GERALD L. MILLER JR.
Sensitivity of *Sclerotinia homoeocarpa* Isolates from Georgia to Propiconazole and other DMI Fungicides
(Under Direction of KATHERINE STEVENSON and LEE BURPEE)

Four hundred and forty-one isolates from six populations of *Sclerotinia homoeocarpa* were collected from creeping bentgrass golf greens in Georgia, and tested for sensitivity to the demethylation inhibitor (DMI) fungicide, propiconazole. Mean ED_{50} values for an unexposed population (baseline) and an exposed population were 0.0049 and 0.0283 μg ml^{-1}propiconazole, respectively. Mean relative mycelial growth on two discriminatory concentrations was significantly (P=0.05) greater for isolates from the populations that had been exposed repeatedly to propiconazole than for isolates from the four unexposed populations. The effective period of control decreased linearly with increasing log ED_{50} value, ranging from a mean of 12.2 days for an isolate with an ED_{50} value of 0.075 μg propiconazole ml^{-1} to 28.2 days for an isolate with an ED_{50} value of 0.005 μg propiconazole ml^{-1}. Based on this study, decreased in vitro sensitivity to propiconazole resulted in reduced dollar spot control.

INDEX WORDS: Fungicide Resistance, Sterol Inhibitors, Fungicide Efficacy, Dollar Spot, Turfgrass

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by

GERALD L. MILLER JR.

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GERALD L. MILLER JR.

Major Professors: Dr. Katherine Stevenson
Dr. Lee Burpee

Committee: Dr. Ed Brown
Dr. Barry Cunfer

Electronic Version Approved:

Gordhan L. Patel
Dean of the Graduate School
The University of Georgia
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DEDICATION

This document is dedicated to the three greatest gifts that God has granted me. To Robin, Mom, and Dad, my greatest level of success in life is linked to making you proud. I love you like crazy, and will continue to put my best foot forward.

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I would like first to give thanks to God for all of the advantages I have gotten throughout life that have allowed me to attain this goal. To Robin, my family, and friends, I thank you for your love, patience, and undying support. I also would like to thank my committee, especially Dr. Stevenson and Dr. Burpee, for their instruction and guidance, and for believing in this project, and in me. Lastly, I would like to extend my gratitude to the entire Plant Pathology Department at The University of Georgia. My development in this profession is forever linked to the instruction and interactions I have had with the faculty and graduate students here at Georgia.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Turfgrass Usage and Disease Impact in Georgia

Turfgrass culture represents an important economic component of horticulture in the United States where annual expenditures for turfgrass management exceeded $25 billion between 1982 and 1993 (3). In Georgia, there are approximately1.6 million acres of turfgrass with a maintenance value of $1.56 billion (9). This turf consists of more than ten species grown for use in home lawns, parks, golf courses, and other athletic facilities.

With a wide variety of climatic conditions, the choice of appropriate turf species in Georgia differs by geographic region. Warm season grasses, such as bermudagrass (*Cynodon dactylon* (L.) Pers.), St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntze.), and zoysia (*Zoysia japonica* Steud.), are used in several regions of the state and almost exclusively in the coastal plain, due to the high heat and humidity in the summer months. Cool season grasses, on the other hand, are more common in the piedmont and mountain regions of the state. These grasses, which include tall fescue (*Festuca arundinacea* Schreb.), creeping bentgrass (*Agrostis palustris* Huds.), and perennial ryegrass (*Lolium perenne* L.), are more difficult to manage effectively during extended periods of high temperatures (2). Approximately 600 acres of creeping bentgrass, which has a high canopy density and an ability to withstand low mowing heights (63), are being managed for use as golf putting greens throughout the mountain and piedmont regions of Georgia (42). Several cultivars exhibit heat tolerance; however, the warm, humid climate of Georgia still causes significant stress on bentgrass, making management difficult.

Due to a warm, humid climate, many pathogens can flourish and cause serious aesthetic and functional losses of turfgrass in the southeastern U.S. In Georgia, these

losses plus the cost for disease control practices was more than $190 million in 1999, with foliar diseases accounting for over $43 million of the total (9).

Symptoms of Dollar Spot and Taxonomy of *Sclerotinia homoeocarpa*

Dollar spot, caused by *Sclerotinia homoeocarpa* F.T. Bennett (4), is the most common turfgrass disease in North America (15). On golf courses, dollar spot can result in considerable reduction in quality of intensively managed turfgrasses, such as creeping bentgrass, bermudagrass, and perennial ryegrass. The disease can also affect a wide variety of other grasses (Table 1.1) used in residential lawns, recreational facilities, or other landscape areas (32,66). On closely mown creeping bentgrass swards, dollar spot is first observed as white to straw-colored spots, 1-2 cm in diameter, consisting of diseased leaf blades and sheaths (57). Tan colored or bleached lesions may be observed on leaf blades at the edges of these spots. If left unchecked, these spots increase in diameter (5 cm), and appear crater-like in the turf canopy. Where many infection centers occur, they can coalesce to affect large areas of turf (54,57).

Sclerotinia homoeocarpa is an inoperculate discomycete in the order *Helatioles* and family *Sclerotiniaceae*, although the taxonomy and nomenclature of this fungus is currently under debate (66). The teleomorph is rare in nature but was characterized as apothecia that arise from microsclerotia or expansive sclerotial flakes or patches (4). These microsclerotia led Bennett (4) to classify the fungus as a *Sclerotinia* species, based on the broad definition of the genus at that time (66). However, the 'sclerotial flakes' or 'microsclerotia' that Bennett described may be better interpreted as darkly pigmented stromal structures (57). It was later concluded that *S. homoeocarpa* does not fit the genus

concept of *Sclerotinia*, and should be classified more appropriately in *Lanzia* Sacc. or *Moellerodiscus* Henn (36). It has also been suggested that dollar spot symptoms are caused by more than one species, in which case, the correct term would be "dollar spot syndrome" (38,54). Due to the lack of fertile apothecia for study, the proper name and classification of this fungus remains unclear (66). Therefore, at the present time the name of the causal agent(s) of dollar spot remains as *S. homoeocarpa*.

Disease Cycle and Epidemiology

Hyphae of *S. homoeocarpa* infect through wounds and stomata on leaf blades, but direct penetration has also been observed (23). The pathogen does not infect roots, but culture filtrates of *S. homoeocarpa* contain a metabolite that is toxic to creeping bentgrass roots. Roots affected by the toxin cease elongating, become thicker, and show a decrease in root hair formation. An increase in the development of adventitious roots has also been observed (44).

Incidence and severity of dollar spot fluctuates in Georgia, with most epidemics occurring in late spring or early summer, and in the fall. The pathogen is thought to overwinter as stroma formed on the margins of dollar spot lesions (15,54), or as dormant mycelium in infected tissue (25). Local distribution of dollar spot occurs when mycelium grows from a diseased leaf to a healthy leaf in close proximity (66). Transport of infected plant parts on implements and footwear serves to spread the pathogen on a larger scale (57).

Once introduced into a turfgrass sward, the pathogen is persistent in the thatch layer, and when favorable conditions are present the disease will develop. Disease can be

initiated at temperatures from 15 ºC to 27 ºC, but *S. homoeocarpa* is most virulent or aggressive when temperatures are between 21 ºC and 27 ºC and atmospheric humidity is >85% (15). The amount and duration of the leaf wetness/dew period is also an important factor determining the severity of a dollar spot epidemic (70).

Stress predisposes turf to dollar spot, and infection is more likely and severe when turf is inadequately fertilized or under moisture stress (30). Since *S. homoeocarpa* can more easily infect a weak or stressed plant, plant health management is an important method of limiting disease incidence (66). Turfgrass suffering from moisture stress in greenhouse studies was more susceptible to *S. homoeocarpa* than irrigated turf (16), implying that disease may be worse on under-irrigated turf or during dry seasons. Adequate fertilization with nitrogen (N) decreases dollar spot severity (30,70), but a proper balance must be maintained between adequate and inadequate N fertility. Over application of nitrogen can enhance other turfgrass diseases and increase thatch accumulation (25).

Disease Management

As a result of the persistent nature of the pathogen, more money is spent on the management of dollar spot than any other turfgrass disease on golf courses (32). Golf course superintendents utilize several methods for dollar spot control. Host resistance is not particularly useful because highly resistant cultivars are not available, although some cultivars of creeping bentgrass are less susceptible to *S. homoeocarpa* and may recover from dollar spot symptoms more quickly than others (13). Blending of susceptible and resistant cultivars does not provide as much disease reduction as a monostand of a

resistant cultivar, but does reduce dollar spot severity as compared to a monostand of a susceptible cultivar (1). Currently, selection of a cultivar is based on agronomic qualities rather than resistance to *S. homoeocarpa* or, in most cases, golf superintendents simply inherit the bentgrass that had been established previously.

Several cultural practices can limit the incidence of dollar spot. The combination of high rates of N with applications of the growth regulator trinexapac-ethyl (Primo, Syngenta Corp., Greensboro, NC) results in dollar spot suppression without increasing the mowing frequency necessary for maintaining creeping bentgrass (30). In addition, removing early morning dew with poling or mowing practices reduced dollar spot incidence by as much as 53% on putting green turf by minimizing the duration of the dew period (70). Lowering humidity in the turf canopy by increasing air circulation is also a recommended practice, and can be achieved by using fans or by pruning and removing trees and shrubs (70).

Several biological control strategies for dollar spot have also been investigated. One approach to facilitate biocontrol is the addition of composts or other carbon sources to enhance antagonistic microbial activity. The addition of sludge or turkey litter composts can suppress dollar spot severity, but the effects have been short-lived and highly variable (45). Another approach is the inundative application of specific bacteria and fungi known to suppress disease (66). Some biocontrol organisms that show promise are *Fusarium heterosporum* (32), *Pseudomonas aureofaciens* (TX-1) (49), *Trichoderma harzianum* strain T22 (43), and hypovirulent isolates of *S. homoeocarpa* (73). Of these, *T. harzianum* T22 is the first and only biological fungicide registered for turf in the United States, but only for use as a preventative measure (66). Efficacy studies of this

product, however, have yielded inconsistent results (43,65). Even though much research has been done, biocontrol must still be considered an emerging technology with several microbial agents in the early stages of development (66).

In golf turf management, cultural practices alone do not provide adequate control of most pathogens. This, combined with the lack of host resistance and the relative infancy of biological control, make regular fungicide applications the most effective and necessary tool for dollar spot management. Results with fungicides are generally predictable, and applications can be made when disease is present (curative) or forthcoming (preventative) (70).

Currently, 14 compounds, representing six chemically unrelated groups, are registered for control of dollar spot in the United States (Table 1.2). These fungicides include contact non-systemic chemicals such as chlorothalonil and PCNB, as well as systemic compounds in the chemical groups dicarboximides, benzimidazoles, and demethylation inhibitors (17,62). It is often necessary for golf course superintendents to apply these fungicides every 14-21 days throughout the growing season to maintain disease-free turf. These frequent applications may result in the selective dominance of resistant strains of pathogens (64), a growing problem in the management of turf disease.

Fungicide Resistance

Fungicide resistance is defined as "the stable, inheritable adjustment of a fungal cell or a fungal population to a fungicide, resulting in a less than normal sensitivity to that fungicide" (19). Application of a fungicide at frequent intervals may select for resistant strains causing the population to become less sensitive. When a high proportion of the

pathogen population becomes resistant, disease control failure occurs. Most problems caused by fungicide resistance have occurred in the past three decades with the advent of selective fungicides. These compounds act systemically in the plant, and inhibit specific sites of fungal metabolism (19). The site-specific mechanism of action is probably the predominant factor that determines the greater risk of resistance associated with these fungicides (7).

A fungal isolate can acquire fungicide resistance either in one step, due to mutation of a major gene, or in multisteps, by the interaction of several mutant genes, each with a small individual effect (29). A resistant pathogen population that develops as the result of a major gene mutation is described as 'qualitative', 'single-step', 'discrete', 'disruptive', or 'discontinuous' resistance, and is characterized by a sudden and marked loss of fungicide efficacy (6). Fungicide concentrations that are 100% effective on the sensitive population often have no effect on the resistant population, making a distinct and separate subpopulation. The fungicide provides a selective advantage for the resistant strains, and this subpopulation increases in proportion to the sensitive strains. The population takes a sudden discontinuous shift towards resistance when the selected resistant subpopulation becomes predominant, resulting in rapid and total loss of fungicide efficacy (29). Because of this, fungicides that can be overcome by a single gene mutation are usually classified in a "high risk" category (7).

In the second type of fungicide resistance, described as 'quantitative', 'multi-step', 'continuous', 'directional' or 'progressive' resistance, the effect of a single mutation in individual phenotypes is generally small (6,29). Because of this, it is not possible to clearly distinguish sensitive from resistant populations. Dose-response curves

from field populations are generally continuous, reflecting the presence of many phenotypic levels of sensitivity (18). In contrast with single-step resistance, resistant individuals generally take longer to dominate the population and cause loss of fungicide efficacy. In addition, the quantitative response obtained with polygenic control provides an indication of reduced performance before complete failure (29). Therefore, fungicides that are only overcome by several gene mutations are usually categorized as "low or moderate risk" fungicides (7).

Factors other than fungicide characteristics and mutation type can influence the amount of resistance risk associated with a fungicide. Disease-associated risk is related to several factors of disease epidemiology that affect the chance of mutation. These factors include the frequency and type (sexual or asexual) of the pathogen's reproductive cycle, abundance of sporulation, and the isolation of pathogen populations (7). The rate, timing and number of fungicide applications can also influence risk. Any reduction in pathogen exposure to the fungicide through the use of different chemistries, or other integrated disease management strategies, lowers the risk of resistance development (6).

History of Fungicide Resistance in *S. homoeocarpa*

From the 1940s to the mid-1960s, heavy metals such as cadmium and mercury were the active ingredients of fungicides that provided excellent control of dollar spot (25). Differing tolerances to cadmium-based fungicides among isolates of *S. homoeocarpa* were first reported in 1967, and by the late 1960s tolerance to the mercuric fungicides was also widespread (14,25,57). The cadmium-resistant populations persisted,

and once resistance developed, it continued even if the fungicides were removed from the management program (68).

