC₅₀) value for each replication of each isolate was estimated by linear regression of the probit-transformed relative inhibition (RI) value (RI = 1 - RG) on log₁₀-transformed fungicide concentration (14). The EC₅₀ value for each isolate was calculated as the mean of the two replicates. A *t* test was performed on the log₁₀-transformed EC₅₀ values to test whether the two populations differed significantly in sensitivity to propiconazole. The resistance factor (RF) was calculated for the exposed population by dividing the mean EC₅₀ value of isolates from the exposed population by the mean EC₅₀ value of isolates from the baseline population.

Based on the low frequency of isolates from the nonexposed population with EC₅₀ values greater than these levels, two discriminatory concentrations of propiconazole (0.02 and 0.2 µg a.i. ml⁻¹) were selected to evaluate differences in sensitivity among isolates representing the six *S. homoeocarpa* populations. To evaluate the accuracy of using these concentrations for this purpose, log₁₀ EC₅₀ values for each isolate from the baseline (HMGC) and exposed (CCC) populations were regressed on mean RG values obtained for each isolate at the two concentrations.

Assay reproducibility. A test was conducted to determine the reproducibility of the mycelial growth assay used for estimating propiconazole sensitivity. Propiconazole sensitivity was assayed five times for 5 isolates that were chosen arbitrarily from the pool of 441 isolates sampled. For each assay, a new stock solution of propiconazole was prepared. The mean of the EC₅₀ values, variance, coefficient of variance, and 95% confidence intervals were calculated for each isolate based upon the five repeated tests using the formulas obtained from Casella and Berger (4) and Gilbert (11) as described by Wong and Wilcox (27). A mean coefficient of variance was calculated for the assay based upon the results from these five isolates.

Comparison of isolates from six populations. Mycelial suspensions of all isolates sampled from the six populations were placed in a 5-mm-diameter well cut in PDA unamended or amended with the

two discriminatory concentrations of propiconazole. Each isolate was tested on two replicate plates of each concentration. Fungicide-amended media were prepared and colony growth was measured as described previously. RG was determined for each isolate by averaging the two replications, and a mean RG was determined for all isolates from each of the six populations. Differences in mean relative growth among populations were determined using analysis of variance (ANOVA), and means were separated using Fisher's protected least significant difference (LSD) (SAS 8.2; SAS Institute, Cary, NC).

Evaluation of cross-sensitivity. The mycelial growth assay described previously was used to calculate EC₅₀ values for triadimefon (Bayer Corp.), myclobutanil (Aventis CropScience, Research Triangle Park, NC), and fenarimol (Dow Agrosciences, Indianapolis, IN) among a subset of 50 isolates of *S. homoeocarpa*. Fungicide-amended PDA was prepared as previously described using technical grade fungicides provided by the respective manufacturers. The subset of isolates, with EC₅₀ values for propiconazole ranging from 0.001 to 0.057 µg ml⁻¹, included 25 isolates from the baseline (nonexposed) population (HMGC) and 25 isolates from a population (CCC) which had been exposed to propiconazole but not to other DMI fungicides. Isolates in the subset were selected to represent the full range of sensitivities to propiconazole in the samples from the two populations. Simple linear correlation coefficients were calculated between each pairwise combination of log₁₀ EC₅₀ values for the four DMI fungicides to assess the potential for cross-resistance.

Greenhouse study. For in planta experiments, seven isolates were selected to represent the entire range of propiconazole sensitivities (0.005 to 0.075 µg ml⁻¹) determined in vitro. The selected isolates included one isolate from the baseline population (HMGC), four isolates from the exposed population (CCC), and the two isolates, S084 and S088, which represented extremes for propiconazole sensitivity in this experiment. To prepare inoculum of each isolate, frozen infested grains were placed in petri dishes containing PDA and incubated for 3 days at room temperature (approximately 25°C). One mycelial plug (6-mm-diameter) was removed from the edge of each actively growing fungal col-

Table 1. Characteristics of *Sclerotinia homoeocarpa* isolates used as propiconazole-resistant and propiconazole-sensitive controls

Isolate no.	Location	Year collected	EC ₅₀ value (µg ml ⁻¹) ^x	EC ₅₀ value (µg ml ⁻¹) ^y	Phenotype ^z
S084	State College, PA	1980	0.03	0.0056	Sensitive
S088	Chicago, IL	1993	0.31	0.0753	Resistant

^x Fifty percent effective concentration (EC₅₀) values for propiconazole estimated by Burpee (3).

