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Determining Dollar Spot Fungicide Resistance in Tennessee and Northern Mississippi

Pamela Rene Baird
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Pamela Rene Baird entitled "Determining Dollar Spot Fungicide Resistance in Tennessee and Northern Mississippi." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Entomology and Plant Pathology.

John Sorocham, Major Professor

We have read this thesis and recommend its acceptance:

Alan Windham, Thomas Samples, Mark Windham, Robert Trigiano

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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Anne Mayhew

Vice Chancellor and
Dean of Graduate Studies

(Original signatures are on file with official student records.)

DETERMINING DOLLAR SPOT FUNGICIDE RESISTANCE IN TENNESSEE AND NORTHERN MISSISSIPPI

A Thesis Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Pamela Rene Baird

May, 2005

DEDICATION

This thesis is dedicated to my fiancé and best friend, Todd Bishop, for his unending support and encouragement, and to my parents, Edward and Barbara Baird, and Carl and Rita Willson for loving and believing in me. And to the Pantses, for comic relief and plenty of red wine when I needed it the most.

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Thank you to all who were involved in this research project, you are much appreciated.

ABSTRACT

Dollar spot, caused by *Sclerotinia homeocarpa*, is the most common turf disease golf course that superintendents deal with on a regular basis. Chemical control has been the front line of defense against this disease; however, most of the fungicides that formerly controlled dollar spot have lost efficacy over time due to development of resistance by the fungus. The objectives of part one of this thesis were to determine the following: (i) if resistance to the fungicides thiophanate-methyl, iprodione, and propiconazole exists in Tennessee, and northern Mississippi isolates of field-collected *S. homeocarpa*; (ii) if isolates collected in Michigan differ in levels of resistance to isolates collected in Tennessee and northern Mississippi; (iii) if multi-resistance to different fungicide classes occurs in these isolates; and (iv) if chemical practices of golf course superintendents have an effect on the occurrence of fungicide resistance in the southern-collected isolates.

Fungicide resistance was observed in nine of the ten southern locations tested. Additionally, three locations from Michigan also exhibited resistance. One location from Michigan known to be sensitive to all fungicides used in this study did not exhibit any resistance. Of the 14 total locations tested, seven exhibited resistance to iprodione and thiophanate-methyl, and two exhibited resistance to propiconazole. One location exhibited multi-resistance to thiophanate-methyl and iprodione, another exhibited multi-resistance to thiophanate-methyl and propiconazole, and another location exhibited multi-

resistance to all fungicides tested. The highest fungicide active ingredient concentration at which resistance occurred to iprodione and thiophanate-methyl was 1000 μg ml^{-1}, and the highest concentration to which resistance occurred to propiconazole was ten μg ml^{-1}. No significant difference was detected among levels of resistance in isolates from the Michigan and southern locations. For all locations, fungicide resistance correlated with fungicide use at that location.

The purpose of the second investigation was to determine the following: (i) if resistance to the fungicide chlorothalonil exists in Tennessee and northern Mississippi by testing isolates of *S. homeocarpa* field-collected in this area; (ii) if the 'wild type' isolate from Michigan is resistant to chlorothalonil, and if so, if it has differing sensitivity from the southern-collected isolates; and (iii) if chemical practices of golf course superintendents have an effect on the occurrence of resistance to chlorothalonil.

All nine isolates tested grew in chlorothalonil amended PDB at rates up to 5 μg active ingredient ml^{-1} PDB. Two isolates grew in 10 μg ml^{-1} PDB, and none grew in 20 μg ml^{-1} PDB. Variation was observed in the relative growths of all isolates at all concentrations, and no correlation was observed between geographic region of collection and fungicide sensitivity. Additionally, no correlation was observed between chlorothalonil use *in situ* and chlorothalonil sensitivity *in vitro*.

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LITERATURE REVIEW

Introduction

Managing turfgrass diseases on golf courses is a full-time job that requires much time and money. Diseases are problematic on golf courses due to close mowing, improper nitrogen fertilization, intense irrigation, and constant bruising from traffic and divots (Beard, 2002). Dollar spot, a disease caused by *Sclerotinia homeocarpa* F.T. Bennett, is the most common disease superintendents deal with on a regular basis (Tani, 1997). More money is spent managing dollar spot in the United States than any other turfgrass disease (Vargas, 1994). In the "zero tolerance" arena of golf course management, symptoms caused by dollar spot can create serious problems with aesthetics and playability. Dollar spot can be a particular nuisance on Tennessee golf courses where the climate allows for growth of both cool- and warm-season turfgrasses. Turfgrasses defined as cool-season grow most actively between 16-24 ^{o}C, while warm-season turfgrasses are most actively between 27-35 ^{o}C (Mazur, 1977). Cool-season turfgrasses include the following: bluegrasses (*Poa*), fescues (*Festuca*), bentgrasses (*Agrostis*), and ryegrasses (*Lolium*) (Christians, 2004). The cool-season turfgrasses are generally less drought tolerant than warm-season turfgrasses, and experience stress at 32-38 ^{o}C. These turfgrasses must be intensely managed in areas where the average daily temperature in July is greater than 24 ^{o}C (Mazur, 1981). Warm-season turfgrasses include: bermudagrass (*Cynodon*), zoysia (*Zoysia*), St. Augustinegrass

(*Stenotaphrum*), bahiagrass (*Paspalum*), centipedegrass (*Eremechloa*), buffalograss (*Buchloe*), and carpetgrass (*Axonopus*) (Christians, 2004). Warm-season turfgrasses are generally very drought tolerant. They become dormant after the first killing frost and extreme low temperatures can injure perennial stands of most species. Warm-season turfgrasses must be intensely managed in areas where the average daily January temperature is less than 4 ^{o}C (Mazur, 1981).

Tennessee is situated in the climatic region referred to as the transition zone, where the average daily temperature in January is often less than 4 ^{o}C, and the average daily July temperature is greater than 24 ^{o}C. The transition zone is defined as an area covering 300 to 700 miles North to South between the cool, humid northern region and warm, humid southern region of the United States (Figure 1.1, all table and figures located in appendix.). Its northern perimeter is roughly defined by interstate 70 from Maryland through eastern Kansas. The southern edge mirrors the contour of Interstate 70, touching parts of Tennessee, Kentucky, and North Carolina (Dunn and Diesburg, 2004). While both warm- and cool-season turfgrasses may grow in this region, temperature-related injury occurs in the winter and summer months, respectively. Warm night temperatures in this region cause high respirational activity that weakens cool-season turfgrasses, increasing susceptibility to disease (Mazur, 1981).

In the transition zone, the most commonly used cool-season turfgrass on golf course putting greens is creeping bentgrass (*Agrostis stolonifera* L.) (Turgeon, 2002). Creeping bentgrass produces a fine-textured, soft, extremely dense sod that tolerates extremely low mowing heights and recuperates from injury relatively quickly (Emmons,

1995). Creeping bentgrass is, however, very susceptible to dollar spot disease. This is especially true in the transition zone where warm summer temperatures weaken cool-season turfgrasses. In the past, chemical control has been an important management strategy against this disease (Walsh *et al.,* 1999). However, several of the chemicals in various classes that formerly controlled dollar spot have lost efficacy over time due to mutating fungal strains with decreased sensitivity (resistance) to them (Vargas, 2002). Fungicide resistance occurs when a growth of a fungal pathogen previously controlled by a fungicide is no longer inhibited by that fungicide's chemistry. Prior to the1970's, fungicide resistance was seldom a problem as nearly all fungicides were multi-site inhibitors with protectant activity. When resistance did occur, it was likely due to nonspecific causes like detoxification or decreased uptake of the fungicide (Kandall *et al.,* 1998). Fungicide resistance became a problem with the widespread use of the systemic fungicide benomyl. An advantage of benomyl was its systemic property that not only protected plants from disease, but also controlled certain diseases when applied at early stages of infection. However, unlike earlier fungicides, the site-specific mode of action in benomyl was quickly overcome by several fungal pathogens (Damicone, 2002). Resistance to site-specific fungicides may be caused by reduced uptake of the fungicide, or by evolutionary metabolic changes in the pathogen, such as an increase in the target enzyme or reduced attraction of the target site (Kendall *et al.,* 1998).

Chemical management of dollar spot includes the use of both multi-site (contact) and site-specific (systemic) fungicides. Contact fungicides, such as chlorothalonil, are used as a protectant coating to prevent infection. Systemic fungicides enter the plant and

are transported through the vascular system to provide protection from within. Systemic fungicides, such as propiconazole, thiophanate methyl and iprodione, are used as preventative controls and to stop early infections (Vargas, 1994). Although resistance to chlorothalonil has not yet been documented, a growing body of evidence suggests that the dollar spot fungus has become resistant to fungicides in many of the other groups (Warren *et al., 1974*; Detweiler *et al.,* 1983; Golembiewski *et al.,* 1995; Hsiang *et al.* 1997; Miller *et al., 2002;* Vargas, 2002).

Fungicides are assigned to classes according to their mode of action. Although the term fungicide is applied to all chemicals that protect plants from fungal diseases, most are actually fungistats. Fungistats work by inhibiting the growth of a fungus by preventing spore germination and/or mycelial growth (Vargas, 1994). Examples of fungistats are the benzimidazoles, such as benomyl and thiophanate methyl, the demethylation inhibitors (DMIs), such as propiconazole and fenarimol, and the dicarboximides, such as iprodione and vinclozolin. Fungistats can be applied to prevent disease and to stop early infections by arresting the growth of the pathogen to allow the infected plant to recover, as well as inhibiting the spread of the pathogen to healthy plants (Emmons, 1995). For the remainder of this paper, all fungicides and fungistats will be referred to as fungicides, in keeping with modern literature (Vargas, 2002; Damicone, 2002; Miller *et al.,* 2002).

The resistance risk for turfgrass pathogens is considered moderate to high for the dicarboximides and benzimidazoles and moderate to low for the DMIs (Wilkinson, 2004). DMIs disrupt the synthesis of sterols (compounds required for growth in many

fungi). Resistance to DMI fungicides develops slowly, and may at first be difficult to detect (Damicone, 2002). Fungal isolates resistant to DMI fungicides have been shown to be less fit than non-resistant strains (Hsiang *et al*., 1997). Benzimidazoles inhibit microtubule assembly within the fungus. This can disrupt a great number of cellular processes such as nuclear cell division, cell migration, and organelle movement, as well as intracellular structure (Davidse, 1982). Benzimidazole-resistant strains of *S. homeocarpa* are present in nature at low frequencies before fungicides are applied. With the selection pressure of fungicides, resistance can develop quickly. Dicarboximides inhibit spore germination and fungal growth. Resistant fungi may be less fit than sensitive strains, and discontinuance of this type of chemical over time usually allows sensitive strains to again dominate the population (Damicone, 2002). Fungicides from each of these classes are commonly used to manage dollar spot infection of golf course greens. These fungicides may be applied at seven-day intervals when disease pressure is high. Frequent application of fungicides is a cause for environmental concern. This, along with documented cases of dollar spot fungicidal resistance, presents a disease management challenge for golf course superintendents. The purpose of this research was to determine if fungicide resistance in *S. homeocarpa* exists to fungicides in four commonly used fungicide classes. This research will serve as a building block to fungicide selection and the eventual reduction of fungicide usage against this pathogen in Tennessee and northern Mississippi.

The Hosts: *Agrostis stolonifera, Poa annua*

Agrostis stolonifera

The genus *Agrostis* is in the kingdom Plantae, phylum Magnoliophyta, order Cyperales, family Poaceae (USDA, 2004). It comprises a cool-season group of turfgrasses known as the bentgrasses, which is composed of about 220 species. Of these species, five are suitable for use as turf (Christians, 2004). All *Agrotis* species used as turfgrasses were introduced to the United States from Europe during the colonial period (Gould and Shaw, 1969). The most commonly used cool-season grass for golf greens today is creeping bentgrass (Turgeon, 2002).

Creeping bentgrass is a densely growing, fine-textured stoloniferous species that tolerates mowing heights as low as 3.2 mm. This, along with its excellent recuperative potential, makes it an excellent surface for putting greens (Emmons, 1995). The species has fair heat tolerance and excellent cold tolerance, although it may suffer winter desiccation in drier climates (Christians, 2004). Optimum pH for growth of bentgrass is between 5.5 and 6.5 in a loose, non-compacted soil texture containing a high percentage of sand (Emmons, 1995).

The intense cultural demands of creeping bentgrass make it a poor candidate for home lawns. The root system of creeping bentgrass is dense, fibrous, and relatively shallow. High temperatures, lack of aerification, and close mowing heights cause the root system to be poorly developed with limited water absorption capacity (Christians and Engelke, 1994). This results in a need for frequent irrigation and mid-day syringing

with water when temperatures are high. Fertility requirement is also high. Greens may be fertilized regularly with granular or liquid products once every three weeks. Additionally, creeping bentgrass is very susceptible to disease at close mowing heights, creating the need for routine fungicide treatments. Strong, vigorous stolon growth contributes to thatch problems that are often corrected with frequent vertical mowing and topdressing. Core cultivation is also necessary to reduce compaction from heavy foot traffic (Emmons, 1995). The stress from constant low mowing, traffic and cultivation practices, coupled with stress associated with growing in an environment warmer than its natural range, causes creeping bentgrass to be quite susceptible to disease in the transition zone (Beard, 2002). Although many disease-resistant cultivars are currently on the market, no variety of bentgrass is completely disease resistant (Graham, 2003). In the transition zone, 'Crenshaw' creeping bentgrass is the one of the most commonly used cultivars because of its greater heat and shade tolerance (Dernoeden, 2000). This is particularly true for Tennessee. 'Crenshaw' is also very susceptible to dollar spot (Dernoeden, 2000; Graham, 2003). Warm temperatures, high humidity, and low nitrogen fertilization on golf courses in Tennessee and surrounding states cause elevated disease pressure during spring, summer, and fall. These optimum conditions allow for severe dollar spot outbreaks on bentgrass golf greens, particularly the 'Crenshaw' cultivar.

Poa annua

The genus *Poa* is in the kingdom Plantae, phylum Magnoliophyta, order Cyperales, family Poaceae (USDA, 2004). It comprises a cool-season group of

turfgrasses known as the bluegrasses, which is composed of more than 500 individual species (Christians, 2004). Of these 500 species, Kentucky bluegrass (*P. pratensis* L.), Canada bluegrass (*P. compressa* L.), rough bluegrass (*P. trivialis* L.), and annual bluegrass *(P. annua* L.) are the ones commonly used for turf (Turgeon, 2002).

Annual bluegrass consists of two subspecies; *P. annua* ssp. *annua,* an annual plant, and *P. annua* ssp. *reptans,* which persists as a perennial in wetter, more intensely managed sites. The perennial type is more common in the cool north, whereas the annual type is dominant in the South (Emmons, 1995).

Annual bluegrass is classified as a 'winter annual' (Christians, 2004). The winter annual life cycle of annual bluegrass ensures its survival in the hot, humid South. Plants avoid summer stress by producing seed and dying in the spring, then producing new plants from seed when the cooler days of fall arrive. Although this is the general life cycle, seeds may germinate soon after dropping from the plant if sufficient moisture is present. Seedheads are formed any time annual bluegrass is actively growing (Christians, 2004). The species produces seedheads at mowing heights as low as 2.54 mm (Christians, 1996). Prolific seed production at low mowing heights, coupled with the moist conditions on a golf course green, makes control of annual bluegrass quite difficult. These stresses may cause an open spot in the turf canopy. Annual bluegrass can germinate and dominate before creeping bentgrass can fill in the hole (Vargas, 1994).

In areas where cool weather and ample moisture are present, such as parts of the Pacific coast, the northern United States, and Canada, annual bluegrass is considered a desirable turfgrass. Because a moist, highly maintained bentgrass turf may contain as

much as 90% of annual bluegrass, the control strategy sometimes involves management of this grass in cooler regions (Emmons, 1995). However, in the warm, humid South, it is treated as a weed that must be controlled (Dunn and Diesburg, 2004). All of the golf course greens in the present study were infested with annual bluegrass.

The seedhead production and lighter yellow color of annual bluegrass not make it aesthetically unappealing on a bentgrass green, but also cause a disruption in the uniformity of the putting surface. Annual bluegrass may take over large portions of a bentgrass green during cool spring months, then die during June or July when foot traffic is heavy (Christians, 2004). Annual bluegrass is susceptible to dollar spot, brown patch, *Pythium* blight, leaf spot, anthracnose, and *Microdochium* patch (Vargas, 1994). These diseases can also affect creeping bentgrass (Tani and Beard, 1997). The senescent nature of annual bluegrass in the summer invites disease, which could spread to creeping bentgrass. Similarly, when disease pressure is high, annual bluegrass will become infected with *S. homeocarpa* along with creeping bentgrass.

The Pathogen: *Sclerotinia homeocarpa*

Sclerotinia homeocarpa, the causal pathogen of dollar spot, has been documented in Australia, Japan, Central America, Europe, New Zealand, and North America (Tani and Beard, 1997). In the United States, dollar spot is widespread, except in the arid regions of the West (Vargas, 1994). The pathogen has an extensive host range, infecting both warm- and cool-season turfgrasses in the family Poaceae. Its major hosts are the bermudagrasses, bentgrasses, and the fine fescues. The pathogen can infect *zoysia*,

bahiagrass, centipedegrass, St. Augustinegrass, the bluegrasses, and tall fescue (Tani and Beard, 1992). In addition to these turfgrasses, *S. homeocarpa* has also infected members of the families Caryophyllaceae (Fenstermacher, 1970), Convolvulaceae (Boesewinkle, 1977), Cyperaceae (Bain, 1964; Whetzel, 1946), and Fabaceae (Hoover and Kucharek, 1995).

Taxonomy

The genus *Sclerotinia* is in the kingdom Fungi, phylum Ascomycota, order Helotiales, family Sclerotiniaceae (USDA, 2004). The causal pathogen of dollar spot is currently classified in this genus; however, its taxonomic status has been a point of discussion among scientists. The fungus rarely produces teleomorph or anamorph structures in culture, making it difficult to determine its true taxonomic classification (Walsh, *et al.,* 1999). Fertile apothecia are not often seen in planta, and cultures on artificial media frequently produce sterile apothecia (Baldwin and Newell, 1992; Fenstermacher, 1970; and Jackson, 1973).

The disease now known as dollar spot was first described by Moneith and Dahl in 1932, although they incorrectly identified the fungus as a species of *Rhizoctonia*, noting the similarity of the disease symptoms to those caused by the turf pathogen *R. solani* (Moneith and Dahl, 1932). Five years later, Bennett examined isolates of the pathogen from Britain, the United States, and Australia and was the first to observe the pathogen's spore stages in culture. He described three separate strains of the fungus as the following: 1) "non-sporing strains" of American, Australian, and British origin that

occasionally produced simple apothecial initials but did not produce ascospores; 2) a "perfect strain" of British origin that formed ascospores and conidia; 3) an "ascigerous strain" of British origin that produced ascospores and microconidia. Despite these differences, he considered all three strains to be a single species. He considered the fungus to belong to the genus *Sclerotinia* because apothecia occasionally developed from small masses of sclerotial cells termed "micro-sclerotia" (Bennett, 1937). At the time, the genus *Sclerotinia* had a broad definition, which included fungi that produced conidia (Walsh *et al.,* 1999). Bennett assigned the species name *homeocarpa* to the fungus because the "perfect strain" formed cupulate or apothecial-shaped structures for the production of both conidia and ascospores (Bennett, 1937).

Whetzel described the family Sclerotiniaceae in 1945. He restricted the genus *Sclerotinia* to include those fungi in which the apothecium arose from a tuberoid sclerotium that was formed free on aerial mycelium (Whetzel, 1945). Based on this definition, *S. homeocarpa* was excluded because true sclerotia have not been observed in dollar spot infections of turfgrass (Baldwin and Newell, 1992). This definition of the family Sclerotiniaceae is still accepted today (Alexopolous, 1996).

In a monographic revision of *Sclerotinia* in 1979, Khon, who considered that dollar spot was caused by more than one organism, specifically excluded the fungus from the genus. He suggested the fungi responsible for dollar spot be classified as *Lanzia Sacc*. (family Rutstroemiaceae) and *Moellerodiscus* Henn. (family Sclerotiniaceae) (Khon, 1979a; Khon, 1979b). Although Khon suggested these genera for the pathogen, he concluded his report stating that more work must be done to delimit the species.

Traditionally, taxonomic clarification of most fungal species has been based on the teleomorphic (sexual) structures (Windham, 2004). Although fertile apothecia in *S. homeocarpa* have been reported, their occurrence is rare (Baldwin and Newell, 1992). The difficulty in finding reproductive structures in *S. homeocarpa* has made assigning it a taxonomic classification difficult. New molecular techniques are currently redefining taxonomic schemes constructed with morphological data (Taylor, *et al.,* 1994; Samuels and Seifert, 1995). In 1997, Vargas and Powell reported an 88% similarity between *S. homeocarpa*, *Rutstroemia henningsiana* and *R. cuniculi* based on nuclear ribosomal internal transcribed spacer 1 sequence (ITS1) alignment, suggesting that *Rutstroemia* may actually be the correct genus for the fungus (Vargas and Powell, 1997). In 1999, Vargas and Powell published another report comparing the ITS1 sequences from *S. homeocarpa* isolates from North America, Britain, and members of the genera *Rutstroemia, Lanzia*, and *Mollerodiscus*. Parsimony analysis revealed that *S. homeocarpa* clustered again within the genus *Rutstroemia* (Vargas and Powell, 1999).