As effectiveness of the heavy metal fungicides decreased, a new and different chemical class, the benzimidazoles, became available and rapidly took the place of the heavy metal fungicides. The benzimidazoles were the first systemic fungicides used on turf and provided excellent control of dollar spot at low dosages with extended application intervals (57). Through a single gene mutation that involved altering the ß-tubulin target site of the fungicide (6), benomyl resistant populations of *S. homoeocarpa* developed rapidly after only a few years of use (55,60,67). To compound the problem, isolates of *S. homoeocarpa* resistant to one benzimidazole, such as benomyl, were also resistant to other members of the benzimidazoles due to a phenomenon called cross-resistance. Like cadmium resistance, the benzimidazole resistant population of *S. homoeocarpa* has been persistent. Benzimidazole resistance has become so widespread in *S. homoeocarpa* populations that it has been suggested that the typical wild type strain of the fungus now has resistance to benomyl, thiophanate-methyl, and thiophanate-ethyl (64).

In the mid-1970s, the dicarboximides vinclozolin and iprodione were registered for use on turf for dollar spot control. These fungicides were used in spray programs designed to delay benzimidazole resistance or to control dollar spot once benzimidazole resistance became a problem (21). Little is known about the mechanism of action of the dicarboximides, but, like the benzimidazoles, resistance problems were encountered for these fungicides about five years after introduction (12,21). A single mutation in a protein kinase gene of *Ustilago maydis* laboratory mutants has conferred resistance to vinclozolin

(46), although this mutation has not been confirmed in other pathogens or resistant populations from the field. Unlike resistance to benzimidazoles, resistance of *S. homoeocarpa* to dicarboximides has not been as widespread and has tended to disappear once the fungicides were no longer used, presumably due to a fitness cost associated with the mutation (6,50). However, with high disease pressure and prolonged use, development of resistant strains still led to reduced performance of the fungicides (59). Some dicarboximide-resistant strains of *S. homoeocarpa* were also found to be resistant to the benzimidazoles, making these strains increasingly difficult to control (21).

DMI Fungicides

In 1979, the sterol demethylation inhibiting (DMI) fungicides became available for dollar spot control in the United States (31). The DMIs consist of the triazoles (largest group), imidazoles, piperazines, pyrimidines, and purines, and comprise a subgroup of a larger class of sterol-inhibiting fungicides (6,53). These compounds bind and inhibit cytochrome P_{450}, a catalyst needed for the oxidative demethylation of eburicol in the biosynthesis of ergosterol, (37,47,60), which is essential in all filamentous fungi (39).

The DMIs represent one third of all of the agricultural fungicides currently in use (41). This popularity stems from several advantages that these fungicides offer, including both protectant and sometimes curative means of plant disease control. In addition, DMIs provide a broad spectrum of fungicidal activity, often controlling several diseases that affect the same crop. They also can be used at lower application rates with greater application intervals compared to several other groups of fungicides (40). This is

especially important to golf course superintendents in northern areas of the United States, who need to treat more extensive areas of turf, such as fairways, and may have limited budgets for disease control (10). Equally important is the ability of the DMIs to control benzimidazole- and dicarboximide-resistant strains of *S. homoeocarpa* and other fungal pathogens.

Decreased DMI sensitivity in pathogens from a variety of crops was first reported approximately 11 years after introduction of the DMIs to the field (6). Examples of the pathogens are *Venturia inaequalis* on apple, *Monilinia fructicola* on peach, *Blumeria graminis* on cereals, *Penicillium digitatum* and *italicum* on citrus, and *Uncinula necator* on grape (35,51,58,71,72). Resistance to the DMIs is manifested as a gradual decrease in sensitivity rather than total control failure as observed in the benzimidazoles and dicarboximides. This is indicative of a quantitative response where more than one gene must undergo mutation in the fungus to decrease sensitivity. Although some cases of monogenic resistance have been found in *Nectria haematococca* var. *cucurbitae* and *Drechslera teres* (20,48), most cases of DMI resistance have been found to be polygenic (37,60). The DMIs have been categorized as "moderate" for risk of fungicide resistance development because of the mostly polygenic nature of resistance combined with a single site of action (7). However, because of the lack of a single genetic model for DMI resistance, resistance information should be evaluated separately for each target pathogen (5,48).

Several biochemical mechanisms have been implicated in DMI resistance, but have not been confirmed in resistant field populations. These include different alterations in the sterol biosynthesis pathway, and alteration in the intercellular concentrations of

DMIs, either as a result of poor penetration across the fungal membrane or due to active efflux systems (11,37,69). These mechanisms, when present as a single molecular event, afford only modest levels of resistance, but when two or more mechanisms are present, problems in disease control are likely to arise (37).

DMI sensitivity is routinely monitored by first establishing a "baseline" sensitivity distribution for a pathogen population that has had no previous exposure to these fungicides. Sensitivity is often expressed as an "effective dose" or ED_{50} value, the fungicide concentration at which growth or development is suppressed by 50% relative to a control. ED_{50} values are determined for each isolate in a sample from this baseline population, based on spore germination, germ tube growth, or mycelial growth as inhibited by a range of fungicide concentrations. The distribution of the baseline ED_{50} values can be used as a basis for comparison of populations and for detecting sensitivity shifts in populations. The difference in sensitivity between populations can be quantified by calculating a resistance factor (RF), expressed as the ratio: mean ED_{50} (resistant population)/mean ED_{50} (sensitive population) (55). Once a baseline has been established, monitoring programs based on the mean relative growth at a single discriminatory fungicide concentration can be used to detect shifts in other populations. This single concentration, instead of the 7-12 concentrations required for calculating ED_{50} values, reduces time and labor required to test isolates and allows for a larger set of isolates to be evaluated (26,55).

DMI Resistance in *S. homoeocarpa*

Using mycelial growth assays to estimate sensitivity, reduced sensitivity to DMIs in *S. homoeocarpa* isolates has been reported from several areas of North America. In Michigan and Ohio, Golembiewski et al. (31) found that the mean ED_{50} value for isolates of *S. homoeocarpa* where DMIs had never been used was 0.002 μg ml^{-1} for propiconazole and 0.03 μg ml^{-1} for fenarimol, compared to ED_{50} values of 0.103 μg ml^{-1} for propiconazole and 0.078 μg ml^{-1} for fenarimol in a population where control failure was observed (31). Resistance factors of 1.8 to ≥10 have been associated with unsatisfactory field control in other crops (55), but in this study the mean resistance factor was 51.5 (31).

In Ontario, Hsiang et al., (36), reported a mean ED_{50} value for five unexposed populations of *S. homoeocarpa* for propiconazole of 0.007 μg ml^{-1} and a mean ED_{50} value of 0.026 μg ml^{-1} for one population with reduced sensitivity. Disease control failure was not observed at the location where the less sensitive population was sampled. This population had a lower mean resistance factor, 3.7, than Michigan populations where control failure was reported (31,36).

In Kentucky, Doney and Vincelli (22), sampled three isolates from two golf courses where triadimefon had been used extensively and DMI resistance was suspected. Based on ED_{50} values, these isolates were significantly ($P \leq 0.05$) less sensitive to triadimefon, cyproconazole, fenarimol, propiconazole, and tebuconazole than two control isolates.

The DMI compounds, triadimefon, myclobutanil, fenarimol, cyproconazole, and propiconazole, are currently labeled for use on dollar spot in the United States. There are

conflicting accounts of cross-resistance among these DMI fungicides in *S. homoeocarpa* populations (22,31,36). Golembiewski et al. (31) showed that ED_{50} values of triadimefon, fenarimol, and propiconazole were highly correlated, and Doney and Vincelli (22) stated that *S. homoeocarpa* can develop cross resistance to all the registered DMI fungicides, including those to which it had not been exposed. Hsiang et al. (36) reported the highest correlation ($r = 0.438$, $P = 0.0001$) between ED_{50} values of myclobutanil and propiconazole, with the second highest correlation ($r = 0.250$, $P = 0.0001$) being between ED_{50} values of tebuconazole and fenarimol (31). In other pathogens, cross-resistance between some DMIs has been shown to be quite common (52,61). However, cross resistance between triazoles such as triadimenol and propiconazole, has not been observed with other triazoles, such as tebuconazole (34), implying some triazoles may be effective for control of resistant strains.

Some strains of *S. homoeocarpa* resistant to the DMI fungicides have been found to be resistant to benzimidazole and dicarboximide fungicides (31,64). These multiple resistant strains severely limit the chemical control options of golf course superintendents and may pose the most difficult resistance control problem turfgrass managers and pathologists have faced to date (64).

Fungicide Resistance Management

In 1998, the Fungicide Resistance Action Committee (FRAC) proposed several recommendations for preventing or delaying the onset of DMI resistance in plant pathogens (28). One recommendation was to avoid repeated application of DMIs on the same crop in one season against a high-risk pathogen in areas of high disease pressure for

that particular pathogen. Secondly, it was recommended to alternate or mix DMIs with an effective non cross-resistant fungicide if repeated spray applications are needed during a season, or if sensitivity testing has confirmed the presence of less sensitive forms of the pathogen. If alternation or tank mixing was not feasible because of lack of effective or compatible partner fungicides, DMI applications were to be reserved for critical parts of growing season. Other recommendations include adhering to manufacturers' recommendations for use of the fungicide, using disease resistant varieties where applicable, and applying good agronomic practices (e.g. sanitation) (28).

Currently, efforts to manage and delay the onset or severity of resistance of *S. homoeocarpa* to the DMI fungicides are being applied in areas under intense disease pressure. Superintendents, under the direction of university turf pathologists currently researching the problem, and in accordance with FRAC guidelines, have been advised to avoid use of DMIs during periods of severe dollar spot pressure, use cultural practices (i.e. poling or removing early morning dew) to reduce disease pressure, and alternate or tank mix DMI fungicides with either contact fungicides, benzimidazoles, or dicarboximides. In alternating and tank mixing, the hope is that other chemistries will help to reduce the number of DMI applications needed per season and therefore reduce selection pressure on the pathogen (64).

Research Objectives

Knowledge of the distribution and impact of fungicide resistant pathogens relies on the monitoring and testing of isolates from field populations for sensitivity to one or more fungicides. Due to the quantitative nature of DMI resistance, sensitivity monitoring

can give an early warning of an impending loss of fungicide efficacy. Sensitivity monitoring also provides valuable information for evaluating the effectiveness of fungicide resistance management strategies, investigating grower complaints of apparent loss of fungicide efficacy, and confirming cases of actual resistance in the field (6).

In Georgia, little is known about the sensitivity of *S. homoeocarpa* populations to the DMI fungicides. In general, use of the DMI fungicides to control dollar spot in the southeastern U.S. is limited to golf course greens where susceptible creeping bentgrass has been established. Disease control failure on bentgrass greens has recently been observed by several superintendents at golf courses with a history of extensive DMI usage. These failures have taken the form of a reduction in the duration of dollar spot control at label rates of fungicide.

This research was initiated with the following specific objectives:

1. Establish the baseline distribution of sensitivity to propiconazole in a *S. homoeocarpa* population from Georgia with no history of exposure to DMI fungicides and compare this distribution to that of a population with a history of extensive DMI use.
2. Compare sensitivity to propiconazole among six populations of *S. homoeocarpa* from bentgrass greens with different histories of exposure to DMI fungicides.
3. Determine the relationship between fungicide sensitivity of *S. homoeocarpa* in vitro and control of dollar spot with fungicide in inoculated bentgrass.
4. Evaluate cross-sensitivity in *S. homoeocarpa* to other DMI fungicides and dual resistance between DMI and benzimidazole fungicides.

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Table 1.1 Turfgrass hosts of *Sclerotinia homoeocarpa*[a]

Latin binomial	Common name	Reference
Agrostis sp.	Bentgrass	(4)
Cynodon sp.	Bermudagrass	(2,8,24,27)
Eremochloa ophiuroides	Centipedegrass	(2,8,15)
Festuca sp.	Fescue	(4)
Holcus lanatus	Common velvetgrass	(15,56)
Lolium multiflorum	Italian ryegrass	(8,27)
Lolium perenne	Perennial ryegrass	(70)
Paspalum notatum	Bahia grass	(2,8,15,24,27)
Poa sp.	Bluegrass	(4)
Puccinellia maritima	Seaside alkali-grass	(33)
Stenotaphrum secundatum	St. Augustinegrass	(2,8,15)
Zoysia sp.	Zoysia grass	(2,8,15)

[a] From Walsh et al. 1999 (66).

Table 1.2. Fungicides registered for use on dollar spot in the United States [a]

Active ingredient	Trade name(s)	Fungicide type	Chemical class
Chlorothalonil	Daconil TwoSome Manicure Thalonil	Contact	Nitrile
PCNB	Defend Engage Revere Terraclor Turfside	Contact	Aromatic Hydrocarbon
Mancozeb	Dithane Mancozeb Fore	Contact	Dithiocarbamate
Thiram	Spotrete	Contact	Dithiocarbamate
Thiophanate	3336 WP	Systemic	Benzimidazole
Thiophanate-methyl	Cleary 3336 Cavalier Scotts	Systemic	Benzimidazole
Iprodione	Chipco Flo Scotts	Systemic	Dicarboximide
Vinclozolin	Curalan Touche Vorlan	Systemic	Dicarboximide
Flutolanil	ProStar	Systemic	Carboxamide
Myclobutanil	Eagle	Systemic	DMI
Fenarimol	Rubigan	Systemic	DMI
Triadimefon	Bayleton	Systemic	DMI
Cyproconazole	Sentinel	Systemic	DMI
Propiconazole	Banner	Systemic	DMI

[a] Cited from Turf and Ornamental Reference for Plant Protection Products (62) and Danneberger (17).

CHAPTER 2

SENSITIVITY OF GEORGIA POPULATIONS OF *SCLEROTINIA HOMOEOCARPA* TO PROPICONAZOLE[1]

[1]Miller, G., K. L. Stevenson, and L. L. Burpee. 2001. To be submitted to Plant Disease.