^y EC₅₀ values for propiconazole estimated in the present study.

^z Reaction to benzimidazoles and demethylation-inhibiting fungicides.

ony and placed in a glass tube (16 by 100 mm) containing approximately 3 g of sterilized rye grains. Grain cultures were incubated for 3 weeks at 20°C to allow the fungus to colonize the rye grains prior to inoculation.

Creeping bentgrass cv. L93 was chosen for use in this experiment based on its moderate susceptibility to dollar spot compared with other cultivars (5). Bentgrass was seeded at a rate of 3.66 g/m² in calcined clay medium (Turface MVP; Profile Products LLC, Buffalo Grove, IL) in Cone-Tainers (Steuwe & Sons, Inc., Corvallis, OR) measuring 3.8 cm in diameter and 21 cm in height. Racks containing 49 Cone-Tainers each were placed in a mist chamber maintained at 100% relative humidity, day and night temperatures of 20 and 26°C, respectively, with a 12-h photoperiod. Seven days after seeding, the Cone-Tainers were transferred to a greenhouse where the bentgrass was allowed to mature for 5 weeks before inoculation. The bent-

grass was fertilized every 14 days with 1.3 g of N/m², and cut to a height of 1.27 cm three times per week. In trial 2, Gnatrol (*Bacillus thuringiensis* var. *israelensis*; Valent BioSciences, Longwood, FL) was applied as a water drench to the bentgrass at a rate of 10.4 ml/liter of water every 2 weeks for control of fungus gnats.

The bentgrass was treated with propiconazole at 0.099 kg a.i. ha⁻¹, the label rate for dollar spot control at a spray interval of 14 to 28 days (Banner Maxx EC, Syngenta), or left untreated as controls. The material was applied in water at a volume rate of 0.082 liters/m² with a handheld, single-nozzle CO₂-pressured sprayer at 248.2 kPa of pressure. The turf in each Cone-Tainer was allowed to dry for 24 h and inoculated by placing a single rye grain infested with an isolate on a matte pin (3.2 cm long) that was positioned in the center of the Cone-Tainer and 0.6 cm below the top of the grass canopy. The experiment was designed as a split plot, with

the fungicide and control treatments applied to the main plots, and seven replications of each isolate arranged in randomized blocks (subplots) within each main plot. Main plots (fungicide) and subplots (isolates) were analyzed with single factor ANOVA to assess the effects of treatments on disease development.

After inoculation, Cone-Tainers were placed in a growth chamber maintained at day and night temperature of 20 and 26°C, respectively, with a 12-h photoperiod. The turf was misted at night for 6 h and then briefly dried with a hair dryer to allow for a total of 10 to 12 h of leaf wetness per day. To counteract the possible adverse effects of repeated wetting and drying on the fungus, inoculum was replaced every 10 days with a new rye grain infested with the same isolate.

Disease severity (Y), measured as the proportion of necrotic turf surface area in each Cone-Tainer, was recorded daily for 36 days. The incubation period was recorded as the number of days between inoculation and first appearance of symptoms. Disease severity values were used to calculate the area under the disease progress curve (AUDPC) for each Cone-Tainer. ANOVA was performed on incubation period, AUDPC, and disease severity at 28 days after inoculation (Y₂₈) to determine differences in virulence among isolates on untreated bentgrass. If differences in virulence were detected for a particular variable, the data for that variable on treated bentgrass were analyzed as a proportion of the untreated control. Differences in incubation period, AUDPC values, and Y₂₈ values among isolates on fungicide-treated bentgrass were analyzed by linear regression on the log₁₀-transformed EC₅₀ values of the isolates. The entire experiment was repeated for a total of two experimental trials.

