2006

Fungicide Sensitivity of *Sclerotinia homoeocarpa* from Golf Courses in Ohio

Young-Ki Jo
Ohio State University

Amy L. Niver
Ohio State University

Joseph W. Rimelspach
Ohio State University

Michael J. Boehm
University of Nebraska-Lincoln, mboehm3@unl.edu

Fungicide Sensitivity of *Sclerotinia homoeocarpa* from Golf Courses in Ohio

Young-Ki Jo, Amy L. Niver, Joseph W. Rimelspach, and **Michael J. Boehm,** Department of Plant Pathology, The Ohio State University, Columbus 43210

ABSTRACT

Jo, Y.-K., Niver, A. L., Rimelspach, J. W., and Boehm, M. J. 2006. Fungicide sensitivity of *Sclerotinia homoeocarpa* from golf courses in Ohio. Plant Dis. 90:807-813.

Managing dollar spot, the most common and chronic disease on intensively cultivated turfgrass, relies on the judicious use of fungicides. The heavy use of fungicides has led to the development of isolates of *Sclerotinia homoeocarpa* insensitive to several classes of fungicides, including benzimidazoles, demethylation-inhibitors, and dicarboximides. In vitro fungicide sensitivity assays using single discriminatory concentrations of thiophanate-methyl, propiconazole, and iprodione were developed in this study for evaluating field efficacy of these fungicides and the prevalence of fungicide insensitivity within *S. homoeocarpa* isolated from golf courses throughout Ohio. Discriminatory concentrations for these fungicides were determined to be: thiophanate-methyl = 1,000 μg a.i. ml^{-1}, propiconazole = 0.1 μg a.i. ml^{-1}, and iprodione = 1.0 μg a.i. ml^{-1}. Effective concentration that produces 50% inhibition (EC_{50}) was estimated based on relative mycelial growth of *S. homoeocarpa* on potato dextrose agar (PDA) versus PDA amended with the discriminatory concentration of each fungicide. Field trials conducted at 3 locations in 2002 and 10 locations in 2003 revealed that the in vitro assays accurately predicted field efficacy for thiophanate-methyl. When used to screen 192 *S. homoeocarpa* isolates collected previously from 55 golf courses throughout Ohio, the in vitro assays revealed that 34 of the golf courses sampled had *S. homoeocarpa* resistant to thiophanate-methyl. *S. homoeocarpa* with reduced in vitro sensitivities was isolated from 18 and 1 golf courses for propiconazole and iprodione, respectively.

Dollar spot, caused by *Sclerotinia homoeocarpa* F. T. Bennett, is one of the most common diseases of turfgrass in temperate and subtropical regions (8). Dollar spot occurs primarily on creeping bentgrass (*Agrostis stolonifera* L.; syn = *A. palustris* Huds.) and annual bluegrass (*Poa annua* L.) putting greens, tees, and fairways on golf courses, but also occurs on Kentucky bluegrass (*P. pratensis* L.) and fescue (*Festuca* spp.) roughs and residential lawns. Symptoms are small (2.5 to 5.0 cm in diameter) straw-colored or necrotic spots on low-cut golf course turfgrass, but appear larger (15 to 30 cm in diameter) and more diffuse on high-cut turfgrass. Necrotic lesions on individual leaves are small and circular in the beginning but ultimately extend the width of the leaf blade, generally resulting in an hourglass-shaped lesion. The disease typically is managed through the combined use of an adequate fertility program and cultural practices designed to minimize the duration of prolonged leaf wetness. Dollar spot management in intensively cultivated turfgrass is heavily dependent on the timely applications of fungicides (30).

Corresponding author: M. J. Boehm
E-mail: boehm.1@osu.edu

Accepted for publication 10 February 2006.

DOI: 10.1094/PD-90-0807

Resistance of *S. homoeocarpa* to numerous fungicides is well documented. The first reports of fungicide resistance in *S. homoeocarpa* were to the broad-spectrum cadmium and mercury-based fungicides in 1968 (7,20). Since then, resistance in *S. homoeocarpa* to most of the systemic and local penetrant fungicides has been described. In the mid 1970s, Warren and coworkers (31,32) reported resistance in *S. homoeocarpa* to several benzimidazole fungicides within 10 years of their introduction into the U.S. market. Resistance in *S. homoeocarpa* to many dicarboximide and demethylation-inhibitor (DMI) fungicides also has been reported (5,9,11,21).

Because of concerns related to decreased field efficacy and the development of fungicide resistance within pathogen populations, many commercial manufacturers include recommendations for minimizing the development of resistance on fungicide labels. In many cases, these recommendations either limit the total amount of an active ingredient that may be applied during a season or reduce the frequency at which fungicide may be applied. In addition to label restrictions, regulatory agencies, based primarily on concerns related to environmental, human, and animal health, also have placed restrictions on turfgrass fungicide use. For example, the United States Environmental Protection Agency (EPA) recently prohibited the use of iprodione (27), vinclozolin (29), and chlorothalonil (28) on residential lawns and limited the maximum application rates of these fungicides on golf course turfgrass. The net result of these restrictions on fungicide use has left many turfgrass managers with a limited means for managing dollar spot.

The ability to accurately assess the fungicide sensitivity of *S. homoeocarpa* in field samples potentially could lead to more effective, economical, and environmentally sound strategies for managing dollar spot. The effective concentration or dose that produces 50% inhibition in growth (EC_{50} or ED_{50}) generally is used as an indication of fungicide sensitivity (4,17). EC_{50} values are calculated by growing fungal pathogens on media amended with serial concentrations of a given fungicide and identifying the concentration that inhibits mycelial growth or spore germination by 50%. This method has been used for determining fungicide sensitivities of numerous fungal pathogens, including *S. homoeocarpa* (5,9,11–13,21,25). This method is time-consuming and labor-intensive and not amenable for processing large numbers of samples. An alternative method was developed to predict fungicide sensitivities based on relative mycelial growth at predetermined discriminatory fungicide concentrations (16,18). This simplified procedure is less cumbersome and more manageable when processing large numbers of isolates, particularly for the purpose of surveys. This approach has been used to assess the diversity in fungicide sensitivities to DMI fungicides in populations of *Venturia inaequalis* in apple orchards (18,25) and *S. homoeocarpa* in golf course turfgrass (11,21).

The goals of this study were to (i) develop in vitro fungicide sensitivity assays using single discriminatory concentrations for thiophanate-methyl, propiconazole, and iprodione to predict fungicide efficacy in the field and (ii) use these assays to determine the prevalence and distribution of fungicide resistance in *S. homoeocarpa* isolated from golf courses in Ohio.