Placement in the proper taxonomic category has not yet been achieved (Rossman, 1987; Tani and Beard, 1997; Christians, 2004). For the purposes of this research, the causal pathogen of dollar spot is referred to as *S. homeocarpa*.

Symptoms and signs

On turfgrasses mown to 15 mm or less, such as those on golf greens, the most distinctive symptom of this disease is small, tan, circular, sunken patches that rarely exceed 60 mm in diameter (Figure 1.2) (Smiley *et al.,* 1992; Vargas, 1994). If a dollar

spot infection is severe, individual patches of blighted grass may coalesce to cover areas up to 150 mm (Tani and Beard, 1997). On turfgrass mown to heights greater than 15 mm, irregularly shaped patches may cover areas 20-150 mm across (Smiley *et al.,* 1992).

Individual leaves infected by *S. homeocarpa* develop a characteristic lesion that is at first chlorotic, then appears water-soaked, and finally a bleached color that is separated from the green portion of the leaf by a reddish-brown band (Figure 1.3) (Joyner and Larson, 1979). This reddish-brown band occurs on all turfgrass species except annual bluegrass (Vargas, 1994). Individual leaf blades may be partially or entirely bleached, or may contain multiple lesions (Joyner and Larson, 1979). The lesions typically take on an hourglass shape (Smiley *et al.,* 1992).

During periods of high humidity or when dew is present on leaves, a white, cobwebby mycelia can be observed on diseased turf with actively growing fungi (Figure 1.4) (Moneith and Dahl, 1932; Smith, 1955). These aerial mycelia disappear as the leaves dry (Smiley *et al.,* 1992).

Although it does not directly affect the roots, *S. homeocarpa* has been associated with a root-damaging toxin (Endo, 1964). At 15.5 ^{o}C, culture filtrates of the fungus contained a heat-stable chemical that caused growth cessation, thickening, and browning of creeping bentgrass roots growing in quartz sand culture (Malca and Endo, 1965).

Disease cycle

Sclerotinia homeocarpa is a facultative saprophyte that overwinters as mycelia on infected plants and as stromata on the margins of lesions from previous infections

(Smiley *et al.,* 1992; Vargas, 1994). The pathogen is distributed locally when mycelium grows from a diseased leaf to a nearby healthy one (Smiley, *et al.,* 1992). Over larger areas, the pathogen is spread by human contact. Infested and diseased material can be transported to other locations on shoes, golf carts, mowers, and other maintenance equipment (Vargas, 1994). Once in contact with a healthy leaf, the fungus may invade through cut leaf tips, stomata, or direct penetration into the leaf (Endo, 1966; Moneith and Dahl, 1932).

Although conidia and ascospores are rarely observed in nature, fertile apothecia have been observed in turf areas, suggesting that they may be an important source of inoculum as well (Baldwin and Newell, 1992).

Epidemiology

There is much variability among geographically separated isolates of *S. homeocarpa* in response to environmental conditions (Bennett, 1937; Endo, 1963). Generally, however, the dollar spot pathogen is most active from late spring through autumn when warm days and cool nights result in heavy dew (Smiley *et al.,* 1992). The optimum growth temperature on potato dextrose agar is reported at 26.8^{o}C. The minimum and maximum temperatures for growth are 4.5 and >32^{o}C, respectively (32^{o}C was the highest temperature tested at which the fungus still grew) (Endo, 1963). In the field, a temperature range of 15 ^{o}C to 30 ^{o}C is favorable for infection and growth (Smiley *et al.,* 1992). Warm, humid weather with cool nights that result in heavy dew increase the incidence of disease (Smiley *et al.,* 1983).

Dew and guttation water play a role in the incidence of the disease as well. The duration of leaf wetness from dew and guttation water is positively correlated to the occurrence of dollar spot (Williams *et al.,* 1996). Guttation water is rich in carbohydrates and amino acids, an excellent food source for the fungus as it grows from leaf to leaf (Vargas, 1994). Nitrogen (N) content is also important. Turf low in N is more likely to develop dollar spot disease than turf that receives adequate nitrogen fertilization (Endo, 1966; Watkins and Wit, 1995). A thick accumulation of thatch may also contribute to disease severity by inhibiting water penetration into the soil and contributing to drought stress (Walsh *et al.,* 1999). Thatch may be decreased through cultural practices such as aerification and vertical mowing.

Cultural control measures

Several cultural practices can be utilized to manage dollar spot in turf. Increased N fertilization can be effective in lessening the damage due to the disease (Vargas, 1994). Adequate N fertilization causes an increase in growth. Vigorous plant growth, along with frequent mowing and subsequent removal of necrotic tissue, decreases the appearance of the disease (Couch and Smith, 1991). Additionally, removal of grass clippings during cutting decreases disease by removing potential sources of secondary inoculum (Walsh *et al.,* 1999).

Because drought stress causes an increase in dollar spot severity, adequate soil moisture also plays a role. Foliar lesions on Kentucky bluegrass grown under low soil moisture conditions were more than double that of plants grown in soil at field capacity.

Maintaining soil moisture above 75% of field capacity reduces disease severity (Couch and Smith, 1991).

Conversely, the presence of guttation water and dew serves to increase dollar spot severity. Many fungi, including *S. homeocarpa*, require free water to maintain cell turgidity and support hyphal growth. Amount and duration of leaf wetness is an important factor in dollar spot development (Williams and Powell, 1995). Additionally, guttation water contains nutrients that are beneficial to the fungus. Exudates from creeping bentgrass make up nearly one-third of dew accumulation on the leaf surface. This exudate contains sugars and amino acids that can be used as a nutrient source for the fungus (Williams *et al.,* 1996). Several cultural practices may be implemented to reduce the amount and duration of leaf wetness caused by dew and guttation water. Poling, lightly irrigating, or mowing greens in the morning hours will displace dew and guttation water and reduce infection (Vargas, 1994). In addition, pruning or removing trees and shrubs to allow more light and air circulation will dry the greens more quickly (Walsh *et al.,* 1999). Additionally, rolling turfgrass greens with a lightweight roller three times per week reduces the incidence of dollar spot by displacing the dew and guttation water from the leaf blade (Nikolai, 2002).

Chemical control measures

Fungicides have been the most widely used tool for managing dollar spot for the last 40 years (Walsh *et al.,* 1999). More money is spent to manage dollar spot outbreaks any other turfgrass disease on golf courses (Goodman and Burpee, 1991). Dollar spot

can be managed with both contact and systemic fungicides. The most commonly used contact fungicide is chlorothalonil (Vargas, 1994). Systemic fungicides effective against the disease fall into the following three chemical classes: the demethylation inhibitors (DMIs), the benzimidazoles, and the dicarboximides (Dernoeden, 2000). Several chemicals within each class can be effective. Within the DMI class are fenarimol, myclobutanil, propiconazole and triadimefon. Thiophanate-methyl and benomyl are benzimidazoles, and the dicarboximides comprise iprodione and vinclozolin (Broder and Samples, 2000). Spot applications provide effective control during times of low disease pressure, however, treatment of the entire turf area is recommended during rainy seasons and other times of high disease pressure (Tani and Beard, 1997). Required application frequency may be as often as every seven to ten days. This frequency of application is one reason for selection of resistant strains over time.

CHAPTER 2

EVALUATION OF THIOPHANATE-METHYL, IPRODIONE, AND PROPICONAZOLE FOR CONTROL OF GROWTH OF *SCLEROTINIA HOMEOCARPA IN VITRO*

Introduction

Fungicide applications are frequently used to manage *S. homeocarpa* on golf courses. Although chemical control is an effective way to manage dollar spot disease, however it is not always efficient because of the incidence of fungicide resistance. *S. homeocarpa* has demonstrated resistance to a number of fungicides including cadmium and mercuric fungicides (Cole *et al.,* 1968; Massie *et al.,* 1968), anilazine (Nicholson *et al.,* 1971), benzimidazoles (Cole *et al.,* 1974; Detweiler *et al.,* 1983; Goldberg and Cole, 1968; Warren *et al.,* 1974), dicarboximides (Detweiler *et al.,* 1983), and DMIs (Golembiewski *et al.,* 1995). Adding to the difficulty of managing the disease with fungicides, strains of *S. homeocarpa* that are resistant to one fungicide chemistry are often resistant to other fungicides that share the same mode of action (Cole *et al.,* 1974; Golembiewski *et al.,* 1995; Warren *et al.,* 1974). Strains of the fungus may also be resistant to more than one fungicide chemistry (Burpee, 1997; Detweiler *et al*., 1983).

Resistance can be defined as a genetic adjustment by a fungus that results in reduced sensitivity to a fungicide. These genetic adjustments are thought to be mutations which occur at frequencies of one in a million or less (Damicone, 2002). Resistant individuals may exist in a fungal population before application of fungicides, or may develop over time after the application of fungicides, however, fungicides do not cause

strains to mutate and become resistant. Resistant strains become dominant when fungicide applications inhibit or destroy sensitive strains in an area (Vargas, 1994).

Resistance may result from single- or multiple-gene mutations. Single-gene mutations confer resistance to site-specific, or systemic, fungicides. Resistance to site-specific fungicides may be caused by a reduction in the uptake of fungicide, or by evolutionary metabolic changes in the pathogen, such as an increase in the target enzyme or reduced attraction of the target site (Kendall *et al.,* 1998). This type of mutation is more likely to occur than multi-site mutations that could confer resistance to multi-site, or contact, fungicides (Damicone, 2002). Neither contact nor systemic fungicides cause mutations. Fungicide treatments may apply selective pressure that allows a mutated (resistant) strain in the population to become dominant as the sensitive strains are eliminated (Vargas, 1994).

The history of fungicide resistance in *S. homeocarpa* is long (Table 2.1). Earliest reports of resistance surfaced in the late 1960's when cadmium tolerance was reported (Cole *et al.,* 1968; Fenstermacher, 1980; Smith *et al.,* 1989). Resistance to cadmium fungicides took many years to establish, and was not considered a major problem at the time since no resistance to other contact fungicides was documented (Vargas, 1994). However, strains resistant to cadmium fungicides possessed increased fitness, dominating fungal populations where cadmium had been used, and surviving long after the final cadmium applications (Warren *et al.*, 1977).

Fungicide resistance in *S. homeocarpa* gained more attention in the early 1970s with reports of resistance to a systemic fungicide (Vargas, 1994). Benomyl, a fungicide

in the benzimidazole class, had been providing excellent systemic disease control at low dosages for several years (Walsh *et al*., 1999). However, benomyl resistance surfaced relatively quickly (Fenstermacher, 1980). One reason for this is that benzimidazole resistant strains of the fungus have been shown to already exist in nature (Damicone, 2002). This, coupled with the frequent spraying with the site-specific fungicide, put high selection pressure on the fungal population and allowed the resistant biotype to quickly become dominant. Like cadmium-resistant strains, benzimidazole-resistant strains have been shown to be more fit compared to non-resistant strains, surviving long after cessation of spraying the benzimidazole fungicide (Vargas, 1994). Disease severity has actually been shown to increase when creeping bentgrass infected with a benzimidazole-resistant strain of *S. homeocarpa* is sprayed with a fungicide from this class (Couch and Smith, 1991).

Resistance to the DMI fungicides has also been documented (Hsiang and Barton, 1997; Golembiewski *et al.*, 1995; Burpee, 1997; Miller and Stevenson, 2002). The DMI fungicides were registered for use on dollar spot in 1979. By 1990, several cases of resistance to this class of fungicides had been reported (Golembiewski *et al.*, 1995). One *S. homeocarpa* isolate with reduced sensitivity to the DMI's was observed in Canada prior to the 1994 release of the fungicide for use on the pathogen, again reinforcing the theory that the fungicide does not cause mutations, but simply selects for strains in the population that are already predisposed to resistance (Hsiang *et al.*, 1997).

Although less information has been published about dicarboximide resistance in *S. homeocarpa*, there has been documentation of its occurrence. A suspected resistant

strain grew on potato dextrose agar amended with up to 1000 µg ml^{-1} iprodione (Detweiler *et al.*, 1983). In vitro, the fitness of dicarboximides resistance strains of pathogens is generally regarded as inferior to strains of the pathogen that are not resistant (Damicone, 2002). Discontinuance of spraying with a dicarboximides fungicide has been shown to significantly reduce the population levels of dicarboximide-resistant strains (Detweiler *et al.*, 1983).

Further complicating the occurrence of fungicide resistance in *S. homeocarpa* is the incidence of strains resistant to more than one fungicide chemistry. This is known as double or multi-resistance (Vargas, 1994). Resistance to both benzimidazole and cadmium fungicides was reported as early as the 1970's (Warren *et al*., 1974). Benzimidazole-resistant strains have also been reported to have multi-resistance to the dicarboximides (Detweiler *et al.*, 1983). Some DMI-resistant strains have been observed resistant to the benzimidazole and dicarboximides fungicides as well (Golembiewski *et al.*, 1995; Vargas *et al.*, 1992).

The purposes of the current investigations were to determine the following: (i) if resistance to the fungicides thiophanate-methyl, iprodione, and propiconazole exists in Tennessee, and Northern Mississippi by testing isolates of *S. homeocarpa* field-collected in these areas; (ii) if isolates collected in Michigan differ in their levels of resistance to isolates collected in Tennessee and Northern Mississippi; (iii) if multi-resistance to different fungicide classes occurs in these isolates; and (iv) if chemical practices of golf course superintendents have an effect on the occurrence of fungicide resistance in the southern-collected isolates.

Materials and Methods

Isolate collection

A total of 284 isolates of *S. homeocarpa* were collected from eight golf courses across Tennessee and in northern Mississippi in the summer of 2003 (Table 2.2). Additionally, isolates were collected from two locations on an additional course where two different creeping bentgrass cultivars were being subjected to separate experimental fungicide applications. Isolates were collected from creeping bentgrass putting greens and a creeping bentgrass nursery exhibiting dollar spot symptoms. The bentgrass (*Agrostis stolonifera*) greens all contained at least a small amount of annual bluegrass (*Poa annua*). Three isolates known to be resistant to either the dicarboximides, benzimidazoles, or DMI's were obtained from Michigan State University and tested as well, along with a known "wild type" isolate sensitive to all three of these fungicide classes (Phillip Dwyer, personal communication).

A small metal spatula was used to extract a small amount of infected leaf blade material from the margins of infection centers. When possible, isolates were collected from various infection sites on the same green. A representative sample was taken from all known affected sites on each course in the study. Isolates were placed individually in sterile plastic bags and labeled with the name of the golf course and location within the course from which the isolates were taken. Bags were stored on ice in a cooler until they reached the lab.

Cultural and chemical practices survey

Superintendents from the 10 locations provided maintenance records for the 2002-2003 growing season. This information was used to asses how these practices might impact the potential development of fungicide resistance in *S. homeocarpa*.

Isolation and growth of pathogen

All collected isolates were surface sterilized for 30 s in a solution containing 10 ml hypochlorite and 5 ml ethanol in 85 ml of sterile deinoized (DI) water, and rinsed once in sterile DI water for 10 s (Miller *et al.,* 2002). Individual leaf blades were placed in Petri dishes (8.5-cm inside diameter) containing 25 ml of potato dextrose agar (PDA) amended with tetracycline HCl and streptomycin sulfate at 8 µg ml^{-1} each, and the cultures were incubated in the dark at 21 ± 1°C for a minimum of 48 h (Golembiewski *et al.,* 1995). The pathogen was identified by visual comparison of colony growth with known cultures of *S. homeocarpa* (Cole *et al.,* 1967; Hsiang *et al.,* 1997; Miller *et al.,* 2002). Single 5-mm-diameter plugs with mycelium of *S. homeocarpa* from an area adjacent to the leaf blades were cut from each plate with a sterile cork borer, transferred to a new Petri dish onto fresh PDA, and sealed with parafilm. Cultures were incubated in the manner previously described until mycelial growth had occurred. The fungi were subcultured in this manner at least three times before testing to ensure that any chemicals from applications made before initial collection at the golf courses were not present on the fungi. Cultures were then stored in a cool chamber at 4°C until testing for fungicide sensitivity (Hsiang *et al.,* 1997).

Isolate selection

Isolate collections from each course were divided into groups according to the location where they were collected. Ten isolates were chosen from each of eight sites to include all locations on the golf course from which the pathogen was collected. Isolates within each location were chosen randomly. Five isolates were chosen from each of the remaining two sites due to a small sample size. Each isolate to be tested was subcultured on 25 ml of fresh PDA for four days before testing.

Treatments

Fungicide solutions were prepared with thiophanate-methyl, propiconazole, and iprodione. Fungicides were commercial grade and contained solvents as well as active ingredients. Individual fungicides in liquid form were mixed directly into autoclaved, partially cooled PDA in a sterile laminar flow hood at concentrations of 1, 10, 100, and 1000 µg active ingredient ml^{-1}. Additionally, a mixture of PDA containing no fungicides was used as a control (Detweiler *et al.,* 1983).

Under sterile conditions,5 mm diameter plugs containing mycelia of *S. homeocarpa* were cut from the actively growing outer edge of four-day-old inoculum dishes with a sterilized cork borer. Each plug was inverted and placed in the center of the dishes containing fungicides. For each course, each of the *S. homeocarpa* isolates was placed on four replicate dishes per fungicide concentration, as well as one control plate containing PDA with no fungicide. Isolates were incubated at 21 ± 1 ^{o}C under a 12 hour light/dark cycle (Detweiler *et al.,* 1983).

Data collection

Isolate radial mycelial growth was measured 24 h after inoculation, as well as the diameter perpendicular to the first. Measurements were subsequently taken every 24 h until the mycelial growth reached the edge of the control dishes. SAS 9.0 Proc Probit was used to calculate the EC_{50} and EC_{90} (estimated fungicide concentration needed to inhibit growth by 50 and 90 percent of control growth, respectively), and SAS 9.0 Proc Mixed was used for analysis of variance of mean fungal growth ($P = 0.05$). Sigmaplot 8.0 was used for regression analysis of relative growth of control against fungicide active ingredient concentration using an exponential decay equation.

Results and Discussion

Fungicide resistance was observed in isolates at nine of the ten southern locations tested. Additionally, three isolates from Michigan also expressed resistance. One isolate from Michigan known to be sensitive to all fungicides used in this study did not express any resistance to the fungicides evaluated (Table 2.3).

Isolates at seven locations exhibited resistance to iprodione and thiophanate-methyl, and isolates at two locations exhibited resistance to propiconazole (Figures 2.1, 2.2, 2.3). One location had isolates with multi-resistance to thiophanate-methyl and iprodione, another had isolates with multi-resistance to thiophanate-methyl and propiconazole, and another location had isolates with multi-resistance to all three fungicides tested. The highest fungicide active ingredient concentration at which resistance occurred was to iprodione and thiophanate-methyl at 1000 $\mu g\ ml^{-1}$, and the

highest concentration to which resistance occurred for propiconazole was 10 µg ml^{-1} (Table 2.4). EC_{50} and EC_{90} values were variable among locations with resistance to the same fungicides (Table 2.5). Most of the resistant isolates grew at concentrations far greater than the recommended application rate for the turf fungicides (Table 2.6). Of the 94 isolates tested 25% were resistant to iprodione, 46% were resistant to thiophanate-methyl, and 11% were resistant to propiconazole. All isolates tested were significantly analyzed separately by location collected.

Analysis by location

Location BM. At location BM, one isolate exhibited resistance to iprodione. All other fungicides inhibited the growth of fungi by 100% at all concentrations. isolate ten was resistant, however, the isolate was resistant up to 1000 µl ml^{-1} of iprodione (Figure 2.4). The interaction effect of the fungicide active ingredient was highly significant for growth of this isolate ($P < 0.0001$). Growth at all concentrations was significantly different, with average relative growth ranging from 17 to 80% of the growth of the control (Figure 2.5).

The dosage-response curve for BM isolate 10 growing on iprodione amended PDA was significant correlated ($r^2 = 0.90$) (Figure 2.6). The EC values were quite high compared to values for most other iprodione-resistant isolates. The EC_{50} value of 230 µl ml^{-1} for isolate 10 was the median value for all isolates, and the EC_{90} value of 1175 µl ml^{-1} was the third highest value for all iprodione-resistant isolates tested (Table 2.5).

Location CH. At location CH, isolates were resistant to thiophanate-methyl. All other fungicides inhibited the growth of the fungi by 100% at all concentrations. All ten isolates were resistant to thiophanate-methyl at all concentrations tested (Figure 2.7). The interaction effect of the fungicide active ingredient concentration with growth of all isolates was highly significant ($P < 0.0001$). Growth at all concentrations was significantly different, with average relative growths ranging from 83 to 100% of the growth of the control isolate (Figure 2.8).

The dosage-response curve for the CH isolate with the highest average relative growth (97%) on thiophanate-methyl amended PDA was significantly correlated ($r^2 = 0.76$) (Figure 2.9). The low r^2 value can be attributed to the fact that no decrease in relative growth occurred between 1 and 10 µl ml^{-1}, with growth at both concentrations near 100% of the control growth. The dosage-response curve for the CH isolate with the lowest average relative growth (89%) on thiophanate-methyl amended PDA was significant ($r^2 = 0.47$) (Figure 2.10). The low r^2 value can again be attributed to the fact that no decrease in relative growth occurred between 1 and 10 µl ml^{-1}, with growth at both concentrations near 100% of the control growth. Additionally, relative growths at 100 and 1000 µl ml^{-1} were also similar in value, with relative growths of 81 and 78%, respectively.

The calculated EC_{50} value for all CH isolates growing on thiophanate-methyl amended PDA was 2089 µl ml^{-1}, which was similar to isolates from four of the other locations with resistance to thiophanate-methyl. The EC_{90} value of 3606 µl ml^{-1} was also similar to the value of three of the locations (Table 2.5).

