Plant Pathology (2007) **56**, 500–507 Doi: 10.1111/j.1365-3059.2007.01573.x

Sensitivity of *Sclerotinia homoeocarpa* to demethylation-inhibiting fungicides in Ontario, Canada, after a decade of use

T. Hsiang*, A. Liao and D. Benedetto

Department of Environmental Biology, University of Guelph, Guelph, Ontario, N1G 2W1, Canada

In late 2003, nine populations of *Sclerotinia homoeocarpa* in Ontario Canada (seven of which had been previously sampled in early 1994, prior to the registration of sterol demethylation-inhibiting (DMI) fungicides for turf disease control in Canada) were sampled and tested for sensitivity to propiconazole. Four of the nine populations had not been treated with DMI fungicides during the intervening years, and isolates from these locations were sensitive to propiconazole (geometric mean EC_{50} values of 0·005–0·012 μg mL^{-1}, compared with 0·005–0·008 μg mL^{-1} for the original 1994 populations). Among the five populations from 2003 that had been exposed to DMI fungicides, mean EC_{50} values were significantly greater, ranging from 0·020 to 0·048 μg mL^{-1}. A significant correlation of determination was found between estimated number of fungicide applications and log EC_{50} (R^2 = 0·832, P = 0·0001), and the equation predicted that 42·3 applications of propiconazole would be needed to bring a sensitive population (EC_{50} < 0·01 μg mL^{-1}) to a resistant level (EC_{50} > 0·10 μg mL^{-1}). Fungicide sensitivity vs. duration of fungicide efficacy was also tested, and it was found that isolates with decreased sensitivity were able to more quickly overcome the inhibitory effects of fungicide application, reducing the duration of control from 3 weeks to 2 weeks.

Keywords: DMI fungicides, dollar spot, fungicide resistance, myclobutanil, propiconazole, turfgrass diseases

Introduction

Dollar spot is a disease of turfgrasses caused by the fungus *Sclerotinia homoeocarpa*. It is very common in eastern Canada and across the USA, and accounts for a large proportion of the fungicides used on amenity turfgrasses for disease control (Ayers & Gilmore, 1991; Anderson *et al*., 1992). The disease has a long period of activity and fungicides are often applied at 7- to 28-day intervals (Ayers & Gilmore, 1991). In southern Ontario, it generally first appears in June and can be active until October. On grass cut at short heights, such as golf course fairways, tees and greens, dollar spot appears as bleached, circular patches up to 6 mm in diameter (Smiley *et al*., 2005). On high-maintenance turfgrasses, there is very low aesthetic tolerance for diseases. In Canada, there are fewer active ingredients registered for control of dollar spot than in the neighbouring USA (Anonymous, 2005; Vincelli & Powell, 2006) and so there are concerns about development of economically significant field resistance and hence the loss of a family of controlling fungicides.

*E-mail: thsiang@uoguelph.ca

Accepted 7 October 2006

Demethylation-inhibiting fungicides (DMIs) were introduced in Canada for use against turfgrass diseases in 1994, whereas they have been available for use on turf in the USA since 1979 (Golembiewski *et al*., 1995). DMI fungicides inhibit oxidative sterol 14α-demethylation in the ergosterol biosynthesis pathway of many fungi (Siegel, 1981). Sterols are important components of membrane lipids in eukaryotic cells. They modulate movement of phospholipids to ensure correct membrane fluidity and to control both permeability and activity of some membrane-bound enzymes (Hollomon *et al*., 1990). Resistance to one DMI fungicide in *S. homoeocarpa* is usually manifested as resistance to other DMI fungicides (Hsiang *et al*., 1997).

The selective pressure of pesticides such as DMI fungicides theoretically allows a higher reproductive rate for strains which are less sensitive, which may lead to a population shift toward resistant strains (Golembiewski *et al*., 1995; Erickson & Wilcox, 1997; Karaoglanidis *et al*., 2002). Gradual evolution towards decreased sensitivity in particular isolates and the increased frequency of less sensitive isolates within populations has decreased the effectiveness of DMI fungicides in regions where their use remains high (Hollomon *et al*., 1990). Development of resistance to DMI fungicides has been

documented for many fungal plant pathogens (Ma & Michailides, 2005).

For dollar spot disease, evidence of field resistance to fungicides was based on a detectable decline in disease control under field conditions associated with decreased sensitivity of the pathogen to fungicide (Miller *et al*., 2002). In documented cases of field resistance to DMI fungicides, *in vitro* mycelial growth assays were employed to detect reduced pathogen sensitivity. Such assays were used to detect resistance to DMI fungicides in field populations of *S. homoeocarpa* in Illinois, Kentucky and Michigan, at sites where control failure had been noted (Vargas *et al*., 1992; Doney & Vincelli, 1993; Golembiewski *et al*., 1995).

Baseline DMI fungicide sensitivity data for Ontario populations of *S. homoeocarpa* were collected in 1994, prior to DMI registration in Canada (Hsiang *et al*., 1997), and a wide range of sensitivity to DMI fungicides was observed. Barton (1999) continued to monitor four of the Ontario populations for sensitivity to propiconazole for the next 2 years after registration. None of the four populations showed decreased sensitivity to propiconazole from 1994 to 1996, even though two of the sites used propiconazole (in the fungicide Banner). Myclobutanil, another DMI fungicide, sold under the trade name Eagle, was registered for use against turfgrass diseases in Canada in 1997. There have been no subsequent studies on DMI sensitivity of Ontario populations of *S. homoeocarpa* since that time, nor any documented cases of control failure of dollar spot disease by DMIs in Ontario. However, anecdotal evidence from turfgrass managers in Ontario indicates that DMI applications do not seem as efficacious as when they were first introduced. The purpose of this study was to assess whether populations of *S. homoeocarpa* in Ontario treated with DMI fungicides such as propiconazole or myclobutanil show reduced sensitivity to DMIs compared to baseline levels.

