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Fungicide Sensitivity of Sclerotinia homoeocarpa from Golf Courses in Ohio

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ABSTRACT

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 (EC₅₀) 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).

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 (EC₅₀ or ED₅₀) generally is used as an indication of fungicide sensitivity (4,17). EC₅₀ 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-
and where fungicide efficacy tests were conducted. 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. Incubated flasks were incubated at 25°C for 2 weeks. Colonized millet seed was air dried and kept at −20°C 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 EC50 values.** EC50 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 amended with the following: thiohanate-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 25°C. The percent relative mycelial growth was calculated as (radial growth on fungicide-amended PDA/radial growth on PDA) × 100. Each isolate was evaluated twice.

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. EC50 values were estimated from the linear regression models. For thiohanate-methyl, EC50 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 thiohanate-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. EC50 values of which were determined previously. For propiconazole and iprodione, regression analyses between EC50 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 EC50 value for each *S. homoeocarpa* isolate solely based on relative mycelial growth on PDA amended with the discriminatory concentrations of these two fungicides (designated EC50(D)). For thiohanate-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 thiohanate-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 thiohanate-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). EC50(D) values for each *S. homoeocarpa* isolate were determined based on the relationship between EC50 values and mycelial growth on PDA amended with the discriminatory concentration. Simple linear regression analysis was used to determine the relationship between EC50 and EC50(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 EC50(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 EC50(D) values of *S. homoeocarpa* isolates recovered. In 2002, field plots (4 m²) 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), 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, 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 CO2 pressure sprayer using TeeJet 6503 nozzles at 275.8 kPa and in a spray volume of 81.5 ml m². In 2002, plots were treated with thiohanate-methyl (2.5 kg a.i. ha⁻¹ applied every 21 days), iprodione (2.8 kg a.i. ha⁻¹, applied every 14 days), and 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 m² of each plot and dividing it by 20 (1 m² = 10,000 cm²; 1 DSIC = approximately 5 cm²; 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 significant 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 (EC50(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**

Detection of EC$_{50}$ values and discriminant 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>
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<th>No. of isolates collected</th>
<th>Host</th>
<th>Abbreviation</th>
<th>Location</th>
</tr>
</thead>
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<tr>
<td>38a  Agrostis stolonifera L. or Poa pratensis L.</td>
<td>OH</td>
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<tr>
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<td>1   A. stolonifera</td>
<td>...</td>
<td>Blackhawk Golf Club, Galena, OH</td>
<td></td>
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<td>...</td>
<td>Blue Ash Golf Course, Blue Ash, OH</td>
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<td>...</td>
<td>Zanesville Country Club, Zanesville, OH</td>
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*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 EC₅₀(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 (EC₅₀ ≤ 1 µg a.i. ml⁻¹) or growth (EC₅₀ > 1,000 µg a.i. ml⁻¹). EC₅₀(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 EC₅₀ values determined by conventional procedures using three or more concentrations of each fungicide (Fig. 3). There was a high correlation between EC₅₀(D) and EC₅₀ values for propiconazole (r = 0.9254, P < 0.0001) and iprodione (r = 0.7523, P < 0.0001). EC₅₀(D) values were identical to EC₅₀ values for thiophanate-methyl.