Location CW. At location CW, isolates exhibited resistance to both iprodione and thiophanate-methyl. Propiconazole inhibited the growth of the fungi by 100% at all concentrations. All 10 isolates were resistant to iprodione at all concentrations tested (Figure 2.11). This was the highest level of resistance to iprodione observed in all locations tested. Less resistance was observed to thiophanate-methyl, with two isolates showing any growth at the lowest concentration tested. (Figure 2.12)

Iprodione. The interaction effect of the fungicide active ingredient concentration with growth of all isolates could not be computed for iprodione because all isolates grew to 100% relative growth of the control at all concentrations tested. Growth at all concentrations was significantly similar (Figure 2.13). Additionally, EC_{50} and EC_{90} values could not be computed because of the total resistance at all concentrations.

The dosage-response curve for the iprodione-resistant CW isolates was a perfect correlation ($r^2 = 1.00$) (Figure 2.14).

Thiophanate-methyl. The interaction of the fungicide concentration was highly significant for the growth of the two isolates that showed resistance ($P < 0.0001$). Relative growth at 1 μl ml^{-1} was significantly different from relative growth at all other concentrations, which was expected because it was the concentration at which growth occurred (Figure 2.15).

The dosage-response curve for the CW isolate with the lowest average relative growth (20%) on thiophanate-methyl amended PDA was significantly correlated ($r^2 = 0.99$) (Figure 2.16). The dosage-response curve for the CW isolate with the highest

average relative growth (24%) on thiophanate-methyl amended PDA was also significantly correlated ($r^2 = 0.99$) (Figure 2.78).

The EC_{50} value for both CW isolates growing on thiophanate-methyl amended PDA was slightly negative (-1), indicating that any concentration could reduce growth to 50% of the control growth. The EC_{90} value of 0.8 µl ml^{-1} was the lowest value for all thiophanate-methyl-resistant isolates. These low EC values indicate that this location had the least resistance to thiophanate-methyl of all locations at which resistance occurred (Table 2.5).

Location GV. At location GV, isolates were observed resistant to thiophanate-methyl. All other fungicides inhibited the growth of the fungi by 100% at all concentrations. All 10 isolates were resistant to thiophanate-methyl at all concentrations tested (Figure 2.18). The interaction effect of the fungicide active ingredient concentration with growth of all isolates was highly significant ($P < 0.0001$). Growth at all concentrations was significantly different, with average relative growths ranging from 52 to 72% of the growth of the control isolate (Figure 2.19).

The dosage-response curve for the GV isolate with the highest average relative growth at all fungicide active ingredient concentrations (66%) on thiophanate-methyl amended PDA was significantly correlated ($r^2 = 0.96$) (Figure 2.20). The dosage-response curve for the GV isolate with the lowest average relative growth (60%) on thiophanate-methyl amended PDA was significantly correlated ($r^2 = 0.52$) (Figure 2.21). The low r^2 value can be attributed to the fact that no decrease in relative growth occurred

between 10 and 100 µl ml^{-1}, with relative growths at these concentrations of 64 and 62%, respectively.

The EC_{50} value for all GV isolates growing on thiophanate-methyl amended PDA was 476 µl ml^{-1}. This was relatively low compared to the other locations with resistance to thiophanate-methyl. The EC_{90} value of 819 µl ml^{-1} was also relatively lower than the values for the other locations (Table 2.5).

Location LG. At location LG, isolates exhibited resistance to iprodione. All other fungicides inhibited the growth of the fungi by 100% at all concentrations. Four isolates were resistant to iprodione. Three of these isolates were resistant at the 1 µl ml^{-1} concentration, and one was resistant up to 10 µl ml^{-1} (Figure 2.22). The interaction effect of the fungicide active ingredient concentration with growth of all isolates that grew was highly significant ($P < 0.0001$). Growth at the 1 µl ml^{-1} was significantly different from all other concentrations, as was growth at 10 µl ml^{-1}. No growth occurred at the 10 and 1000 µl ml^{-1} concentrations, making them significantly similar (Figure 2.23).

The dosage-response curve for the LG isolate with the highest average relative growth at all fungicide active ingredient concentrations (9%) on iprodione-amended PDA was highly correlated ($r^2 = 0.99$) (Figure 2.24). The dosage-response curve for the LG isolate with the lowest average relative growth (2%) on thiophanate-methyl amended PDA was also highly correlated ($r^2 = 0.99$) (Figure 2.25).

The EC_{50} value for all LG isolates growing on iprodione amended PDA was negative (-24), indicating that any amount of fungicide active ingredient could reduce the

growth of the isolates at this location to 50% of the control growth. This was the lowest value compared to the other locations with resistance to iprodione. The EC_{90} value of 7 µl ml^{-1} was the median value for all iprodione-resistant locations (Table 2.5).

Location LT1. At location LT1, isolates exhibited resistance to iprodione. All other fungicides inhibited the growth of the fungi by 100% at all concentrations. All five of the isolates tested were resistant to iprodione up to the 10 µl ml^{-1} concentration (Figure 2.26). The interaction effect of the fungicide active ingredient concentration with growth of all isolates was highly significant ($P < 0.0001$). Growth at the 1 µl ml^{-1} was significantly different from all other concentrations, as was growth at the 10 µl ml^{-1}. No growth occurred at the 100 and 1000 µl ml^{-1} concentrations, making them significantly similar (Figure 2.27).

The dosage-response curve for the LT1 isolate with the highest average relative growth at all fungicide active ingredient concentrations (22%) on iprodione-amended PDA was perfectly correlated ($r^2 = 1.00$) (Figure 2.28). The dosage-response curve for the LT1 isolate with the lowest average relative growth (12%) on thiophanate-methyl amended PDA was also perfectly correlated ($r^2 = 1.00$) (Figure 2.29).

The EC_{50} value for all LT1 isolates growing on iprodione amended PDA was negative (-11), indicating that any amount of fungicide active ingredient could reduce the growth of the isolates at this location to 50% of the control growth. This was the second lowest value compared to the other locations with resistance to iprodione. The EC_{90}

value of 6 µl ml^{-1} was the third lowest value for all iprodione-resistant locations (Table 2.5).

Location LT2. At location LT2, isolates were observed resistant to iprodione. All other fungicides inhibited the growth of the fungi by 100% at all concentrations. Three of the five isolates tested were resistant to iprodione at the 1 µl ml^{-1} concentration (Figure 2.30). The interaction effect of the fungicide active ingredient concentration with growth of all isolates that grew was highly significant ($P < 0.0001$). Growth at 1 µl ml^{-1} was significantly different from all other concentrations. No growth occurred at the 1, 10 and 1000 µl ml^{-1} concentrations, making them significantly similar (Figure 2.31).

The dosage-response curve for the LT2 isolate with the highest average relative growth at all fungicide active ingredient concentrations (7%) on iprodione-amended PDA was perfectly correlated ($r^2 = 1.00$) (Figure 2.32). The dosage-response curve for the LT2 isolate with the lowest average relative growth (4%) on thiophanate-methyl amended PDA was highly correlated ($r^2 = 0.99$) (Figure 2.33).

The EC_{50} value for all LG isolates growing on iprodione amended PDA was zero, indicating that any amount of fungicide active ingredient could reduce the growth of the isolates at this location to 50% of the control growth. This was the third lowest value compared to the other locations with resistance to iprodione. The EC_{90} value of 2 µl ml^{-1} was the lowest value for all iprodione-resistant locations (Table 2.5).

Location MN. At location MN, no resistance was observed. All fungicides inhibited the growth of the fungi by 100% at all concentrations.

Location WS. At this location, isolates exhibited resistance to all three fungicides tested. All ten isolates were resistant to thiophanate-methyl at all concentrations tested (Figure 2.34). Less resistance was observed to iprodione, with five isolates showing any growth at the lowest concentration tested (Figure 2.35). Resistance was also observed to propiconazole (Figure 2.36). All ten isolates grew at the 1 µl ml^{-1} propiconazole concentration, but two grew at the 10 µl ml^{-1} concentration, and none grew at the higher concentrations.

Iprodione. The interaction of the fungicide active ingredient concentration was highly significant for growth of the two isolates that showed resistance ($P < 0.0001$). Growth at all concentrations except 1 µl ml^{-1} was significantly similar (Figure 2.37).

The dosage-response curve for the WS isolate with the highest average relative growth (17%) on iprodione-amended PDA was highly correlated ($r^2 = 0.99$) (Figure 2.38). The dosage-response curve for the WS isolate with the lowest average relative growth (11%) on iprodione-amended PDA was also highly correlated ($r^2 = 0.99$) (Figure 2.39).

The EC_{50} value for the WS isolates growing on iprodione-amended PDA of 1 µl ml^{-1} was the median value for all iprodione-resistant locations, although it was quite a bit lower than the three highest EC_{50} values. The EC_{90} value of 3 µl ml^{-1} was also the median value in its category, yet was also lower than the highest three values (Table 2.5).

Thiophanate-methyl. The interaction of the fungicide active ingredient concentration was highly significant for growth of the two isolates that showed resistance ($P < 0.0001$). Relative growth at 1 and 10 µl ml^{-1} was 100% for both concentrations, and was significantly different from relative growth at all other concentrations Relative growths at 100 and 1000 µl ml^{-1} were also significantly different from relative growths at all other concentrations (Figure 2.40).

The dosage-response curve for the WS isolate with the highest average relative growth (99%) on thiophanate-methyl amended PDA was not highly correlated as an exponential decay ($r^2 = 0.04$) (Figure 2.41). The low r^2 value can be attributed to the fact that relative growth changed very little as the fungicide active ingredient concentration increased. The dosage-response curve for the WS isolate with the lowest average relative growth (95%) on thiophanate-methyl amended PDA was significantly correlated ($r^2 = 0.92$) (Figure 2.42).

The EC_{50} value for all WS isolates growing on thiophanate-methyl amended PDA was 2309 µl ml^{-1}, the second highest for thiophanate-resistance at all locations. The EC_{90} value of 3469 µl ml^{-1} was the second highest value for thiophanate-methyl-resistance at all locations as well (Table 2.5).

Propiconazole. The interaction of the fungicide active ingredient concentration was highly significant for growth of all isolates ($P < 0.0001$). Relative growths at 1 and 10 µl ml^{-1} were significantly different from relative growth at all other concentrations. No fungal growth occurred at the100 and 1000 µl ml^{-1} concentrations (Figure 2.43).

The dosage-response curve for the WS isolate with the highest average relative growth (15%) on iprodione-amended PDA was highly correlated (r^2 = 0.99) (Figure 2.44). The dosage-response curve for the WS isolate with the lowest average relative growth (9%) on iprodione-amended PDA was also highly correlated (r^2 = 0.99) (Figure 2.45).

The EC_{50} value of 0 µl ml^{-1} for the WS isolates growing on propiconazole-amended PDA was similar to the EC_{50} value for the other location showing resistance to propiconazole. The EC_{90} value of 8 µl ml^{-1} was lower than the EC_{90} value of the other location (Table 2.5).

Location WW. At location WW, isolates exhibited resistance to thiophanate-methyl. All other fungicides inhibited the growth of the fungi by 100% at all concentrations. All ten isolates were resistant to thiophanate-methyl at all concentrations tested (Figure 2.46). The interaction effect of the fungicide active ingredient concentration with growth of all isolates was highly significant ($P < 0.0001$). Growth at each concentration was significantly different from growth at all other concentrations (Figure 2.47).

The dosage-response curve for the WW isolate with the highest average relative growth at all fungicide active ingredient concentrations (94%) on thiophanate-methyl-amended PDA was correlated (r^2 = 0.78) (Figure 2.48). The dosage-response curve for the WW isolate with the lowest average relative growth (90%) on thiophanate-methyl amended PDA was not as significantly correlated (r^2 = 0.59) (Figure 2.49). The low r^2

value can be attributed to the fact that growths at the 1 and 10 µl ml^{-1} concentrations were very similar (100 and 96%, respectively), and that growth at the 100 and 1000 µl ml^{-1} concentrations were also very similar (84 and 80%, respectively).

The EC_{50} value for all WW isolates growing on thiophanate-methyl-amended PDA was 2183 µl ml^{-1}, the third highest value in its category. The EC_{90} value of 3766 µl ml^{-1} was the second highest in its category. Isolates at the WW location had the second highest incidence of resistance of all thiophanate-methyl-resistant locations (Table 2.5).

Location MIB. Testing for fungicide resistance in location MIB consisted of one isolate from Michigan with known resistance to the benzimidazole class fungicides (Phillip Dwyer, personal communication). At location MIB, the isolate exhibited resistance to thiophanate-methyl, a fungicide in the benzimidazole class. All other fungicides inhibited the growth of the fungi by 100% at all concentrations. The interaction effect of the benzimidazole active ingredient concentration with the growth of the isolate was highly significant ($P < 0.0001$). The isolate was resistant to thiophanate-methyl at all concentrations, and growth at each concentration was significantly different from growth at all other concentrations tested (Figure 2.50).

The dosage-response curve for the MIB isolate on iprodione-amended PDA was correlated ($r^2 = 0.64$) (Figure 2.51). The low r^2 value can be attributed to the fact that growths at 1 and 10 µl ml^{-1} were very similar (88 and 80%, respectively), and growths at 100 and 1000 µl ml^{-1} were also very similar (62 and 54%, respectively).

The EC_{50} value for the MIB isolate growing on thiophanate-methyl-amended PDA was 1100 µl ml^{-1}, the third lowest value for all thiophanate-methyl-resistant locations. The EC_{90} value of 2896 µl ml^{-1} was also the third lowest value for all locations at which thiophanate-methyl resistance was observed (Table 2.5).

Location MIDMI. Testing for fungicide resistance in location MIDMI consisted of one isolate from Michigan with known resistance to demethylation-inhibiting fungicides (Phillip Dwyer, personal communication). At location MIDMI, the isolate exhibited resistance to both propiconazole and thiophanate-methyl. Iprodione inhibited the growth of the fungi by 100% at all concentrations. The isolate was resistant to propiconazole at concentrations of 1 and 10 µl ml^{-1}. It was also resistant to thiophanate-methyl at all concentrations tested.

Propiconazole. The interaction effect of the propiconazole active ingredient concentration with growth of the isolate was highly significant ($P < 0.0001$). Growth at the 1 µl ml^{-1} concentration was significantly different from growth at all other concentrations, as was growth at 10 µl ml^{-1}. No growth occurred at concentrations of 100 and 1000 µl ml^{-1} (Figure 2.52).

The dosage-response curve for the MIDMI isolate on propiconazole-amended PDA was highly correlated ($r^2 = 0.99$) (Figure 2.53).

The EC_{50} value for the MIDMI isolate growing on propiconazole-amended PDA was zero, indicating that any concentration of propiconazole could reduce fungal growth

to 50% of the control. The EC_{90} value of 19 µl ml^{-1} was the higher of the two locations at which propiconazole resistance was observed (Table 2.5).

Thiophanate-methyl. The interaction effect of the iprodione active ingredient concentration with the growth of the isolate was highly significant ($P < 0.0001$). Growths at the 1, 10 and 100 µl ml^{-1} concentrations were not significantly different, as were growths at 100 and 1000 µl ml^{-1} (Figure 2.54).

The dosage-response curve for the MIDMI isolate on thiophanate-methyl-amended PDA was correlated ($r^2 = 0.84$) (Figure 2.55).

The EC_{50} value for the MIDMI isolate growing on thiophanate-methyl-amended PDA was 3309 µl ml^{-1}, the highest value for all thiophanate-methyl resistant locations. The EC_{90} value of 6185 µl ml^{-1} was also the highest value for all thiophanate-methyl-resistant locations (Table 2.5).

Location MID. Testing for fungicide resistance in location MID consisted of one isolate from Michigan with known resistance to dicarboximide class fungicides (Phillip Dwyer, personal communication). At location MID, the isolate exhibited resistance to iprodione, a fungicide in the dicarboximide class. All other fungicides inhibited the growth of the fungi by 100% at all concentrations. The interaction effect of the propiconazole active ingredient concentration with the growth of the isolate was highly significant ($P < 0.0001$). The isolate was resistant to iprodione at all concentrations, and growth at each concentration was significantly different from growth at all other concentrations tested (Figure 2.56).

The dosage-response curve for the MID isolate on iprodione-amended PDA was correlated ($r^2 = 0.87$) (Figure 2.57).

The EC_{50} value for the MID isolate growing on iprodione-amended PDA was 850 $\mu l\ ml^{-1}$, the second highest value for all iprodione resistant locations. The EC_{90} value of 2047 $\mu l\ ml^{-1}$ was also the second highest value for all locations at which iprodione resistance was observed (Table 2.5).

Location MIW. Testing for fungicide resistance in location MIW consisted of one isolate from Michigan with no known resistance to any fungicides (Phillip Dwyer, personal communication). At location MIW, all fungicides inhibited the growth of the fungi by 100% at all concentrations. No EC values could be computed for this isolate.

Conclusion

Fungicide resistance was exhibited by isolates in 12 of the 14 locations tested. Isolate MIW, known to be sensitive to all fungicides used in this study, did not show any resistance. Isolates at another location, MN, also showed no resistance.

Isolates at seven locations exhibited resistance to iprodione or thiophanate-methyl, and isolates at two locations exhibited resistance to propiconazole. Isolates at three locations exhibited multi-resistance to thiophanate-methyl and iprodione, thiophanate-methyl and propiconazole, or multi-resistance to all fungicides tested.

Isolates tested against thiophanate-methyl exhibited the greatest overall relative growth, making it the fungicide to which most resistance was observed (Figure 2.58).

Isolates tested against iprodione exhibited the next highest level of resistance, and propiconazole had the least.

The highest fungicide active ingredient concentration at which isolate resistance occurred to iprodione and thiophanate-methyl was 1000 μg ml^{-1}, and the highest concentration to which resistance occurred to propiconazole was 10 μg ml^{-1}. EC_{50} and EC_{90} values were variable for locations with resistance to the same fungicides. Most of the resistant isolates grew at rates far greater than the recommended application rate for the fungicides tested. Of the 94 isolates tested, 26% were resistant to iprodione, 47% were resistant to thiophanate-methyl, and 12% were resistant to propiconazole.

Variation in the growth of different isolates at the same location to the same fungicide concentrations could be attributed to varying degrees of fitness in the genetic makeup of the individual isolates.

Fungicide resistance at all locations could be correlated with past or present use of the fungicide to which resistance was observed. This suggests a direct link between use of a fungicide to combat dollar spot and build-up of resistance to that fungicide in the *S. homeocarpa* population.

Propiconazole use was reported for locations CH, GV, and WW, although resistance to this fungicide was not observed at these locations. This is probably because the phytotoxic effects of propiconazole limit its use in the South. This, along with the high cost of this fungicide, may cause this fungicide to be used with little frequency at these locations. This could explain why isolates resistant to propiconazole had not been selected on this course.

Iprodione use was reported at locations GV and WW, although isolates resistant to this fungicide were not observed at these locations. This could be because of one of the following: iprodione was not applied with enough frequency to eliminate the sensitive *S. homeocarpa* strains; iprodione-resistant strains were not selected in the sample population; or because iprodione-resistant strains to not exist in nature at these locations.

CHAPTER 3

EVALUATION OF CHLOROTHALONIL FOR CONTROL OF GROWTH OF *SCLEROTINIA HOMEOCARPA IN VITRO*

Introduction

Contact fungicides are used as a preventative measure to protect plants from disease. They are applied to foliage where they form a protective coating that prevents a pathogen from entering a plant (Vargas, 1994). Chlorothalonil is a commonly used contact turf fungicide used to help prevent dollar spot outbreaks. The most commonly used formulation of chlorothalonil is the trade fungicide Daconil. It is used frequently because of its merits as a low-cost fungicide with broad-spectrum activity; however, the Environmental Protection Agency (EPA) has recently placed restrictions on the maximum allowable application rates per year for this fungicide because of its toxicity to aquatic animals. The maximum seasonal amount of chlorothalonil allowed on golf course greens is 33 kg active ingredient per acre per year. At the highest recommended product rate of 141 g/93 m^2, this product may be sprayed at 14 day intervals, but twice during the growing season. At the lowest rate, 91 g/93 m^2, it may be sprayed every 7 days, but must not exceed 907 g/1000 ft^2 annually (Vincelli, 2003). Because of its excellent reputation for controlling dollar spot disease and limited availability for use, it is important to know if resistance to chlorothalonil has developed.

One study observed a significant difference in the effective concentrations (EC) of chlorothalonil required to inhibit growth of two different isolates of *S. homeocarpa* in vitro by 50%. One isolate had an EC_{50} of 0.56 µg active ingredient ml^{-1} and an EC_{90}

value of 4.31 μg ml^{-1}, while the other isolate had an EC_{50} of 3.41μg ml^{-1} and an EC_{90} of 34.27 μg ml^{-1} (Burpee, 1997). No other studies of this nature have been published.

The purposes of the current investigation were to determine the following: (i) if resistance to the fungicide chlorothalonil exists in Tennessee and Northern Mississippi by testing isolates of *S. homeocarpa* field-collected in this area; (ii) if the 'wild type' isolate from Michigan State University is resistant to chlorothalonil, and if so, if it has differing sensitivity from the southern-collected isolates; and (iii) if chemical practices of golf course superintendents have had an effect on the occurrence of resistance to chlorothalonil.