ABSTRACT

Miller, G., Stevenson, K. L., and Burpee, L.L. 2001. Sensitivity of Georgia populations of *Sclerotinia homoeocarpa* to propiconazole. To be submitted to Plant Disease.

Four hundred and forty-one isolates from six populations of *Sclerotinia homoeocarpa* were collected from creeping bentgrass golf greens in Georgia and tested for sensitivity to the demethylation inhibitor (DMI) fungicide propiconazole. Mean ED_{50} values for an unexposed population (baseline) and an exposed population were 0.0049 and 0.0283 μg ml^{-1}, respectively. Based on these values, two discriminatory concentrations of propiconazole (0.02 and 0.2 μg ml^{-1}) were chosen and used to compare isolates from all six populations. Mean relative mycelial growth on these concentrations was significantly ($P \leq 0.05$) greater for isolates from two populations that had been exposed repeatedly to propiconazole than for isolates from four unexposed populations. Additionally, sensitivity to triadimefon, fenarimol, myclobutanil, and thiophanate-methyl was estimated for a subset of 50 isolates with ED_{50} values for propiconazole ranging from 0.001 to 0.057 μg ml^{-1}. Correlation analysis of ED_{50} values for the different fungicides showed a moderate, but statistically significant, positive correlation between propiconazole and myclobutanil ($r = 0.635$ and $P < 0.0001$), fenarimol and myclobutanil ($r = 0.623$ and $P < 0.0001$), and propiconazole and fenarimol ($r = 0.437$ and $P = 0.0015$). Pairwise correlations with triadimefon were not significant, providing evidence that cross resistance in field populations of *S. homoeocarpa* may not exist among all DMI fungicides.

Introduction

Dollar spot, caused by *Sclerotinia homoeocarpa* F.T. Bennett, is the most common turfgrass disease in North America (4), causing considerable reduction in quality of high amenity turfgrasses. In Georgia, the disease is most severe and economically damaging on creeping bentgrass (*Agrostis palustris* Huds) managed for golf putting greens, with most epidemics occurring in late spring, early summer, and fall. Since cultural practices alone are not sufficient to control the disease on bentgrass (17), and disease thresholds on golf courses are particularly low, fungicides are applied preventatively, usually on a 2-week schedule, when conditions are favorable for the disease (13). This intensive fungicide use can result in selection for resistant strains in pathogen populations (8,14), and a decrease in fungicide efficacy over time.

The sterol demethylation inhibitor (DMI) fungicides, available for dollar spot control in the U. S. since 1979 (8), offer several advantages. These fungicides provide a broad spectrum of activity, controlling many important turfgrass diseases with a single application. In addition, the DMIs can be used at low application rates and at wide application intervals because of their systemic activity (10). Equally important is the ability of the DMIs to control strains of *S. homoeocarpa* resistant to the benzimidazole (16) and dicarboximide (5) fungicides.

In vitro mycelial growth assays have been used to detect resistance to the DMI fungicides in field populations of *S. homoeocarpa* in Illinois, Kentucky, and Michigan, at sites where control failure had been observed (6, 8,14). Similar assays were used to detect reduced sensitivity to the DMIs in a population in Ontario, Canada, at a site where field resistance to the DMIs had not been observed (9). Reports such as these, indicate

the need for confirmation of in vivo sensitivity testing with in planta loss of fungicide efficacy prior to confirming a fungicide resistance problem.

Little is known about the sensitivity of *S. homoeocarpa* populations in Georgia to the DMI fungicides. DMI use on turf in the southeastern U.S. is generally limited to golf course greens where susceptible creeping bentgrass has been established. Recently, a reduction in fungicide efficacy on bentgrass greens has been observed at golf courses with a history of extensive DMI usage. These failures have taken the form of significantly shorter intervals of dollar spot control on golf greens treated with propiconazole than the intervals listed on the fungicide label. The research reported here was initiated to investigate these reports. The objectives of this research were to (i) establish a baseline sensitivity for monitoring propiconazole sensitivity of *S. homoeocarpa* populations in Georgia, (ii) compare propiconazole sensitivity in six populations of *S. homoeocarpa* in Georgia with differing histories of exposure to the fungicide, and (iii) evaluate the potential for cross-sensitivity in *S. homoeocarpa* to other DMI fungicides and dual resistance in the benzimidazoles.

Materials and Methods

Sampling locations and fungal isolations. Isolates of *S. homoeocarpa* were collected from diseased creeping bentgrass (*Agrostis palustris* Huds.) putting greens exhibiting dollar spot symptoms at six golf courses across northern Georgia in the fall of 1999 and spring and fall of 2000. A representative sample of at least 50 isolates was collected from each location. DMI fungicide use varied among the sampling locations (Table 2.1), with four of the sites having no documented history of exposure to DMIs and

two of the sites having a history of DMI exposure. Population AAC had been exposed to propiconazole (Banner Maxx EC, Syngenta Crop Protection, Greensboro, NC) at 0.025 kg a.i. ha^{-1} twice a year for a minimum of 6 years prior to sampling. Population CCC had been exposed to the same rate of propiconazole at least twice a year for a minimum of 4 years prior to sampling.

Each isolate was obtained from a single dollar spot, and in heavy infestations (>5 spots/m^2), one isolate was collected per 30 cm^2. Individual leaf blades were removed from the margins of an infection center, surface sterilized for 30 sec in a 70% ethanol solution and a 0.5% sodium hypochlorite solution, rinsed once in sterile water, and placed in Petri dishes (9-cm diameter) containing potato dextrose agar (PDA, Difco Laboratories, Detroit). The Petri dishes were sealed with Parafilm and incubated at room temperature (25ºC) for three days. The pathogen was identified by visual comparison of colony growth with known cultures of *S. homoeocarpa* (3,9). Mycelial plugs from each isolate were placed on sterilized rye grains, allowed to grow at 25ºC for 21 days, and frozen at -20ºC until needed. Two isolates, S084 and S088, with known *in vitro* sensitivities and phenotypic levels of resistance to the DMI and benzimidazole fungicides (1), were used as standards in each of the experiments (Table 2.2).

Baseline sensitivity determination. The sensitivities of 59 isolates of *S. homoeocarpa* from a population (HMGC) not exposed to propiconazole and 69 isolates from a population (CCC) exposed to propiconazole were determined using a modification of the mycelial growth assay (12). Hyphal plugs (6 mm diameter) from the edge of actively growing colonies on PDA were homogenized in 5 ml of sterile potato dextrose broth (PDB) to provide a uniform suspension of mycelial fragments. Petri dishes (9-cm

diameter) of PDA amended with propiconazole at a concentration of 0, 0.0002, 0.0006, 0.002, 0.006, 0.02, 0.06, 0.2, 0.6, 2, 6, or 20 μg a.i. ml^{-1} were prepared. Technical grade propiconazole (97.2% a.i., Syngenta Crop Protection, Greensboro, NC) was dissolved in acetone to make fungicide concentrations. Fungicide solutions were added to autoclaved PDA cooled to 60 ºC, such that the final concentration of acetone was 0.1% (v/v) in all treatments, including the non-amended control. Acetone at this concentration does not inhibit growth of *S. homoeocarpa* (9). A 5-mm diameter plug of agar was removed from the center of each dish to form a well in the solidified medium. Fifty microliters of the mycelial suspension of each isolate was transferred to the well in each of 2 replicate plates per fungicide concentration.

Cultures were incubated at room temperature (25ºC), and radial growth of colonies was measured when colony diameters of controls reached at least 50 mm (3-4 days of incubation). The diameter of each colony was measured in two perpendicular directions and the mean diameter was adjusted by subtracting the diameter of the well. Relative growth [RG = (the mean adjusted colony diameter on propiconazole-amended medium divided by the mean adjusted colony diameter on non-amended medium) X 100%] was determined for each isolate and fungicide concentration.

The ED_{50} value for each replication of each isolate was estimated by linear regression of the probit-transformed relative inhibition value (RI = 1 - RG) on $\log_{10}$ – transformed fungicide concentration (9). The ED_{50} value for each isolate was calculated as the mean of the two replicates. A t-test was performed on the $\log_{10}$-transformed ED_{50} values of the two populations to test whether mean ED_{50} values were significantly different. The resistance factor (RF) was calculated for the exposed population by

dividing the mean of all isolates from the exposed population by the mean ED_{50} value of all isolates from the baseline population.

Based on the frequency distribution of the baseline sensitivity data, two discriminatory concentrations (0.02 and 0.2 μg ml^{-1}, technical grade propiconazole) were arbitrarily selected and used to evaluate differences in sensitivity among isolates representing the six *S. homoeocarpa* populations. To evaluate the accuracy of using these concentrations for this purpose, log ED_{50} values for each isolate from the baseline (HMGC) and exposed (CCC) populations were regressed on mean RG values obtained for each isolate at the two concentrations.

Assay reproducibility. To determine the reproducibility of the mycelial growth assay used for estimating propiconazole sensitivity, a test was conducted with methods described by Wong and Wilcox (18). Propiconazole sensitivity was assayed five times for five isolates that were chosen randomly from the pool of 441 isolates sampled. For each repeat of the test in which all five isolates were assayed, a new stock solution of propiconazole was prepared. The mean of the ED_{50} values, variance, coefficient of variance, and 95% confidence intervals were calculated for each isolate based upon the five repeated tests. Since the data were log-normally distributed, the formulas obtained from Casella and Berger (2) and Gilbert (7) were used to calculate these values (Table 2.3). A mean co-efficient of variance was calculated for both of the propiconazole assays based upon the results from these five isolates.

Comparison of isolates from six populations. Mycelial suspensions of each isolate were placed in a 5-mm-diameter well cut in PDA unamended or amended with the two discriminatory concentrations of propiconazole. Each isolate was tested on two

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

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

ED_{50} values of isolates resistant to the benzimidazoles are difficult to estimate since mycelial growth is not limited by high doses of the fungicide (4, 17). Therefore, sensitivity to thiophanate-methyl was assessed using only two concentrations of fungicide in amended PDA: 20 μg ml^{-1} and 60 μg ml^{-1}. Mycelial growth assays were conducted as

previously described, except that the technical grade active ingredient (thiophanate-methyl, Cleary's Corp. Somerset, NJ) was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St. Louis, MO) instead of acetone, but at the same final concentration of 0.10% (v/v). At this concentration, DMSO, like acetone, did not significantly affect mycelial growth (unpublished data). For each isolate, relative growth on each of the two concentrations of thiophanate-methyl was compared to the ED_{50} value for propiconazole by linear regression.

Results

Assay reproducibility. For the five isolates evaluated in five repeated runs of the assay, the coefficients of variance for each isolate ranged from 0.02% to 0.2%. The mean coefficient of variance for these assays was 0.09% (Table 2.4). The 95% confidence intervals ranged from 84% to 122% of the mean ED_{50} values for individual isolates subjected to the propiconazole assay. On average, the 95% confidence intervals ranged from 89% to 115% of the mean ED_{50} values for the propiconazole assay.

Baseline sensitivity. Using a modification of the mycelial growth assay commonly used for estimation of propiconazole sensitivity, ED_{50} values of 0.0056 and 0.0753 μg ml^{-1} were estimated for the standard isolates S084 and S088 respectively. These values are lower than sensitivity values for propiconazole reported by Burpee (1), (0.03 and 0.31 μg ml^{-1}), using a standard mycelial growth assay (Table 2.2).

The frequency distributions of ED_{50} values for the baseline (HMGC) and exposed (CCC) populations were log-normal. ED_{50} values for propiconazole for isolates from the baseline population ranged from 0.0006 to 0.0102 μg ml^{-1} with a mean of 0.0049 μg ml^{-1}

(Fig. 2.1). The range of ED_{50} values for isolates from the exposed population was wider (0.005 to 0.057 μg ml^{-1}) and the mean ED_{50} value (0.0283 μg ml^{-1}) was significantly greater ($P<0.0001$) than that of the unexposed isolates. The resistance factor (RF) for the exposed population was 5.8.

Comparison of isolates from six populations. A significant correlation (r = 0.903, $P<0.0001$) was detected between the log ED_{50} value of the baseline (HMGC) and exposed (CCC) populations and the mean RG of each isolate at 0.02 μg ml^{-1} (Fig. 2.2). At the discriminatory concentration of 0.2 μg ml^{-1}, a significant, but more variable relationship (r^2 = 0.26, $P<0.0001$) was detected between RG and log ED_{50} values presumably because a large proportion of isolates failed to grow at this concentration. However, mean RG values on both concentrations were significantly ($P\leq0.05$) greater for the two exposed populations than for the four unexposed populations (Table 2.5). Mean RG values for the two exposed populations, CCC and AAC, were significantly different at the 0.02 μg ml^{-1} concentration, but not at the 0.2 μg ml^{-1} concentration. In the unexposed populations, mean RG was significantly greater for population FGC and HMGC than for population TC and ML on both discriminatory concentrations (Table 2.5).

Evaluation of cross-sensitivity. For the DMI fungicides tested, correlation analysis of ED_{50} values from the subset of 50 isolates showed a significant positive correlation between propiconazole and myclobutanil, fenarimol and myclobutanil, and propiconazole and fenarimol (Fig. 2.3). There was no significant correlation between the sensitivities to propiconazole, myclobutanil, or fenarimol and the sensitivity of the isolates to triadimefon (Table 2.6).

Only one isolate out of the 50 isolates tested was inhibited completely by thiophanate-methyl at 20 and 60 μg ml^{-1}, with mycelial growth of the remaining isolates being unaffected by the fungicide concentration. This single isolate with sensitivity to thiophanate-methyl was from the baseline population.

Discussion

Reduced sensitivity to propiconazole was found in isolates of *S. homoeocarpa* that were collected from a site in Georgia (CCC) with a history of DMI use. The mean ED_{50} value of isolates sampled from this population was 0.0283 μg ml^{-1}. The RF (5.8) for this population was approximately 10 H lower than the mean RF (51.5) reported by Golembiewski et al. (8) for three Michigan populations of *S. homoeocarpa* that had been exposed to DMIs. The higher resistance factor for the Michigan populations is consistent with a greater intensity of dollar spot in northern states and a higher frequency of fungicide use (14).