The specific objectives of this study were as follows: (i) to revisit the same golf courses which were sampled in 1994 (Hsiang *et al*., 1997; Barton, 1999) and collect samples from the same locations; (ii) to compare sensitivity to propiconazole of isolates from locations that used DMI fungicides with that of those from locations that did not; and (iii) to conduct field tests on the duration of efficacy of DMI fungicides for isolates of *S. homoeocarpa* with varying fungicide sensitivity.

Materials and methods

Sample collection

In 2003, a minimum of 80 samples of turfgrass with dollar spot symptoms were collected from each of nine locations in southern Ontario (Table 1). Samples were collected from the same fairways sampled by Hsiang *et al.* (1997), except for one site (the Cambridge Research Farm) which no longer exists. Two new sites were chosen to complement the original seven. To provide a uniform distribution of sampling, a grid pattern averaging 3 m × 5 m was used, and up to 30 leaf blades were collected from single infection centres within a 1-m radius of each sampling point. These samples were placed in microfuge tubes and transported back to the laboratory on ice. The samples were then allowed to dry with the lids open in a laminar flow hood overnight, and then stored at 4°C for isolation within a week.

Isolates of *S. homoeocarpa* were obtained by placing four leaf blades from each sample onto a plate of potato dextrose agar (PDA) amended with 0·1 g L^{-1} each of tetracycline hydrochloride and streptomycin sulphate to inhibit bacterial growth. The antibiotic stock solutions were added to molten PDA after cooling to 60°C. After 48 h at room temperature (23°C), the plates were examined, and those with colonies resembling *S. homoeocarpa* were subcultured onto fresh, unamended PDA. After another 72 h, the subcultured plates were examined, and a single isolate typical of *S. homoeocarpa* was chosen from each sampling point. These isolates were transferred to PDA slants for short-term storage at 4°C, and into 15-mL vials containing autoclaved mixed grain for long-term

Table 1 Sampling sites for *Sclerotinia homoeocarpa* in Ontario, Canada, with host genera (major one first) and DMI fungicide use at each site

			DMI applications per season										
Location	Host species	DMI fungicide used[a]	94	95	96	97	98	99	00	01	02	03	Total[b]
Barrie	*Poa*, *Agrostis*	None	0	0	0	0	0	0	0	0	0	0	0
Guelph	*Poa*, *Agrostis*	None	0	0	1	0	0	0	0	0	0	0	1
London	*Agrostis*	None on fairways	0	0	0	0	0	0	0	0	0	0	0
Mississauga	*Agrostis*, *Poa*	Banner, Eagle	–	–	–	–	1	1	1	2	3	3	18·3
Oshawa	*Agrostis*, *Poa*	None	0	0	0	0	0	0	0	0	0	0	0
Point Pelee	*Poa*, *Agrostis*	Banner	–	–	–	–	–	–	2	2	2	2	20
St. Catherines	*Poa*, *Agrostis*	Banner, Eagle	–	–	–	–	–	–	1	2	4	3	25
Toronto	*Agrostis*, *Poa*	Banner, Eagle	1	2	2	6	6	5	3	4	2	0	31
Windsor	*Agrostis*, *Poa*	Banner, Eagle	1	3	5	4	5	2	5	3	3	3	34

[a]Banner or Banner MAXX containing propiconazole was generally applied at a rate of 7·3 g a.i. per 100 m^2. Eagle containing myclobutanil was generally applied at a rate of 8 g a.i. per 100 m^2.

[b]The total number of applications during 10 seasons of use was extrapolated from the data available as the sum of known applications divided by number of years of known application times 10. '–' refers to missing data.

storage. The vial cultures were first grown at room temperature until mycelium covered half of the surface and then held at –20°C.

Preparation of propiconazole-amended media

Technical-grade propiconazole (97·7%; Syngenta) was dissolved in 100% acetone, 0·55 g in 50 mL, and then further diluted 10-fold in deionized water for the first stock solution. A series of dilutions were made in 10% acetone to obtain the required stock concentrations. These propiconazole solutions were added to molten PDA that had been cooled to 60°C to obtain final concentrations of 0, 0·001, 0·01, 0·10, 1·0 and 10·0 μg mL^{-1}, while maintaining an equal calculated final concentration of acetone (0·10% v/v). Acetone at this concentration did not inhibit growth of *S. homoeocarpa* (Hsiang *et al.*, 1997). Fungicide-amended PDA was kept well mixed with an autoclaved magnetic stir bar, and 20-mL aliquots dispensed into 9-cm-diameter plates. After the agar had solidified, it was cut with six blades mounted on a 9-cm-diameter plexiglass holder, and agar was removed to leave three 1-cm-wide strips following Hsiang *et al.* (1997).