RESULTS

Baseline sensitivity. Using a modification of the mycelial growth assay commonly used for estimation of propiconazole sensitivity, EC₅₀ values of 0.0056 and 0.0753 µg ml⁻¹ were estimated for the standard isolates S084 and S088, respectively. These values are lower than sensitivity values for propiconazole for the same two isolates reported by Burpee (3), (0.03 and 0.31 µg ml⁻¹) using a standard mycelial growth assay (Table 1). This provides evidence that a modification in the technique used to derive in vitro sensitivity can produce substantial differences in sensitivity values.

The frequency distributions of EC₅₀ values for the baseline (HMGC) and exposed (CCC) populations were log-normal. EC₅₀ values for propiconazole for isolates from the baseline (nonexposed) population ranged from 0.0006 to 0.0102 µg ml⁻¹ with a mean of 0.0049 µg ml⁻¹ (Fig. 1). The range of EC₅₀ values for isolates from the

Fig. 1. Frequency distributions of sensitivities to propiconazole for *Sclerotinia homoeocarpa* isolates from the baseline (HMGC) and demethylation-inhibiting fungicide-exposed (CCC) populations in Georgia.

Table 2. Reproducibility of mycelial growth assay for determining 50% effective concentration (EC₅₀) values for propiconazole in five isolates of *Sclerotinia homoeocarpa*

Isolate	EC ₅₀ value (µg ml ⁻¹)		
	Mean ^x	CI ^y	CV (%) ^z
AAC 34	0.064	0.073 – 0.058	0.096
AAC 50	0.038	0.041 – 0.036	0.020
FGC 51	0.065	0.079 – 0.056	0.217
ML 1	0.025	0.029 – 0.021	0.062
TC 1	0.025	0.029 – 0.022	0.046
Mean	...	...	0.088

^x Mean fifty percent effective concentration (EC₅₀) value based upon ln-transformed EC₅₀ values obtained from five repeated assays.

^y 95% confidence interval based upon ln-transformed EC₅₀ values.

^z CV = coefficient of variance based upon ln-transformed EC₅₀ values.

exposed population was wider (0.005 to 0.057 $\mu\text{g ml}^{-1}$) and the mean EC_{50} value (0.0283 $\mu\text{g ml}^{-1}$) was significantly greater ($P < 0.0001$) than that of the nonexposed isolates. The RF for the exposed population was 5.8.

Assay reproducibility. For the five isolates evaluated in five repeated runs of the mycelial growth assay, the coefficients of variance for each isolate ranged from 0.02 to 0.2%. The mean coefficient of variance for these assays was 0.09% (Table 2). The

95% confidence intervals ranged from 84 to 122% of the mean EC_{50} values for individual isolates subjected to the propiconazole assay. On average, the 95% confidence intervals ranged from 89 to 115% of the mean EC_{50} values for the propiconazole assay.

Comparison of isolates from six populations. A significant correlation ($r = 0.90$, $P < 0.0001$) was detected between the log EC_{50} value of the baseline (HMGC) and exposed (CCC) populations and the mean

RG of each isolate at 0.02 $\mu\text{g ml}^{-1}$ (Fig. 2). At the discriminatory concentration of 0.2 $\mu\text{g ml}^{-1}$, a significant, but more variable relationship ($r = 0.51$, $P < 0.0001$) was detected between RG and log₁₀ EC_{50} values, presumably because a large proportion of isolates failed to grow at this concentration. This higher correlation ($r = 0.90$) supports the use of 0.02 $\mu\text{g ml}^{-1}$ for separation of propiconazole sensitivities among *S. homoeocarpa* populations, and for estimation of the mean EC_{50} value of a population with the equation of the regression line ($y = -2.88 + 2.35x$; Fig. 2). However, mean RG values on both concentrations were significantly ($P \leq 0.05$) greater for the two exposed populations than for the four nonexposed populations (Table 3). In the two exposed populations, the CCC population had a significantly greater mean RG value than the AAC population at the 0.02 $\mu\text{g ml}^{-1}$ concentration, but mean RG values were not significantly different between the two at the 0.2 $\mu\text{g ml}^{-1}$ concentration. In the nonexposed populations, mean RG was significantly greater for population FGC and HMGC than for population TC and ML on both discriminatory concentrations (Table 3).