Materials and Methods

Isolate selection

Previous experiments testing *S. homeocarpa* isolates for growth on chlorothalonil-amended potato dextrose agar gave inconclusive results. Measurement of mycelial growth was difficult because growth in the Petri plate was an irregular, uneven shape with much aerial mycelia (Figures 3.1, 3.2). A total of nine isolates from eight locations were chosen for testing. Five of the chosen isolates showed apparent growth at some levels of chlorothalonil concentration on potato dextrose agar, and four showed no growth (Table 3.1).

Treatments

Potato dextrose broth (PDB) was amended with chlorothalonil at concentrations of 0, 0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 µg active ingredient ml^{-1}. Twenty ml of the fungicide amended PDB was then pipetted into autoclaved 150 ml flasks.

Plugs 5 mm in diameter were extracted from the edge of actively growing colonies of *S. homeocarpa*. Each of the *S. homeocarpa* isolates was placed in four replicate flasks per fungicide concentration, as well as four control flasks containing PDB with no fungicide. Flasks were then sealed and the fungi were incubated at 21 ± 1 ^{o}C on a rotary shaker at 120 rpm for seven days.

Data collection

Individual flasks were emptied onto pre-weighed filter paper, and the PDB was drained off using a flask connected to a vacuum line, leaving the mycelial mat (Figure 3.3). The original PDA plug was extracted from the mycelial mat using a metal spatula. The mycelial mat and filter paper were then transferred to a drying oven for 24 h to remove excess moisture (Coursen and Sisler, 1960). Dry mycelial mat and filter paper were then placed on a scale and weighed. The weight of the filter paper before addition of the mycelial mat was subtracted from the weight of both the filter paper and the mycelial mat together to determine the weight of the mycelial mat. SAS 9.0 Proc Probit was used to perform the EC_{50} and EC_{90} (estimated fungicide concentration needed to inhibit growth by 50 and 90 percent of control growth, respectively), and SAS 9.0 Proc Mixed was used for analysis of variance of fungal growth (P = 0.05). Sigmaplot 8.0 was

used for regression analysis of relative growth of control against fungicide active ingredient concentration using an exponential decay equation.

Results and Discussion

All isolates tested were analyzed separately by location collected. All nine isolates grew in chlorothalonil amended PDA at rates up to 5 µg active ingredient ml^{-1} PDA. Two isolates grew in 10 µg ml^{-1}, and none grew in 20 µg ml^{-1}. Relative growth of isolates at each concentration was charted for visual comparison across locations. Relative growth at discrete concentrations was variable by location, and variability increased as the concentration of active ingredient increased (Figure 3.4).

The isolate with the least sensitivity had an EC_{50} of 3 µg ml^{-1} and an EC_{90} of 6 µg ml^{-1}, while the isolate with the most sensitivity had an EC_{50} µg ml^{-1} value of 9 and an EC_{90} µg ml^{-1} value of 15 µg ml^{-1}. The mean EC_{50} and EC_{90} for all isolates was 5 and 8 µg ml^{-1}, respectively. The median EC_{50} and EC_{90} for all isolates was 4 and 8 µg ml^{-1}, respectively. No correlation was observed between isolate geographic region of collection (East Tennessee, Middle Tennessee, West Tennessee/Northern Mississippi, and Michigan) and EC values.

Translated to the area of a Petri dish, the lowest recommended application rate of 28 g/93 m^2 the chlorothalonil-containing fungicide used in this experiment to control dollar spot disease is 1 µg active ingredient ml^{-1} PDA per plate, and the highest recommended rate of 142 g/93 m^2 translates to 5 µg ml^{-1} per plate. All but two of the EC_{50} values in this experiment were less than the highest recommended rate, but none

were less than the lowest recommended rate. None of the EC_{90} values were less than the highest recommended rate (Table 3.2). Although this data could indicate that chlorothalonil does not sufficiently inhibit growth of *S. homeocarpa* at recommended rates, no turf managers from locations at which fungi were collected reported any resistance to the product. This indicates that *in vitro* and *in situ* application of this fungicide may not have the same effect on the fungus.

Analysis by isolate

Isolate BM. The interaction between isolate growth and fungicide active ingredient concentration was highly significant for this isolate ($P < 0.0001$). Growths at concentrations of 0.05 and 1 μg ml^{-1} were significantly similar, with average growth rates of fungi in fungicide-amended PDB between 67 and 83% of the average control growth rate. Growths at concentrations of 1, 2, and 5 μg ml^{-1} were also significantly similar, with average growth rates of fungi in fungicide-amended PDB between 50 and 68% of the average control growth rate. No growth occurred for this isolate at rates of 10 and 20 μg ml^{-1} (Figure 3.5).

The dosage-response curve for the BM isolate growing in chlorothalonil amended PDB was significantly correlated ($r^2 = 0.82$) (Figure 3.6). The EC_{50} value of 3 μg ml^{-1} was below average for all isolates tested, and the EC_{90} value of 8 μg ml^{-1} was near average (Table 3.2).

Isolate CW1. The interaction between isolate growth and fungicide active ingredient was highly significant for this isolate ($P < 0.0001$). Growths at concentrations of 0.05 and 1 μg ml^{-1} were significantly similar, with average growth rates of fungi in fungicide-amended PDB between 79 and 90% of the control growth rate. Growths at concentrations of 2 and 5 μg ml-1 were also significantly similar, with average growth rates of fungi in fungicide-amended PDB between 53 and 62% of the average control growth rates. No growth occurred for this isolate at rates of 10 and 20 μg ml^{-1} (Figure 3.7).

The dosage-response curve for the CW1 isolate growing in chlorothalonil amended PDB was significantly correlated ($r^2 = 0.94$) (Figure 3.8). The EC_{50} value of 4 μg ml^{-1} was below the average for all isolates tested, but very close to the median value. The EC_{90} value of 8 μg ml^{-1} was near average and median EC_{90} values for all isolates tested (Table 3.2).

Isolate CW2. The interaction between isolate growth and fungicide active ingredient concentration was highly significant for this isolate ($P < 0.0001$). Growths at all concentrations at which this isolate grew were significantly different. At concentrations of 0.05, 1, 2, and 5 μg ml^{-1}, the average growth rates of fungi in fungicide-amended PDB were 89, 78, 50, and 41%, respectively. No growth occurred for this isolate at rates of 10 and 20 μg ml^{-1} (Figure 3.9).

The dosage-response curve for the CW2 isolate growing in chlorothalonil amended PDB was highly correlated ($r^2 = 0.96$) (Figure 3.10). The EC_{50}value of 3 μg ml^{-}

^{1} was below average for all isolates tested, but near the mean EC_{50} for all isolates. The EC_{90} value of 7 μg ml^{-1} was slightly below the average and median EC_{90} values for all isolates (Table 3.2).

Isolate GV. The interaction between isolate growth and fungicide active ingredient concentration was highly significant for this isolate ($P < 0.0001$). Growths at fungicide active ingredient concentrations of 0.05 and 1 μg ml^{-1} were significantly similar, with average growth rates of fungi in fungicide-amended PDB between 88 and 95% of the average relative growth of the control fungi. All other concentrations at which the fungi grew were significantly different, with average growth rates at 2 and 5 μg ml^{-1} of 53 and 18 percent of the average growth of the control, respectively. No growth occurred for this isolate at rates of 10 and 20 μg ml^{-1} (Figure 3.11).

The dosage-response curve for the GV isolate growing in chlorothalonil amended PDB was significantly correlated ($r^2 = 0.96$) (Figure 3.12). The EC_{50} value of 3 μg ml^{-1} was the lowest value in its category, well below average for all isolates tested, and slightly below the median EC_{50} for all isolates. The EC_{90} value of 6 μg ml^{-1} was also the lowest value in its category, well below the average and median EC_{90} values for all isolates (Table 3.2).

Isolate LG. The interaction between isolate growth and fungicide active ingredient concentration was highly significant for this isolate ($P < 0.0001$). Growths at fungicide active ingredient concentrations of 0.05, 1, and 2 μg ml^{-1} were significantly

similar, with average growth rates of fungi in fungicide-amended PDB between 89 and 99% of the average relative growth of the control fungi. Average growth at 10 μg ml^{-1} was 43% of the average growth of the control fungi, and was significantly different from all other concentrations at which the fungi grew. No growth occurred for this isolate at 20 μg ml^{-1} (Figure 3.13).

The dosage-response curve for the LG isolate growing in chlorothalonil amended PDB was significantly correlated ($r^2 = 0.90$) (Figure 3.14). The EC_{50} value of 9 μg ml^{-1} was the highest value in its category, and thus above average for all isolates tested, and slightly above the median EC_{50} for all isolates. The EC_{90} value of 15 μg ml^{-1} was also the highest value in its category, almost twice the average and median EC_{90} values for all isolates (Table 3.2).

Isolate LT1. The interaction between isolate growth and fungicide active ingredient concentration was highly significant for this isolate ($P < 0.0001$). Growths at fungicide active ingredient concentrations of 0.05 and 1 μg ml^{-1} were significantly similar, with average growth rates of fungi in fungicide-amended PDB between 90 and 93% of the average relative growth of the control fungi. Average growth at 2 μg ml^{-1} was 76% of the average growth of the control fungi, and was significantly different from all other concentrations at which the fungi grew. Average growth at 5 μg ml^{-1} was 34% of the average growth of the control fungi, and was also significantly different from all other concentrations at which the fungi grew. No growth occurred for this isolate at either 10 or 20 μg ml^{-1} (Figure 3.15).

The dosage-response curve for the LT1 isolate growing in chlorothalonil amended PDB was significantly correlated ($r^2 = 0.90$) (Figure 3.16). The EC_{50} value of 4 μg ml^{-1} was below average for all isolates tested, and slightly below the median EC_{50} for all isolates. The EC90 value of 7 μg ml^{-1} was below the average and median EC_{90} values for all isolates (Table 3.2).

Isolate MN. The interaction between isolate growth and fungicide active ingredient concentration was highly significant for this isolate ($P < 0.0001$). Growths at all fungicide active ingredient concentrations at which the fungi grew were significantly different. Average growths at 0.05, 1, 2, 5, and 10 μg ml^{-1} were 100%, 93%, 69%, 54%, and 32% of the average growth of the control fungi, respectively. No growth occurred for this isolate at 20 μg ml^{-1} (Figure 3.17).

The dosage-response curve for the MN isolate growing in chlorothalonil amended PDB was highly significant ($r^2 = 0.97$) (Figure 3.18). The EC_{50} value of 7 μg ml^{-1} was above average for all isolates tested, and above the median EC_{50} for all isolates. The EC_{90} value of 13 μg ml^{-1} was the second highest in its category, well above the average and median EC_{90} values for all isolates (Table 3.2).

Isolate WS. The interaction between isolate growth and fungicide active ingredient concentration was highly significant for this isolate ($P < 0.0001$). Growths at fungicide active ingredient concentrations of 0.05, 1, and 2 were all significantly similar with average growth rates of 99, 94, and 89% of the growth rate of the control,

respectively. Average growth at 5 μg ml^{-1} was significantly different from growth at all other concentrations, with an average growth rate of 44% of the average growth of the control fungi. No growth occurred for this isolate at either 10 or 20 μg ml^{-1} (Figure 3.19).

The dosage-response curve for the WS isolate growing in chlorothalonil amended PDB was significantly correlated ($r^2 = 0.96$) (Figure 3.20). The EC_{50} value of 5 μg ml^{-1} was below average for all isolates tested, and above the median EC_{50} for all isolates. The EC_{90} value of 8 μg ml^{-1} was below the average and median EC_{90} values for all isolates (Table 3.2).

Isolate MIW. This isolate was collected in Michigan. The interaction between isolate growth and fungicide active ingredient concentration was highly significant for this isolate ($P < 0.0001$). Growths at fungicide active ingredient concentrations 0.5 and 1 were significantly similar, with average growth of fungi in fungicide-amended PDB between 85 and 91% of the average growth rate of the control fungi. Growths at fungicide active ingredient concentrations 1and 2 μg ml^{-1} were also significantly similar, with average growth of fungi in fungicide-amended PDB 86 and 77% of the average growth rate of the control fungi, respectively. Average growth at 5 μg ml^{-1} was significantly different from the average growth rate at all other concentrations at which the fungi grew, with an average growth rate of 54% of the average growth rate of the control fungi. No growth occurred for this isolate at either 10 or 20 μg ml^{-1} (Figure 3.21).

The dosage-response curve for the MIW isolate growing in chlorothalonil amended PDB was highly significant ($r^2 = 0.92$) (Figure 3.22). The EC_{50} value of 4 μg ml^{-1} was below average for all isolates tested, but above the median EC_{50} for all isolates. The EC_{90} value of 8 μg ml^{-1} was equal to the average for all isolates tested, and was the median EC_{90} value for all isolates (Table 3.2).

Conclusion

The interaction between isolate growth and chlorothalonil active ingredient concentration was highly significant for all isolates tested. Regression analysis showed a negative correlation between chlorothalonil concentration and relative growth of isolates in fungicide-amended PDB for all isolates.

Variation was observed among isolate growth rates at each chlorothalonil concentration. At the 0.5 μg ml^{-1} concentration, the difference in relative growth between the highest and lowest growth value was 17%. As concentration increased, the variation between the highest and lowest growth value increased for all concentrations at which all isolates grew, indicating more variability in fungicide sensitivity at higher concentrations (Table 3.3). The difference between the highest and lowest growth rates at the 5 μg ml^{-1} concentration was 70%. Although two isolates grew at the 10 μg ml^{-1} concentration, none grew at the 20μg ml^{-1} concentration.

The MIW isolate from Michigan grew at concentrations up to 5 μg ml^{-1}. Although this isolate did not show any significant difference in relative growth rate from

the southern-collected isolates as a whole, it was, like all other isolates, variable in its relative growth from other isolates at each concentration.

Translated to the area of a Petri dish, the lowest recommended application rate of 28 g/93 m^2 the chlorothalonil-containing fungicide used in this experiment to control dollar spot disease is 1.5 μg ml^{-1} per plate, and the highest recommended rate of 138 g/93 m^2 translates to 4.9 μg ml^{-1} per plate. All but two of the EC_{50} values in this experiment were less than the highest recommended rate, but none were less than the lowest recommended rate. None of the EC_{90} values were less than the highest recommended rate (Table 3.2). Although this data could indicate that chlorothalonil does not sufficiently inhibit growth of *S. homeocarpa* at the recommended rates for this fungicide, no turf managers from locations at which the fungi were collected reported any resistance to the product. This indicates that *in vitro* and *in situ* application of this fungicide may not have the same effect on the fungus.

The isolate with the least sensitivity had an EC_{50} of 3 μg ml^{-1} and an EC_{90} of 6 μg ml^{-1}, while the isolate with the most sensitivity had an EC_{50} μg ml^{-1} value of 9 and an EC_{90} μg ml^{-1} value of 15 μg ml^{-1}. The mean EC_{50} and EC_{90} for all isolates was 5 and 8 μg ml^{-1}, respectively. The median EC_{50} and EC_{90} for all isolates was 4 and 8 μg ml^{-1}, respectively. No correlation was observed between isolate geographic region of collection (East Tennessee, Middle Tennessee, West Tennessee/Northern Mississippi, and Michigan) and EC values.

The mean EC_{50} rate of 5μg ml^{-1} for all isolates tested in this study was slightly higher than the highest mean EC_{50} rate of 3.41 μg ml^{-1} for the S088 isolate in the Georgia

study. Four of the isolates in this study had a difference of less than 0.55 μg ml^{-1} from the S088 isolate mean EC_{50}. Two of the isolates in this study had a difference of less than 1.05 μg ml^{-1} from the S088 isolate mean EC_{50} (Burpee, 1997). Both the EC values of isolates in this study and the Georgia study had great variability.

A survey conducted at the time of isolate collection gave information about the chemical practices for each location (Table 3.4). Regression of rate of chlorothalonil application *in situ* against the EC_{50} and EC_{90} values for each isolate for which chlorothalonil application rate was known showed a very weak correlation for both the EC_{50} values ($r^2 = 0.39$) and the EC_{90} values ($r^2 = 0.56$) (Figures 3.23, 3.24). This data suggests that an *in situ* chlorothalonil application does not correlate well to an *in vitro* fungal sensitivity to the fungicide.

The data indicate that much variation exists in sensitivity to chlorothalonil by isolate, but does not indicate that a trend in sensitivity exists for isolates collected in different geographic locations, or for isolates exposed to varying degrees of chlorothalonil *in situ*. Growth of most isolates was suppressed by 10 μg ml^{-1}, and growth of all isolates was suppressed by 20 μg ml^{-1}. The study in Georgia observed that reduced sensitivity to *in vitro* treatment was not evident in field trials with the same isolates (Burpee, 1997). A possible explanation for this could be that the sterile environment in which the *in vitro* studies were conducted could be missing an element from the environment, such as temperature fluctuation, microbial activity, or ultraviolet light, which causes chlorothalonil to effectively inhibit fungal growth. This suggests that the

varying rates to which the isolates in this study grew in the discrete concentrations of chlorothalonil in vitro may not translate into reduced sensitivity in the field.

The differences in sensitivity to chlorothalonil could be attributed to differences in overall fitness of the individual strains. Some strains may grow more vigorously than others. These strains would have a higher rate of growth than other strains under the same circumstances, such as contact with fungicides.

THESIS SUMMARY

Discoveries from this research will provide valuable insight into the study of fungicide resistance in turfgrass pathogens. Hopefully, the knowledge that fungicide resistance in *S. homeocarpa* is developing in Tennessee and northern Mississippi will cause turf managers to be more aware of chemical control practices when managing dollar spot. Following are conclusions for both experiments.

Experiment One—Evaluation of thiophanate-methyl, iprodione, and propiconazole for control of growth of *Sclerotinia homeocarpa.*

Fungicide resistance was present isolates from nine of the 10 southern locations tested. Additionally, three isolates from Michigan showed resistance. One isolate from Michigan known to be sensitive to all fungicides used in this study did not show any resistance. Isolates exhibited resistance to iprodione, thiophanate-methyl, and propiconazole at seven, seven, and two locations, respectively. Isolates at one location exhibited multi-resistance to thiophanate-methyl and iprodione, isolates at another location had multi-resistance to thiophanate-methyl and propiconazole, and isolates at another location had multi-resistance to all three fungicides tested. The highest fungicide active ingredient concentration at which resistance occurred to iprodione and thiophanate-methyl was 1000 $\mu g\ ml^{-1}$, and the highest concentration to which resistance occurred to propiconazole was 10 $\mu g\ ml^{-1}$. For all locations, fungicide resistance correlated with fungicide use at that location.

Experiment Two—Evaluation of chlorothalonil for control of growth of *Sclerotinia homeocarpa.*

Results for this experiment demonstrated that isolate sensitivity to chlorothalonil varied from location to location. All nine isolates grew in chlorothalonil amended PDA at rates of up to 5 μg active ingredient ml^{-1} PDA. Two isolates grew in 10 μg ml^{-1}, and none grew in 20 μg ml^{-1}. Relative growth at discrete concentrations was variable by location. Variability increased as the concentration of active ingredient increased. No correlation was observed between isolate geographic region of collection (East Tennessee, Middle Tennessee, West Tennessee/Northern Mississippi, and Michigan) and sensitivity to chlorothalonil. Additionally, no correlation existed between chlorothalonil use *in situ* and chlorothalonil sensitivity *in vitro*.

The Next Step—Finding the mechanism of resistance.

The discovery that fungicide resistance in *S. homeocarpa* exists in the Tennessee/Northern Mississippi area is the first step toward management of resistance. The next step will be to examine resistant and non-resistant isolates to determine if any molecular markers are common to isolates resistant to the same fungicide formulation. Research of this nature is currently underway at the University of Tennessee.

Representative fungal isolates from both the Tennessee/Northern Mississippi collection and the Michigan collection will be selected for DNA analysis. DNA profiling, or genetic analysis, will be accomplished using DNA Amplification Fingerprinting. Data from individual fungal profiles will be analyzed using cluster and

principal coordinate analyses. In addition, similarity and relatedness indices will be developed. The data will be analyzed for potential molecular markers correlated to fungicidal resistance. This research will provide valuable information for development of best management practices for chemical control of dollar spot, and for development a test kit for identifying fungicide-resistant isolates of *S. homeocarpa*.

BIBLIOGRAPHY

BIBLIOGRAPHY

2002. Turf and Ornamental Reference for Plant Protection Products, 11th ed. C&P Press, NY.

Alexopolous, C.J., Mims, C.W., and Blackwell, M. 1996. Introductory Mycology, 4th ed. John Wiley and Sons, NY.

Beard, J.B. 2002. Turfgrass Management for Golf Courses. Ann Arbor Press, Chelsea, MI.

Bain, D.C. 1964. *Sclerotinia* blight on nutgrass in Mississippi. Plant Dis. Rptr. 48:742.

Baldwin, N.A. and Newell, A.J. 1992. Field production of fertile apothecia by *Sclerotinia homeocarpa* in festuca turf. J. Sports Turf Res. Inst. 68:73-76.

Bennett, F.T. 1937. Dollar spot disease on turf and its causal organism *Sclerotinia homeocarpa* n. sp. Ann. Appl. Biol. 24:236-257.

Boesewinkle, H.J. 1977. New plant disease records in New Zealand. Record in the period 1969-1976. New Zealand J. Agr. Res. 20:583-589.

Broder, M.F. and Samples, T. 2000. Tennessee Handbook for Golf Course Environmental Management. Tennessee Department of Agriculture.

Burpee, L.L. 1997. Control of dollar spot of creeping bentgrass caused by an isolate of *Sclerotinia homeocarpa* resistant to benzimidazole and demethylation-inhibitor fungicides. Plant Dis. 81:1259-1263.