DMI sensitivity assay

Up to 60 isolates were selected from each site for sensitivity assays (Table 2). Only vigorously growing cultures less than 2 weeks old, with the colony margin at least 1 cm from the edge of the plate, were used for testing. Plugs 5 mm in diameter were cut from colony margins, and a plug was placed in the center of an agar strip on each treatment plate, with the mycelium contacting the agar surface. Plugs of each isolate were placed onto three different plates at each fungicide concentration. In each test, two of the less sensitive (EC_{50} values of 3·54 and 0·57 μg mL^{-1}) and one of the more sensitive isolates (EC_{50} 0·013 μg mL^{-1} propiconazole) from the earlier baseline study (Hsiang *et al.*, 1997) were included as standards to ensure consistent results. These standard isolates had been stored on mixed cereal grains at –20°C, and preliminary testing showed that they had retained their original level of sensitivity to propiconazole.

Treatment plates were placed in an incubator at 24°C. The extent of radial colony growth was marked at 24 h and 48 h and recorded with three replicates per isolate. Only the mycelium in contact with the agar surface was considered as growth, since there were sometimes aerial hyphae hanging over the medium which seemed to avoid contact with the agar. To reduce the number of plates used in testing, the isolates were tested initially at four propiconazole concentrations: 0, 0·001, 0·01 and 0·1 μg mL^{-1}. Only those isolates showing significant growth at 0·1 μg mL^{-1}, i.e. diameter > 25% of the growth on unamended PDA, were further tested at propiconazole concentrations of 1·0 and 10·0 μg mL^{-1}.

Field tests for fungicide-efficacy duration

Isolates of *S. homoeocarpa* collected in 2003 with calculated EC_{50} values ranging from 0·001 to 0·4 μg mL^{-1} were selected for field testing (Table 3). Isolates were grown separately in jars of wheat bran to which an equal volume of water had been added and autoclaved twice. The inoculum was grown at 25°C for up to 2 weeks, after which it was dried at room temperature for 2 days, then ground using a domestic blender to break up large clumps. The inoculum was diluted in nine parts fresh wheat bran for easier application to field plots.

In summer 2005, plots of 0·5 × 0·5 m were laid out in a randomized complete block design with four replicate plots per isolate on a sand-based green established in 1994 and seeded with Penncross creeping bentgrass (*Agrostis stolonifera*). The green was maintained at 5-mm mowing height with regular fertilization (2 kg sulphur-coated urea m^{-2} applied three times a year) and no pesticide use. Another set of plots was laid out on the same green with the same design, but not treated with propiconazole during this trial. All the plots were first inoculated with 5 g m^{-2} of the diluted inoculum (9 June), followed a day later by

Table 2 Population number, location in Ontario, and geometric mean and range of EC_{50} values from sites where isolates of *Sclerotinia homoeocarpa* were collected in 1994 and 2003 and tested for sensitivity to propiconazole

	Number of isolates		Mean EC_{50} (μg mL^{-1})[a]		Range EC_{50} (μg mL^{-1})[b]	
Location	1994	2003	1994	2003	1994	2003
Barrie	52	53	0·007 ef	0·011 d	0·003–0·030	0·002–0·032
Guelph	60	44	0·005 g	0·005 g	0·002–0·010	0·004–0·010
London	58	42	0·008 e	0·012 d	0·002–0·028	0·001–0·081
Mississauga	None	52	NA	0·023 bc	NA	0·004–0·084
Oshawa	None	47	NA	0·007 ef	NA	0·001–0·060
Point Pelee	71	59	0·008 e	0·048 a	0·003–0·046	0·008–0·378
St. Catherines	57	55	0·006 fg	0·020 c	0·002–0·014	0·006–0·044
Toronto	41	58	0·008 e	0·027 b	0·004–0·012	0·007–0·070
Windsor	21	55	0·022 bc	0·047 a	0·005–0·069	0·009–0·326

[a]Log EC_{50} values were subjected to analysis of variance. Means were separated using the test of least significant difference at $P = 0·05$, and back-transformed (antilog) means are shown. Means from the 1994 and 2003 collections followed by a letter in common are not significantly different.
[b]Ranges show the lowest and highest EC_{50} values for individual isolates within each location.

Table 3 Origin and propiconazole sensitivity of *Sclerotinia homoeocarpa* isolates used in field experiments with dollar spot counts taken 1, 2, and 3 weeks after fungicide application

Isolate	Population	EC_{50} (μg mL^{-1})	Group[a]	Test[b]	Untreated/treated spots[c]		
					1 week	2 weeks	3 weeks
04096	Barrie	0·0018	S	Aug	32·0	2·0	1·2
04182	Oshawa	0·0022	S	Jun	5·0	2·6	0·7
04127	Barrie	0·0023	S	Jun	13·0	4·2	3·1
04691	London	0·0036	S	Aug	10·8	3·5	1·3
04768	Guelph	0·0036	S	Jun	16·0	2·1	0·5
04185	Oshawa	0·0042	S	Aug	13·7	5·3	1·2
04717	Guelph	0·0093	S	Aug	13·3	3·4	0·6
04669	London	0·0102	M	Jun	6·9	2·0	1·0
04013	Windsor	0·0113	M	Jun	18·0	1·9	1·5
04251	St. Catherines	0·0163	M	Aug	13·0	3·8	1·2
04253	St. Catherines	0·0164	M	Aug	16·8	3·9	1·5
04236	St. Catherines	0·0441	M	Aug	14·2	2·4	0·8
04236	St. Catherines	0·0441	M	Jun	2·2	0·6	0·4
04293	Mississauga	0·0497	M	Aug	15·0	5·2	1·0
04528	Toronto	0·0503	M	Jun	17·5	1·4	0·5
04021	Windsor	0·0658	M	Aug	8·4	2·3	1·9
04533	Toronto	0·0870	M	Aug	26·5	8·2	1·1
04517	Toronto	0·0892	M	Aug	24·0	6·7	1·0
04596	Point Pelee	0·1755	R	Aug	10·1	1·1	0·9
04588	Point Pelee	0·2229	R	Jun	9·6	1·0	1·3
04036	Windsor	0·2384	R	Aug	27·5	1·0	0·9
04594	Point Pelee	0·3780	R	Jun	6·0	1·0	1·3