Although population CCC exhibited reduced sensitivity to propiconazole, and a reduction in fungicide efficacy was observed, an in planta test is required to confirm field resistance. In Ontario, a population of *S. homoeocarpa* with reduced sensitivity to propiconazole had a mean ED_{50} value of 0.026 μg ml^{-1} and amean RF of 3.7 (9). The Ontario population was sampled from a site where propiconazole was not yet labeled for turf use, and no disease control failure had been observed. The site was close enough to the U.S. border to experience ingress of less sensitive isolates or the turf may have been exposed to non-labeled use of a DMI fungicide (9). Compared to the high mean RF

values (>50) found in Michigan, propiconazole sensitivity of population CCC may not be resistant enough to initiate an economic loss associated with fungicide control failure.

Further evidence that population CCC may not exhibit fungicide resistance in the field comes from our mycelial growth assay. An ED_{50} value of 0.073 μg propiconazole ml^{-1} was estimated for an isolate (S088) with known field resistance to propiconazole (1). This value is approximately 2 to 2.5 H higher than the mean ED_{50} value for population CCC, and only four isolates sampled from population CCC had a reduction in sensitivity close to the level of this resistant isolate.

The mean ED_{50} value of 0.005 μg ml^{-1} for the population not exposed to propiconazole was similar to mean ED_{50} values of 0.002 and 0.007 μg ml^{-1} for non-exposed populations reported by Golembiewski et al. (8) and Hsiang et al. (9) respectively,. This confirms that at the time of sampling, little, if any, selective change toward propiconazole insensitivity had occurred within population HMGC. Therefore, this population can act as a suitable baseline to which future populations of *S. homoeocarpa* from Georgia can be compared. In addition, the frequency distribution of sensitivities of isolates from the HMGC and CCC populations provides a basis for the selection of discriminatory doses that can be used to test other *S. homoeocarpa* populations for propiconazole sensitivity.

The predictive value for log ED_{50} values of populations HMGC and CCC was statistically greater for relative mycelial growth at the 0.02 μg ml^{-1} discriminatory concentration (r^2=0.815) than at the 0.2 μg ml^{-1} discriminatory concentration (r^2=0.26). This supports the use of 0.02 μg ml^{-1} for separation of propiconazole sensitivities among dollar spot populations, and for estimation of the mean ED_{50} value of a population with

the equation of the regression line (Y = -2.88 + 2.35x) shown in this report. However, mean relative growth was statistically greater (LSD, P≤0.05) at both concentrations for populations that had been exposed to propiconazole than for unexposed populations, showing a definitive difference in sensitivity to propiconazole in these populations.

There are conflicting accounts of the potential for cross-resistance among the DMI compounds currently labeled for use on dollar spot in the United States (6,8,9). Cross-resistance in the DMI fungicides was thought to be universal in *S. homoeocarpa* (6,8), but Hsiang et al. (9) found distinct groupings between propiconazole and myclobutanil (r=0.438), and tebuconazole and fenarimol (r=0.25), in Ontario populations. In this study, the strongest correlation was found between sensitivity to propiconazole and myclobutanil, which was consistent with the previous study (9). Weaker, but statistically significant correlations, were also found between fenarimol and myclobutanil and fenarimol and propiconazole, implying that resistance to all three of these fungicides may be linked. This result agrees with Köller and Wudden (11) who found high correlations ($0.86 \leq r \leq 0.99$) in sensitivities to these three fungicides in one wild-type sensitive and four lab-generated resistant isolates of *Ustilago avenae*. Potential for cross-resistance to triadimefon and any of the other three DMIs tested in this study was not statistically confirmed. This does not agree with cross-resistance data found for *Uncinula necator*, in which significant correlations ($0.60 \leq r \leq 0.83$) in sensitivities were reported for triadimefon, myclobutanil, and fenarimol (19). Cross-resistance to DMI fungicides appears to vary by fungicide and fungal species, and may need to be evaluated for each individual pathosystem.

In our analysis of cross-resistance, 50 isolates were selected from sampled isolates collected from an exposed and unexposed population to represent the range of propiconazole sensitivity in a sample of 128 isolates. The exposed population used in this study had documented exposure to propiconazole only, so presumably the apparent cross-resistance to other DMIs in these isolates is related to this previous exposure. In contrast, Hsiang et al. (9) used over 300 isolates to assess the potential for cross-resistance, with 21 of those isolates coming from a population with reduced sensitivity to propiconazole and fenarimol. Golembiewski et al. (8) reported cross-resistance between triadimefon, fenarimol, and propiconazole based on 150 isolates from a site where the DMIs had been used extensively and did not provided adequate control of dollar spot. In comparing the results of our study and previous studies (6,8,9), several methods differ and must be considered. By modifying the mycelial growth assay used previously for DMI sensitivity assessment in *S. homoeocarpa*, we may have significantly altered our estimated ED_{50} values, yielding results too different from values found in other studies to allow for adequate comparison. Evidence of this is the difference between ED_{50} values found in this study for control isolates S084 and S088, and the ED_{50} values previously published for these isolates by Burpee (1). Differences in sample size and range of sensitivities may also account for the differences between the cross-resistance results found in this study and previous reports.

Resistance to the benzimidazole class of fungicides has become so widespread in *S. homoeocarpa* that it has been suggested that the resistant strain is the typical wild type strain of the fungus (14). Therefore it was not surprising to find that 49 of the 50 *S. homoeocarpa* isolates tested in this study had no measurable reduction in growth by

thiophanate-methyl at 20 and 60 μg ml^{-1} when compared to growth on unamended media. DMI resistance, if compounded with benzimidazole and dicarboximide resistance, leaves golf course superintendents with few control options for dollar spot and "may pose the most difficult resistance control problem we (turfgrass managers and pathologists) may yet have to face" (14).

Currently, efforts to manage and delay the development of resistance of *S. homoeocarpa* populations to DMI fungicides are being pursued at turfgrass sites under intense disease pressure. Due to the climate in Georgia, dollar spot had not previously been considered a severe problem, but golf course superintendents have found it increasingly more difficult to manage the disease on creeping bentgrass. Some superintendents have relied heavily on DMI fungicides to manage dollar spot. The results of this study reveal a shift in propiconazole sensitivity in *S. homoeocarpa* populations in Georgia where propiconazole has been used. This information emphasizes the need to employ fungicide resistance management strategies to extend the potential effectiveness of the DMIs and other at-risk fungicides for control of dollar spot.

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Table 2.1. Number of isolates, host cultivar, and DMI exposure history of *Sclerotinia homoeocarpa* populations sampled in 1999 and 2000

				Usage History	
Population	**No. of isolates**	**Bentgrass cultivar**	**DMI used**	**Years of DMI use[x]**	**No. of applications[y]**
AAC	50	Crenshaw	propiconazole, triadimefon	6	2
CCC	69	Crenshaw	propiconazole	4	2
HMGC	59	Dominant	none	-	-
FGC	100	Crenshaw	none	-	-
ML	64	Crenshaw	none	-	-
TC	99	Penncross	none	-	-

[x] Minimum number of years a DMI fungicide had been used prior to sampling.
[y] Average number of treatments of a DMI fungicide applied per year.

Table 2.2. Characteristics of *Sclerotinia homoeocarpa* isolates used as DMI resistant and sensitive controls

Isolate number	Location	Year collected	ED_{50} value [x] (:g ml^{-1})	ED_{50} value [y] (:g ml^{-1})	Known phenotype
S084	State College, PA	1980	0.03	0.0056	Sensitive to benzimidazoles and DMIs
S088	Chicago, IL	1993	0.31	0.0753	Resistant to benzimidazoles and DMIs

[x] from Burpee (1).
[y] ED_{50} values for propiconazole estimated in this study.

Table 2.3. Formulas used for calculations of assay reproducibility[y]

Term	Formula	Reference
Mean	$e^{\mu+\sigma^2}$	Casella (2)
Variance	$e^{2(\mu+\sigma^2)} - e^{2\mu+\sigma^2}$	Casella (2)
Coefficient of variance	$\frac{e^{2(\mu+\sigma^2)} - e^{2\mu+\sigma^2}}{e^{\mu+\sigma^2}}$	Casella (2)
Confidence interval upper limit	$e^{\mu+\frac{\sigma^2}{2}+\frac{\sigma H_{1-\alpha}}{\sqrt{n-1}}}$	Gilbert (7)
Confidence interval lower limit	$e^{\mu+\frac{\sigma^2}{2}+\frac{\sigma H^{\alpha}}{\sqrt{n-1}}}$	Gilbert (7)

[y] As previously described by Wong and Wilcox (18).
[z] μ and σ^2 are the mean and variance, respectively of the natural log of the ED_{50} values of individual isolates determined from the mycelial growth assays. $H_{1-\alpha}$ and H_{α} are the Land's coefficients for calculating the upper and lower confidence intervals for the specified level of $\forall$ (= 0.05).

Table 2.4. Reproducibility[w] of mycelial growth assay for determining ED_{50} values for propiconazole against five isolates of *Sclerotinia homoeocarpa*

	ED_{50} value (:g ml^{-1})		
Isolate	**Mean[x]**	**95% Ci[y]**	**CV[z]**
AAC 34	0.064	(0.073 – 0.058)	9.6 H 10^{-4}
AAC 50	0.038	(0.041 – 0.036)	2.0 H 10^{-4}
FGC 51	0.065	(0.079 – 0.056)	2.2 H 10^{-3}
ML 1	0.025	(0.029 – 0.021)	6.2 H 10^{-4}
TC 1	0.025	(0.029 – 0.022)	4.6 H 10^{-4}
Mean			8.8 H 10^{-4}

[w] As previously described by Wong and Wilcox (18)
[x] Mean ED_{50} value based upon ln-transformed ED_{50} values obtained from five repeated assays.
[y] 95% confidence interval based upon ln-transformed ED_{50} values.
[z] The coefficient of variance based upon ln-transformed ED_{50} values.

Table 2.5. Mean relative growth of isolates from six populations of *Sclerotinia homoeocarpa* exposed to two discriminatory concentrations of propiconazole

			Discriminatory concentrations			
			0.02 :g ml^{-1}		**0.2 µg ml^{-1}**	
Population	**No. of isolates**	**DMI history**[x]	**Range**	**Mean**	**Range**	**Mean**
CCC	50	+	0.18 – 0.77	0.52 a[y]	0 – 0.27	0.09 a
AAC	69	+	0.10 – 0.82	0.45 b	0 – 0.58	0.09 a
FGC	59	-	0.03 – 0.78	0.38 c	0 – 0.34	0.06 b
HMGC	100	-	0 – 0.56	0.24 d	0 – 0.17	0.06 b
TC	64	-	0 – 0.22	0.12 e	0 – 0.03	0.0003 c
ML	99	-	0 – 0.33	0.11 e	0	0 c

[x] Isolates in population were collected from bentgrass that had a history of treatment with DMI fungicides (+) or no history of DMI use (-).
[y]Within a column, means followed by the same letter are not significantly different (P > 0.05) based on Fisher's protected LSD.

Table 2.6. Correlation coefficients and P-values from pairwise comparison of sensitivities among four DMI fungicides on a subset of 50 *Sclerotinia homoeocarpa* isolates from Georgia

	Propiconazole		**Myclobutanil**		**Fenarimol**		**Triadimefon**	
	r	**P-value**	**r**	**P-value**	**r**	**P-value**	**r**	**P-value**
Propiconazole	1.000	-	0.635	<0.0001	0.437	0.0015	0.078	0.6048
Myclobutanil			1.000	-	0.623	<0.0001	0.115	0.4479
Fenarimol					1.000	-	0.195	0.1931
Triadimefon							1.000	-

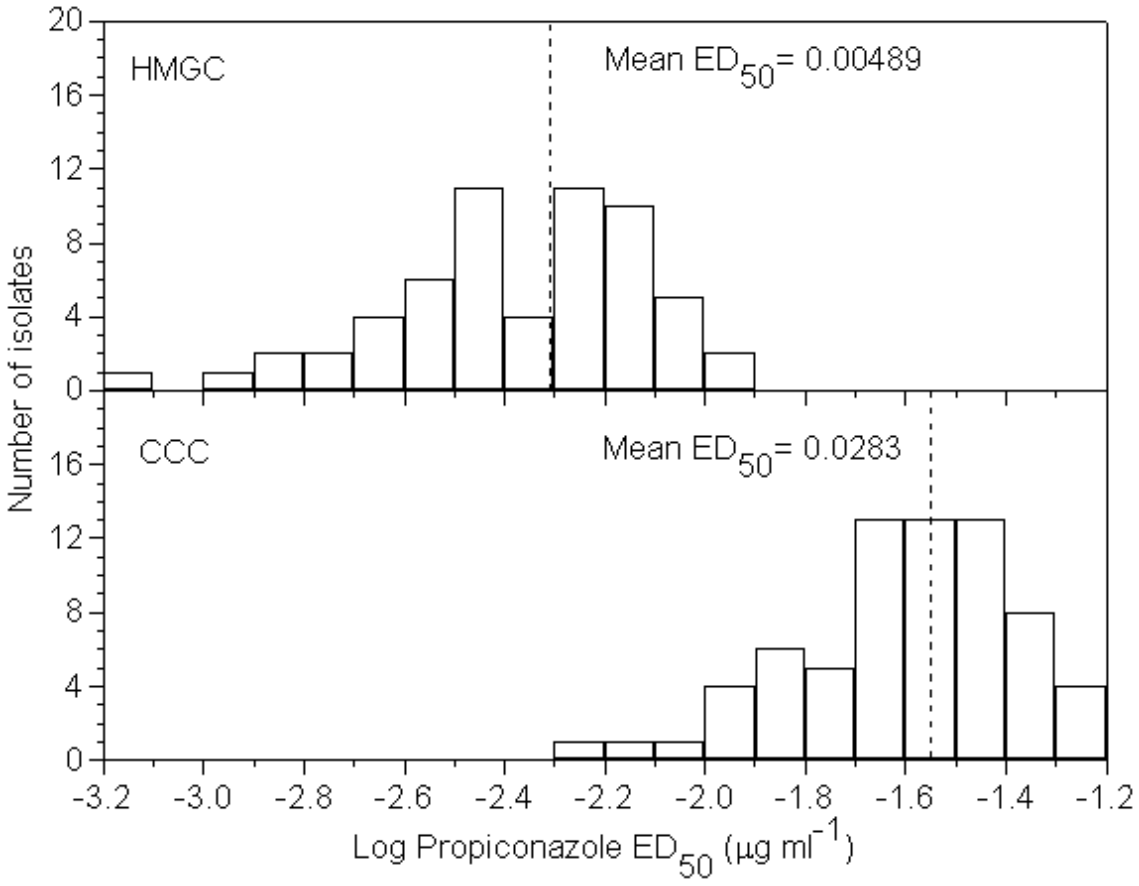

Figure 2.1. Frequency distribution of *Sclerotinia homoeocarpa* isolates generated from log propiconazole ED_{50} values for the baseline (HMGC) and DMI exposed (CCC) populations from Georgia.