Cole, H., Taylor, B., and Duich, J. 1968. Evidence of differing tolerances to fungicides among isolates of *Sclerotinia homeocarpa*. Phytopathology 58:683-686.

Couch, H.B., and Bloom, J.R. 1960. Influence of environment on diseases of turfgrasses. II. Effect of nutrition, pH, and soil moisture on *Sclerotinia* dollar spot. Phytopathology 50:761-763.

Couch, H.B. 1962. Diseases of Turfgrasses. Reinhold Publishing Corporation, NY.

Couch, H.B., and Smith, B.D. 1991. Increase in incidence and severity of target turfgrass diseases by certain fungicides. Plant Dis. 75:1064-1067.

Coursen, B.W., and Sisler, H.D. 1960. Effect of the antibiotic cyclohexamide on the metabolism and growth of *Saccharomyces pastorianus*. Amer. J. Bot. 47:532.540.

Christians, N.E., and Engelke, M.C. 1994. Choosing the right grass to fit the environment. Pages 99-112 in: Handbook of Integrated Pest Management for Turfgrass and Ornamentals. Lewis Publishers, Boca Raton, FL.

Christians, N.E. 1996. A history of *Poa annua* control. Golf Course Mgmt. 64:49-57.

Christians, N.E. 2004. Fundamentals of Turfgrass Management, 2nd ed. John Wiley and Sons, Inc., Hoboken, NJ.

Damicone, J. Fungicide resistance management. Oklahoma Cooperative Extension Publication #F-7663. http://pearl.agcomm.okstate.edu/plantdiseases/f-7663.html.

Davidse, L. 1981. Resistance to acylalanine fungicides in *Phytophthora megasperma* f. sp. *medicaginis*. Netheralands J. of Plant Pathol. 87:11-24.

Dernoeden, P.H. 2000. Creeping Bentgrass Management: Summer Stresses, Weeds, and Selected Maladies. Ann Arbor Press, Chelsea, MI.

Dernoeden, P.H. 2000. Dollar spot: getting tougher to manage in creeping bentgrass. Turfax of the Intl. Sports Turf Res. Inst. 8:1-6.

Detweiler, A.R., Vargas, J.M., Jr., and Denneberger, T.K. 1983. Resistance of *Sclerotinia homeocarpa* to iprodione and benomyl. Plant Dis. 67:627-630.

Doney, J.C., Jr. and Vincelli, P. 1993. Cross resistance in *Sclerotinia homeocarpa* to DMI fungicides. Phytopathology 83:1338 (Abstr.)

Dunn, J. and Diesburg, K. 2004. Turf Management in the Transition Zone. John Wiley & Sons, Inc., New York.

Endo, R.M., Malca, I., and Krausman, E.M. 1964. Degeneration of the apical meristem and apex of bentgrass roots by a fungal toxin. Phytopathology 54:1175-1176.

Endo, R.M. 1966. Control of dollar spot of turfgrass by nitrogen and its probable bases. Phytopathology 56:877 (Abstr.)

Emmons, R.D. 1995. Turfgrass Science and Management, 2nd ed. Delmar Publishers, New York.

Fenstermacher, J.M. 1970. Variation within *Sclerotinia homeocarpa* F.T. Bennett. MSc

Thesis, Univ. of Rhode Island, Kingston.

Fenstermacher, J.M. 1979. Certain features of dollar spot disease and its causal organism, *Sclerotinia homeocarpa*. Pages 49-53 in: Advances in Turfgrass Pathology. B.G. Joyner and P.O Larsen, eds. Harcourt Brace Jovanovich, Duluth, MN.

Golembiewski, R.C., Vargas, J.M., Jones, A.L., and Detweiler, A.R. 1995. Detection of demethylation inhibitor (DMI) resistance in *Sclerotinia homeocarpa* populations. Plant Dis. 79:491-493.

Goodman, D.M. and Burpee, L.L. 1991. Biological control of dollar spot disease of creeping bentgrass. Phytopathology 81:1438-1446.

Gould, F.W. and Shaw, R.B. 1969. Grass Systematics, 2nd ed. McGraw-Hill Book Co., NY.

Graham, D.D. 2003. Resistance is (not) futile. Grounds Maintenance. http://grounds-mag.com/mag/grounds_maintenance_resistance_not_futile/.

Hoover, R.J. and Kucharek, T.A. 1995. First report of leaf spot on perennial peanut caused by *Sclerotinia homeocarpa*. Plant Dis. 79:1249.

Hsiang, T., Yang, L., and Barton, W. 1997. Baseline sensitivity and cross-resistance to demethylation-inhibiting fungicides in Ontario isolates of *Sclerotinia homeocarpa*. Eur. J. Plant Pathol. 103:406-416.

Joyner, B.G. and Larson, P.O. 1979. Advances in Turfgrass Pathology. Harcourt Brace Jovanovich, Duluth, Minnesota.

Jackson, N. 1973. Apothecial production in *Sclerotinia homeocarpa* F.T. Bennett. J. Sports Turf Res. Inst. 49:58-63.

Jiang, H., Fry, J, and Tisserat, N. 1998. Assessing irrigation management for its effects on disease and weed levels in perennial ryegrass. Crop Sci. 38:440-445.

Kendall, S.J. and Holloman, D.W. 1998. Fungicide resistance. Pages 87-108 in: Fungicidal Activity. D.H. Hutson and J. Miyamoto, eds. John Wiley and Sons, Ltd., New York.

Kohn, L.M. 1979a. A monographic revision of the genus *Sclerotinia*. Mycotaxon. 9:365-444.

Kohn, L.M. 1979b. Delimitation of the economically important plant pathogenic *Sclerotinia* species. Phytopathology 69:881-886.

Malca, I. and Endo, R.M. 1965. Identification of galactose in cultures of *Sclerotinia homeocarpa* as the factor toxic to bentgrass roots. Phytopathology 55:775-780.

Mazur, A.R. 1977. Southern turfgrasses: the struggle for survival. The Golf Superintendent. 45:18-20.

Mazur, A.R. 1981. Cool-season/warm-season turfgrass management in the transition zone. USGA Green Section Record. Volume: 10-12.

Massie, L.B., Cole, H., and Duich, J. 1968. Pathogen variation in relation to disease severity and control of *Sclerotinia* dollar spot of turfgrass by fungicides. Phytopathology 58:1616-1619.

Miller, G.L., Stevenson, K.L., and Burpee, L.L. Sensitivity of *Sclerotinia homeocarpa* isolates to propiconazole and impact on control of dollar spot. Plant Dis. 36:1240-1246.

Nicholson, J.F., Meyer, W.A., Sinclair, J.B., and Butler, J.D. 1971. Turf isolates of *Sclerotinia homeocarpa* tolerant to dyrene. Phytopathology 72:169-172.

Nikolai, T. A. 2002. Effects of rolling and fertility on putting green root zone mixes. Ph.D. Diss., Michigan State University, Ann Arbor, MI.

Powell, J.F., and Vargas, J.M., Jr. 1999. Taxonomic clarification of the dollar spot pathogen: *Sclerotinia homeocarpa* Bennett. Phytopathology 89:s61 (Abstr.)

Powell, J.F., and Vargas, J.M., Jr. 2000. Management of dollar spot on creeping bentgrass with metabolites of Pseudomonas aureofaciens (TX-1). Plant Dis. 84:19-24.

Rossman, A.Y., Palm, M.E., and Spielman, L.J. 1987. A literature guide for the identification of plant pathogenic fungi. APS Press, St. Paul, MN.

Samuels, G.J. and Seifert, K.A. 1995. The impact of molecular characters on systematics of filamentous Ascomycetes. Annu. Rev. Phytopathol. 33:37-67.

Smiley, R.W., Dernoeden, P.H., and Clarke, B.B. 1992. Compendium of Turfgrass Diseases, 2nd ed. APS Press, St. Paul, MN.

Smith, J.D. 1955. Fungi and turf diseases. J. Sports Res. Inst. 9:35-59.

Smith, J.D. and Gossen, B.D. 2001. First report of dollar spot, caused by *Sclerotinia homeocarpa*, on Poa pratensis in Saskatchewan, Canada. Plant Dis. 85:803.

Smith, J.D., Jackson, N., and Woolhouse, A.R. 1989. Fungal diseases of amenity turf turfgrasses, 3rd ed. E. & F.N. Spoon, New York.

Tani, T. and Beard J.B. 1997. Color Atlas of Turfgrass Diseases. Ann Arbor Press, Ann Arbor, MI.

Taylor, J.W., Swann, E., and Berbee, M.L. 1994. Molecular evolution of ascomycete fungi: Phylogeny and conflict in: Ascomycete Systematics: Problems and Perspectives in the Nineties. Hawksworth, D.L., ed. Plenum Press, NY.

Turgeon, A.J. 2002. Turfgrass Management, 6th ed. Prentice Hall, New Jersey.

United States Department of Agriculture. ITIS Report. 2004. http://www.itis.usda.gov.

Vargas, J.M., Jr., Golembiewski, R., and Detweiler, A.R. 1992. Dollar spot resistance to DMI fungicides. Golf Course Manage. 60 (3):50-54.

Vargas, J.M., Jr. 1994. Management of Turfgrass Diseases, 2nd ed. Lewis Publishers, Ann Arbor, MI.

Vargas, J.M., Jr. 1998. Managing resistance—Part 1: Fungicides. Grounds Maint.

http://groundsmag.com/mag/grounds_maintenance_managing_resistance_part/index.html.

Vargas, J.M., Jr. 2002. To rotate or not to rotate? Golf Course Manage. 70:35-40.

Vincelli, P. 2003. EPA Restrictions on Chlorothalonil Make Sense. Turfgrass Trends. http://www.turfgrasstrends.com/turfgrasstrends/article/articleDetail.jsp?id=48313.

Walsh, B. and Boland, J. 1998. Epidemiology and disease forecasting of dollar spot

Walsh, B., Ikeda, S.S., and Boland, G.J. 1999. Biology and management of dollar spot (*Sclerotinia homeocarpa*); an important disease of turfgrass. Hort. Sci. 34:13-21.

Warren, C.G., Sanders, P., and Cole, H. 1974. *Sclerotinia homeocarpa* tolerance to benzimidazole configuration fungicides. Phytopathology 64:1139-1142.

Warren, C.G., Sanders, P.L., Cole, H., Jr., and Duich, J.M. 1977. Relative fitness of benzimidazole- and cadmium-tolerant populations of *Sclerotinia homeocarpa* in the absence and presence of fungicides. Phytopathology 67:704-708.

Whetzel, H.H. 1946. The cypericolous and juncicolous species of *Sclerotinia*. Farlowia 2:385-437.

Wilkinson, H.T. 2004. Understanding fungicides. Grounds Maint. http://grounds-mag.com/mag/grounds_maintenance_understanding_fungicides/index.html.

Williams, D.W. and Powell, A.J., Jr. 1995. Dew removal and dollar spot on creeping

bentgrass. Golf Course Manage. 63:49-52.

Windham, M.T. 2004. Introduction to plant pathogens. Pages 11-15 in: Plant Pathology Concepts and Laboratory Exercises. R.N. Trigiano, M.T. Windham, A.S. Windham, Eds. CRC Press, New York.

APPENDIX

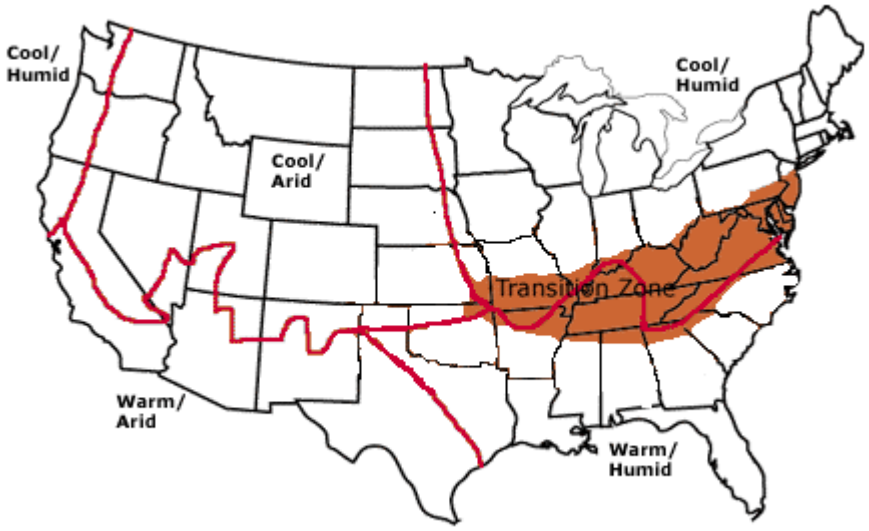

Figure 1.1. Turfgrass adaptation zones in the United States.

Figure 1.2. Tan, sunken areas caused by dollar spot infection on creeping bentgrass.

Figure 1.3. Close up of dollar spot lesion with red banding on individual leaf blade of creeping bentgrass.

Figure 1.4. Close up of *S. homeocarpa* aerial mycelial growth on Kentucky bluegrass during high humidity.

Table 2.1. Reported cases of fungicide resistance in *S. homeocarpa*.

Fungicide Class	Host	Origin	Author	Year
Benzimidazole	Putting Green	Eastern/Mid-western U.S.	C.G. Warren, P.L. Sanders, and H. Cole	1974
Dicarboximide				
Benzimidazole	Creeping bentgrass	Michigan	A.R. Detweiler. J.M. Vargas, Jr., and T.K. Danneberger	1983
DMI[z]	Bentgrass/ annual bluegrass	Michigan	J.M. Vargas, Jr., R.C.Golembiewski, and A.R. Detweiler	1992
DMI	Putting Green	Kentucky	J.C. Doney, and P.C. Vincelli	1993
DMI	Creeping bentgrass/ annual bluegrass	Michigan	R.C. Golwembiewski, J.M. Vargas, Jr., A.L. Jones, and A.R. Detweiler	1995
Benzimidazole				
DMI	Creeping bentgrass	Pennsylvania, Illinois	L.L. Burpee	1997
DMI	Creeping bentgrass/ annual bluegrass	Ontario	T. Hsiang, L. Yang, and W. Barton	1997
DMI	Creeping bentgrass	Georgia	G.L. Miller, K.L. Stevenson, L.L. Burpee	2002

[z]DMI = Demethylation inhibitor

Table 2.2. Number of test isolates, host plants, and fungicide information for each location assayed for fungicide resistance in *S. homeocarpa*.

Population	No. of Isolates	Origin	Bentgrass Cultivar	Fungicides Used	Resistance
BM	10	Seymour, TN	Penncross, L 93, Crenshaw	Chlorothalonil Iprodione Triademifon	Iprodione
CH	10	Olive Branch, MS	Crenshaw	Chlorothalonil Propiconazole Thiophanate-methyl	Thiophanate-methyl
GV	10	Knoxville, TN	Crenshaw	Etridiazole Iprodione Propiconazole Mancozeb Metalaxyl Thiophanate-methyl	Thiophanate-methyl
LG	10	Franklin, TN	Pennlinks	Chlorothalonil Flutolanil Iprodione Metalaxyl	Iprodione
LT1	5	Franklin, TN	18th Green	Propiconazole Pyracostrobin	Iprodione
LT2	5	Franklin, TN	SR 1020	Boscalid Propiconazole	Iprodione
MN	10	Memphis, TN	Crenshaw	No data available.	None
CW	10	Robinsville, MS	Crenshaw	Chlorothalonil Iprodione Thiophanate-methyl	Iprodione Thiophanate-methyl
WS	10	Athens, TN	Crenshaw	Chlorothalonil Iprodione Propiconazole Thiophanate-methyl	Iprodione Propiconazole Thiophanate-methyl
WW	10	Olive Branch, MS	Crenshaw and L93	Chlorothalonil Iprodione Mancozeb Propiconazole Thiophanate-methyl	Thiophanate-methyl

Table 2.3. Incidence of *S. homeocarpa* resistance to fungicides by location.

Location	Treatment		
	Propiconazole	**Iprodione**	**Thiophanate-methyl**
BM	NS	*	NS
GV	NS	NS	*
WS	*	*	*
LG	NS	*	NS
LT1	NS	*	NS
LT2	NS	*	NS
CH	NS	NS	*
MN	NS	NS	NS
CW	NS	*	*
WW	NS	NS	*
MIB	NS	NS	*
MID	NS	*	NS
MIDMI	*	NS	*
MIW	NS	NS	NS

* -- Significant at the 0.01 probability level.
NS – Not significant at the 0.01 probability level.

Table 2.4. Greatest level of fungicide active ingredient to which resistance occurred in *S. homeocarpa* by location.

Location	Treatment		
	Iprodione[z]	Thiophanate-methyl	Propiconazole
BM	1000	0	0
CH	0	1000	0
GV	0	1000	0
LG	10	0	0
LT1	10	0	0
LT2	1	0	0
MN	0	0	0
CW	1000	1	0
WW	0	1000	0
WS	1	1000	10
MID	1000	0	0
MIB	0	1000	0
MIDMI	0	1000	10
MIW	0	0	0

[z] All fungicide concentrations given in μl ml^{-1}.

Table 2.5. EC_{50} and EC_{90} values for all *S. homeocarpa* isolates exhibiting resistance to ioprodione, propiconazole, and/or thiophanate-methyl at each location.

Fungicide	**Population**	**EC_{50}[y]**	**EC_{90}**
Iprodione	BM	230	1175
	CW	*	*
	LG	-24[z]	7
	LT1	-11	6
	LT2	0	2
	WS	1	3
	MID	850	2047
Propiconazole	WS	0	8
	MIDMI	0	19
Thiophanate-methyl	CH	2089	3606
	CW	-1	0.8
	GV	476	819
	WW	2183	3766
	WS	2309	3469
	MIB	1100	2896
	MIDMI	3309	6185

[y]All EC values given in μl ml^{-1}.
[z] A negative number indicates that any concentration of the fungicide would reduce the growth of the fungus to 50% of the growth of the control.
* = Value could not be computed because all isolates grew to 100% relative growth.

Table 2.6. Comparison of recommended fungicide application rates to equivalent rates in the area of Petri dishes.

Fungicide	Field Application Rate[y]	Equivalent Rate in Petri Plate[z]
Propiconazole	15	1.30
Thiophanate-methyl	59-118	1.50-3.00
Iprodione	89-118	1.26-1.69

[y] Field application rates given in ml fungicide per 93 m^2
[z]Equivalent rates in Petri plate give in µL a.i. per .06134 ft^2

Figure 2.1. Example of growth of *S. homeocarpa* on PDA amended with (left to right) 0, 1, 10, 100 and 1000 µl ml^{-1} of thiophanate-methyl. Three days after inoculation

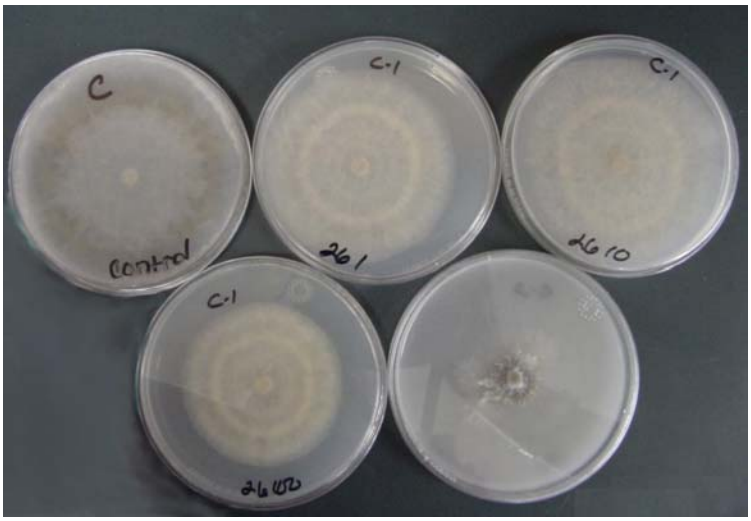

Figure 2.2. Example of growth of *S. homeocarpa* on PDA amended with (top row, left to right) 0, 1, 10, (bottom row, left to right) 100 and 1000 µl ml^{-1} of iprodione. Four days after inoculation.

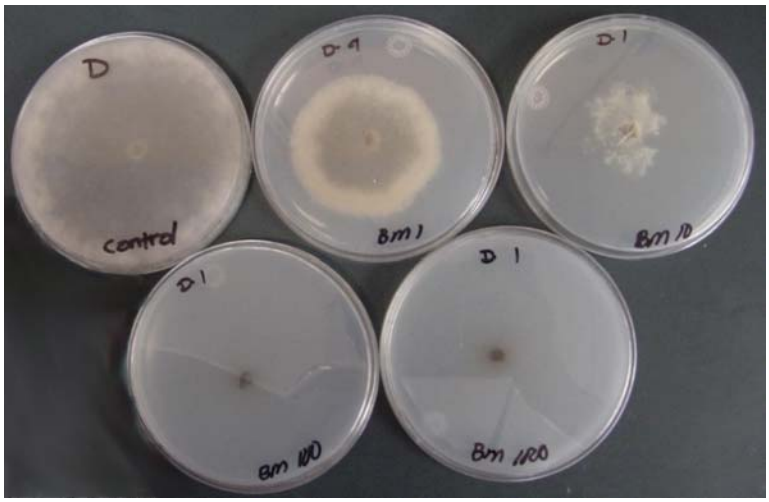

Figure 2.3. Example of growth of *S. homeocarpa* on PDA amended with (top row, left to right) 0, 1, 10, (bottom row, left to right) 100 and 1000 µl ml^{-1} of propiconazole. Four days after inoculation.