MATERIALS AND METHODS

Collection of *S. homoeocarpa* isolates. *S. homoeocarpa* was isolated from leaf tissue with symptoms of dollar spot. Diseased leaf tissue was surface disinfested for 1 min in a 3% sodium hypochlorite solution, rinsed twice in sterile water, and placed on acidified potato dextrose agar (APDA) prepared by adding 0.75 ml of 85% lactic acid (Fisher Scientific, Fair Lawn, NJ) per 1 liter of PDA (Difco Labo-

ratories, Detroit) after autoclaving. Culture plates were incubated at 25ºC and *S. homoeocarpa* was purified by making repeated hyphal tip transfers as required. Identification of *S. homoeocarpa* was based on cultural morphology and microscopic observation as originally described by Bennett (3). Millet grain inoculum of *S. homoeocarpa* was produced using a modified protocol described previously by Burpee and Goulty (6) and Miller et al. (21). Five mycelial plugs (6 mm in diameter) from the margin of actively growing *S. homoeocarpa* cultures were transferred to 250-ml Erlenmeyer flasks containing 40 g of sterilized millet seed. Prior to inoculation, the millet seed was soaked in 50 ml of sterile water for 12 h and then autoclaved twice for 30 min within 24 h. Inoculated flasks were incubated at 25ºC for 2 weeks. Colonized millet seed were air dried and kept at –20ºC for long-term storage. Viability of *S. homoeocarpa*–millet inoculum was verified by plating five colonized millet seed on PDA prior to use.

Determination of EC_{50} values. EC_{50} values were determined for 74 *S. homoeocarpa* isolates as described by Detweiler et al. (9) and Golembiewski et al. (11). In all, 36 isolates were collected from 28 golf courses in Ohio and 38 additional isolates were collected from research plots at The Ohio State University (OSU) Turfgrass Research and Education Facility in Columbus, where all fungicides effectively reduce dollar spot. These 38 isolates were used to determine baseline fungicide sensitivities, the importance of which previously was emphasized by Russell (24) and Smith et al. (25). Agar plugs (6 mm in diameter) containing actively growing mycelium were transferred to PDA and PDA amended with the following: thiophanate-methyl at 0.1, 1, 10, 500, and 1,000 µg a.i. ml^{-1} (Cleary's 3336 4F; Cleary Chemical Corporation, Dayton, NJ); propiconazole at 0.0001, 0.001, 0.01, and 0.1 µg a.i. ml^{-1} (Banner MAXX 1.3ME; Syngenta Crop Protection, Greensboro, NC); or iprodione at 0.1, 0.5, and 1.0 µg a.i. ml^{-1} (Chipco 26GT 2SC; Aventis Professional Products, Montvale, NJ). Radial mycelial growth was measured after 60 h of incubation at 25ºC. The percent relative mycelial growth was calculated as (radial growth on fungicide-amended PDA/radial growth on PDA) × 100. Each isolate was evaluated twice.

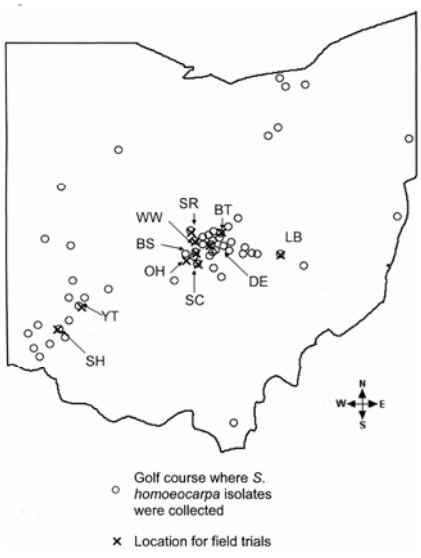

Fig. 1. Location of golf courses in Ohio from which *Sclerotinia homoeocarpa* was collected and where fungicide efficacy tests were conducted.

For propiconazole and iprodione, linear regression was used to determine the relationship between mycelial growth and fungicide concentration using PROC REG (SAS 9.1; SAS Institute, Cary, NC). Fungicide concentrations were log transformed. EC_{50} values were estimated from the linear regression models. For thiophanate-methyl, EC_{50} values were qualitatively estimated based on growth (>50% relative mycelial growth) or lack of growth on PDA amended with the various concentrations of this fungicide because fungicide sensitivities were not normally distributed.

Determination of discriminatory fungicide concentrations. Single discriminatory concentrations for thiophanate-methyl, propiconazole, and iprodione were determined using the 36 *S. homoeocarpa* isolates collected from golf courses in Ohio and a representative baseline sensitive isolate from the OSU research facility, EC_{50} values of which were determined previously.

For propiconazole and iprodione, regression analyses between EC_{50} values and relative mycelial growth of these isolates were performed at various concentrations of fungicides: propiconazole at 0.0001, 0.001, 0.01, and 0.1 µg a.i. ml^{-1} or iprodione at 0.1, 0.5, and 1.0 µg a.i. ml^{-1}. The fungicide concentration at which the regression model yielded the highest coefficient of determination (r) value was selected as the discriminatory concentration. The regression models then were used to predict an EC_{50} value for each *S. homoeocarpa* isolate solely based on relative mycelial growth on PDA amended with the discriminatory concentrations of these two fungicides (designated $EC_{50(D)}$).

For thiophanate-methyl, each isolate was grown on PDA amended with the fungicide at 0.1, 1, 10, 500, and 1,000 µg a.i. ml^{-1}. The discriminatory concentration for thiophanate-methyl was determined as the concentration at which *S. homoeocarpa* isolates clearly could be separated into two groups: those inhibited or those not inhibited by the presence of thiophanate-methyl. The mean relative mycelial growth of *S. homoeocarpa* on PDA amended with the discriminatory concentration was compared between these two groups using a one-way analysis of variance (ANOVA; PROC GLM; SAS). $EC_{50(D)}$ values for each *S. homoeocarpa* isolate were estimated based on the relationship between EC_{50} values and mycelial growth on PDA amended with the discriminatory concentration. Simple linear regression analysis was used to determine the relationship between EC_{50} and $EC_{50(D)}$ values for propiconazole and iprodione.

Relationship of in vitro fungicide sensitivity assay results with fungicide efficacy in the field. To determine the relationship between $EC_{50(D)}$ values and fungicide efficacy in the field, fungicide trials were conducted at various locations throughout southwest and central Ohio in 2002 and 2003 (Fig. 1). Locations were selected based on $EC_{50(D)}$ values of *S. homoeocarpa* isolates recovered. In 2002, field plots (4 m^2) were established on mixed *A. stolonifera* and *P. annua* golf course fairways at locations BS and WW (Table 1), and on a sward of *A. stolonifera* and *P. annua* maintained at 1.3 cm at the OSU research facility (designated location OH). In 2003, fungicide efficacy trials were established on mixed *A. stolonifera* and *P. annua* fairways at nine golf courses (BS, BT, DE, LB, SC, SH, SR, WW, and YT; Table 1; Fig.1), and at location OH. Plots were established on different locations within the same fairways for golf courses BS and WW and within the same sward for location OH in 2002 and 2003. All fungicides were applied with a CO_2 pressure sprayer using TeeJet 6503 nozzles at 275.8 kPa and in a spray volume of 81.5 ml m^{-2}. In 2002, plots were treated with thiophanate-methyl (2.5 kg a.i. ha^{-1} applied every 21 days), iprodione (2.8 kg a.i. ha^{-1} applied every 14 days), or propiconazole (0.4 or 0.8 kg a.i ha^{-1} applied every 21 days). In 2003, the same fungicide treatments were used except propiconazole was applied at only 0.4 kg a.i. ha^{-1}.