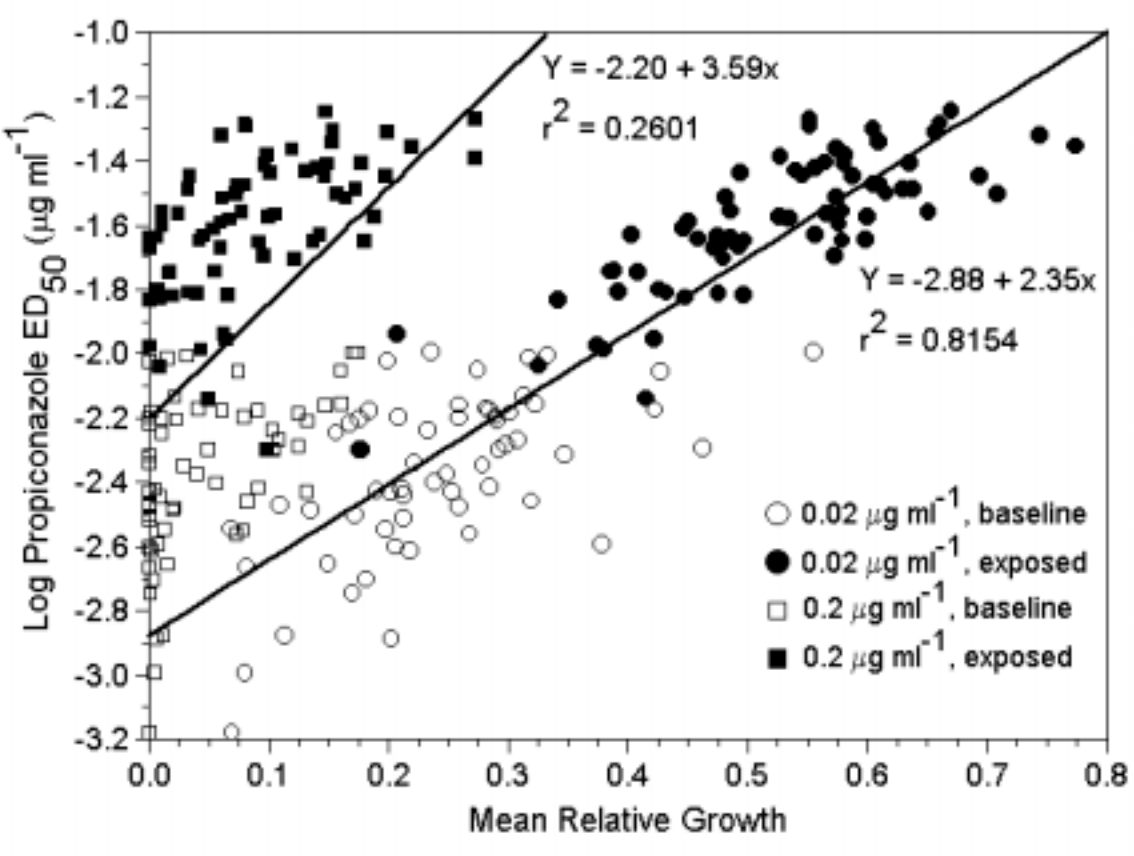

Figure 2.2. Relationship between relative growth on discriminatory concentrations of propiconazole and the log ED_{50} values for isolates from a baseline and exposed population of *Sclerotinia homoeocarpa*.

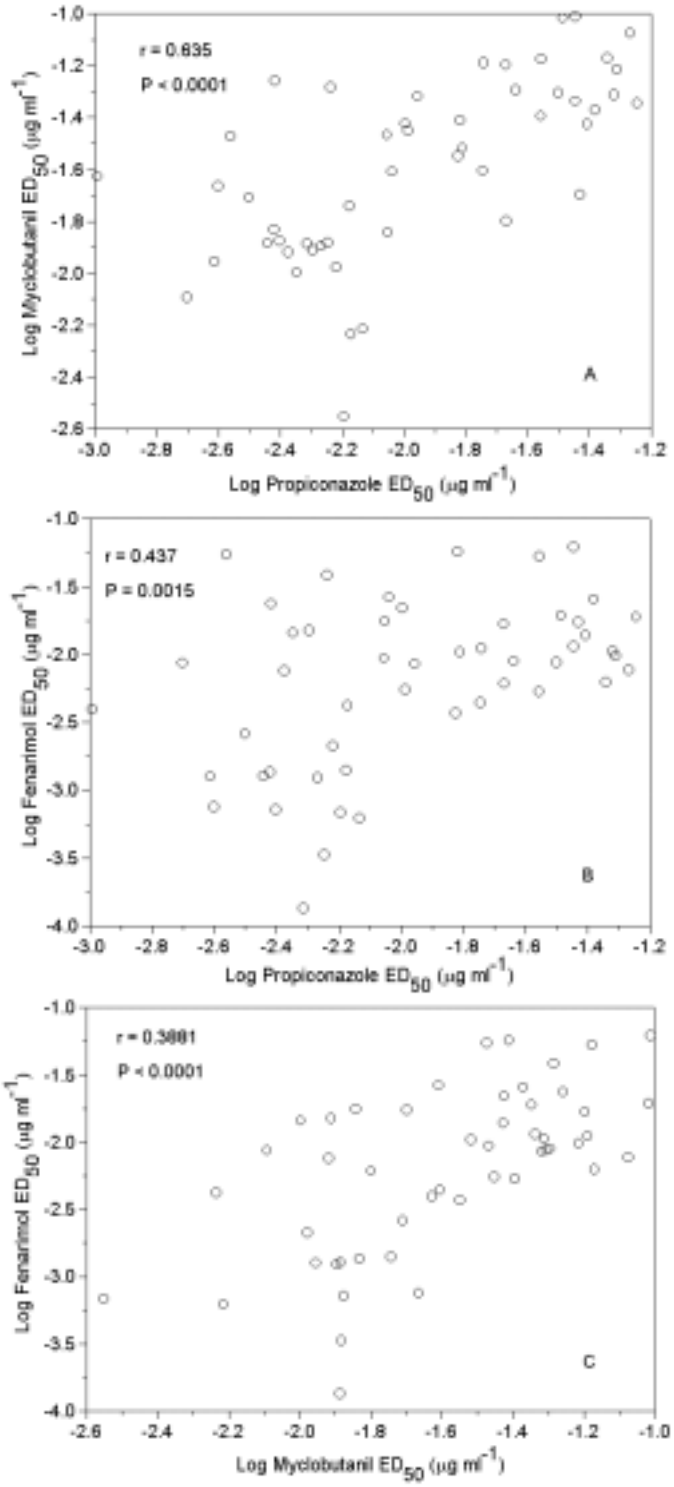

Figure 2.3. Correlation between sensitivities to DMI fungicides in a subset of 50 *Sclerotinia homoeocarpa* isolates from baseline and exposed populations in Georgia. ED_{50} values were estimated by modified mycelial growth assay: (A) myclobutanil vs. propiconazole, (B) fenarimol vs. propiconazole, (C) fenarimol vs. myclobutanil.

CHAPTER 3

EFFECT OF FUNGICIDE SENSITIVITY ON CONTROL OF DOLLAR SPOT WITH PROPICONAZOLE[1]

[1]Miller, G., K. L. Stevenson, and L. L. Burpee. 2001. To be submitted to Plant Disease.

ABSTRACT

Miller, G., Stevenson, K. L., and Burpee, L.L. 2001. Effect of fungicide sensitivity on control of dollar spot with propiconazole. To be submitted to Plant Disease.

Reduced fungicide sensitivity in *Sclerotinia homoeocarpa* populations is an important problem in the turfgrass industry and can result in reduced or total loss of fungicide efficacy and unsatisfactory control of dollar spot. The objective of this study was to establish the relationship between propiconazole sensitivity based on mycelial growth assays in vitro and symptom development on propiconazole-treated bentgrass. Creeping bentgrass (var. L93) established in Cone-Tainers was treated with propiconazole at a rate of 0.099 kg a.i. ha^{-1}. Bentgrass was inoculated 24 h later with each of seven isolates of *S. homoeocarpa* with ED_{50} values ranging from 0.005 to 0.075 :g ml^{-1}. Dollar spot severity was visually assessed daily for 36 days. The incubation period decreased linearly with increasing log ED_{50} value, ranging from a mean of 12.2 days for an isolate with an ED_{50} value of 0.075 :g ml^{-1} to 28.2 days for an isolate with an ED_{50} value of 0.005 :g ml^{-1}. Area under the disease progress curve and disease severity values increased linearly with increasing log ED_{50} value. Decreased in vitro sensitivity to propiconazole resulted in reduced dollar spot control.

Introduction

Dollar spot, caused by *Sclerotinia homoeocarpa* F.T. Bennett, is the most common turfgrass disease in North America, causing considerable reduction in quality of intensively managed turfgrasses. Disease can be initiated at temperatures from 15ºC to 27ºC, but *S. homoeocarpa* is most virulent or aggressive when temperatures are between 21ºC and 27ºC, and atmospheric humidity is >85% (3). On closely mown turfgrass swards, infection results in white to straw-colored spots, 1-5 cm in diameter, which can appear crater-like in the turf canopy and coalesce to affect large areas of turf (15,16).

Since cultural practices alone are not sufficient to control dollar spot on bentgrass (19), and disease thresholds on golf courses are particularly low, it is often necessary to apply fungicides every 14-21 days throughout the growing season to maintain disease-free turf. This intensive fungicide use can select for resistant strains of pathogen populations (1), yielding unsatisfactory control by the fungicide.

The sterol demethylation inhibiting (DMI) fungicides represent one third of all of the agricultural fungicides currently in use (11) and were first available for dollar spot control in 1979 (6). This popularity stems from several advantages that these fungicides offer, including both protectant and curative plant disease control (9). In addition, DMIs provide a broad spectrum of fungicidal activity, controlling many important turfgrass diseases with a single application. They also can be used at lower application rates with greater application intervals compared to several other groups of fungicides (11). Equally important is the ability of the DMIs to control benzimidazole- and dicarboximide-resistant strains of *S. homoeocarpa* and other fungal pathogens (18).

Using mycelial growth assays to estimate sensitivity, resistance to the DMI fungicides in field populations of *S, homoeocarpa* has been reported in Illinois, Kentucky, and Michigan, at sites where control failures had been observed (4,6,18). Using a similar assay, reduced sensitivity to the DMIs was found in a population of *S. homoeocarpa* in Ontario, Canada, at a site where field resistance to the DMIs had not been observed (8). This report shows that isolates with reduced sensitivity can be detected using an in vitro sensitivity growth assay with no sign of reduced fungicide efficacy and demonstrates the need for confirmation of in vitro sensitivity testing with in planta loss of fungicide efficacy prior to confirming a fungicide resistance problem.

In Georgia, little is known about the sensitivity of *S. homoeocarpa* populations to the DMI fungicides. According to the manufacturer's label, propiconazole (Banner Maxx, Syngenta Crop Protection, RTP, NC) applied correctly at 0.099 kg a.i. ha^{-1} should provide 14-28 days of disease control. Disease control failures of propiconazole have been reported on several golf courses in Georgia with an extensive history of DMI usage, taking the form of shorter intervals of dollar spot control than the intervals listed on the fungicide label. Using an in vitro mycelial growth assay, a reduction in sensitivity to propiconazole in one of these exposed populations was demonstrated when compared to propiconazole sensitivity in a population that had never been exposed to DMI fungicides (Chapter 2).

A better understanding is required of the relationship between results of in vitro DMI sensitivity assays and in planta suppression of dollar spot development by these fungicides. Therefore, the objective of this study was to determine the relationship

between dollar spot severity on propiconazole-treated bentgrass and the in vitro propiconazole sensitivity of *S. homoeocarpa* isolates used as inoculum.

Materials and Methods

In a previous study using a mycelial growth assay, propiconazole sensitivity was assessed for 128 isolates of *S. homoeocarpa* collected from diseased creeping bentgrass (*Agrostis palustris* Huds.) putting greens that differed in exposure to propiconazole (Chapter 2). Five of these isolates were selected for further greenhouse study to represent the range of sensitivities detected in vitro (Table 3.1). Two standard isolates were also included in the study. One of these isolates (S084) was highly sensitive to propiconazole (ED_{50} = 0.005 :g ml^{-1}) and the other (S088) exhibited significantly reduced sensitivity to propiconazole (ED_{50} = 0.0753 :g ml^{-1}) and tested positive for field resistance to the DMIs (2).

Isolates were collected in the fall of 1999 and spring and fall of 2000. Each isolate was obtained from a single dollar spot, and in heavy infestations (>5 spots/m^2) one isolate was collected per 30 cm^2 of turf surface area. Individual leaf blades were removed from the margins of an infection center, surface sterilized for 30 sec in a 70% ethanol solution and a 0.5% sodium hypochlorite solution, rinsed once in sterile water, and placed in Petri dishes containing potato dextrose agar (PDA, Difco Laboratories, Detroit). The Petri dishes were sealed with Parafilm and cultures were incubated at room temperature (~25ºC) for 3 days. The pathogen was identified by visual comparison of colony growth and morphology with known cultures of *S. homoeocarpa* (8). Mycelial plugs from each

isolate were placed on sterilized rye grains, allowed to grow at 25ºC for 21 days, and infested grains were frozen at -20ºC until needed.