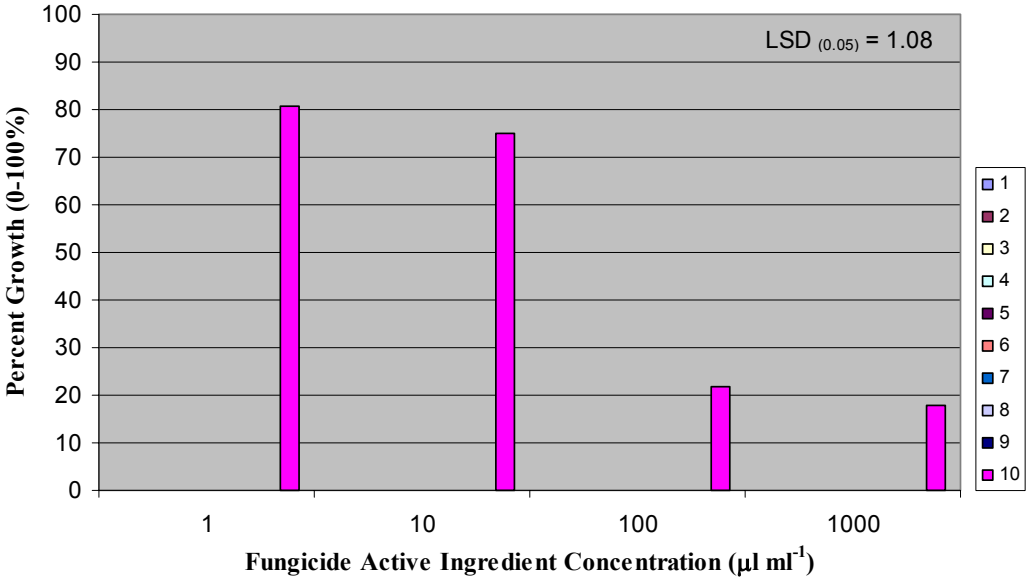

Figure 2.4. Relative growth interaction for iprodione concentration by *S. homeocarpa* isolate for location BM.

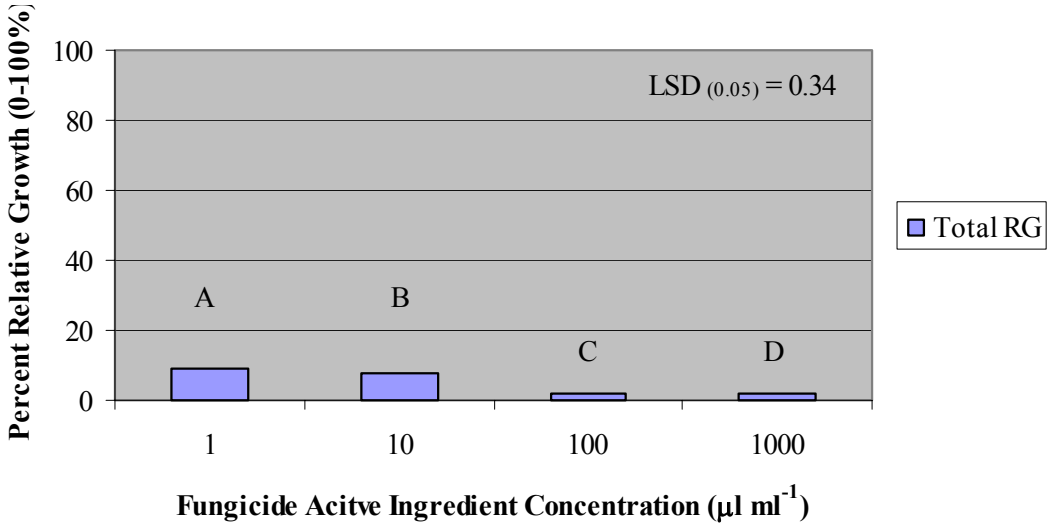

Figure 2.5. Relative growth (RG) interaction for iprodione concentration by total *S. homeocarpa* growth for location BM.

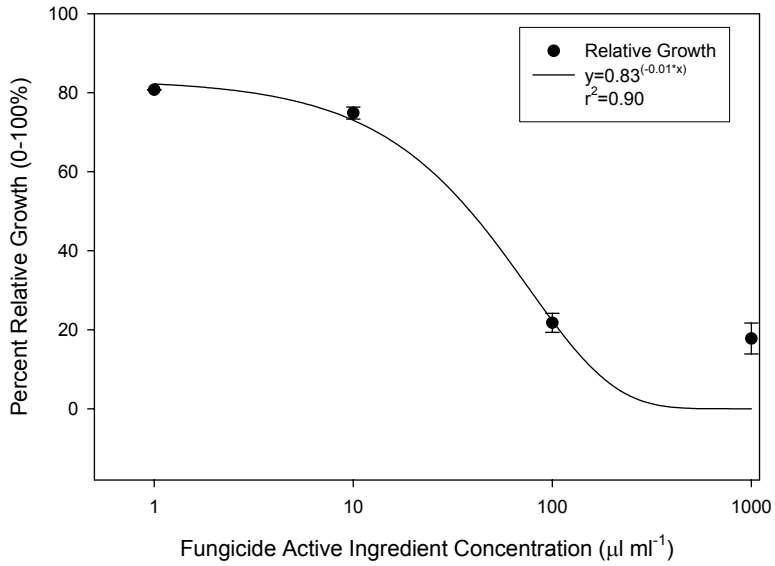

Figure 2.6. Dosage-response curve for *S. homeocarpa* isolate showing resistance to iprodione at location BM.

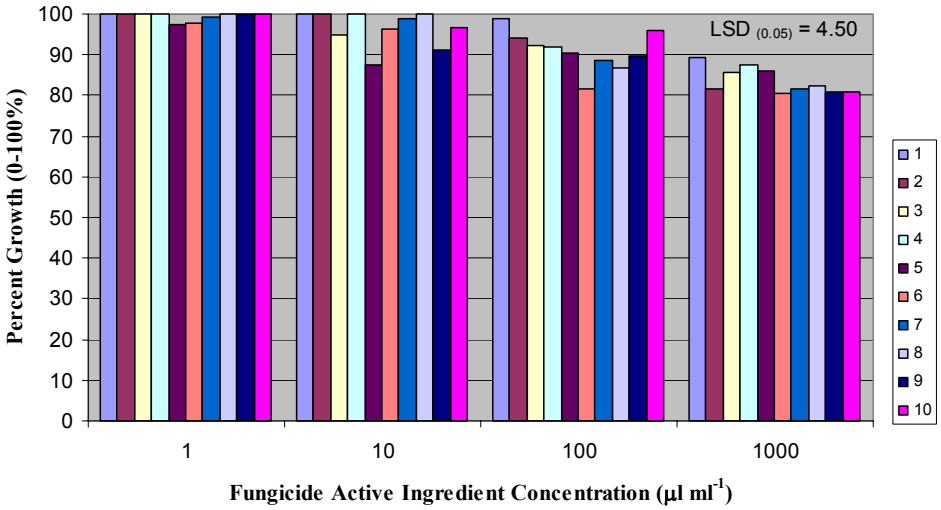

Figure 2.7. Relative growth interaction for thiophanate-methyl concentration by *S. homeocarpa* isolate for location CH.

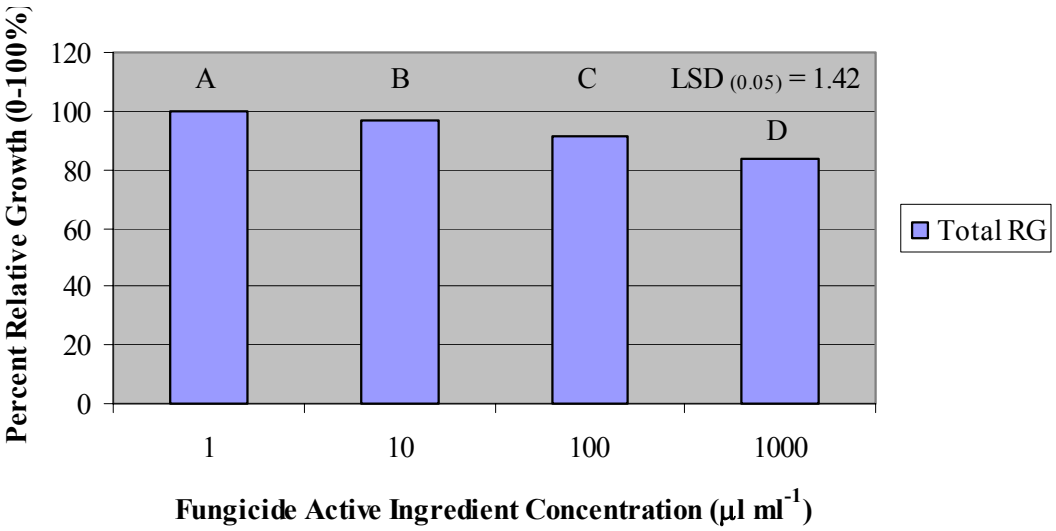

Figure 2.8. Relative growth (RG) interaction for thiophanate-methyl concentration by total *S. homeocarpa* growth for location CH.

.

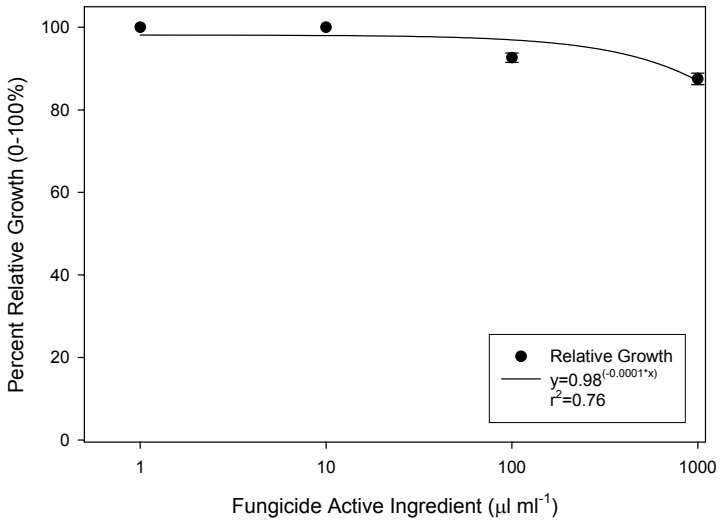

Figure 2.9. Dosage-response curve for *S. homeocarpa* isolate with greatest resistance to thiophanate-methyl at location CH.

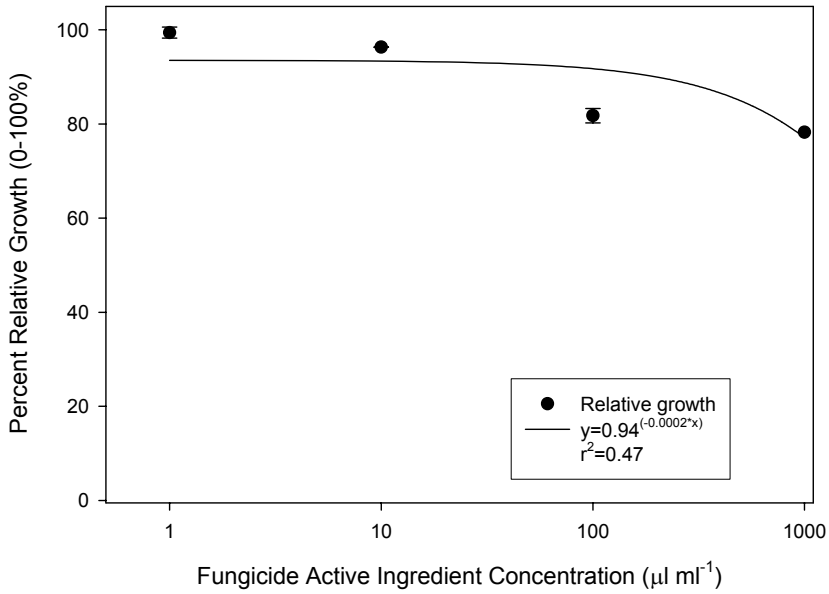

Figure 2.10. Dosage-response curve for *S. homeocarpa* isolate with least resistance to thiophanate-methyl at location CH.

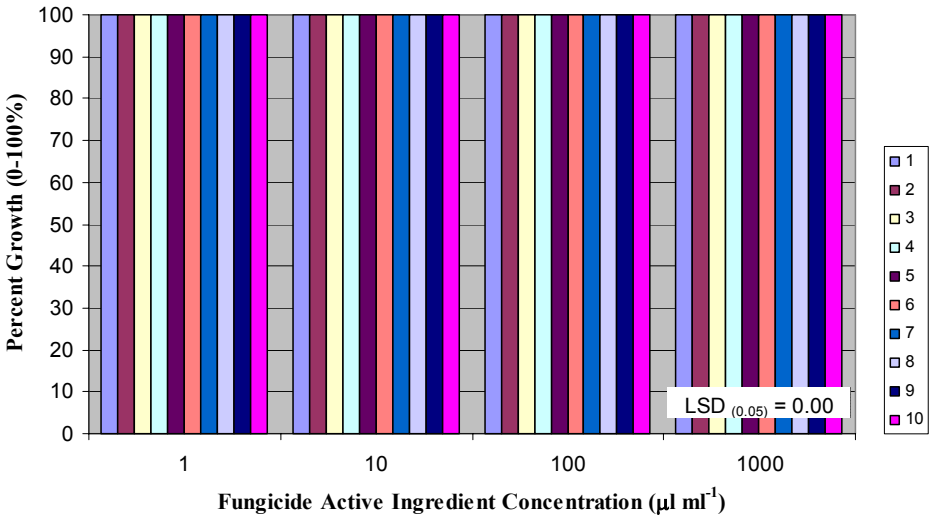

Figure 2.11. Relative growth interaction for iprodione concentration by *S. homeocarpa* isolate for location CW.

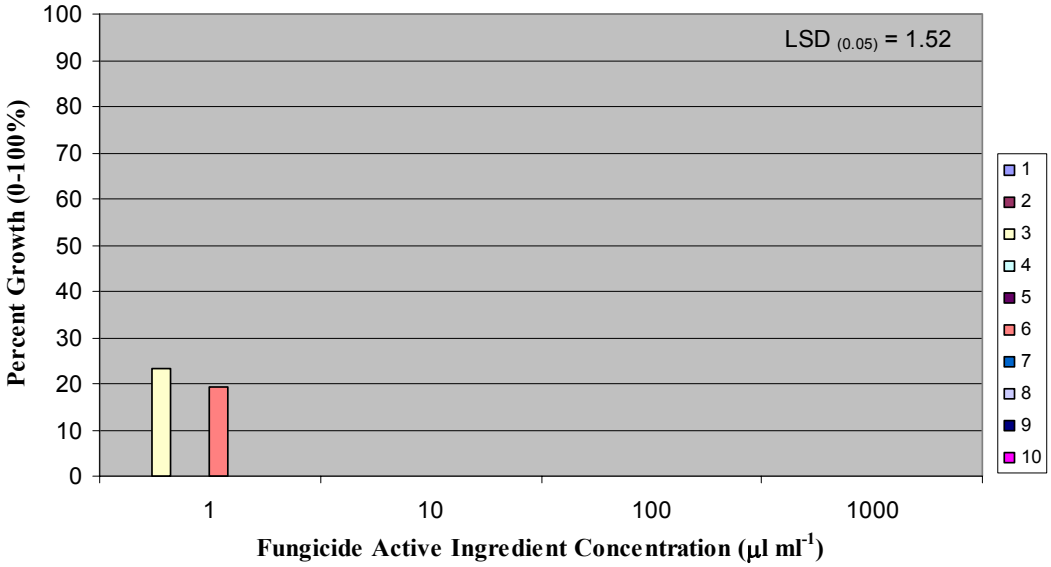

Figure 2.12. Relative growth interaction for thiophanate-methyl concentration by *S. homeocarpa* isolate for location CW.

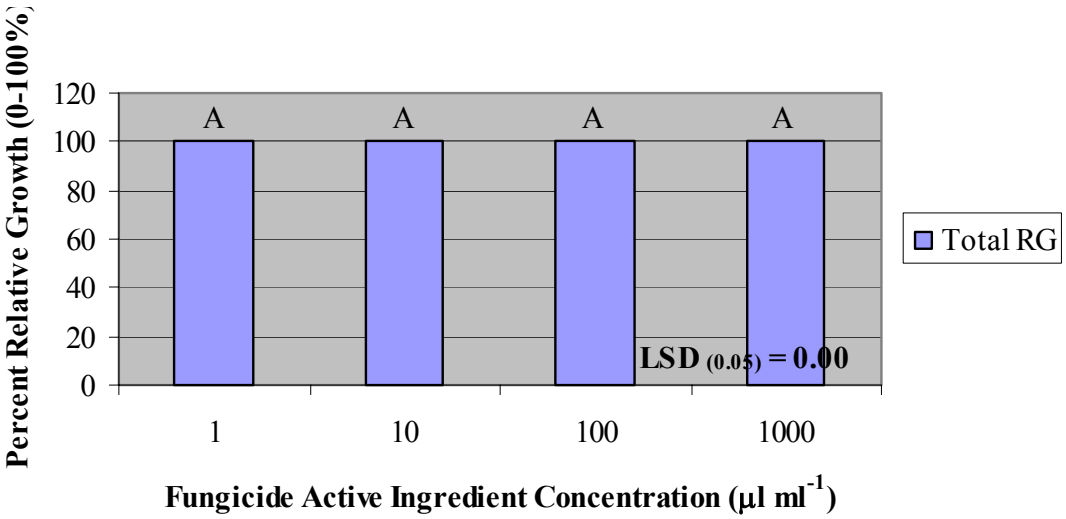

Figure 2.13. Relative growth (RG) interaction for iprodione concentration by total *S. homeocarpa* growth for location CW.

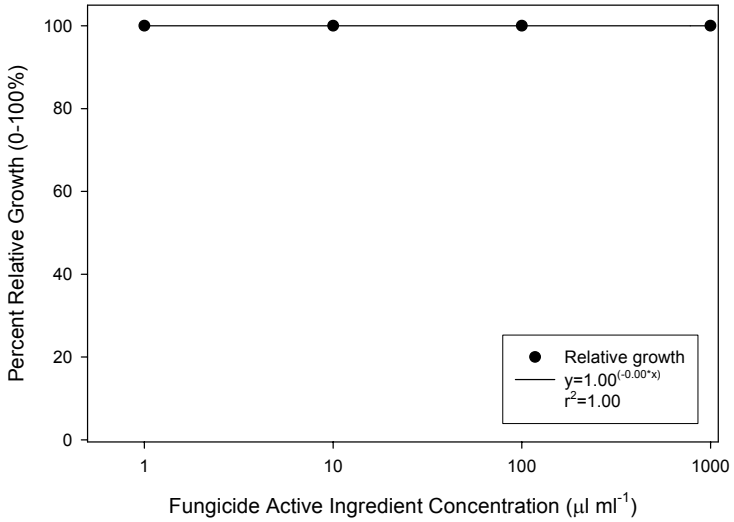

Figure 2.14. Dosage-response curve for all *S. homeocarpa* isolates with resistance to iprodione at location CW.

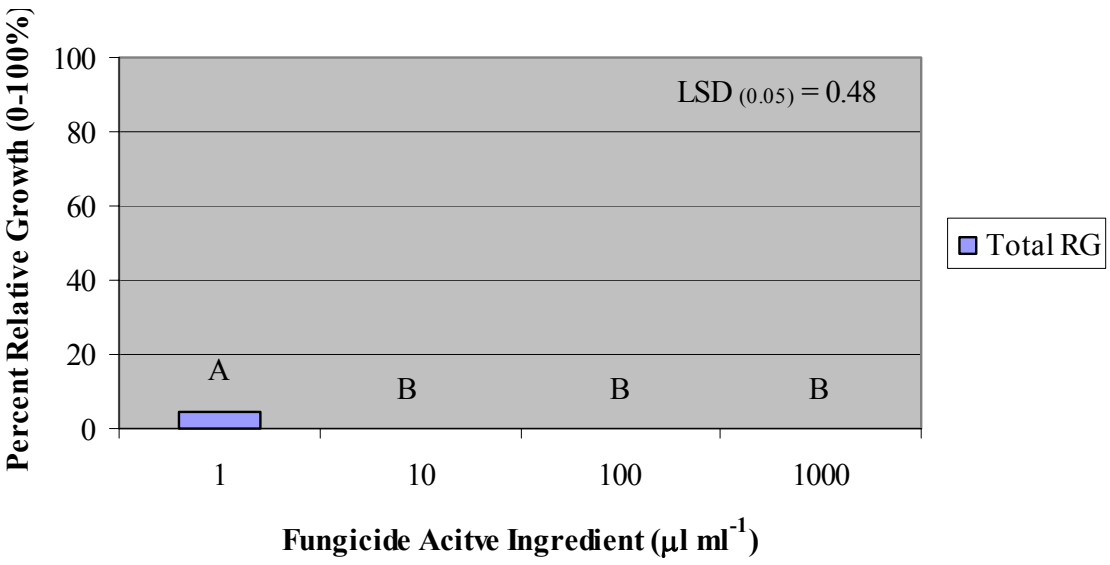

Figure 2.15. Relative growth (RG) interaction for thiophanate-methyl concentration by total *S. homeocarpa* growth for location CW.