Dollar spot severity was assessed visually between June and July. The percent area with dollar spot was determined by counting the number of dollar spot infection centers (DSICs) within the center 1 m^2 of each plot and dividing it by 20 (1 m^2 = 10,000 cm^2; 1 DSIC = approximately 5 cm^2; 20 DSICs = 1% disease severity). In plots with ≥200 DSICs (≥10% disease severity), the percent diseased turfgrass was estimated visually. Disease severity on fungicide-treated plots at each location was normalized as (percent disease on the fungicide-treated plot)/(percent disease on the nontreated control plot). Randomized complete block designs (n = 4) were used for all field trials. Fungicide efficacy was analyzed with a one-way ANOVA. Differences among treatment means were determined using Fisher's protected least significance difference (LSD) at P = 0.05.

S. homoeocarpa was isolated from 10 DSICs within the four nontreated control plots at each field location in August 2003 and their in vitro fungicide sensitivities ($EC_{50(D)}$) were determined. The intraloca-

tion variability of fungicide sensitivities among the 10 isolates recovered from each location was assessed. Mean $EC_{50(D)}$ values of nine golf courses for propiconazole and iprodione were compared with those of location OH (in vitro baseline sensitivity) with a one-way ANOVA. The relationship between $EC_{50(D)}$ values of the 10 isolates recovered from each location and normalized dollar spot severity was determined.

Screening of *S. homoeocarpa* isolates collected from golf courses in Ohio. In all, 192 *S. homoeocarpa* isolates were recovered from symptomatic leaf tissue collected from 55 of Ohio's 768 golf courses (The National Golf Foundation, Jupiter, FL; *personal communication*) between 1999 and 2004 (Table 1; Fig.1). Although most of the diseased turfgrass samples used to make isolations were collected in the field, some of the samples were received through The OSU's plant and pest diagnostic clinic. Most of the samples collected were from golf course fairways whose age, sward composition, and history of fungicide use were not characterized. Sensitivities ($EC_{50(D)}$) of each isolate for thiophanate-methyl, propiconazole, and iprodione were estimated twice using the in vitro assays developed in this study.

RESULTS

Determination of EC_{50} values and discriminatory fungicide concentrations. In vitro baseline sensitivities (EC_{50}) determined using the 38 *S. homoeocarpa* isolates collected from the OSU research facility for thiophanate-methyl, propiconazole, and

Table 1. *Sclerotinia homoeocarpa* isolates used in this study

No. of isolates collected	Host	Abbreviation	Location
38[a]	*Agrostis stolonifera* L. or *Poa pratensis* L.	OH	Ohio State University Research & Education Facility, Columbus, OH
1	*P. pratensis*	…	Arrowhead Lakes Golf Club, Galena, OH
1	Unknown	…	Ashland University, Ashland, OH
12	*A. stolonifera* or *Poa annua* L.	BT	Bent Tree Golf Club, Sunbury, OH
1	*A. stolonifera*	…	Big Walnut Golf Club, Sunbury, OH
1	*A. stolonifera*	…	Blackhawk Golf Club, Galena, OH
1	*A. stolonifera*	…	Blue Ash Golf Course, Blue Ash, OH
15	*A. stolonifera* or *P. annua*	BS	Brookside Golf & Country Club, Columbus, OH
1	*A. stolonifera* or *P. annua*	…	Columbus Country Club, Columbus, OH
2	*A. stolonifera* or *P. annua*	…	Deer Track Golf Club, Elyria, OH
11	*A. stolonifera*	DE	Double Eagle Club, Galena, OH
1	*A. stolonifera* or *P. annua*	…	East Palestine Country Club, Negley, OH
1	*A. stolonifera* or *P. annua*	…	Forrest Hills Golf Course, Elyria, OH
1	*A. stolonifera*	…	Granville Country Club, Greenville, OH
2	*A. stolonifera* or *P. annua*	…	Hamilton Elks, Hamilton, OH
17	*A. stolonifera* or *P. annua*	…	Hickory Hills Golf Club, Grove City, OH
2	*A. stolonifera* or *P. annua*	…	Hyde Park Golf & Country Club, Cincinnati, OH
2	*A. stolonifera*	…	Legends at Locust Lane, Alexandria, OH
12	*A. stolonifera* or *P. annua*	LB	Longaberger Golf Club, Newark, OH
1	Unknown	…	Miami University, Miami, OH
2	*A. stolonifera* or *P. annua*	…	Miami Valley Golf Club, Dayton, OH
1	*A. stolonifera* or *P. annua*	…	Mohican Hills Golf Club, Jeromesville, OH
1	*A. stolonifera* or *P. annua*	…	Moraine Country Club, Kettering, OH
2	*A. stolonifera*	…	New Albany Links, New Albany, OH
1	*A. stolonifera* or *P. annua*	…	Oberlin Golf Club, Oberlin, OH
2	*A. stolonifera* or *P. annua*	…	Pepper Pike Club, Pepper Pike, OH,
4	*A. stolonifera* or *P. annua*	…	Pipe Stone Golf Club, Miamisburg, OH
11	*A. stolonifera*	…	Piqua Country Club, Piqua, OH
1	*A. stolonifera* or *P. annua*	…	Prairie View Golf Course, Waynesfield, OH
1	*A. stolonifera*	…	Raccoon International Golf Club, Granville, OH
1	*A. stolonifera*	…	Rattlesnake Ridge Golf Club, Sunbury, OH
1	*A. stolonifera* or *P. annua*	…	Red Hawk Run Golf Club, Findlay, OH
1	*A. stolonifera* or *P. annua*	…	Royal American Links, Galena, OH
12	*A. stolonifera*	SC	Scioto Country Club, Columbus, OH
6	*A. stolonifera* or *P. annua*	SR	Scioto Reserve Golf & Athletic Club, Powell, OH
1	*A. stolonifera* or *P. annua*	SH	Shaker Run Golf Club, Lebanon, OH
2	*A. stolonifera* or *P. annua*	…	Steubenville Country Club, Steubenville, OH
3	*A. stolonifera* or *P. annua*	…	Stillwater Valley Golf Club, Bradford, OH
1	*A. stolonifera*	…	Sugar Valley Country Club, Bellbrook, OH
1	*A. stolonifera* or *P. annua*	…	Sunbury Golf Course, Sunbury, OH
1	*A. stolonifera*	…	Table Rock Golf Club, Centerburg, OH
4	*A. stolonifera* or *P. annua*	…	The Golf Center at Kings Island, Mason, OH
1	*A. stolonifera*	…	The Golf Club, New Albany, OH
1	*A. stolonifera*	…	The Lakes Golf & Country Club, Galena, OH
1	*A. stolonifera* or *P. annua*	…	The Medallion Club, Westerville, OH
1	*A. stolonifera*	…	The Ohio State University Golf Course, Columbus, OH
4	*A. stolonifera* or *P. annua*	…	TPC Rivers Bend, Maineville, OH
14	*A. stolonifera*	WW	Wedgewood Golf & Country Club, Powell, OH
5	*P. pratensis*	…	Westchester Golf Club, Canal Winchester, OH
2	*A. stolonifera* or *P. annua*	…	Wetherington Golf & Country Club, West Chester, OH
1	*A. stolonifera*	…	Willow Run Golf Club, Westerville, OH
1	*A. stolonifera*	…	Winding Hollow Country Club, New Albany, OH
4	*A. stolonifera* or *P. annua*	…	Worthington Hills Country Club, Columbus, OH
1	*A. stolonifera*	…	Wyandot Golf Course, Centerburg, OH
9	*A. stolonifera*	YT	Yankee Trace Golf Club, Centerville, OH
2	*A. stolonifera* or *P. annua*	…	Zanesville Country Club, Zansville, OH