To prepare inoculum of each isolate, frozen infested grains were placed in Petri dishes containing PDA and incubated for 3 days at room temperature (~25ºC). One mycelial plug (6-mm diameter) was removed from the edge of each actively growing fungal colony and placed in a glass tube (16H100mm) containing approximately 3 g of sterilized rye grains. Prior to inoculation, grain cultures were incubated for 3 weeks at 20ºC to allow the fungus to colonize the rye grains.

Bentgrass cv. L93 was seeded at a rate of 3.66 g/m^2, in calcined clay medium (Turface MVP, Profile Products LLC, Buffalo Grove, IL) in Cone-Tainers (Steuwe & Sons, Inc., Corvallis, OR) measuring 3.8 cm in diameter and 21 cm long. Racks containing 49 Cone-Tainers each were placed in a mist chamber maintained at 100% humidity, 20/26ºC day/night with a 12-h photoperiod. Seven days after seeding, the Cone-Tainers were transferred to a greenhouse where the bentgrass was allowed to mature for 5 weeks before inoculation. The bentgrass was fertilized every 14 days with 1.3 g N/m^2, and cut to a height of 1.27 cm three times per week. In trial 2, Gnatrol (*Bacillus thurengiensis* var. *israelensis*, Valent BioSciences, Longwood, FL) was applied as a water drench to the bentgrass at a rate of 10.4 ml/L of water every 2 weeks for control of fungus gnats.

The bentgrass was treated with propiconazole at a label rate of 0.099 kg a.i. ha^{-1} (Banner Maxx EC, Syngenta, Greensboro, NC), or left untreated as controls. The material was applied in water at a volume rate of 0.082 L/m^2 with a handheld, single-nozzle CO_2-pressured sprayer at 248.2 kPA pressure. The turf in each Cone-Tainer was

allowed to dry for 24 h and inoculated by placing a single rye grain infested with an isolate on a matte pin (3.2 cm long) which was positioned in the center of the Cone-tainer and 0.6 cm below the top of the grass canopy. The experiment was designed as a split plot, with the fungicide and control treatments applied to the main plots, and seven replications of each isolate arranged in randomized blocks (subplots) within each main plot. Main plots (fungicide) and subplots (isolates) were analyzed with single factor analysis of variance to assess the affects of treatments on disease.

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

Disease severity (Y), measured as the proportion of necrotic turf/area in each Cone-Tainer, was recorded daily for 36 days. The incubation period in days was recorded as the number of days between inoculation and first appearance of symptoms. Area under the disease progress curve (AUDPC) was also calculated for each isolate. Differences among isolates in DOC, AUDPC values, disease severity at 28 days after inoculation (Y_{28}), final disease severity (Y_{36}), and maximum disease severity (Y_{max}) were analyzed by linear regression on the $\log_{10}$-transformed ED_{50} values of the isolates. The entire experiment was repeated for a total of two experimental trials.

Results

Based on ANOVA analysis, fungicide treatment had a significant effect on dollar spot for all variables tested (Appendix). Dollar spot symptoms appeared on untreated bentgrass an average of 2-3 days after inoculation for all the isolates tested, although symptoms appeared sooner in Trial 2 than in Trial 1 (Table 3.1). AUDPC values ranged from 11.5-14.4 in Trial 1 on untreated bentgrass, a significantly lower range of values than those found in Trial 2 (15.1-18.0). Y_{28} was also significantly higher on untreated bentgrass in Trial 2 than in Trial 1, but Y_{36} and Y_{max} values were not different between the two trials (Table 3.1). Small differences in virulence among the isolates in the untreated bentgrass were also observed (Table 3.1).

On fungicide-treated bentgrass in both trials, disease severity was consistently highest on bentgrass inoculated with the least sensitive (S088) and consistently lowest on bentgrass inoculated with the most sensitive isolate (S084) (Table 3.2). However, disease severity was more variable for isolates with intermediate sensitivity to propiconazole, particularly in Trial 1. These inconsistencies may have been related to presence of fungus gnats, which were better managed in Trial 2 than in Trial 1. In both trials, all measures of disease severity (Y_{28}, Y_{36}, and Y_{max}) increased linearly with ED_{50} value (Figs. 3.1, 3.2, 3.3).

AUDPC values showed a similar pattern. On fungicide-treated bentgrass in both trials, the mean AUDPC was highest on bentgrass inoculated with the least sensitive isolate (S088) and was lowest on bentgrass inoculated with the most sensitive isolate (S084) (Table 3.2). As was observed for disease severity, AUDPC values were more

variable for isolates with intermediate sensitivity values, especially for Trial 1. In both trials, AUDPC increased linearly with increasing ED_{50} value (Fig. 3.4).

On propiconazole-treated bentgrass in both trials, incubation period was consistently longest on bentgrass inoculated with the most sensitive isolate (S084) and shortest on bentgrass inoculated with the least sensitive isolate (S088) (Table 3.2). There was some variation in the incubation period for isolates with intermediate sensitivity to propiconazole. However, in both trials, incubation period decreased linearly with increasing ED_{50} value (Fig. 3.5).

Discussion

Interpretation of results of fungicide monitoring has proven difficult in the past and has occasionally resulted in misleading over-prediction of resistance problems (1). Therefore, evidence of the correlation of fungicide sensitivity measured in vitro to actual disease control provided by that fungicide is a necessary and vital part of resistance monitoring. Many estimates of DMI sensitivities of pathogen populations can be found in the literature (7,13,14,17,20,21) , but few studies, (2,5,6,10,12), have included a correlation of in vitro sensitivities to fungicide efficacy with an in planta test.

The present study provides evidence of significant quantitative (linear) relationships between the sensitivity of isolates of *S. homoeocarpa* to propiconazole and efficacy of dollar spot control in bentgrass with propiconazole. These results further validate the use of mycelial growth assays for estimating propiconazole sensitivity in pathogen populations to detect potential erosion of disease control efficacy. Franke et al. (5) found no significant correlation ($P>0.05$) between disease control with tebuconazole

and differing in vitro sensitivities of 20 *Sclerotium rolfsii* isolates. The authors state that the absence of significant correlations may have resulted from low in vitro sensitivities of the isolates used in the study which would have all been adequately controlled by the label rate of tebuconazole (5) .

Propiconazole efficacy was highest for the most sensitive isolate of *S. homoeocarpa* used in this study and lowest for the least sensitive isolate. Köller et al. (10) used a greenhouse infection study to find that apple scab severity on leaves treated with fenarimol or myclobutanil was substantially higher for an isolate with an ED_{50} value representing a population with reduced sensitivity to fenarimol than for an isolate with an ED_{50} value representing a baseline population. Under field conditions, Burpee (2) observed significantly less control of dollar spot on propiconazole-treated bentgrass (a reduction of about 12-15 days) caused by isolate S088, which had reduced in vitro sensitivity to propiconazole, than control of disease caused by S084, a sensitive isolate.

Wide ranges of DMI sensitivities have been detected in populations of *S. homoeocarpa* with differences in mean ED_{50} values between baseline and exposed populations that were not always associated with confirmed field resistance (4,6,8). The results of this study provide an indication of the quantitative loss in efficacy that might occur in *S. homoeocarpa* populations based on the sensitivities of the isolates in that population. A mean ED_{50} value for propiconazole of 0.0283 μg ml^{-1} was reported for *S. homoeocarpa* isolates from a location in Georgia with a history of exposure to propiconazole (Chapter 2). Based on the regression from Trial 2 (Fig. 1), application of propiconazole at the full rate of 0.099 kg a.i. ha^{-1} would provide 19.5 days of control, a reduction in effective length of control of approximately 8 days compared to control of

disease caused by a sensitive isolate. This confirms that observed reduction in disease control by propiconazole in the field may be due to reduced sensitivity in this population, providing evidence to document the occurrence of field resistance at this location.

It is important to point out that these trials were conducted in the greenhouse and not the field. However, it is encouraging to note that the mean DOC values for isolate S084 and S088 (Table 2) found in this greenhouse study are similar to DOC values previously reported for these isolates under field conditions (24 to >29 days and 11.5 to 13.8 days for isolates S084 and S088, respectively) (2). There are numerous differences in environmental conditions and cultural practices between our research greenhouse and the field. Further experimentation is needed to confirm the results found here under field conditions and, therefore, care should be taken before extrapolating these results to a field situation.

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Table 3.1. Incubation period (IP), area under the disease progress curve (AUDPC), and disease severity values[v] on untreated bentgrass and inoculated with isolates of *Sclerotinia homoeocarpa* with different sensitivities to propiconazole.

		IP (days)		AUDPC		Y_{28}[x]		Y_{36}[y]		Y_{max}[z]	
		Trial		Trial		Trial		Trial		Trial	
Isolate	ED_{50} (µg /ml)	1	2	1	2	1	2	1	2	1	2
S084	0.0054	3	2	12.1	15.1	0.42	0.50	0.61	0.48	0.60	0.59
HMGC 46	0.0104	3.29	2	12.0	15.1	0.44	0.53	0.59	0.52	0.59	0.61
CCC 32	0.0202	2	2	12.3	16.3	0.44	0.55	0.63	0.55	0.63	0.65
CCC 61	0.0306	3	2	12.8	16.5	0.45	0.56	0.66	0.53	0.66	0.62
CCC 64	0.0409	3	2	11.5	17.4	0.39	0.59	0.60	0.59	0.60	0.67
CCC 56	0.0511	3	2	13.2	15.9	0.40	0.59	0.64	0.58	0.70	0.66
S088	0.0753	3	2	14.4	18.0	0.47	0.64	0.77	0.61	0.77	0.74

[v]Severity measured as a proportion of diseased turf area
[x] Severity at 28 days after inoculation
[y] Severity at 36 days after inoculation
[z] Maximum disease severity

Table 3.2. Incubation period (IP), area under the disease progress curve (AUDPC), and disease severity values[y] on bentgrass treated with propiconazole and inoculated with isolates of *Sclerotinia homoeocarpa* with different sensitivities to propiconazole

		IP (days)		AUDPC		Y_{28}[x]		Y_{36}[y]		Y_{max}[z]	
		Trial		Trial		Trial		Trial		Trial	
Isolate	ED_{50} (µg/ml)	1	2	1	2	1	2	1	2	1	2
S084	0.0054	27.3	29.4	1.22	1.24	0.11	0.06	0.13	0.25	0.18	0.28
HMGC 46	0.0104	22.0	23.4	2.92	3.11	0.24	0.21	0.22	0.34	0.32	0.39
CCC 32	0.0202	17.7	21.0	4.13	4.96	0.30	0.36	0.28	0.51	0.33	0.53
CCC 61	0.0306	21.9	19.6	2.56	4.82	0.19	0.32	0.26	0.47	0.28	0.52
CCC 64	0.0409	19.3	17.4	4.49	5.28	0.37	0.33	0.36	0.44	0.38	0.46
CCC 56	0.0511	20.3	19.0	3.21	5.69	0.28	0.38	0.29	0.48	0.31	0.54
S088	0.0753	13.3	10.7	7.53	10.3	0.48	0.52	0.48	0.57	0.51	0.62

[y]Severity measured as a proportion of diseased turf area
[x] Severity at 28 days after inoculation
[y] Severity at 36 days after inoculation
[z] Maximum disease severity

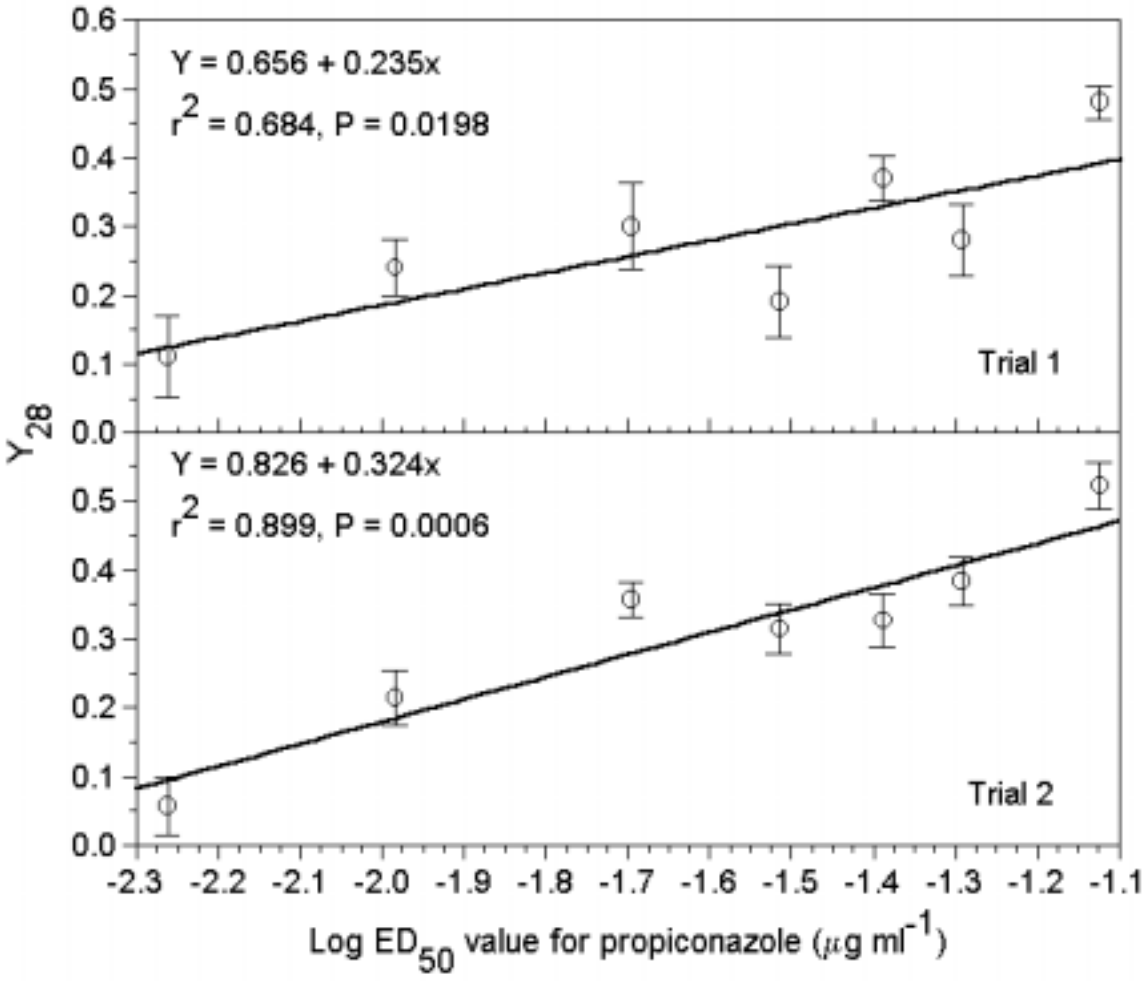

Figure 3.1. Relationship between sensitivity to propiconazole and disease severity 28 days after inoculation on bentgrass treated with propiconazole and inoculated with different isolates of *S. homoeocarpa*.