Relationship between EC₅₀(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 ≤ EC₅₀(D) ≤ 0.005 µg a.i. ml⁻¹; standard deviation of EC₅₀(D) ≤ 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 ≤ EC₅₀(D) ≤ 0.390 µg a.i. ml⁻¹; 0.007 ≤ standard deviation of EC₅₀(D) ≤ 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 EC₅₀(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 (EC₅₀(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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate (kg ha⁻¹)</th>
<th>Interval (days)</th>
<th>Dollar spot severity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>OH</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>July 10</td>
</tr>
<tr>
<td>Thiophanate-methyl</td>
<td>2.5 ± 0.45</td>
<td>21</td>
<td>0.63</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>0.4 ± 0.05</td>
<td>21</td>
<td>0.75</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>0.8 ± 0.06</td>
<td>21</td>
<td>1.50</td>
</tr>
<tr>
<td>Iprodione</td>
<td>2.8 ± 0.07</td>
<td>14</td>
<td>0.50</td>
</tr>
<tr>
<td>Nontreated control</td>
<td>...</td>
<td>...</td>
<td>47.50</td>
</tr>
<tr>
<td>Treatment F value</td>
<td>...</td>
<td>...</td>
<td>52.53</td>
</tr>
<tr>
<td>P value</td>
<td>...</td>
<td>...</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LSD (P = 0.05)</td>
<td>...</td>
<td>...</td>
<td>8.67</td>
</tr>
</tbody>
</table>

a Severity was determined by counting the number of dollar spot infection centers (DSICs) within the center 1 m² 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 to 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 collected 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)} ≥ 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 55 golf courses had a reduced in vitro sensitivity for iprodione (EC_{50(D)} ≥ 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. homoeocarpa isolates is persistent even after benzimidazole fungicides are not used (17).
Thiophanate-methyl 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 EC50(D) values (>1,000 µg a.i. ml⁻¹) accurately corresponded to failure of 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 EC50(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 EC50(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 EC50(D) values for propiconazole revealed that fungicide efficacy tended to decrease in the field as EC50(D) values increased (Fig. 4B).

The positive correlation between dollar spot severity and mean EC50(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 EC50 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 EC50 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 on 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 Michigan.

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

<table>
<thead>
<tr>
<th>County</th>
<th>Number of Golf courses</th>
<th>Isolates</th>
<th>Thiophanate-methyl</th>
<th>Propiconazole</th>
<th>Iprodione</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;0.002⁴&lt;1,000</td>
<td></td>
<td>&lt;0.19&lt;1.00</td>
</tr>
<tr>
<td>Ashland</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Auglaize</td>
<td>1</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Butler</td>
<td>3</td>
<td>3</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Columbiana</td>
<td>1</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Cuyahoga</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Darke</td>
<td>1</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Delaware</td>
<td>11</td>
<td>45</td>
<td>16 (6)</td>
<td>29 (5)</td>
<td>...</td>
</tr>
<tr>
<td>Franklin</td>
<td>11</td>
<td>65</td>
<td>9 (4)</td>
<td>56 (6)</td>
<td>...</td>
</tr>
<tr>
<td>Greene</td>
<td>1</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Hamilton</td>
<td>3</td>
<td>5</td>
<td>2</td>
<td>3 (3)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Hancock</td>
<td>1</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
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<td>Jefferson</td>
<td>2</td>
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<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Knox</td>
<td>2</td>
<td>2</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Lawrence</td>
<td>1</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Licking</td>
<td>4</td>
<td>5</td>
<td>3 (3)</td>
<td>2 (2)</td>
<td>...</td>
</tr>
<tr>
<td>Lorain</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3 (2)</td>
<td>2</td>
</tr>
<tr>
<td>Miami</td>
<td>1</td>
<td>1</td>
<td>...</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Montgomery</td>
<td>4</td>
<td>16</td>
<td>5 (2)</td>
<td>11 (2)</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Muskingum</td>
<td>2</td>
<td>14</td>
<td>1</td>
<td>13 (2)</td>
<td>7 (2)</td>
</tr>
<tr>
<td>Warren</td>
<td>3</td>
<td>9</td>
<td>5 (2)</td>
<td>4 (3)</td>
<td>7 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>192</td>
<td>50 (27)</td>
<td>142 (34)</td>
<td>7 (7)</td>
</tr>
</tbody>
</table>

⁴ Discriminatory effective concentration that produces 50% inhibition (EC50(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).

⁵ 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 ≤ EC_{50(D)} ≤ 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 1.5-fold greater for iprodione compared with baseline (sensitivities of S. homoeocarpa) using the 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.

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LITERATURE CITED