[a] *S. homoeocarpa* isolates used to determine baseline sensitivities for thiophanate-methyl, propiconazole, and iprodione based on previous field efficacy that all fungicides effectively reduced dollar spot.

iprodione were <1, 0.003 ± 0.002, and 0.15 ± 0.08 μg a.i. ml^{-1}, respectively.

For propiconazole and iprodione, the discriminatory concentrations were determined to be 0.1 and 1.0 μg a.i. ml^{-1}, respectively (Fig. 2), based upon the results from simple linear regression analysis of mean $EC_{50(D)}$ values and relative mycelial growth. For thiophanate-methyl, the discriminatory concentration was determined as 1,000 μg a.i. ml^{-1}. Based on relative mycelial growth on PDA amended with this discriminatory concentration, *S. homoeocarpa* isolates were separated into two significantly different groups ($P < 0.0001$): lack of growth ($EC_{50} < 1$ μg a.i. ml^{-1}) or growth ($EC_{50} > 1{,}000$ μg a.i. ml^{-1}).

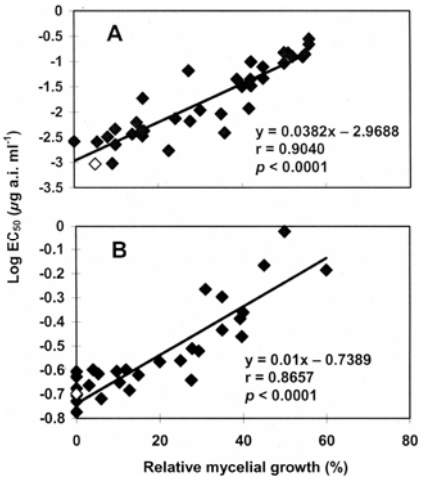

Fig. 2. Relationship between EC_{50} values and relative mycelial growth of *Sclerotinia homoeocarpa* on potato dextrose agar amended with single discriminatory concentrations of **A,** propiconazole (0.1 μg a.i. ml^{-1}) and **B,** iprodione (1 μg a.i. ml^{-1}). ◆ = Total of 36 isolates collected from 28 golf courses in Ohio and ◇ = a representative fungicide sensitive isolate collected from the Ohio State University research facility.

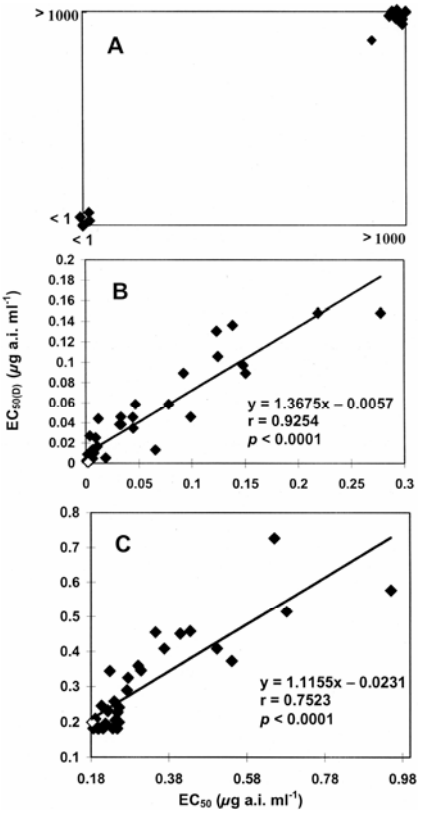

Fig. 3. Relationship between EC_{50} and $EC_{50(D)}$ values for **A,** thiophanate-methyl, **B,** propiconazole, or **C,** iprodione. ◆ = Total of 36 *Sclerotinia homoeocarpa* isolates collected from 28 golf courses in Ohio and ◇ = a representative fungicide sensitive isolate collected from the Ohio State University research plots.

$EC_{50(D)}$ values based on relative mycelial growth of *S. homoeocarpa* on PDA amended with the single discriminatory concentration of thiophanate-methyl, propiconazole, or iprodione were associated with EC_{50} values determined by conventional procedures using three or more concentrations of each fungicide (Fig. 3). There was a high correlation between $EC_{50(D)}$ and EC_{50} values for propiconazole ($r = 0.9254$, $P < 0.0001$) and iprodione ($r = 0.7523$, $P < 0.0001$). $EC_{50(D)}$ values were identical to EC_{50} values for thiophanate-methyl.

Relationship between $EC_{50(D)}$ values and fungicide efficacy in the field. In 2002, thiophanate-methyl significantly reduced dollar spot severity at location OH but not at location BS compared with nontreated controls (Table 2). Thiophanate-methyl also significantly reduced dollar spot at location WW; however, disease severity in these plots was significantly higher than those treated with propiconazole or iprodione. In 2003, thiophanate-methyl significantly reduced dollar spot at locations OH, DE, and SR but not at the other seven locations (WW, BT, SC, YT, BS, SH, and LB). Propiconazole and iprodione significantly reduced dollar spot at all locations in both 2002 and 2003 (Tables 2 and 3).

In vitro sensitivities of 10 *S. homoeocarpa* isolates recovered from each location in 2003 revealed intralocation variability for propiconazole and iprodione but not for thiophanate-methyl (Table 4). The 10 isolates from locations OH and SR showed a limited range in the in vitro sensitivities for propiconazole ($0.001 \leq EC_{50(D)} \leq 0.005$ μg a.i. ml^{-1}; standard deviation of $EC_{50(D)} \leq 0.001$ μg a.i. ml^{-1}), and in vitro sensitivities were not significantly different between OH and SR ($P = 0.97$). In contrast, *S. homoeocarpa* isolates recovered from the other eight locations (WW, BT, SC, YT, BS, SH, DE, and LB) showed a relatively high degree of the intralocation variability in propiconazole sensitivities ($0.001 \leq EC_{50(D)} \leq 0.390$ μg a.i. ml^{-1}; 0.007 ≤ standard deviation of $EC_{50(D)} \leq 0.121$ μg a.i. ml^{-1}). In vitro sensitivities at six (WW, BT, YT, BS, SH, and LB) of the eight locations were significantly reduced ($P < 0.02$) compared with location OH; however, those at the other two locations (SC and DE), were not ($P > 0.08$). Intralocation variability in in vitro sensitivities for iprodione was present, albeit not as diverse as that observed for propiconazole. Iprodione sensitivities at four locations (BT, BS, SH, and LB) were significantly reduced ($P < 0.008$) but those at the other five locations (WW, SR, SC, DE, and YT) were not ($P > 0.10$) compared with location OH. There was no intralocation variability in the in vitro sensitivities for thiophanate-methyl at any location. The 10 *S. homoeocarpa* isolates recovered from locations OH, DE, and SR did not grow on PDA amended with the discriminatory concentration of thiophanate-methyl (1,000 μg a.i. ml^{-1}). In contrast, all 10 isolates collected from the remaining seven locations (WW, BT, SC, YT, BS, SH, and LB) had mycelial growth in excess of 50% on PDA amended with thiophanate-methyl at 1,000 μg a.i. ml^{-1}.