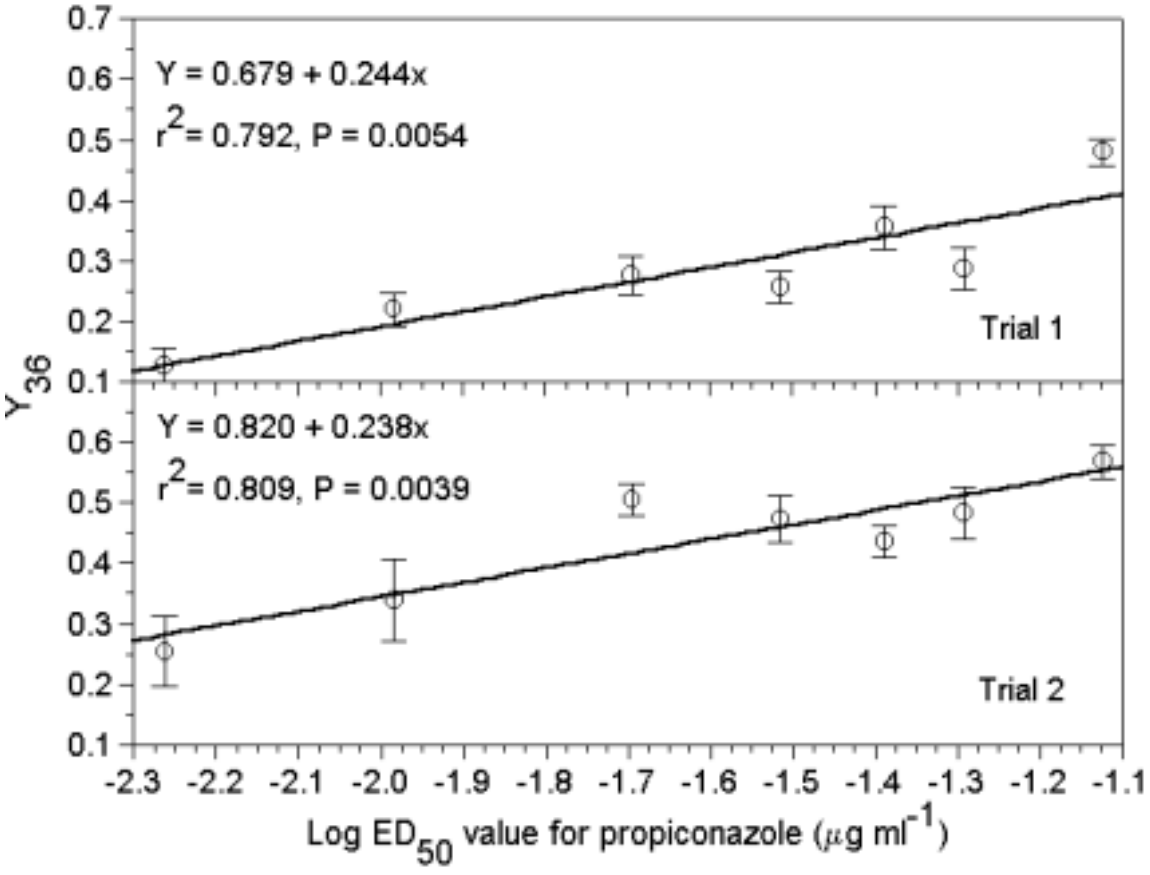

Figure 3.2. Relationship between sensitivity to propiconazole and disease severity 36 days after inoculation on bentgrass treated with propiconazole and inoculated with different isolates of *S. homoeocarpa*.

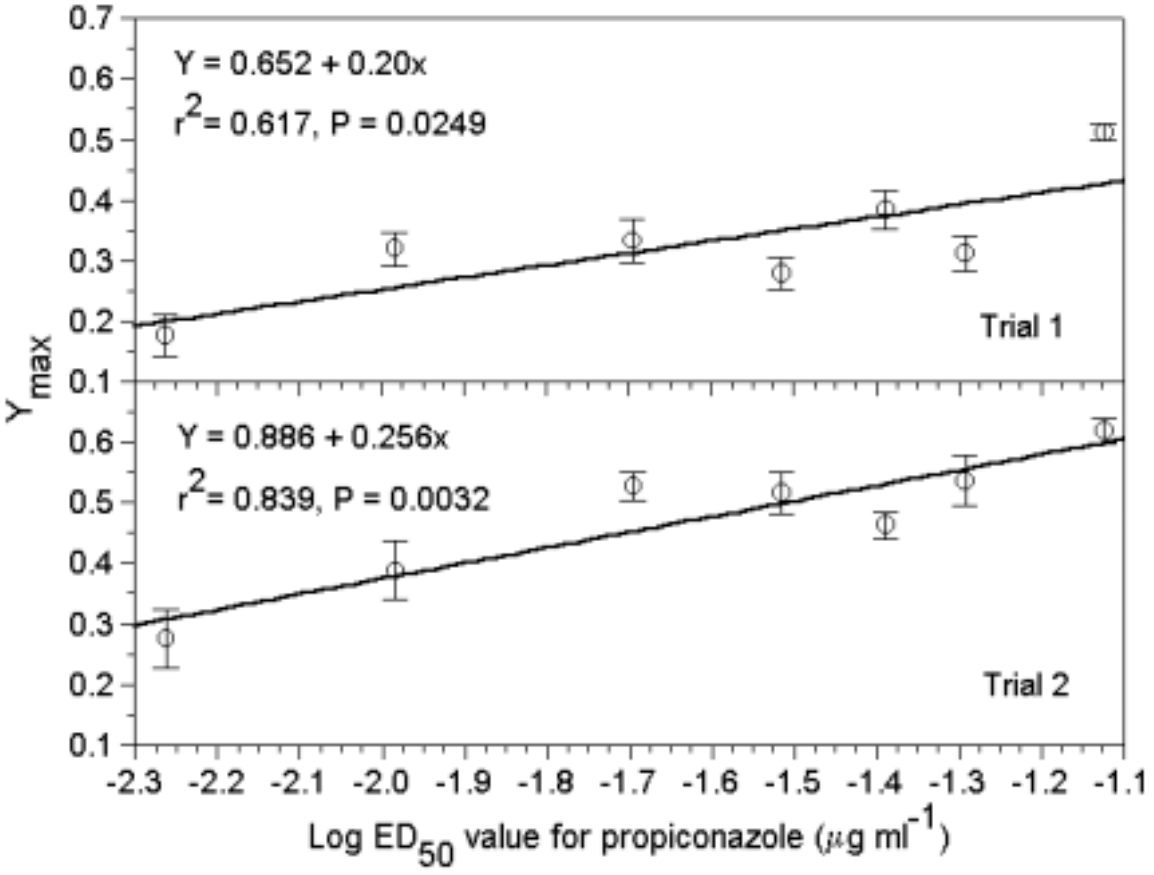

Figure 3.3. Relationship between sensitivity to propiconazole and maximum disease severity on bentgrass treated with propiconazole and inoculated with different isolates of *S. homoeocarpa*.

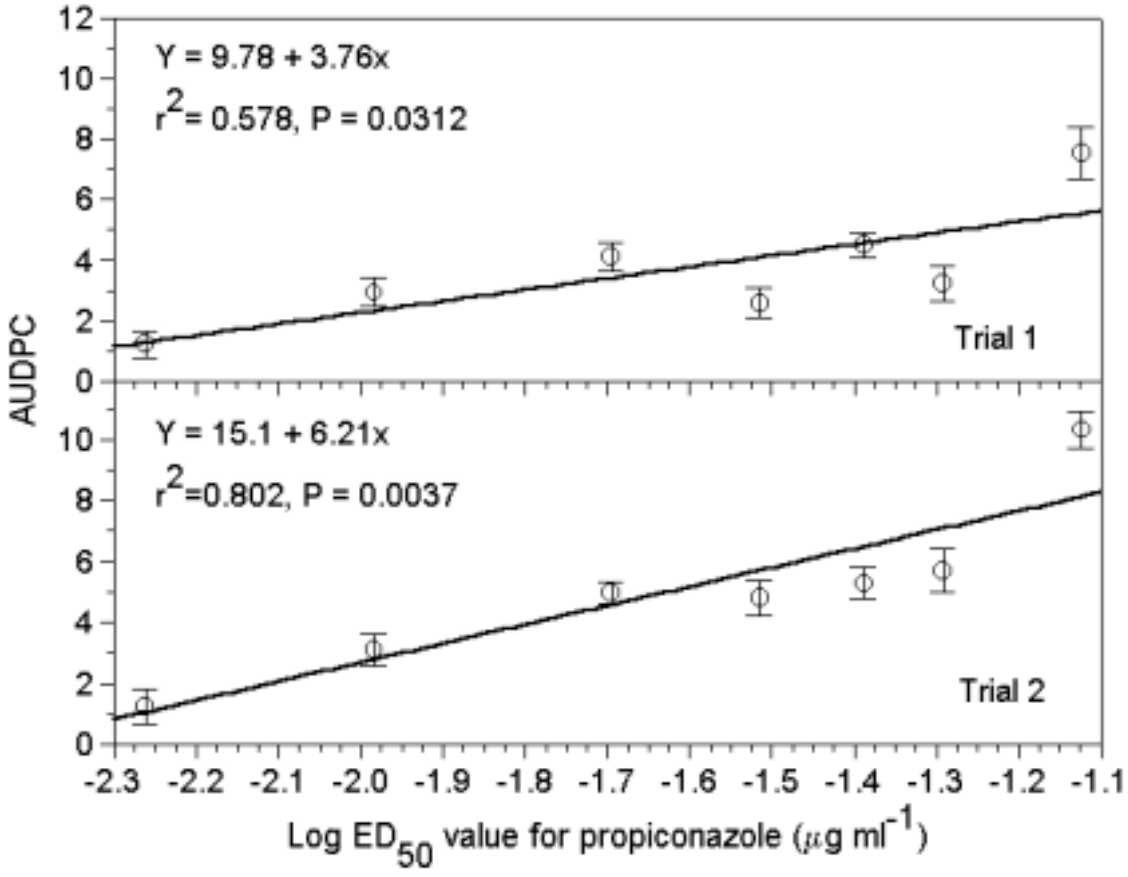

Figure 3.4. Relationship between sensitivity to propiconazole and area under the disease progress curve (AUDPC) on bentgrass treated with propiconazole and inoculated with different isolates of *S. homoeocarpa*

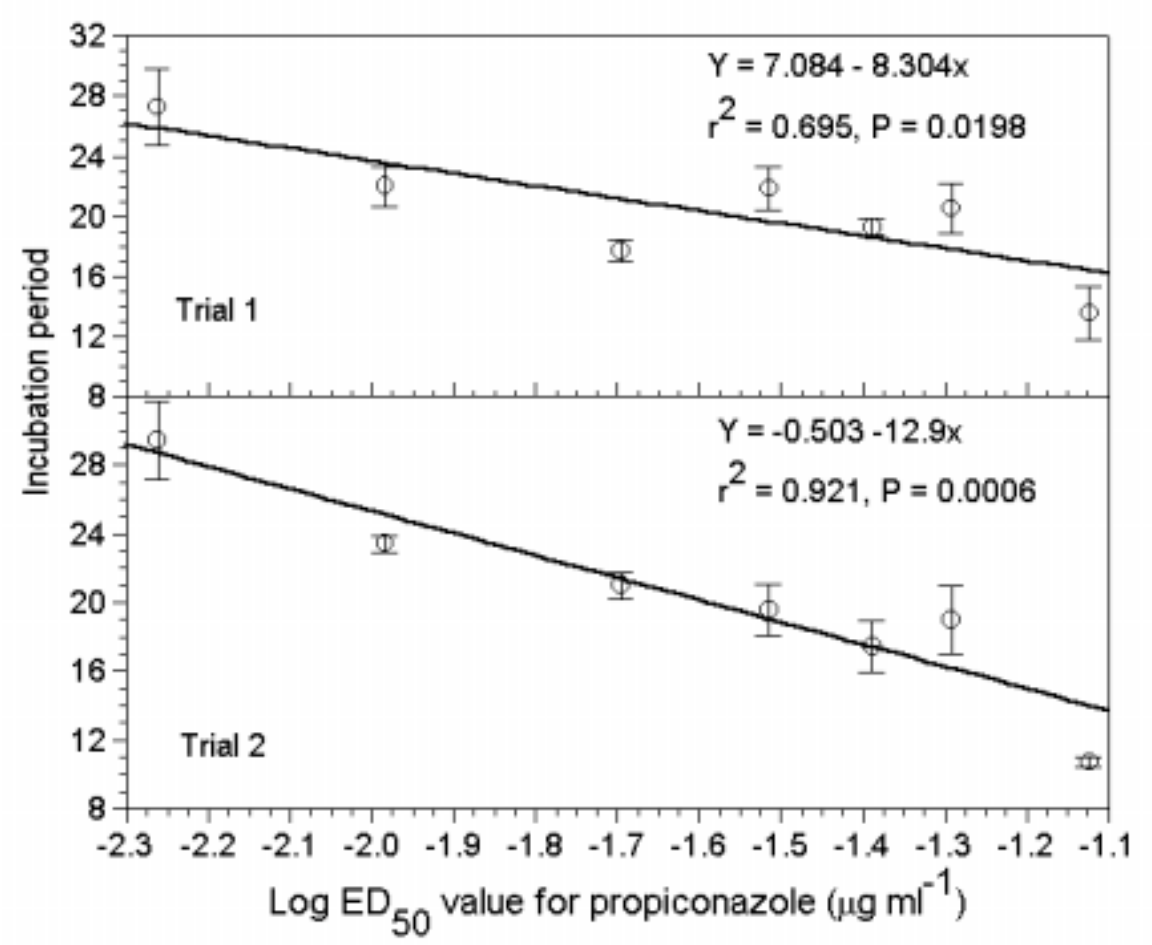

Figure 3.5. Relationship between sensitivity to propiconazole and incubation period of *S. homoeocarpa* isolates on bentgrass treated with propiconazole.