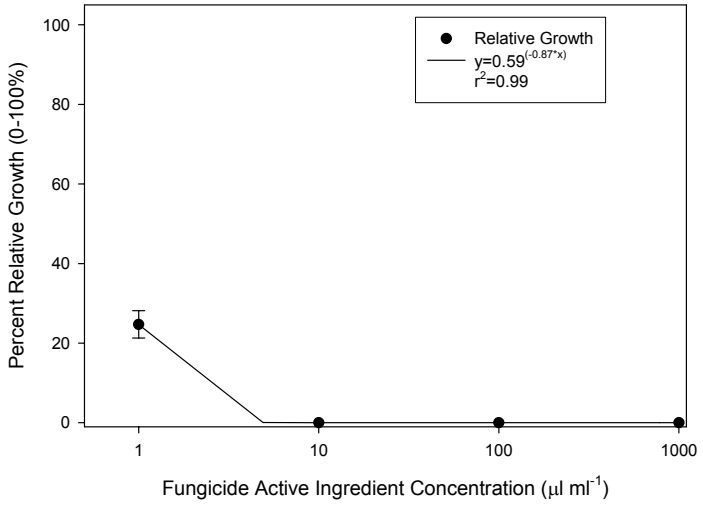

Figure 2.16. Dosage-response curve for *S. homeocarpa* isolate with greatest resistance to thiophanate-methyl at location CW.

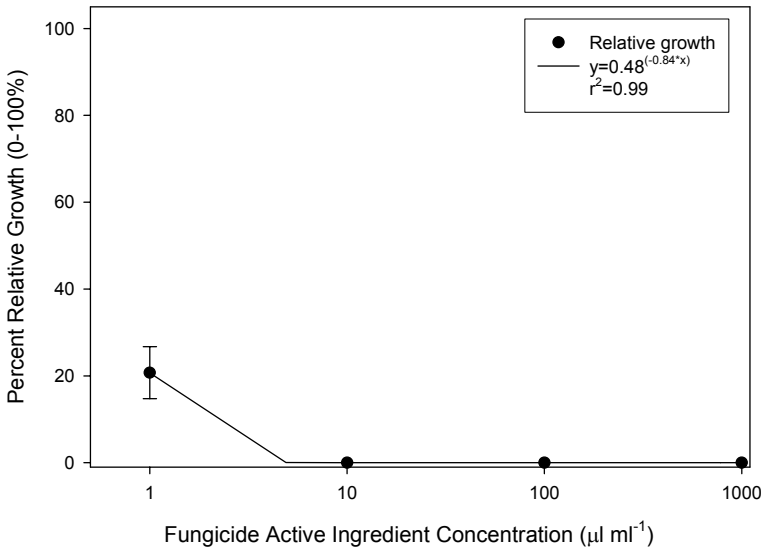

Figure 2.17. Dosage-response curve for *S. homeocarpa* isolate with least resistance to thiophanate-methyl at location CW.

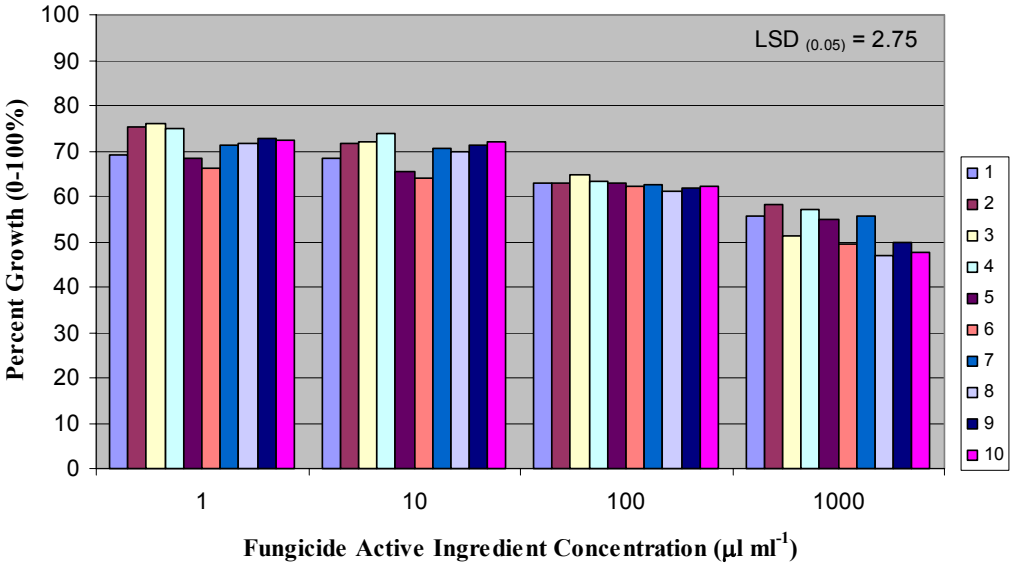

Figure 2.18. Relative growth interaction for thiophanate-methyl concentration by *S. homeocarpa* isolate for location GV.

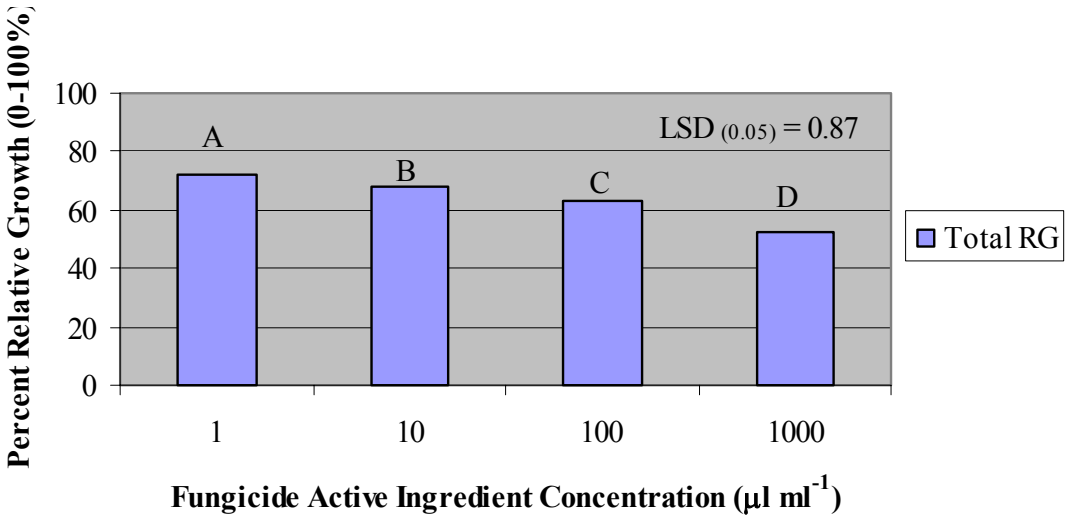

Figure 2.19. Relative growth (RG) interaction for thiophanate-methyl concentration by total *S. homeocarpa* growth for location GV.

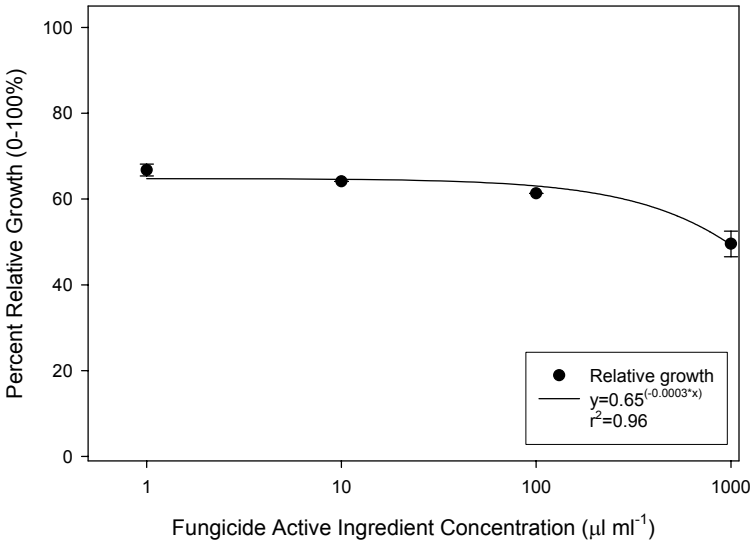

Figure 2.20. Dosage-response curve for *S. homeocarpa* isolate with greatest resistance to thiophanate-methyl at location GV.

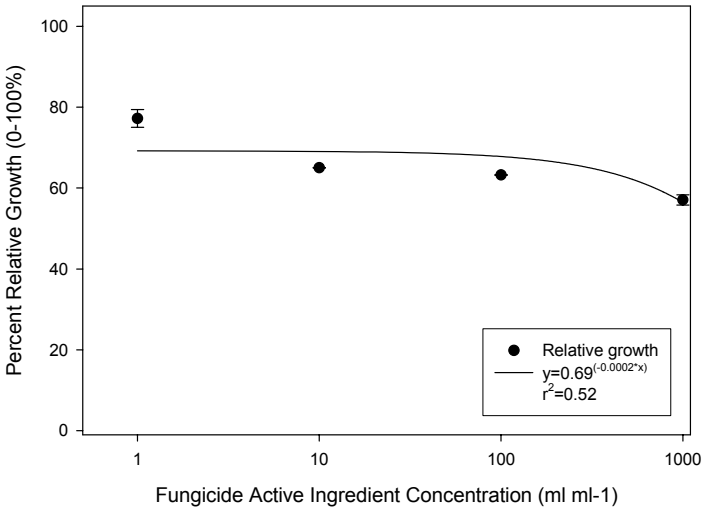

Figure 2.21. Dosage-response curve for *S. homeocarpa* isolate with least resistance to thiophanate-methyl at location GV.

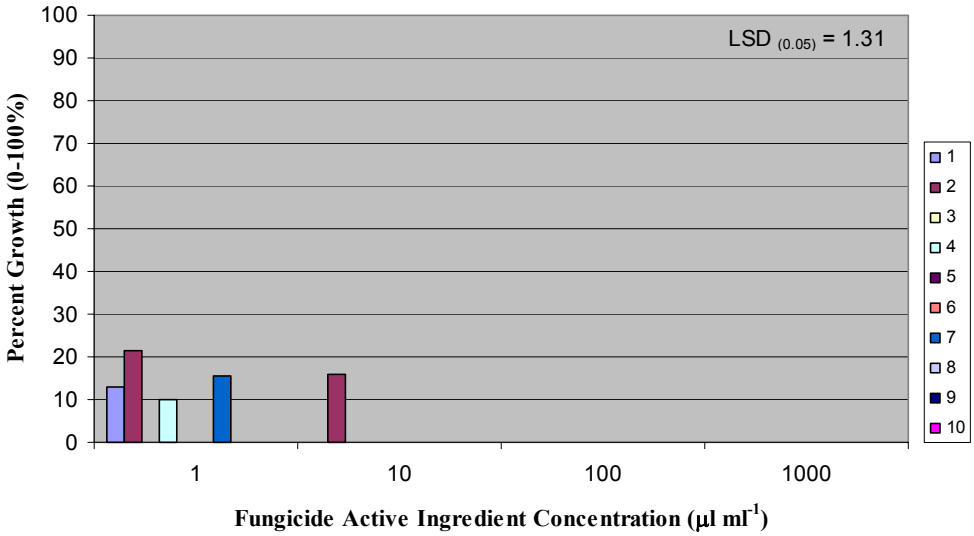

Figure 2.22. Relative growth interaction for iprodione concentration by *S. homeocarpa* isolate for location LG.

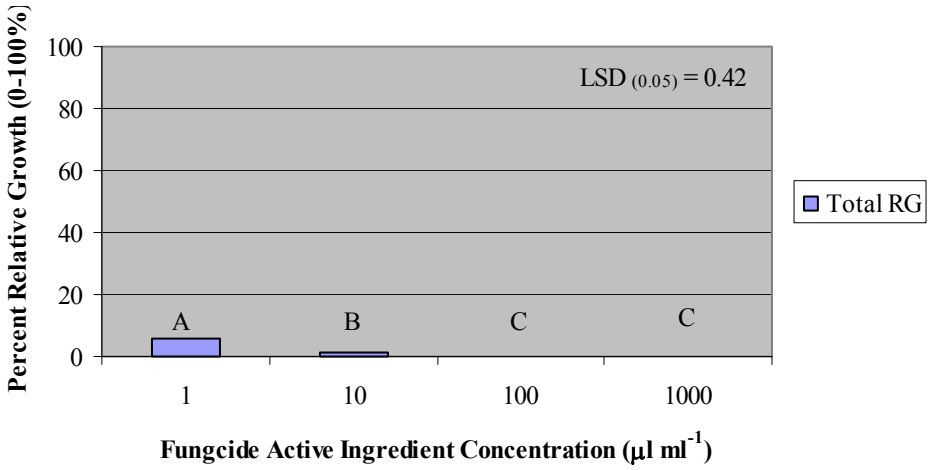

Figure 2.23. Relative growth (RG) interaction for iprodione concentration by total *S. homeocarpa* growth for location LG.

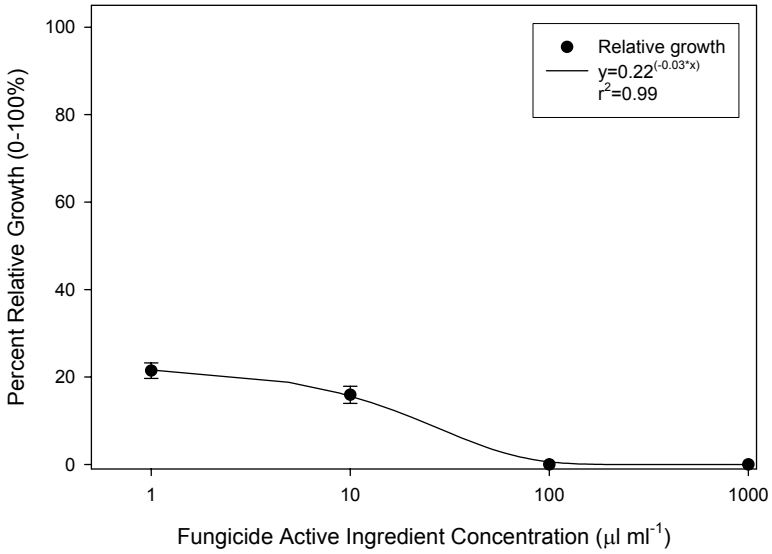

Figure 2.24. Dosage-response curve for *S. homeocarpa* isolate with greatest resistance to iprodione at location LG.

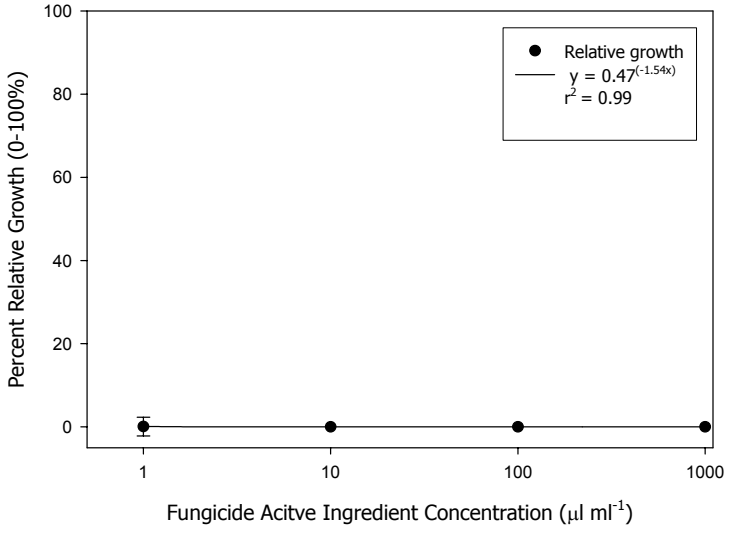

Figure 2.25. Dosage-response curve for *S. homeocarpa* isolate with least resistance to iprodione at location LG.

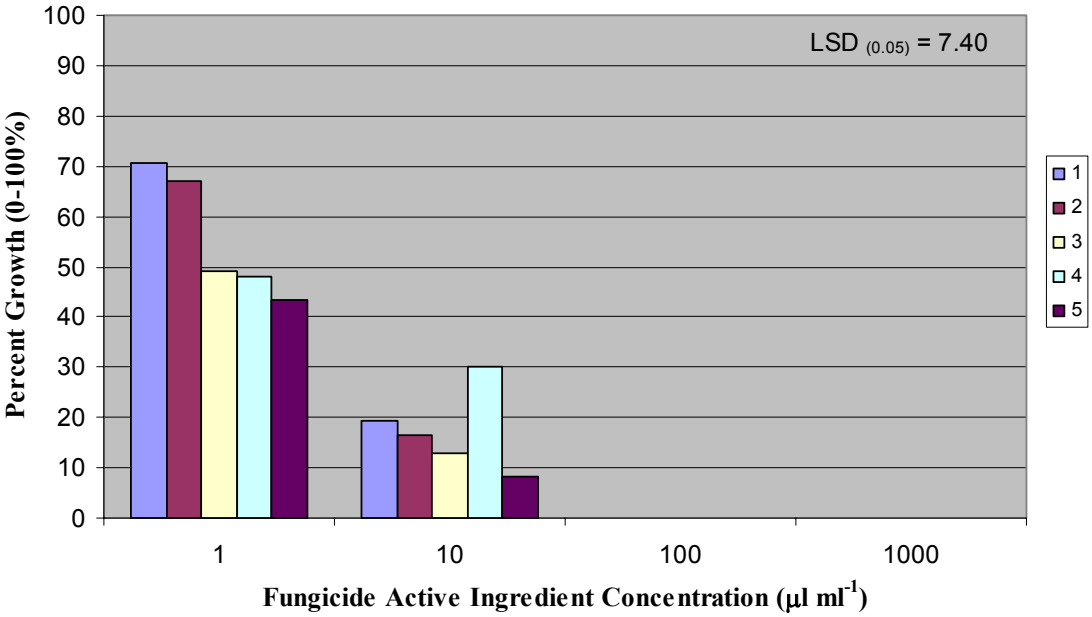

Figure 2.26. Relative growth interaction for iprodione concentration by *S. homeocarpa* isolate for location LT1.

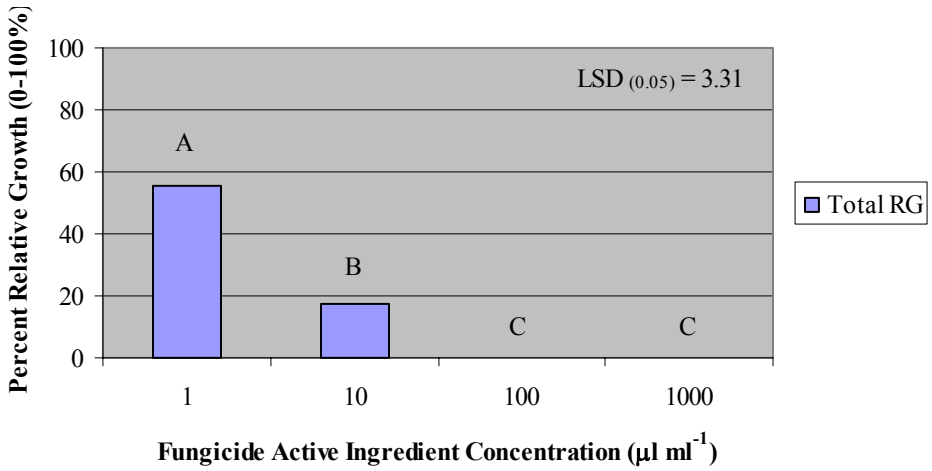

Figure 2.27. Relative growth (RG) interaction for iprodione concentration by total *S. homeocarpa* growth for location LT1.

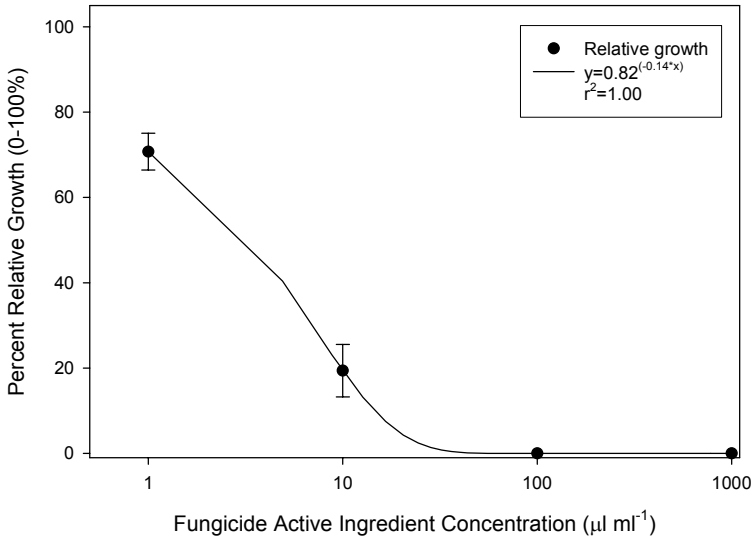

Figure 2.28. Dosage-response curve for *S. homeocarpa* isolate with greatest resistance to iprodione at location LT1.

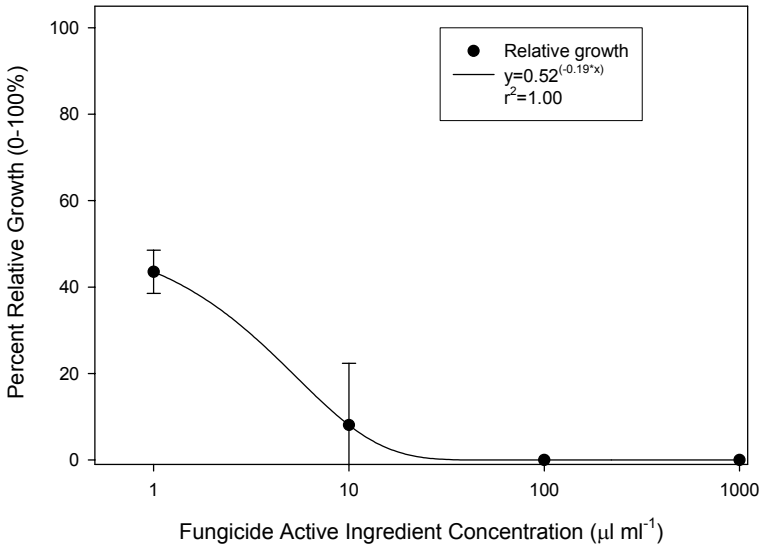

Figure 2.29. Dosage-response curve for *S. homeocarpa* isolate with least resistance to iprodione at location LT1.