[a]Group refers to fungicide sensitivity grouping: S = sensitive (< 0·01 μg mL^{-1}), M = moderate (0·01–0·1 μg mL^{-1}) and R = reduced sensitivity (EC_{50} > 0·1 μg mL^{-1}).

[b]Test refers to the field test starting in either June or August 2005.

[c]The ratio of untreated to treated spots was calculated by comparing the mean numbers of spots on untreated plots vs. fungicide-treated plots, and the data are presented by number of weeks after fungicide application.

the first fungicide application containing Banner MAXX (Syngenta) at 51 mL per 100 m^2 (propiconazole at 7·3 g per 100 m^2), with a wheel-mounted compressed air boom sprayer (140 kPa in water at 11 L per 100 m^2 using Lurmark 03-F110 nozzles).

Plots were evaluated weekly for the number of dollar spot patches. The first spots were seen 3 weeks after the start of the experiment (30 June), but because of the continuing low disease pressure, more inoculum was applied in week 5 (14 July). Weekly disease evaluations were made until week 8 (4 August).

Because of possible complications from multiple sets of inoculations in the first field trial, another experiment was set up on 4 August 2005, on another research green approximately 100 m away. This green had been constructed in 1994 on a soil base of 80% sand and 20% organic matter to USGA specifications, and was also seeded with Penncross creeping bentgrass. At the start of this second trial, there were no spots visible on this green. Except for one isolate where anomalous results were seen in the first test (isolate 04 236, Table 3), a new set of 12 other isolates was chosen for this trial (Table 3), and these isolates were inoculated onto the plots on 12 August. Fungicide application occurred 1 week later, and the plots were evaluated weekly for the next 3 weeks for number of dollar spots.

Statistical analyses

For the fungicide plate assays, only the growth in diameter which occurred between 24 h and 48 h was used for analysis since the first 24 h of growth could show variability as a result of establishment effects. Percentage inhibition was calculated as [1 - (mean colony diameter on propiconazole-amended medium divided by mean colony diameter on unamended medium)] × 100%. SAS version 6·12 (SAS Institute) PROC PROBIT was used to calculate EC_{50} values (the concentration required to inhibit diameter growth by 50%). An example of the SAS program statements is available upon request from the first author.

The log EC_{50} values were then subjected to analysis of variance using PROC GLM. The isolates were grouped by their population of origin, with a population comprising isolates collected from the same year and same location. In addition to the EC_{50} values from samples obtained in 2003, data from the original populations of the baseline study in 1994 (Hsiang *et al.*, 1997) were also used in this analysis. When a significant effect was observed in the analyses, the geometric mean population EC_{50} values were compared using Fisher's protected LSD (least significant difference) at $P = 0·05$. A regression analysis was performed to assess the relationship between the estimated number of DMI fungicide applications at each

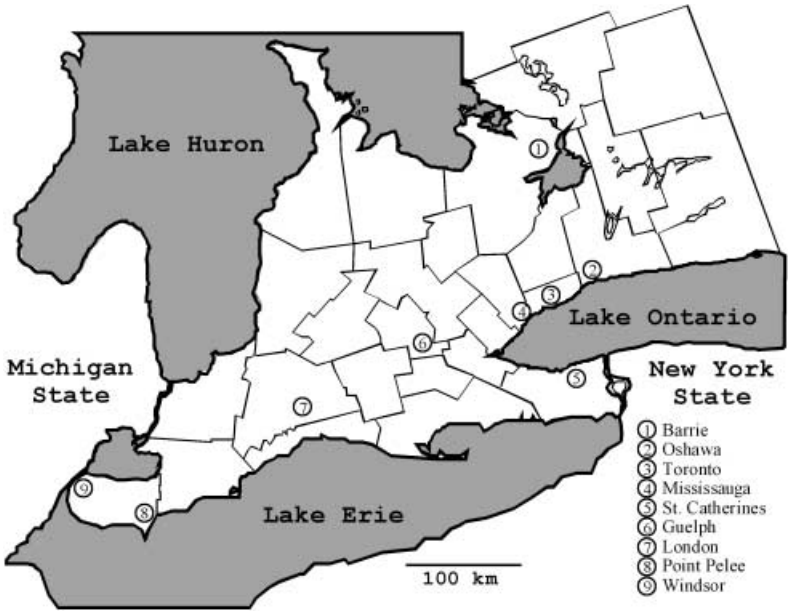

Figure 1 Map of the locations of sample sites where *Sclerotinia homoeocarpa* was collected in late 2003.

site and the geometric mean EC_{50} values, and the coefficient of determination (R^2) was calculated using SAS PROC REG.