Evaluation of cross-sensitivity. For the DMI fungicides tested, correlation analysis of EC_{50} values from the subset of 50 isolates showed a significant positive correlation between sensitivity to propiconazole and myclobutanil, fenarimol and myclobutanil, and propiconazole and fenarimol. There was no significant correlation between the sensitivities to propiconazole, myclobutanil, or fenarimol and the sensitivity of the isolates to triadimefon (Table 4).

Fig. 2. Relationship between relative growth on discriminatory concentrations of propiconazole and the log₁₀ 50% effective concentration values for isolates from a baseline (HMGC) and demethylation-inhibiting fungicide-exposed (CCC) population of *Sclerotinia homoeocarpa*.

Table 3. Mean relative growth of isolates from six populations of *Sclerotinia homoeocarpa* exposed to two discriminatory concentrations of propiconazole

Population	No. of isolates	Location	Bentgrass cultivar	DMI history ^x			Relative growth at discriminatory concentrations ^w			
				DMIs used	Appl./year ^y	Years of use ^z	0.02 $\mu\text{g ml}^{-1}$		0.2 $\mu\text{g ml}^{-1}$	
							Range	Mean	Range	Mean
CCC	69	Roswell, GA	Crenshaw	Propiconazole	2	4	0.18–0.77	0.52 a	0–0.27	0.087 a
AAC	50	Atlanta, GA	Crenshaw	Propiconazole	2	6	0.10–0.82	0.45 b	0–0.58	0.087 a
				Triadimefon	2	6	...	...	...	...
FGC	100	Duluth, GA	Crenshaw	None	...	...	0.03–0.78	0.38 c	0–0.34	0.057 b
HMGC	59	Dacula, GA	Dominant	None	...	...	0–0.56	0.24 d	0–0.24	0.048 b
TC	99	Alpharetta, GA	Penncross	None	...	...	0–0.22	0.12 e	0–0.03	0.0003 c
ML	64	Villa Rica, GA	Crenshaw	None	...	...	0–0.33	0.11 e	0	0 c

^w Within a column, means followed by the same letter are not significantly different ($P > 0.05$) based on Fisher's protected least significant difference.

^x DMI = demethylation-inhibiting fungicides.

^y Average number of applications per year.

^z Minimum number of years a DMI fungicide had been used prior to sampling.

Table 4. Pairwise correlations of sensitivities (50% effective concentration) to four demethylation-inhibiting fungicides of a subset of 50 isolates of *Sclerotinia homoeocarpa* from Georgia

	Propiconazole		Myclobutanil		Fenarimol		Triadimefon	
	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value	<i>r</i>	<i>P</i> value
Propiconazole	1.000	...	0.635	<0.0001	0.437	0.0015	0.078	0.6048
Myclobutanil	...	...	1.000	...	0.623	<0.0001	0.115	0.4479
Fenarimol	...	...	...	...	1.000	...	0.195	0.1931
Triadimefon	...	...	...	...	...	...	1.000	...

Greenhouse study. Fungicide treatment had a significant effect on dollar spot for all variables tested. Differences in virulence among isolates on untreated bent-

grass were not detected for incubation period or Y_{28} values, but were significant for AUDPC in trial 2, with isolate S088 having a significantly higher AUDPC

value than the other six isolates. To account for this difference in virulence, relative AUDPC values (proportion of untreated control) were calculated for subsequent analysis of this variable.

Significant linear relationships were detected between in vitro sensitivity and disease severity in propiconazole-treated bentgrass. In both trials, incubation period decreased linearly with increasing $\log_{10}$ EC_{50} value (Fig. 3). Relative AUDPC values (Fig. 4) and Y_{28} values (Fig. 5) increased linearly with increasing $\log_{10}$ EC_{50} value in both trials.

DISCUSSION

The mean EC_{50} value of $0.005 \mu\text{g ml}^{-1}$ for the HMGC population, which had not been exposed to propiconazole, was similar to mean EC_{50} values of 0.002 and $0.007 \mu\text{g ml}^{-1}$ for nonexposed populations reported by Golembiewski et al. (12) and Hsiang et al. (14), respectively. This confirms that, at the time of sampling, little, if any, selective change toward propiconazole insensitivity had occurred within population HMGC. Therefore, this population can serve as a suitable baseline to which future populations of *S. homoeocarpa* from Georgia can be compared. In addition, the frequency distributions of sensitivities of isolates from the HMGC and CCC populations provide a basis for the selection of discriminatory doses that can be used to test other *S. homoeocarpa* populations for propiconazole sensitivity.