Dollar spot severity was significantly lower in field plots treated with thiophanate-methyl than in nontreated control plots when sensitivities determined in the in vitro assays were high (thiophanate-methyl $EC_{50(D)} < 1$ μg a.i. ml^{-1}). In contrast, dollar spot severity was not reduced or significantly higher in locations where *S. homoeocarpa* isolates were recovered with low sensitivities for thiophanate-methyl ($EC_{50(D)} > 1{,}000$ μg a.i. ml^{-1}). For example,

Table 2. Efficacy of thiophanate-methyl, propiconazole, and iprodione on dollar spot severity at three locations in 2002

Treatment	Rate (kg ha^{-1})	Interval (days)	Dollar spot severity (%)[a] OH July 10	BS July 22	WW July 23
Thiophanate-methyl	2.5	21	0.63	4.13	2.75
Propiconazole	0.4	21	0.75	0.63	0.25
Propiconazole	0.8	21	1.50	0.00	0.19
Iprodione	2.8	14	0.50	0.00	0.13
Nontreated control	…	…	47.50	4.75	5.00
Treatment *F* value	…	…	52.53	4.37	11.21
P value	…	…	<0.0001	0.0153	0.0002
LSD ($P = 0.05$)[b]	…	…	8.67	2.89	1.95

[a] Severity was determined by counting the number of dollar spot infection centers (DSICs) within the center 1 m^2 of each plot and dividing it by 20. In plots with ≥200 DSICs (≥10% disease severity), the percent disease was assessed visually.

[b] LSD = least significant difference.

dollar spot was significantly reduced in plots treated with thiophanate-methyl at location OH where *S. homoeocarpa* had an $EC_{50(D)}$ value < 1 μg a.i. ml^{-1}. In contrast, thiophanate-methyl had no impact on dollar spot severity compared with the nontreated controls at locations BS and WW, in which *S. homoeocarpa* with high $EC_{50(D)}$ values (>1,000 μg a.i. ml^{-1}) was found.

Normalized dollar spot severity on fungicide-treated plots was associated with mean $EC_{50(D)}$ values of 10 *S. homoeocarpa* isolates recovered from each location for thiophanate-methyl and propiconazole but not for iprodione (Fig. 4). Normalized disease severity on propiconazole-treated plots increased as mean $EC_{50(D)}$ values for propiconazole increased (r = 0.8819, P = 0.007). In the case of thiophanate-methyl, normalized disease severity was close to zero when $EC_{50(D)}$ < 1 μg a.i. ml^{-1} but normalized disease severity was >1 when $EC_{50(D)}$ > 1,000 μg a.i. ml^{-1} (Fig. 4A).

Prevalence and distribution of fungicide insensitive *S. homoeocarpa* isolates from golf courses in Ohio. Screening 192 *S. homoeocarpa* isolates from 55 golf courses throughout Ohio revealed that reduced in vitro fungicide sensitivities existed (Table 5). Mean $EC_{50(D)}$ values of isolates from 55 golf courses sampled were 0.034 to 0.065 μg a.i. ml^{-1} for propiconazole and 0.24 to 0.32 μg a.i. ml^{-1} for iprodione. For thiophanate-methyl, 62% of golf courses sampled had *S. homoeocarpa* with highly reduced sensitivities ($EC_{50(D)}$ > 1,000 μg a.i. ml^{-1}).

In all, 185 *S. homoeocarpa* isolates collected from 55 golf courses had a reduced in vitro sensitivity for propiconazole ($EC_{50(D)} \geq 0.002$ μg a.i. ml^{-1}). For 53 isolates from 18 golf courses, in vitro sensitivity for propiconazole was reduced 100- to 200-fold ($EC_{50(D)}$ > 0.171 μg a.i. ml^{-1}) compared with baseline (sensitive) isolates. Isolates from 35 golf courses had a reduced in vitro sensitivity for iprodione ($EC_{50(D)} \geq 0.19$ μg a.i. ml^{-1}). Only one isolate had an iprodione $EC_{50(D)}$ > 1.00 μg a.i. ml^{-1}, which was more than fivefold greater than the mean $EC_{50(D)}$ value of the sensitive isolates.

Reduced in vitro sensitivities to more than one fungicide also existed. Twenty-two *S. homoeocarpa* isolates from 16 golf courses showed reduced sensitivities to both thiophanate-methyl and propiconazole. Thirty-three isolates of *S. homoeocarpa* from 15 golf courses showed reduced sensitivities to both propiconazole and iprodione. One isolate showed reduced sensitivity to both thiophanate-methyl and iprodione. In all, 117 isolates from 25 golf courses showed reduced in vitro sensitivities to all three fungicides.

DISCUSSION

The use of EC_{50} values determined by relative mycelial growth on PDA amended with a single discriminatory concentration of a given fungicide ($EC_{50(D)}$) was an effective means for making predictions about efficacy of thiophanate-methyl in the field and may provide insights about the potential development of resistance to propiconazole and iprodione in the future. It was a useful tool for screening a large number of *S. homoeocarpa* isolates collected from golf courses throughout Ohio and making predictions about prevalence and distribution of fungicide resistance.

$EC_{50(D)}$ values determined based on growth of *S. homoeocarpa* on PDA amended with a single discriminatory concentration (thiophanate-methyl at 1,000 μg a.i. ml^{-1}) were a good predictor of thiophanate-methyl efficacy in the field. Field efficacy trials revealed that thiophanate-methyl effectively reduced dollar spot at locations from which *S. homoeocarpa* isolates with low $EC_{50(D)}$ values were recovered. In contrast, thiophanate-methyl was ineffective in managing dollar spot at locations where *S. homoeocarpa* isolates with high $EC_{50(D)}$ values were found. Therefore, *S. homoeocarpa* isolates showing a high $EC_{50(D)}$ value (>1,000 μg a.i. ml^{-1}) are resistant but isolates showing a low $EC_{50(D)}$ value (<1 μg a.i. ml^{-1}) are sensitive to thiophanate-methyl in the field.