For the field trials, the mean number of spots was compared between the two sets of plots: one treated with fungicide and one without fungicide. These data were then examined with further clustering of isolates into EC_{50} groupings defined as follows: reduced sensitivity (> 0·1 μg mL^{-1}), moderate (0·01–0·1 μg mL^{-1} and sensitive (< 0·01 μg mL^{-1}). These sensitivity criteria were based on propiconazole EC_{50} values for *S. homoeocarpa* from Hsiang *et al.* (1997), which set the range for full sensitivity, and results from Jo *et al.* (2006) who placed the discriminatory concentration for resistance at 0·1 μg mL^{-1}. PROC GLM was then used to assess differences between groups, and LSD was used to separate means when significant treatment effects were observed ($P < 0·05$).

Results and discussion

Isolates of *S. homoeocarpa* were collected from nine golf course fairways in southern Ontario in late 2003 (Fig. 1). The history of DMI fungicide use was also collected from these sites, although some of the records were incomplete (Table 1). Among these nine sites, seven had been sampled previously in 1994, and those isolates had been tested for sensitivity to several DMI fungicides at the time of collection (Hsiang *et al.*, 1997).

The isolates collected in 2003 were tested for sensitivity to propiconazole, and they showed a wider range of such sensitivity than the results for the 1994 isolates (Table 2). Among the seven sites where isolates were collected in both 1994 and 2003, the four sites (Point Pelee, St. Catherines, Toronto and Windsor) which had been exposed to DMI fungicides showed EC_{50} values in 2003 which were significantly greater ($P = 0·05$) than those in 1994 (Table 2), whereas the three sites (Barrie, Guelph and London) that reportedly had not been exposed had mean EC_{50} values in both 1994 and 2003 which showed high sensitivity to propiconazole (0·005–0·015 μg mL^{-1}), with no statistically significant differences between the 1994 and 2003 mean EC_{50} values (Table 2).

The distribution of fungicide sensitivity for populations from 2003 that were treated with DMI fungicides (mean EC_{50} 0·031 μg mL^{-1}) showed a shift towards decreased sensitivity compared to the untreated populations (mean EC_{50} 0·008 μg mL^{-1}) (Fig. 2). Miller *et al.* (2002) reported a propiconazole EC_{50} range of 0·005–0·057 μg mL^{-1}, with a mean of 0·028 μg mL^{-1} for two *S. homoeocarpa* populations in Georgia which had been exposed to DMI fungicides twice a year for at least 4 years. The maximum EC_{50} values for DMI-treated populations in

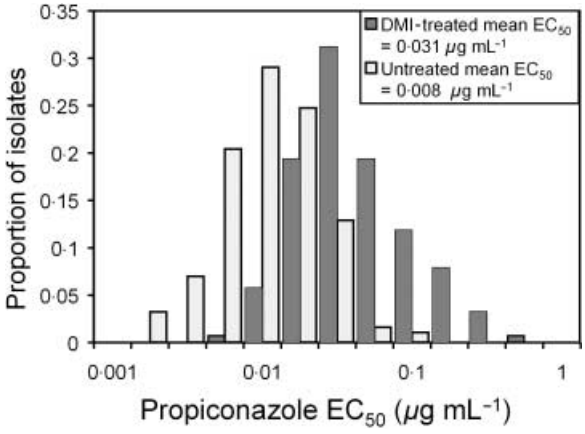

Figure 2 Distribution of fungicide sensitivity of isolates from five DMI-treated populations (279 isolates) and four untreated populations (186 isolates) of *Sclerotinia homoeocarpa* in southern Ontario, 2003.

the current study (Table 2) were higher than those of Miller *et al.* (2002), possibly because of a longer or more intensive history of DMI exposure (Table 1).

Jo *et al.* (2006) reported that among 41 isolates of *S. homoeocarpa* from 36 golf courses in Ohio, 54% had EC_{50} values less than 0·05 μg mL^{-1}, while 17% had EC_{50} values greater than 0·08 μg mL^{-1}. For the four populations in Ontario sampled in 2003 that were not treated with DMI fungicides, only three isolates out of 190 had EC_{50} > 0·05 μg mL^{-1}, whereas for the five 2003 populations that had been treated with DMI fungicides, 71 out of 279 isolates had EC_{50} > 0·05 μg mL^{-1}. Jo *et al.* (2006) used a discriminatory concentration of 0·10 μg propiconazole mL^{-1} to determine that among 192 isolates from 55 golf courses in Ohio, propiconazole resistance was prevalent at 18 courses. For the 471 isolates collected in Ontario in 2003, only 33 showed EC_{50} > 0·10 μg mL^{-1}, and nearly all of these were from either the Windsor or Point Pelee populations, which were the two most southerly populations and closest to the Michigan border. These results imply that while the distribution of isolates with reduced sensitivity may be slightly more widespread in Ontario than in Ohio, the incidence of resistant isolates (as defined by Jo *et al.*, 2006) is much more limited in Ontario.