CHAPTER 4

SUMMARY

In Georgia, there are approximately 1.6 million acres of turfgrass with a maintenance value of $1.56 billion (3). Dollar spot, caused by the fungus *Sclerotinia homoeocarpa* F. T. Bennett (1), can result in considerable reduction in quality of intensively managed turfgrasses, such as creeping bentgrass (*Agrostis palustris* Huds), bermudagrass (*Cynodon dactylon* (L.) Pers.), and perennial ryegrass (*Lolium perenne* L.), which are commonly used on golf courses in Georgia. As a result of the persistent nature of the pathogen in turfgrass swards, more money is spent on the management of dollar spot than any other turfgrass disease on golf courses (9).

In Georgia, the disease is most severe and economically damaging on creeping bentgrass managed for putting greens, with most epidemics occurring in late spring or early summer and in the fall. The pathogen is thought to overwinter as stroma formed on the margins of dollar spot lesions (4,12) or as dormant mycelium in infected tissue (7). Local distribution of dollar spot occurs when mycelium grows from a diseased leaf to a healthy leaf in close proximity (15), with distribution on a larger scale occurring due to transport of infected plant parts on implements and footwear (13). Disease can be initiated at temperatures from 15ºC to 27ºC, but *S. homoeocarpa* is most virulent or aggressive when temperatures are between 21ºC and 27ºC, and atmospheric humidity is >85% (4). The amount and duration of the leaf wetness/dew period is also an important factor determining the severity of a dollar spot epidemic (17).

Since cultural practices alone are not sufficient to control dollar spot on creeping bentgrass (17) and disease thresholds on golf course greens are particularly low, fungicides are applied preventatively, usually on a 2-week schedule, when conditions are favorable for the disease (14). This intensive fungicide use can result in selection of

resistant strains in pathogen populations (8,14), and over time, yield unsatisfactory suppression of disease.

The sterol demethylation inhibitor (DMI) fungicides, available for dollar spot control in the U. S. since 1979 (8), offer several advantages. These fungicides offer a broad spectrum of activity, controlling many important turfgrass diseases with a single application. In addition, the DMIs can be used at lower application rates and at greater application intervals because of their systemic activity (11). Equally important is the ability of the DMIs to control strains of *S. homoeocarpa* resistant to the benzimidazole (16) and dicarboximide (5) fungicides.

In vitro mycelial growth assays have been used to detect resistance to the DMI fungicides in field populations of *S. homoeocarpa* in Illinois, Kentucky, and Michigan, at sites where control failures had been observed (6,8,14). Similar assays were used to detect reduced sensitivity to the DMIs in a population in Ontario, Canada, at a site where field resistance to the DMIs had not been observed (10). Reports such as these express the need for confirmation of in vitro sensitivity testing with in planta loss of fungicide efficacy prior to confirming a fungicide resistance problem.

The most important step to detect shifts toward resistance is the establishment of a baseline sensitivity for the pathogen system, which represents the sensitivity distribution of the population prior to exposure to a new fungicide (2). Prior to this study, little was known about the sensitivity of *S. homoeocarpa* populations from Georgia to the DMI fungicides. Therefore, the first objective was to establish a baseline sensitivity distribution for a *S. homoeocarpa* population from Georgia with no history of exposure to DMI fungicides. Using a modification of a mycelial growth assay, a non-exposed

population was estimated to have a mean ED_{50} value of 0.005 μg propiconazole ml^{-1}, which was similar to mean ED_{50} values of 0.002 and 0.007 μg propiconazole ml^{-1} for non-exposed populations reported by Golembiewski et al. (8) and Hsiang et al. (10), respectively. This confirms that at the time of sampling little, if any, selective change toward propiconazole insensitivity had occurred within this population, and it could act as a suitable baseline.

With the same procedure used for baseline sensitivity determination, ED_{50} values were estimated for a population of *S. homoeocarpa* that had been exposed to propiconazole. This population had a mean ED_{50} value for propiconazole of 0.0283 μg ml^{-1}, a significant reduction in sensitivity when compared to the propiconazole sensitivity of the baseline population. At the site of this sampled population, the golf course superintendent complained of propiconazole control failure, which took the form of shorter intervals of dollar spot control than intervals listed on the fungicide label.

The frequency distributions of the exposed (CCC) and baseline populations (HMGC) served as a guide for the selection of two discriminatory concentrations of propiconazole (0.02 and 0.2 μg ml^{-1}) which were used to compare sensitivities among a total of six sampled populations from Georgia. Regression analysis showed a stronger relationship between the log ED_{50} value of an isolate and the mean relative growth (RG) of that isolate on 0.02 μg ml^{-1} than on 0.2 μg ml^{-1}. This result supports the use of mean RG at this single concentration as an accurate predictor of in vitro sensitivity, significantly reducing the amount of work required to monitor future propiconazole sensitivity shifts in populations of *S. homoeocarpa* from Georgia.

Propiconazole sensitivities of six sampled populations with different exposure histories to the DMIs were compared with mean RG at both discriminatory concentrations. Two of these populations had been exposed to propiconazole and were from sites where complaints of propiconazole control failure had been received, while the other four populations had no reported exposure to the DMIs. Mean RG values were significantly greater (LSD, $P \leq 0.05$) at both concentrations for populations that had been exposed to propiconazole than for non-exposed populations, indicating a reduction in sensitivity to propiconazole in these populations resulting from previous exposure to the fungicide.

Potential for cross-sensitivity among four DMI compounds (propiconazole, myclobutanil, fenarimol, and triadimefon) was also evaluated. There are conflicting accounts of the extent of cross-resistance among the DMI fungicides currently labeled for use on dollar spot in the United States (6,8,10). Cross-resistance in *S. homoeocarpa* was thought to be consistent for all DMI fungicides (6,8), but Hsiang et al. (10) found distinct sensitivity groupings between propiconazole and myclobutanil (r=0.438), and tebuconazole and fenarimol (r=0.250) in Ontario populations. In our analysis of cross-resistance, the strongest correlation was found between sensitivity to propiconazole and myclobutanil (r=0.635), which was consistent with the previous study (10). Weaker, but statistically significant correlations, were also found between fenarimol and myclobutanil (r=0.623), and propiconazole and fenarimol (r=0.437), implying resistance to all three of these fungicides may be linked. Potential for cross-resistance between triadimefon and propiconazole, myclobutanil, or fenarimol was not confirmed, providing further evidence

that potential cross-resistance in field populations of *S. homoeocarpa* may not be the same for all DMI fungicides.

A better understanding of the relationship between the monitoring results gained from an in vitro assay and dollar spot development in planta is essential for applying what can be learned about fungicide sensitivity in a laboratory to fungicide efficacy in a field situation. To serve this purpose, experiments were designed to assess the effects of propiconazole on dollar spot of creeping bentgrass caused by seven isolates of *S. homoeocarpa* selected to represent the range of propiconazole sensitivities measured in this study. Significant linear relationships were detected for length and degree of dollar spot control, and log ED_{50} values of individual isolates. These results provide information on the extent of propiconazole efficacy loss associated with *S. homoeocarpa* populations based on in vitro sensitivity values of isolates from that population. They also support observations by golf course superintendents that reductions in sensitivity in the two exposed populations of *S. homoeocarpa* sampled in this study may be responsible for the observed reduction in disease control by propiconazole.

DMI resistance, if compounded with benzimidazole and dicarboximide resistance, leaves golf course superintendents with few control options for dollar spot and "may pose the most difficult resistance control problem we (turfgrass managers and pathologists) may yet have to face" (14). This study provides evidence of a significant reduction in propiconazole sensitivity in *S. homoeocarpa* populations in Georgia that is the probable cause of a loss of efficacy of the fungicide. This efficacy loss is not to the extent of that seen in resistant populations found in northern states (6,8,14), which is consistent with the higher use rates of the DMIs in these areas. However, it does express the need for

further monitoring of the sensitivities of *S. homoeocarpa* populations in Georgia with the baseline and discriminatory dose data established in this study. The information gained from this report is also useful to university research and extension personnel for making more efficient and knowledgeable fungicide recommendations to golf course superintendents and for emphasizing the need for implementation of fungicide resistance management strategies in Georgia.

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APPENDIX

RESULTS OF STATISTICAL ANALYSIS FOR CHAPTER 3

Table 1. Results of analysis of variance to determine effects of fungicide application, sensitivity of isolate, and the interaction of fungicide application and sensitivity of isolate, on the days until dollar spot symptoms were observed. Experimental trials were analyzed separately.

Trial	Source	df	Mean Square	F-value	P-value
1	Replication	6	9.95	0.42	0.8409
	Treatment (main plot)	1	6898	292.83	<0.0001
	Main-plot error	6	23.6	---	---
	Isolate (sub-plot)	6	68.5	5.18	0.0006
	Treat*isolate	6	67.4	5.09	0.0007
	Subplot error	36	13.2	---	---
2	Replication	6	13.1	1	0.500
	Treatment (main plot)	1	8010	610.73	<0.0001
	Main-plot error	6	13.1	---	---
	Isolate (sub-plot)	6	114.1	17.98	<0.0001
	Treat*isolate	6	114.1	17.98	<0.0001
	Subplot error	36	228.5	---	---

Table 2. Results of analysis of variance to determine effects of fungicide application, sensitivity of isolate, and the interaction of fungicide application and sensitivity of isolate, on area under the disease progress curve (AUDPC). Experimental trials were analyzed separately.

Trial	Source	df	Mean Square	F-value	P-value
1	Replication	6	7.99	0.34	0.8897
	Treatment (main plot)	1	1820	78.59	0.0001
	Main-plot error	6	23.2	---	---
	Isolate (sub-plot)	6	26.1	7.78	<0.0001
	Treat*isolate	6	8.18	2.43	0.0445
	Subplot error	36	6.35	---	---
2	Replication	6	3.17	1.56	0.3017
	Treatment (main plot)	1	3186	1566	<0.0001
	Main-plot error	6	2.03	---	---
	Isolate (sub-plot)	6	58.5	15.82	<0.0001
	Treat*isolate	6	9.18	2.48	0.0411
	Subplot error	36	133.1	---	---

Table 3. Results of analysis of variance to determine effects of fungicide application, sensitivity of isolate, and the interaction of fungicide application and sensitivity of isolate, on disease severity 28 days after inoculation (Y_{28}). Experimental trials were analyzed separately.

Trial	Source	df	Mean Square	F-value	P-value
1	Replication	6	0.021	0.71	0.6551
	Treatment (main plot)	1	1.14	39.04	0.0008
	Main-plot error	6	0.029	---	---
	Isolate (sub-plot)	6	0.063	5.28	0.0005
	Treat*isolate	6	0.052	4.43	0.0019
	Subplot error	36	0.012	---	---
2	Replication	6	0.011	2.38	0.1579
	Treatment (main plot)	1	1.58	336.9	<0.0001
	Main-plot error	6	0.005	---	---
	Isolate (sub-plot)	6	0.124	11.2	<0.0001
	Treat*isolate	6	0.038	3.44	0.0087
	Subplot error	36	0.011	---	---

Table 4. Results of analysis of variance to determine effects of fungicide application, sensitivity of isolate, and the interaction of fungicide application and sensitivity of isolate, on disease severity 36 days after inoculation (Y_{36}). Experimental trials were analyzed separately.

Trial	Source	df	Mean Square	F-value	P-value
1	Replication	6	0.008	0.48	0.805
	Treatment (main plot)	1	1.11	63.43	0.0002
	Main-plot error	6	0.0175	---	---
	Isolate (sub-plot)	6	0.073	12.02	<0.0001
	Treat*isolate	6	0.028	4.61	0.0014
	Subplot error	36	2.24	---	---
2	Replication	6	0.0277	1.88	0.2301
	Treatment (main plot)	1	0.308	20.22	0.0041
	Main-plot error	6	0.015	---	---
	Isolate (sub-plot)	6	0.075	8.40	<0.0001
	Treat*isolate	6	0.0186	2.09	0.0792
	Subplot error	36	0.0089	---	---

Table 5. Results of analysis of variance to determine effects of fungicide application, sensitivity of isolate, and the interaction of fungicide application and sensitivity of isolate, on the maximum disease severity (Y_{max}). Experimental trials were analyzed separately.

Trial	Source	df	Mean Square	F-value	P-value
1	Replication	6	0.028	0.58	0.7407
	Treatment (main plot)	1	2.35	48.01	0.0004
	Main-plot error	6	0.049	---	---
	Isolate (sub-plot)	6	0.080	7.05	<0.0001
	Treat*isolate	6	0.02	1.73	0.143
	Subplot error	36	0.011	---	---
2	Replication	6	0.015	1.29	0.3834
	Treatment (main plot)	1	0.759	65.97	0.0002
	Main-plot error	6	0.012	---	---
	Isolate (sub-plot)	6	0.086	12.74	<0.0001
	Treat*isolate	6	0.02	2.95	0.0190
	Subplot error	36	0.006	---	---