Consistency between in vitro sensitivities and field efficacy for thiophanate-methyl may be explained by population dynamics of *S. homoeocarpa* and the mode of action of this fungicide. The mode of action for benzimidazole fungicides is to restrict cell division by inhibiting microtubule assembly (14,22,26). Once fungicide resistance develops, it causes complete resistance to all benzimidazole fungicides without compromising the fitness for survival of fungi (15). Resistant isolates are persistent even after benzimidazole fungicides are not used (17). Because resistance of *S. homoeocarpa* to benzimidazole fungicides was first reported in the mid-1970s (31,32), the development of benzimidazole-resistant *S.*

Table 4. In vitro fungicide sensitivities ($EC_{50(D)}$) of 10 *Sclerotinia homoeocarpa* isolates collected from each field location in August 2003[a]

		Propiconazole		Iprodione	
Location	Thiophanate-methyl[b]	Mean	Range	Mean	Range
OH	<1	0.001	0.001–0.002	0.18	0.18–0.23
BS	>1,000	0.082	0.025–0.222	0.55	0.29–0.81
WW	>1,000	0.136	0.025–0.390	0.19	0.18–0.23
DE	<1	0.064	0.002–0.210	0.24	0.23–0.27
BT	>1,000	0.134	0.073–0.206	0.31	0.20–0.39
SC	>1,000	0.012	0.001–0.023	0.26	0.22–0.33
SR	<1	0.003	0.001–0.005	0.24	0.20–0.28
YT	>1,000	0.133	0.004–0.380	0.23	0.18–0.39
SH	>1,000	0.171	0.063–0.327	0.60	0.40–0.93
LB	>1,000	0.140	0.045–0.331	0.56	0.26–0.91

[a] Discriminatory effective concentration that produces 50% inhibition ($EC_{50(D)}$) values (μg a.i. ml^{-1}) were determined by relative mycelial growth of *S. homoeocarpa* on potato dextrose agar (PDA) amended with single discriminatory concentrations of thiophanate-methyl, propiconazole, and iprodione.

[b] Mean and range were not determined because all 10 isolates grew >50% ($EC_{50(D)}$ > 1,000 μg a.i. ml^{-1}) or did not grow ($EC_{50(D)}$ < 1 μg a.i. ml^{-1}) on PDA amended with the discriminatory concentration of thiophanate-methyl (1,000 μg a.i. ml^{-1}).

Table 3. Efficacy of thiophanate-methyl, propiconazole, and iprodione on dollar spot severity at 10 locations in 2003

			Dollar spot severity (%)[a]									
Treatment	Rate (kg ha^{-1})	Interval (days)	OH June 17	BS June 16	WW June 16	DE June 9	BT June 19	SC June 17	SR June 16	YT June 12	SH June 12	LB July 9
Thiophanate-methyl	2.5	21	0.06	16.50	0.58	0.00	13.50	2.01	0.00	0.25	5.00	2.48
Propiconazole	0.4	21	0.15	1.53	0.05	0.00	1.23	0.01	0.00	0.01	0.76	0.39
Iprodione	2.8	14	0.00	0.10	0.08	0.00	1.38	0.01	0.00	0.00	0.01	0.10
Nontreated control	…	…	14.34	14.00	0.48	0.27	7.14	1.89	0.83	0.16	4.25	1.94
Treatment F value	…	…	3.78	6.33	5.10	41.00	16.49	13.39	10.93	3.72	5.60	16.59
P value	…	…	0.0406	0.0081	0.0167	<0.0001	0.0001	0.0004	0.0010	0.0422	0.0123	0.0001
LSD (P = 0.05)[b]	…	…	11.31	10.31	0.37	0.07	4.42	0.95	0.38	0.19	3.23	0.80

[a] Severity was determined by counting the number of dollar spot infection centers (DSICs) within the center 1 m^2 of each plot and dividing it by 20. In plots with ≥200 DSICs (≥10% disease severity), the percent disease was visually assessed.

[b] LSD = least significant difference.

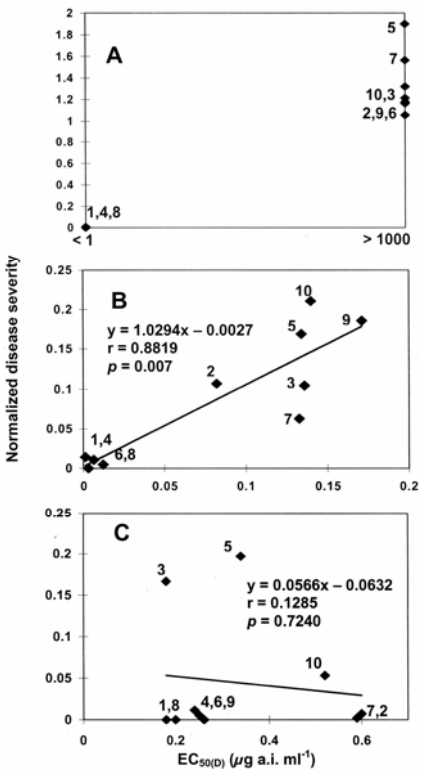

Fig. 4. Relationship between mean $EC_{50(D)}$ values and normalized dollar spot severity on plots treated by **A,** thiophanate-methyl, **B,** propiconazole, or **C,** iprodione at 10 locations. Dollar spot severity at each location was normalized as (percent disease on the fungicide-treated plot)/(percent disease on the nontreated control plot). Numbers denote 10 field locations: 1 = OH, 2 = BS, 3 = WW, 4 = DE, 5 = BT, 6 = SC, 7 = SR, 8 = YT, 9 = SH, and 10 = LB.

homoeocarpa has been known to be fast and widespread due to easy mutation and long persistence (30). In this study, no intralocation variability on sensitivities for thiophanate-methyl was observed and high $EC_{50(D)}$ values (>1,000 µg a.i. ml^{-1}) accurately corresponded to failure of this fungicide in the field.

The results of the in vitro assays with propiconazole and iprodione did not completely match fungicide efficacy in the field trials. Mean $EC_{50(D)}$ values used in this field study ranged from 0.001 to 0.171 µg a.i. ml^{-1} for propiconazole and from 0.18 to 0.60 µg a.i. ml^{-1} for iprodione. It was impossible to clearly delineate exact cutoff $EC_{50(D)}$ values for effective versus ineffective responses of these fungicides in the field. Propiconazole and iprodione significantly reduced dollar spot severity compared with the nontreated control at all locations in both years of this study, even though reduced in vitro sensitivities were observed.

Resistance to DMI fungicides, including propiconazole, is known to develop through the accumulation of mutations at multiple genes (17). Development of resistance to DMIs is gradual and directional, so that complete resistance is more difficult to acquire compared with benzimidazole fungicides. A failure of propiconazole was not observed in this study, although in vitro sensitivities of *S. homoeocarpa* tested were reduced >100-fold compared with the baseline sensitivity. However, the regression analysis between normalized dollar spot severity on propiconazole-treated plots and mean $EC_{50(D)}$ values for propiconazole revealed that fungicide efficacy tended to decrease in the field as $EC_{50(D)}$ values increased (Fig. 4B).