Brownback & Latin (2002) commented that at some golf courses in midwestern American states, there was a noticeable decrease in the performance of systemic fungicides, including DMI fungicides. Golembiewski *et al.* (1995) found a resistance factor (RF) of over 50 (EC_{50}-treated/EC_{50}-sensitive) for *S. homoeocarpa* at sites exhibiting field resistance (EC_{50} = 0·10 μg mL^{-1}) compared to sites which were not treated with DMIs (EC_{50} = 0·002 μg mL^{-1}). Miller *et al.* (2002) reported a propiconazole RF of 5·8 for *S. homoeocarpa* populations exposed to DMIs (EC_{50} = 0·0283 μg mL^{-1}) vs. populations not treated with DMIs (EC_{50} = 0·0049 μg mL^{-1}), and the exposed populations showed reduced efficacy of DMI fungicides and control intervals were shorter than the application interval on the fungicide label. For the Ontario populations sampled in 2003, the RF of 4·2 (treated populations vs. untreated populations) demonstrated a shift toward decreased sensitivity at DMI-treated sites, yet there was no decreased performance of DMIs nor disease control failure reported at these sites.

To quantify the relationship between DMI fungicide use and reduced sensitivity, a regression of log EC_{50} values (Table 2) on the number of fungicide applications (Table 1) was performed using data from the 1994 and 2003 samples. The coefficient of determination (R^2 = 0·832, P = 0·0001) showed a significant relationship and yielded the predictive equation: Applications = 42·3(log EC_{50}) + 90·6 (Fig. 3). This equation predicted that to shift a population from sensitive (EC_{50} < 0·01 μg mL^{-1}) to resistant (EC_{50} > 0·1 μg mL^{-1}) would require 42·3 DMI fungicide applications, with lower and upper standard error values of 36·8 and 49·7 applications, respectively. Since one of the populations had been exposed to DMI fungicides prior to registration (Table 2; Hsiang *et al.*, 1997), the data were re-analysed omitting

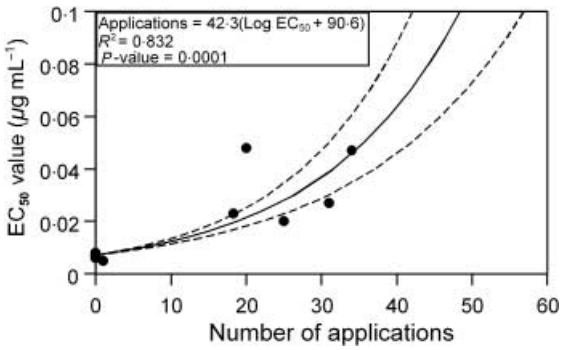

Figure 3 Relationship between the estimated number of DMI fungicide applications during 10 years (Table 1) and the mean EC_{50} value (Table 2) for five DMI-treated populations of *Sclerotinia homoeocarpa* sampled in late 2003, and seven populations sampled in early 1994 in southern Ontario. R^2 is the coefficient of determination as calculated in SAS PROC REG.

this population. The relationship between fungicide applications and log EC_{50} then became slightly weaker (R^2 = 0·779, P = 0·0007), and number of applications required to reach resistance increased slightly to 42·6 (with 35·9 and 52·5 as the lower and upper limits, respectively).

For two of the populations from 2003, exposure to DMI fungicides was estimated at over 30 applications (Table 1), yet no indication of field resistance to DMI fungicides, not even decreased sensitivity, was reported at these sites. Hsiang *et al.* (1998) found a significant relationship between propiconazole sensitivity and field 'virulence' for *S. homoeocarpa*, and proposed that there were fitness costs related to fungicide resistance. Perhaps the decreased fitness of isolates with lowered fungicide sensitivity reduced the occurrence and impact of field resistance at these sites.

Another possible factor for the lack of field resistance would be the transport or invasion of sensitive isolates into treated sites. In this study, there was some evidence of local transport of isolates from DMI-treated to untreated areas, and presumably vice versa. For example, the sampled golf course fairway at London reportedly had no fungicide use, at least in the eight seasons prior to collection, yet there were isolates detected on this fairway with EC_{50} > 0·080 μg mL^{-1}. This is a maximum EC_{50} value similar to that found for Mississauga, to which DMI fungicides had been regularly applied. The most sensitive isolates at the London site had EC_{50} = 0·001 μg mL^{-1}, while the most sensitive isolates at the five DMI-treated sites had EC_{50} values ranging from 0·004 to 0·009 μg mL^{-1} (Table 2), implying that the London sample site had not been directly exposed to DMI fungicide selection, since such highly sensitive isolates were still found. However, at the London golf course, DMI fungicides had been applied to adjacent putting greens, which could account for the higher mean EC_{50} value at this site (0·015 μg mL^{-1}) in 2003 than at the other three golf courses which had not been exposed to DMI fungicides at all (Table 2). More

research on the persistence and composition of *S. homoeocarpa* isolates on a fairway from year to year is needed.

To experimentally assess the relationship between EC_{50} values and field efficacy, two field experiments were conducted in 2005 using sensitive (EC_{50} < 0·01 μg mL^{-1}), moderately sensitive, and reduced-sensitivity (EC_{50} > 0·10 μg mL^{-1}) isolates collected in 2003 (Table 3). Results of the first field trial showed that propiconazole application at the labelled rate of 7·3 g per 100 m^2 significantly suppressed the development of dollar spot for at least 2 weeks after application for the highly sensitive isolates, but for only 1 week for moderately sensitive and reduced-sensitivity groups of isolates (Fig. 4). For the second field trial (Fig. 5), the plots were inoculated on 12 August and fungicide applied on 18 August. By 1 week after fungicide treatment, the number of spots was reduced from an average of 13 per 0·25-m^2 plot for all isolates to two. By the end of the second week after fungicide application, the moderately sensitive and sensitive isolates were still suppressed by the fungicide, with less than five spots per plot, while the reduced-sensitivity isolates averaged 15 spots per plot. By the end of the third week, all three groups averaged 18 spots per plot (Fig. 5). These two field tests demonstrated that isolates with decreased sensitivity were able to more quickly overcome the inhibitory effects of fungicide application, reducing the duration of control from 3 weeks to 2 weeks.