Reduced sensitivity to propiconazole was found in isolates of *S. homoeocarpa* that were collected from a site in Georgia (CCC) with a history of propiconazole use. The RF (5.8) for this population was approximately 10 times lower than the mean RF (51.5) reported for three Michigan populations of *S. homoeocarpa* that had been exposed to DMIs (12). The higher resistance factor for the Michigan populations is consistent with a greater intensity of dollar spot in northern states and a higher frequency of fungicide use (24).

Compared with the high mean RF values found in Michigan, the RF for the CCC population may not indicate a sensitivity reduction large enough to initiate an economic loss associated with fungicide control failure, although the precise RF value where failure occurs is not known. In Ontario, a population of *S. homoeocarpa* with reduced sensitivity to propiconazole had a mean EC_{50} value of $0.026 \mu\text{g ml}^{-1}$ and a mean RF of 3.7 (14). The Ontario population was sampled from a site where propiconazole was not yet labeled for turf use, and no disease control failure had been observed. The site was close enough to the U.S. border that ingress of less sensitive isolates may have occurred or the turf may have been exposed to nonlabeled use of a DMI fungicide (14).

Interpretation of results of fungicide monitoring has proven difficult in the past

Fig. 3. Relationship between sensitivity to propiconazole and incubation period (inoculation until symptoms) of *Sclerotinia homoeocarpa* isolates on bentgrass treated with propiconazole and inoculated with each isolate. Each point represents the mean incubation period of a single isolate over seven replications. Error bars represent the standard errors about the means.

Fig. 4. Relationship between sensitivity to propiconazole and relative area under the disease progress curve (AUDPC) for isolates of *Sclerotinia homoeocarpa* on bentgrass treated with propiconazole and inoculated with each isolate. AUDPC values are calculated as a proportion of the untreated controls. Each point represents the mean relative AUDPC caused by a single isolate over seven replications. Error bars represent the standard errors about the means.

and has occasionally resulted in misleading over-prediction of resistance problems (2). Therefore, evidence of the correlation of fungicide sensitivity measured in vitro to actual disease control provided by that fungicide is a necessary and vital part of resistance monitoring. Many estimates of DMI sensitivities of pathogen populations can be found in the literature (13,19,20, 22,28,29), but few studies (3,10,12,16,18) have included a correlation of in vitro sensitivities to fungicide efficacy with an in planta test.

The present study provides evidence of a significant positive linear relationship between the sensitivity of isolates of *S. homoeocarpa* to propiconazole and efficacy of propiconazole for dollar spot control in bentgrass. These results further validate the use of mycelial growth assays for estimating propiconazole sensitivity in pathogen populations to detect potential erosion of disease control efficacy. Franke et al. (10) found no significant correlation between disease control with tebuconazole and virulence of 20 isolates of *Sclerotium rolfsii* that differed in sensitivity to the fungicide in vitro. The authors state that the sensitivities of the isolates used in the study may not have been low enough to cause inadequate control by the labeled rate of tebuconazole (10).

Propiconazole efficacy was highest for dollar spot caused by the most sensitive isolate of *Sclerotinia homoeocarpa* used in this study and lowest for the least sensitive isolate. In a similar greenhouse study,

Köller et al. (16) found that apple scab severity on leaves treated with fenarimol or myclobutanil was substantially higher for an isolate with an EC_{50} value representing a population with reduced sensitivity to fenarimol than for an isolate with an EC_{50} value representing a baseline population. Under field conditions, Burpee (3) observed significantly less control of dollar spot on propiconazole-treated bentgrass (a reduction in incubation period of about 12 to 15 days) caused by isolate S088, which had reduced in vitro sensitivity to propiconazole, than control of disease caused by S084, a sensitive isolate.