The positive correlation between dollar spot severity and mean $EC_{50(D)}$ values for propiconazole indicated the potential development of resistance reaching the level of inefficacy of propiconazole, as shown in previous studies (5,11,21). Golembiewski et al. (11) first validated that dollar spot on field plots inoculated with DMI-resistant *S. homoeocarpa* isolates, whose mean EC_{50} values were more than 50-fold greater than those of sensitive isolates, was not reduced by DMI fungicides in 2 of 3 years. The field and greenhouse studies by Burpee (5) and Miller et al. (21) revealed that disease caused by a DMI-resistant isolate, whose mean EC_{50} value was 5- to 10-fold greater than that of a baseline sensitive isolate, was not effectively controlled with applications of DMI fungicides.

Resistance to dicarboximide fungicides, including iprodione, may develop easily based the relative ease with which mutants have been generated under laboratory conditions (1). However, this mutation is likely to be associated with alteration of the osmotic regulation and, thus, compromise fitness in the field (1). Therefore, dicarboximide-resistant isolates are less persistent because they are less competitive than wild-type isolates in nature when the use of dicarboximides is discontinued (9,30). Resistance to dicarboximide fungicides has been observed in several plant pathogens but occurred sporadically without causing practical problems (2,19,23). Field resistance to iprodione in *S. homoeocarpa* first was observed in 1983 on a creeping bentgrass putting green in Michi-

Table 5. In vitro fungicide sensitivities of *Sclerotinia. homoeocarpa* isolates collected from golf courses in Ohio between 1999 and 2004

			$EC_{50(D)}$ (µg a.i. ml^{-1})[a]							
	Number of		Thiophanate-methyl		Propiconazole			Iprodione		
County	Golf courses	Isolates	<1[b]	>1,000	<0.002[b]	0.002–0.171	<0.400	<0.19[b]	0.19–1.00	>1.00
Ashland	2	2	1	1	1	1	…	2 (2)	…	…
Auglaize	1	1	…	1	…	…	1	…	1	…
Butler	2	3	…	3 (2)	…	2	1	1	2 (2)	…
Columbiana	1	1	…	1	…	1	…	1	…	…
Cuyahoga	1	2	1	1	1	1	…	1	1	…
Darke	1	3	…	3	…	3	…	…	3	…
Delaware	11	45	16 (6)	29 (5)	1	25 (9)	19 (5)	7 (7)	38 (5)	…
Franklin	11	65	9 (4)	56 (8)	1	54 (9)	10 (5)	18 (9)	47 (8)	…
Greene	1	1	1	…	1		…	1	…	…
Hamilton	3	5	2	3 (2)	1	3 (3)	1	2	2 (2)	1
Hancock	1	1	1	…	…	1	…	1	…	…
Jefferson	1	2	…	2	…	2	…	…	2	…
Knox	2	2	2 (2)	…	1	1	…	1	1	…
Lawrence	1	1	1	…	…	1	…	…	1	…
Licking	4	5	3 (3)	2 (2)	…	5 (4)	…	1	4 (3)	…
Lorain	2	3	2	1	…	3 (2)	…	2	1	…
Miami	1	11	…	11	…	5	6	…	11	…
Montgomery	4	16	5 (2)	11 (2)	…	10 (4)	6	9 (3)	7 (3)	…
Muskingum	2	14	1	13 (2)	…	7 (2)	7	2	12	…
Warren	3	9	5 (2)	4 (3)	…	7 (3)	2 (2)	4 (3)	5 (2)	…
Total	55	192	50 (27)	142 (34)	7 (7)	132 (46)	53 (18)	53 (34)	138 (34)	1

[a] Discriminatory effective concentration that produces 50% inhibition ($EC^{50(D)}$) values were determined by relative mycelial growth of *S. homoeocarpa* on potato dextrose agar amended with single discriminatory concentrations of thiophanate-methyl (1,000 µg a.i. ml^{-1}), propiconazole (0.1 µg a.i. ml^{-1}), and iprodione (µg a.i. ml^{-1}). Numbers listed indicated the number of isolates (the number of golf courses).

[b] Baseline sensitivities.

gan (9). Since then, few cases of dicarboximide field resistance in *S. homoeocarpa* have been documented. Therefore, it was expected that *S. homoeocarpa* isolates with reduced iprodione sensitivities ($0.31 \leq EC_{50(D)} \leq 0.61$ μg a.i. ml^{-1}) detected in the current study would be effectively controlled by iprodione.

Another possible explanation for significant disease reduction in the field by propiconazole and iprodione regardless of the in vitro fungicide sensitivities is that the labeled rates of the fungicides applied in the field may be higher than $EC_{50(D)}$ values of *S. homoeocarpa* as discussed previously (10,21). Concentrations of propiconazole and iprodione used in the field are equivalent to 490 μg a.i. ml^{-1} (converted from 0.4 kg a.i. ha^{-1} in a spray volume of 81.5 ml m^{-2}) and 3,436 μg a.i. ml^{-1} (converted from 2.8 kg a.i. ha^{-1} in a spray volume of 81.5 ml m^{-2}). These concentrations are much greater (>3,000 times) than the range of $EC_{50(D)}$ values for propiconazole and iprodione, even considering differing conditions between field and in vitro experiments. More work is needed to define the relationship between fungicide concentrations used in these in vitro assays and those used in the field.

The development of resistance in *S. homoeocarpa* to current systemic and locally penetrant fungicides is thought to be prevalent in the United States. (30). Distribution of fungicide sensitivities in *S. homoeocarpa* from golf courses throughout Ohio first was investigated in a large scale using the in vitro assays developed in this study. In vitro sensitivity assays revealed that thiophanate-methyl resistance is widespread and found in 34 of 55 golf courses sampled throughout Ohio. Reduced in vitro sensitivities to propiconazole and iprodione also are widespread and found in 55 and 35 golf courses in Ohio, respectively. Mean $EC_{50(D)}$ values of isolates from 55 golf courses were 50-fold greater for propiconazole and 1.5-fold greater for iprodione compared with baseline (sensitive) isolates.

This study could not determine fungicide efficacy in the field for *S. homoeocarpa* isolates with mean propiconazole $EC_{50(D)}$ values higher than 0.171 μg a.i. ml^{-1} and mean iprodione $EC_{50(D)}$ higher than 1.0 μg a.i. ml^{-1}. For propiconazole, the positive correlation of mean $EC_{50(D)}$ values with dollar spot severity on propiconazole-treated plots suggests that *S. homoeocarpa* isolates with mean $EC_{50(D)} > 0.171$ μg a.i. ml^{-1} collected from 18 golf courses may lead to decreased fungicide efficacy in the field. Further field studies using *S. homoeocarpa* with greater reduced sensitivity to propiconazole will complement field validation of this in vitro assay. For iprodione, efficacy trials in the field indicated that isolates of *S. homoeocarpa* with $EC_{50(D)}$ values ranging from 0.19 to 1.00 μg a.i. ml^{-1} were still sensitive. Periodic monitoring using the field-validated in vitro assays developed in this study will enable us to detect the alteration of fungicide sensitivities of *S. homoeocarpa* populations in a given site. This information can be used for developing effective programs that reduce the risk of fungicide resistance and, ultimately, extend the viability of these fungicides for managing dollar spot.