Another way of analysing these field data was to examine the ratio between spots on the untreated plots compared to spots on the corresponding plots in the treated area

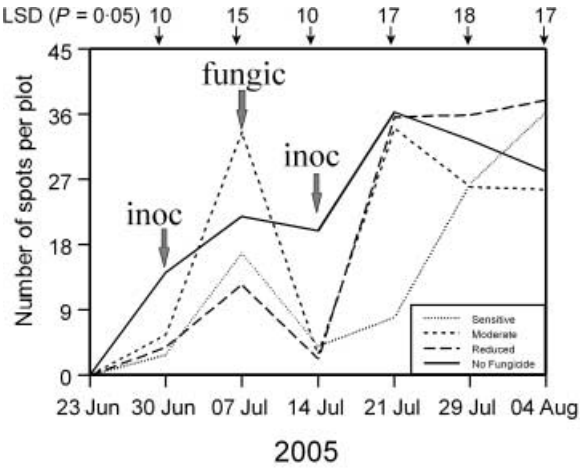

Figure 4 Mean dollar spot counts per 0·25-m^2 plot in the first field trial starting 23 June 2005, arrows showing dates of inoculation with nine isolates (three sensitive, four moderate, two reduced sensitivity), and fungicide application (propiconazole at 7·3 g per 100 m^2), based on four replicate plots per isolate. An adjacent set of plots were inoculated but not treated with fungicide, and the mean dollar spot count for this 'no fungicide' treatment resulted from pooling results of all 9 isolates. LSD values are shown above each weekly mean count per sensitivity grouping, and means differing by at least this amount are significantly different at P = 0·05.

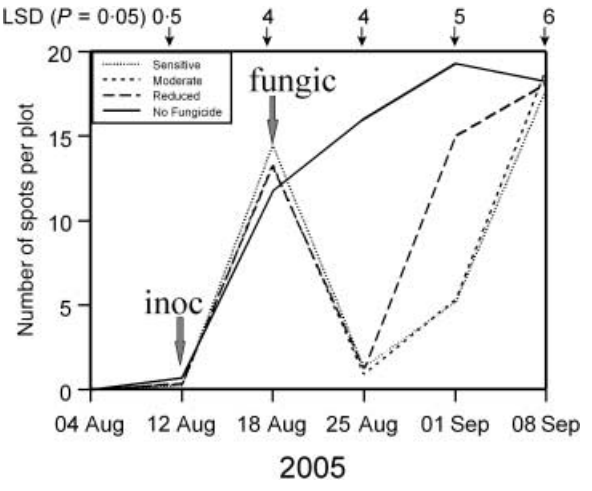

Figure 5 Mean dollar spot counts per 0·25-m^2 plot in the second field trial starting 4 August 2005, showing dates of inoculation with 13 isolates (four sensitive, seven moderate, two2 reduced sensitivity), and fungicide application (propiconazole at 7·3 g per 100 m^2), based on four replicate plots per isolate. An adjacent set of plots were inoculated but not treated with fungicide, and the mean dollar spot count for this 'no fungicide' treatment resulted from pooling results of all 13 isolates. LSD values are shown above each weekly mean count per sensitivity grouping, and means differing by at least this amount are significantly different at P = 0·05.

(Table 3). With both field tests pooled, there were no significant differences between the three sensitivity groups in weeks 1 and 3, but during week 2 the isolates with reduced sensitivity showed an average ratio of 1·0, indicating that reduced-sensitivity isolates on treated plots were producing the same number of spots as those on untreated plots much sooner than the sensitive or moderately sensitive isolates, which showed ratios of 3·3 and 3·5, respectively (Table 3). Similarly, Burpee (1997) found that a propiconazole-resistant isolate (EC_{50} = 0·31 μg mL^{-1}) of *S. homoeocarpa* was able to reach a 5% disease level much more quickly on propiconazole-treated plots than a sensitive isolate (EC_{50} = 0·03 μg mL^{-1}), and Miller *et al.* (2002) also showed that the higher the EC_{50}, the shorter the latent period for DMI-resistant *S. homoeocarpa*.

Future observation and testing is required to determine whether the DMI EC_{50} values are stable or increasing with DMI usage at sites in southern Ontario, and whether full field resistance will occur with continued use. As mentioned above, Hsiang *et al.* (1998) found a slight fitness cost associated with DMI resistance in *S. homoeocarpa* and speculated that if propiconazole applications were halted or curtailed, then theoretically, isolates with reduced sensitivity would be out-competed by sensitive isolates. To delay the development of DMI resistance in *S. homoeocarpa*, Vargas *et al.* (1992) suggested reducing the propiconazole dose, while Gilstrap *et al.* (1997) suggested tank mixes with non-DMI fungicides or alternating with other fungicides. The results from the present study demonstrated that continued use of DMI fungicides selects against sensitive isolates and for resistant isolates,

resulting in populations with reduced sensitivity. The number of propiconazole applications estimated to be required over a 10-year period for a shift from a sensitive population with $EC_{50} < 0{\cdot}005\ \mu g\ mL^{-1}$ to a resistant population with $EC_{50} > 0{\cdot}10\ \mu g\ mL^{-1}$ was 48. It is not known whether the same number of applications over a shorter or longer period would result in the same level of reduced sensitivity. The continued efficacy of DMI fungicides for *S. homoeocarpa* in Ontario will depend on patterns of fungicide use by turf managers.