The results of this study provide an indication of the quantitative loss in fungicide efficacy that might occur in populations of *S. homoeocarpa* isolates with reduced fungicide sensitivity. By substituting the $\log_{10}$ -transformed mean EC_{50} value for population CCC into the regression equation for trial 2 (Fig. 3), it was possible to predict the average incubation period (effectively equivalent to "days of control") that one might expect following application of propiconazole at the same rate and under the same conditions as trial 2. Based on this equation, application of propiconazole at a label rate of 0.099 kg a.i. ha⁻¹ would provide an average of 19.5 days of control of population CCC (mean EC_{50} = 0.0283 $\mu\text{g ml}^{-1}$). This is a reduction in effective length of control of approximately 8 days compared with control of disease caused by a sensitive population (mean EC_{50} = 0.0049 $\mu\text{g ml}^{-1}$).

In previous reports (12,14), loss of fungicide efficacy in DMI-resistant populations of *S. homoeocarpa* has been associated with high RF values (>50). This differs from other pathogen populations for which resistance factors of 1.8 to 10 or more were linked to reduced fungicide efficacy in the field (1,21). In the present study, a reduction in propiconazole efficacy was detected for *S. homoeocarpa* populations with an RF value of only 5.8, indicating that shifts in sensitivity may be associated with small reductions in fungicide efficacy, which may not be noticed by turfgrass managers.

It is important to point out that our trials were conducted in the greenhouse under conditions that differ substantially from the field. However, the mean incubation periods for isolates S084 and S088 on propiconazole-treated turf in the greenhouse study were similar to the incubation periods previously reported for these isolates under field conditions (24 to >29 days and 11.5 to 13.8 days for isolates S084 and S088, respectively) (3).

There are conflicting accounts of the potential for cross-resistance among the DMI compounds currently labeled for use on dollar spot in the United States (9,12,14). Cross-resistance among the DMI fungicides was thought to be universal in *S. homoeocarpa* (9,12), but Hsiang et al. (14) found distinct groupings between propiconazole and myclobutanil ($r = 0.438$, $P = 0.0001$), and tebuconazole and fenarimol ($r = 0.25$, $P = 0.0001$) in populations of *S. homoeocarpa* from Ontario. In the present study, the strongest correlation was found between sensitivity to propiconazole and myclobutanil, which was consistent with the previous study (14). Weaker, but statistically significant correlations, also were found between fenarimol and myclobutanil and fenarimol and propiconazole, implying that resistance to all three of these fungicides may be linked. This result agrees with Köller and Wudden (17), who found strong correlations ($0.86 \leq r \leq 0.99$) in sensitivities to these three fungicides in one wild-type sensitive and four lab-generated resistant isolates of *Ustilago avenae*. Cross-resistance in *S. homoeocarpa* to triadimefon and any of the other three DMIs tested in this study was not confirmed. This is in contrast to results reported for *Uncinula necator*, in which significant correlations ($0.60 \leq r \leq 0.83$) between sensitivities were found for triadimefon, myclobutanil, and fenarimol (28). Cross-resistance to DMI fungicides appears to vary by fungicide and fungal species and may need to be evaluated for each individual pathosystem.

Currently, efforts to manage and delay the development of resistance of *S. homoeocarpa* populations to DMI fungicides are being pursued at turfgrass sites under intense disease pressure. Due to the climate in Georgia, dollar spot previously had not

Fig. 5. Relationship between sensitivity of isolates of *Sclerotinia homoeocarpa* to propiconazole and disease severity 28 days after inoculation of propiconazole-treated bentgrass with each isolate. Each point represents the mean severity 28 days after inoculation caused by a single isolate over seven replications. Error bars represent the standard errors about the means.

been considered a severe problem, but golf course superintendents have found it increasingly more difficult to culturally or chemically manage the disease on creeping bentgrass. Some superintendents have relied heavily on DMI fungicides to manage dollar spot. The results of this study reveal a shift in propiconazole sensitivity in *S. homoeocarpa* populations in Georgia where propiconazole has been used. This information emphasizes the need to employ fungicide resistance management strategies (24) to extend the potential effectiveness of the DMIs and other at-risk fungicides for control of dollar spot.

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