ACKNOWLEDGMENTS

Support for this project was provided by the Ohio Turfgrass Foundation, and state and federal funds appropriated to the Ohio Agricultural Research and Development Center. We thank the superintendents and staff of Brookside Golf & Country Club, Double Eagle Club, Bent Tree Golf Club, Hickory Hills Golf Club, Longaberger Golf Club, Scioto Country Club, Scioto Reserve Golf & Athletic Club, Wedgewood Golf & Country Club, and Yankee Trace Golf Club for their support in the field trials.

LITERATURE CITED

1. Beever, R. E. 1983. Osmotic sensitivity of fungal variants resistant to dicarboximide fungicides. Trans. Br. Mycol. Soc. 80:327-332.
2. Beever, R. E., and Brien, H. M. R. 1983. A survey of resistance to the dicarboximide fungicides in *Botrytis cinerea*. N. Z. J. Agric. Res. 26:391-400.
3. Bennett, F. T. 1937. Dollar spot disease of turf and its causal organism *Sclerotinia homoeocarpa* n. sp. Ann. Appl. Biol. 24:236-257.
4. Brent, K. J. 1988. Monitoring for fungicide resistance. Pages 9-11 in: Fungicide Resistance in North America. C. J. Delp, ed. The American Phytopathological Society, St. Paul, MN.
5. Burpee, L. L. 1997. Control of dollar spot of creeping bentgrass caused by an isolate of *Sclerotinia homoeocarpa* resistant to benzimidazole and demethylation-inhibitor fungicides. Plant Dis. 81:1259-1263.
6. Burpee, L. L., and Goulty, L. G. 1984. Suppression of brown patch disease of creeping bentgrass by isolates of nonpathogenic *Rhizoctonia* spp. Phytopathology 74:692-694.
7. Cole, H., Taylor, B., and Duich, J. 1968. Evidence of differing tolerances to fungicides among isolates of *Sclerotinia homoeocarpa.* Phytopathology 58:683-686.
8. Couch, H. B. 1995. Diseases of Turfgrasses, 3rd ed. Krieger Publishing Co., Malabar, FL.
9. Detweiler, A. R., Vargas, J. M., Jr., and Danneberger, T. K. 1983. Resistance of *Sclerotinia homoeocarpa* to iprodione and benomyl. Plant Dis. 67:627-630.
10. Franke, M. D., Brenneman, T. B., and Stevenson, K. L. 1998. Stem rot of peanut: relationship between in vitro fungicide sensitivity and field efficacy of fungicides. Peanut Sci. 25:76-80.
11. Golembiewski, R. C., Vargas, J. M., Jr., Jones, A. L., and Detweiler, A. R. 1995. Detection of demethylation inhibitor (DMI) resistance in *Sclerotinia homoeocarpa* populations. Plant Dis. 79:491-493.
12. Hsiang, T., Yang, L., and Barton, W. 1997. Baseline sensitivity and cross-resistance to demethylation-inhibiting fungicides in Ontario isolates of *Sclerotinia homoeocarpa*. Eur. J. Plant Pathol. 103:409-416.
13. Hsiang, T., Yang, L., and Barton, W. 1998. Relative virulence of isolates of *Sclerotinia homoeocarpa* with varying sensitivity to propiconazole. Eur. J. Plant Pathol. 104:163-169.
14. Jung, M. K., and Oakley, B. R. 1990. Identification of an amino acid substitution in the *ben*A, beta-tubulin gene of *Aspergillus nidulans* that confers thiabendazole resistance and benomyl supersensitivity. Cell Motil. Cytoskelet. 17:87-94.
15. Koenraadt, H., Somerville, S. C., and Jones, A. L. 1992. Characterization of mutations in the beta-tubulin gene of benomyl-resistant field strains of *Venturia inaequalis* and other plant pathogenic fungi. Phytopathology 82:1348-1354.
16. Köller, W., Parker, D. M., and Reynolds, K. L. 1991. Baseline sensitivities of *Venturia inaequalis* to sterol demethylation inhibitors. Plant Dis. 75:726-728.
17. Köller, W., and Scheinpflug, H. 1987. Fungal resistance to sterol biosynthesis inhibitors: a new challenge. Plant Dis. 71:1066-1074.
18. Köller, W., Wilcox, W. F., Barnard, J., Jones, A. L., and Braun, P. G. 1997. Detection and quantification of resistance of *Venturia inaequalis* populations to sterol demethylation inhibitors. Phytopathology 87:184-190.
19. Littley, E. R., and Rahe, J. E. 1984. Specific tolerance of *Sclerotium cepivorum* to dicarboximide fungicides. Plant Dis. 68:371-374.
20. Massie, L. B., Cole, H., and Duich, J. 1968. Pathogen variation in relation to disease severity and control of Sclerotinia dollarspot of turfgrass by fungicides. Phytopathology 58:1616-1619.
21. Miller, G. L., Stevenson, K. L., and Burpee, L. L. 2002. Sensitivity of *Sclerotinia homoeocarpa* isolates to propiconazole and impact on control of dollar spot. Plant Dis. 86:1240-1246.
22. Orbach, M. J., Porro, E. B., and Yanofsky, C. 1986. Cloning and characterization of the gene for tubulin from a benomyl-resistant mutant of *Neurospora crassa* and its use as a dominant selectable marker. Mol. Cell. Biol. 6:2452-2461.
23. Ritchie, D. F. 1982. Effect of dichloran, iprodione, procymidone and vinclozolin on the mycelial growth, sporulation, and isolation of resistant strains of *Monilinia fructicola*. Plant Dis. 66:484-486.
24. Russell, P. E. 2004. Sensitivity baselines in fungicide resistance research and management. FRAC Monograph No. 3, GIFAP, Brussels.
25. Smith, F. D., Parker, R. D. M., and Köller, W. 1991. Sensitivity distribution of *Venturia inaequalis* to the sterol demethylation inhibitor flusilazole: baseline sensitivity and implications for resistance monitoring. Phytopathology 81:392-396.
26. Thomas, J. H., Neff, N. F., and Botstein, D. 1985. Isolation and characterization of mutations in the beta-tubulin gene of *Saccharomyces cervisiae*. Genetics 111:715-734.
27. United States Environmental Protection Agency. 1998. Reregistration eligibility decision (RED) iprodione, EPA 738-R-98-019. Washington, DC.
28. United States Environmental Protection Agency. 1999. Reregistration eligibility decision (RED) chlorothalonil, EPA 738-R-99-004. Washington, DC.
29. United States Environmental Protection Agency. 2000. Reregistration eligibility decision (RED) vinclozolin, EPA 738-R-00-023. Washington, DC.
30. Vargas, J. M., Jr. 1994. Management of Turfgrass Diseases. Lewis Publishers, Boca Raton, FL.
31. Warren, C. G., Sanders, P., and Cole, H. 1974. *Sclerotinia homoeocarpa* tolerance to benzimidazole configuration fungicides. Phytopathology 64:1139-1142.
32. Warren, C. G., Sanders, P., Cole, H. J., and Duich, J. M. 1977. Relative fitness of benzimidazole- and cadmium-tolerant populations of *Sclerotinia homoeocarpa* in the absence and presence of fungicides. Phytopathology 67:704-708.