Acknowledgements

This work was supported by funding from Syngenta and the Natural Sciences and Engineering Research Council of Canada. We are grateful for the technical assistance of Sandra Cook.

References

Anderson A, Dean S, Courneya M, Charbonneau P, 1992. 1990 Turf industry survey. In: *1992 Annual Report*. Guelph, Canada: Guelph Turfgrass Institute, 146–50.

Anonymous, 2005. *Turfgrass Management Recommendations*. Toronto, Canada: Ontario Ministry of Agriculture and Food: Publication no. 384.

Ayers JE, Gilmore BK, 1991. *Fungicide Benefits Assessment, Turf*. Washington, USA: USDA: States National Agricultural Pesticide Impact Assessment Program Fungicide Assessment Project.

Barton W, 1999. *Sensitivity of* Sclerotinia homoeocarpa *to the DMI Fungicide Propiconazole*. Guelph, Ontario, Canada: University of Guelph, MSc thesis.

Brownback LE, Latin R, 2002. Survey of isolates of *Sclerotinia homoeocarpa* in Indiana for sensitivity to three fungicides. *Phytopathology* **92**, S10 (Abstract).

Burpee LL, 1997. Control of dollar spot of creeping bentgrass caused by an isolate of *Sclerotinia homoeocarpa* resistant to benzimidazole and demethylation-inhibitor fungicides. *Plant Disease* **81**, 1259–63.

Doney JC, Vincelli P, 1993. Cross resistance in *Sclerotinia homoeocarpa* to DMI fungicides. *Phytopathology* **83**, 1338.

Erickson EO, Wilcox WF, 1997. Distributions of sensitivities to three sterol demethylation inhibitor fungicides among populations of *Uncinula necator* sensitive and resistant to triadimefon. *Phytopathology* **87**, 784–91.

Gilstrap DM, Vargas JM Jr, Golembiewski RC, Jones AL, Schabenberger O, 1997. Fungicide efficacy on demethylation inhibition (DMI) resistant *Sclerotinia homoeocarpa*. *International Turfgrass Society Research Journal* **8**, 875–81.

Golembiewski RC, Vargas JM, Jones AL Jr, Detweiler AR, 1995. Detection of demethylation inhibitor (DMI) resistance in *Sclerotinia homoeocarpa* populations. *Plant Disease* **79**, 491–3.

Hollomon DW, Butters J, Hargreaves JA, 1990. Resistance to sterol biosynthesis – inhibiting fungicides. In: Green MB, LeBaron HM, Moberg WK, eds. *Managing Resistance to Agrochemicals – from Fundamental Research to Practical Strategies*. Washington, USA: American Chemical Society, 199–214.

Hsiang T, Yang L, Barton W, 1997. Baseline sensitivity and cross-resistance to demethylation-inhibiting fungicides in Ontario isolates of *Sclerotinia homoeocarpa*. *European Journal of Plant Pathology* **103**, 409–16.

Hsiang T, Yang L, Barton W, 1998. Relative virulence of isolates of *Sclerotinia homoeocarpa* with varying sensitivity to propiconazole. *European Journal of Plant Pathology* **104**, 163–9.

Jo Y, Niver A, Rimelspach J, Boehm M, 2006. Fungicide sensitivity of *Sclerotinia homoeocarpa* isolates from golf courses in Ohio. *Plant Disease* **90**, 807–13.

Karaoglanidis GS, Ioannidis PM, Thanassoulopoulous CC, 2002. Changes in sensitivity of *Cercospora beticola* populations to sterol-demethylation-inhibiting fungicides during a 4-year period in northern Greece. *Plant Pathology* **51**, 55–62.

Ma Z, Michailides TJ, 2005. Advances in understanding molecular mechanisms of fungicide resistance and molecular detection of resistant genotypes in phytopathogenic fungi. *Crop Protection* **24**, 853–63.

Miller GL, Stevenson KL, Burpee LL, 2002. Sensitivity of *Sclerotinia homoeocarpa* isolates to propiconazole and impact on control of dollar spot. *Plant Disease* **86**, 1240–6.

Siegel MR, 1981. Sterol-inhibiting fungicides: effects on sterol biosynthesis and sites of action. *Plant Disease* **65**, 986–9.

Smiley RW, Dernoeden PH, Clarke BB, eds, 2005. *Compendium of Turfgrass Diseases*, 3rd Edition. St. Paul, MN, USA: APS Press.

Vargas JM, Golembiewski RC, Detweiler AR, 1992. Dollar spot resistance to DMI fungicides. *Golf Course Management* **60**, 50–4.

Vincelli P, Powell AJ, 2006. *Chemical Control of Turfgrass Diseases 2006*. Kentucky, USA: University of Kentucky Cooperative Extension Service: Publication PPA-1.