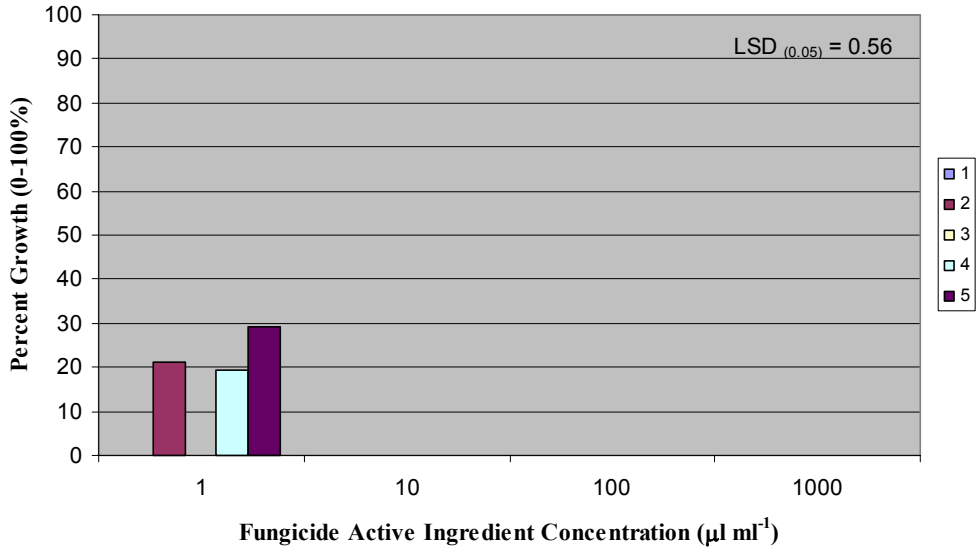

Figure 2.30. Relative growth interaction for iprodione concentration by *S. homeocarpa* isolate for location LT2.

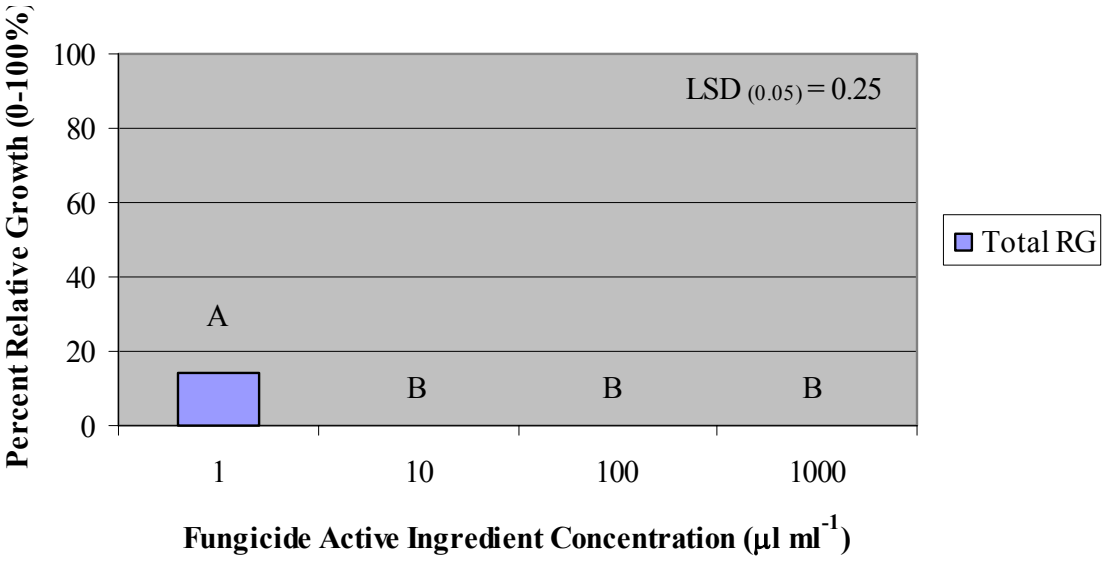

Figure 2.31. Relative growth (RG) interaction for iprodione concentration by total *S. homeocarpa* growth for location LT2.

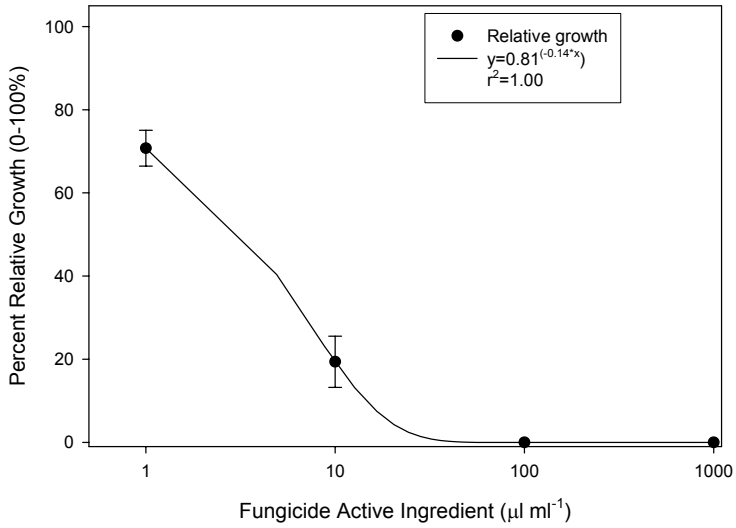

Figure 2.32. Dosage-response curve for *S. homeocarpa* isolate with greatest resistance to iprodione at location LT2.

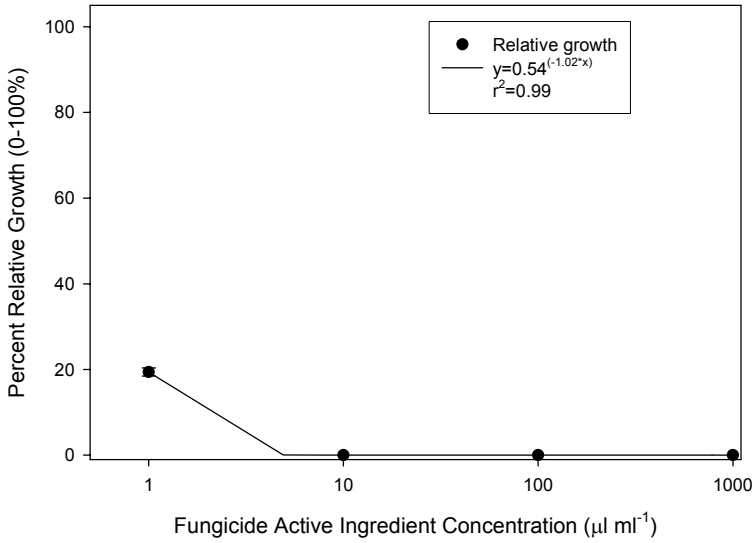

Figure 2.33. Dosage-response curve for *S. homeocarpa* isolate with least resistance to iprodione at location LT2.

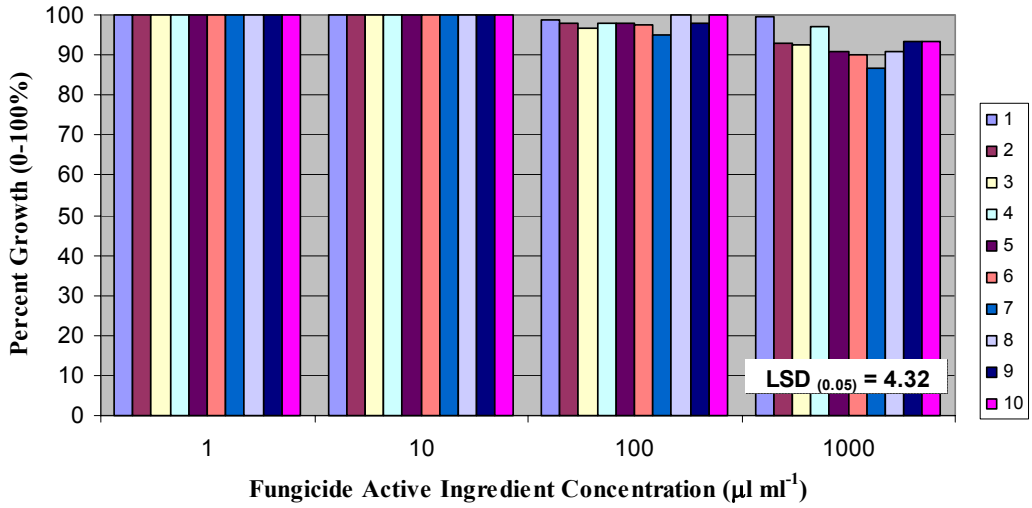

Figure 2.34. Relative growth interaction for thiophanate-methyl concentration by *S. homeocarpa* isolate for location WS.

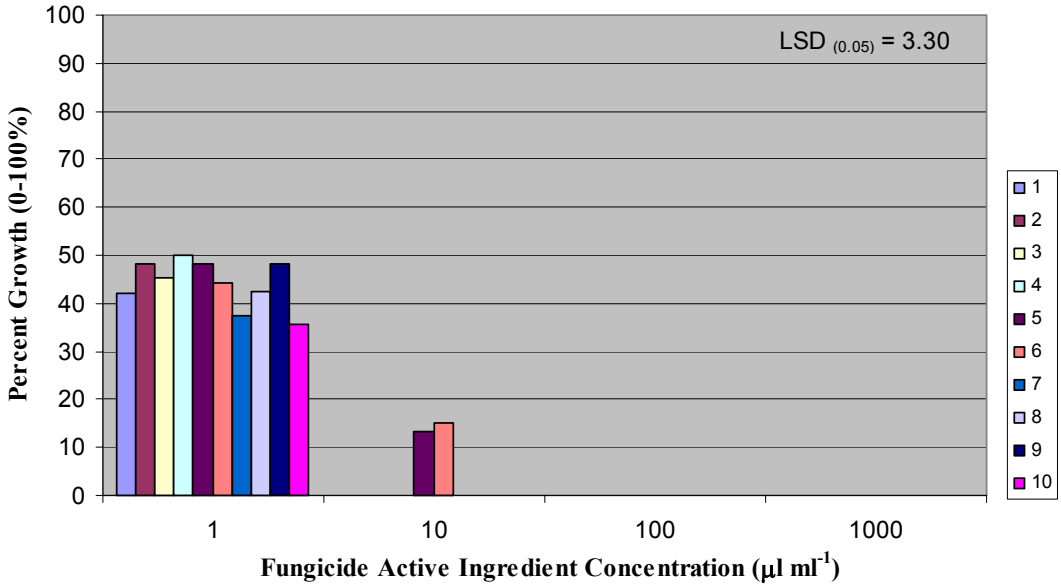

Figure 2.35. Relative growth interaction for iprodione concentration by *S. homeocarpa* isolate for location WS.

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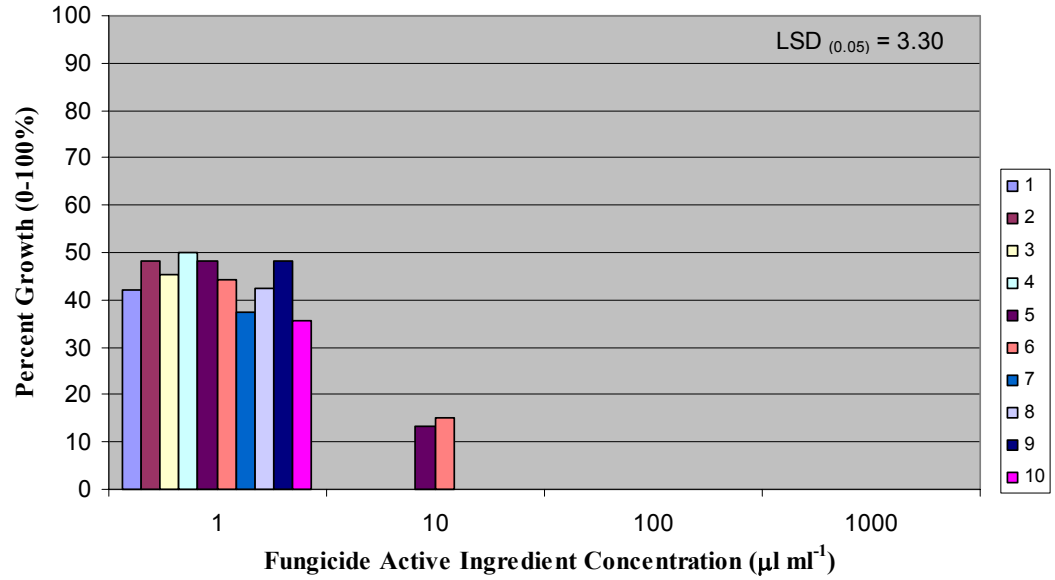

Figure 2.36. Relative growth interaction for propiconazole concentration by *S. homeocarpa* isolate for location WS.

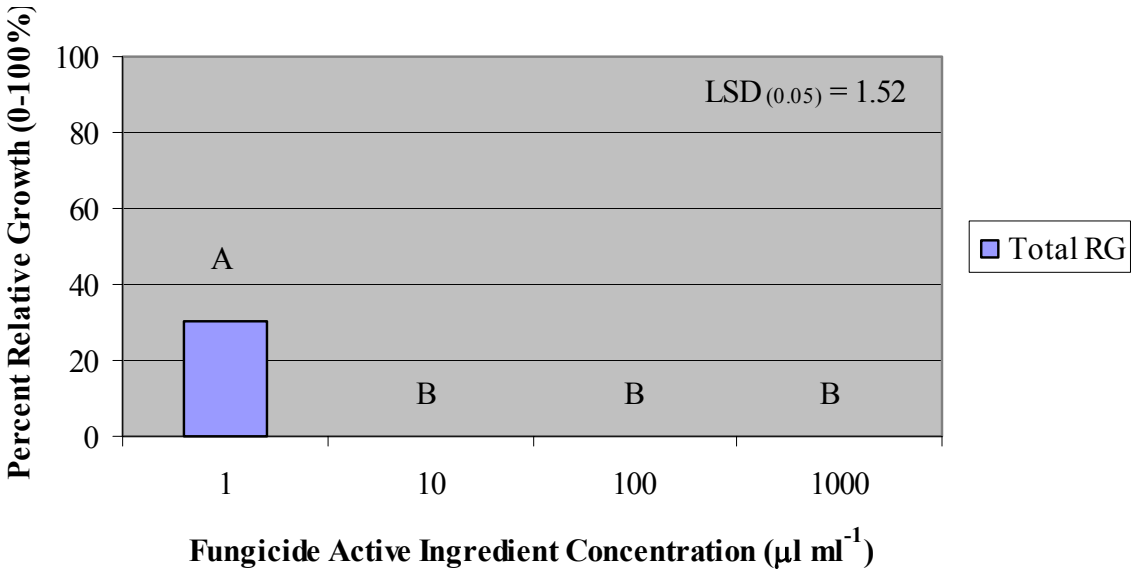

Figure 2.37. Relative growth (RG) interaction for iprodione concentration by total *S. homeocarpa* growth for location WS.

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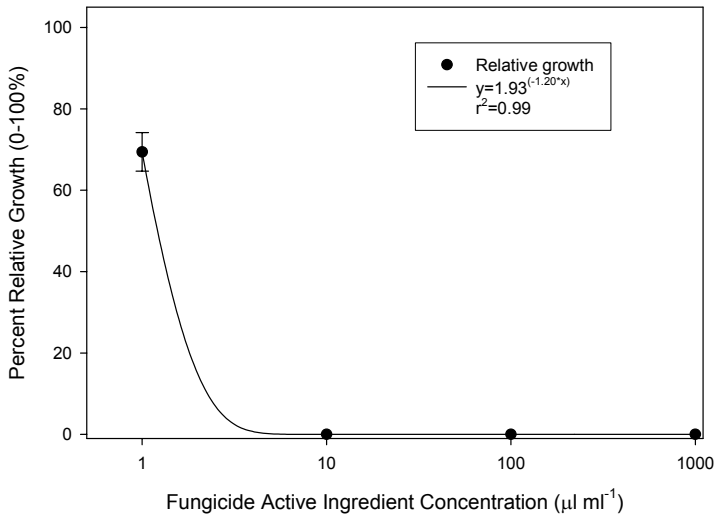

Figure 2.38. Dosage-response curve for *S. homeocarpa* isolate with greatest resistance to iprodione at location WS.

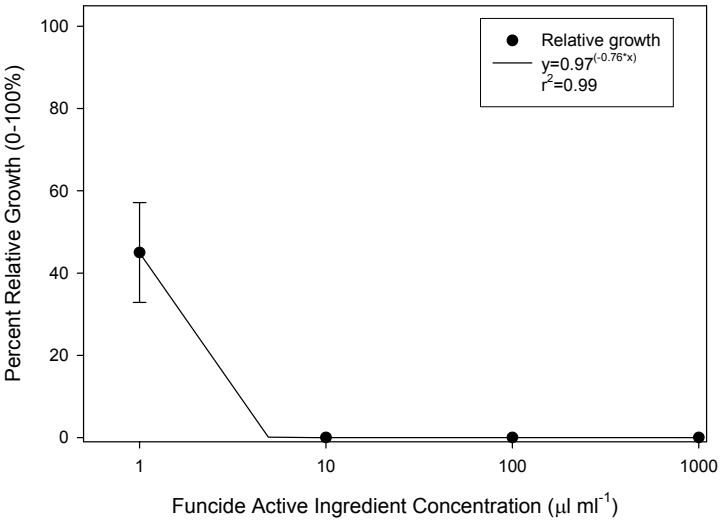

Figure 2.39. Dosage-response curve for *S. homeocarpa* isolate with least resistance to iprodione at location WS.

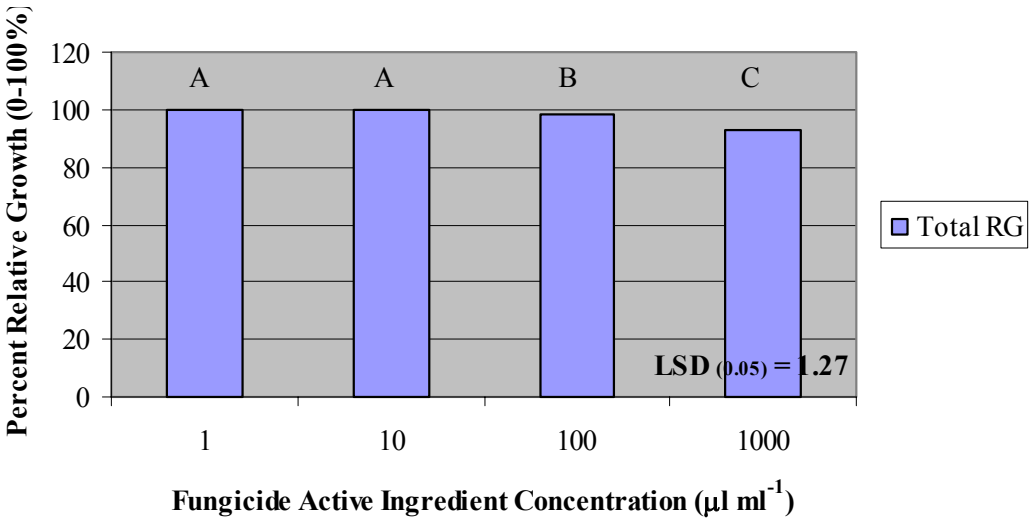

Figure 2.40. Relative growth (RG) interaction for thiophanate-methyl concentration by total *S. homeocarpa* growth for location WS.

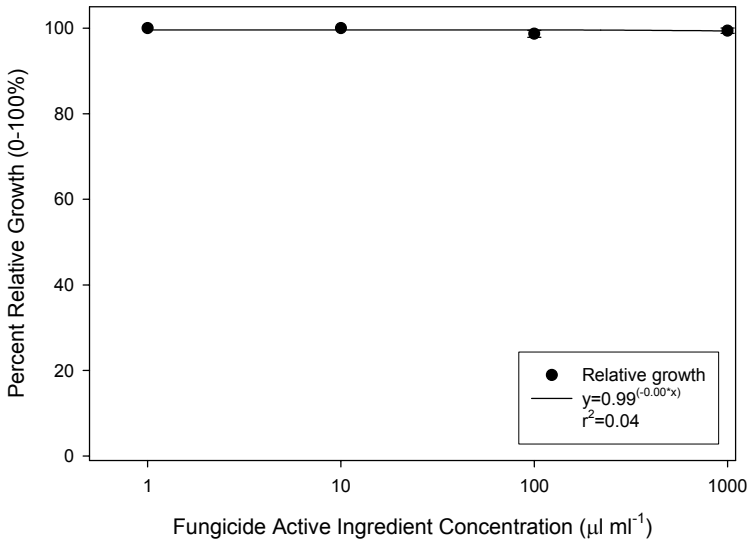

Figure 2.41. Dosage-response curve for *S. homeocarpa* isolate with greatest resistance to thiophanate-methyl at location WS.

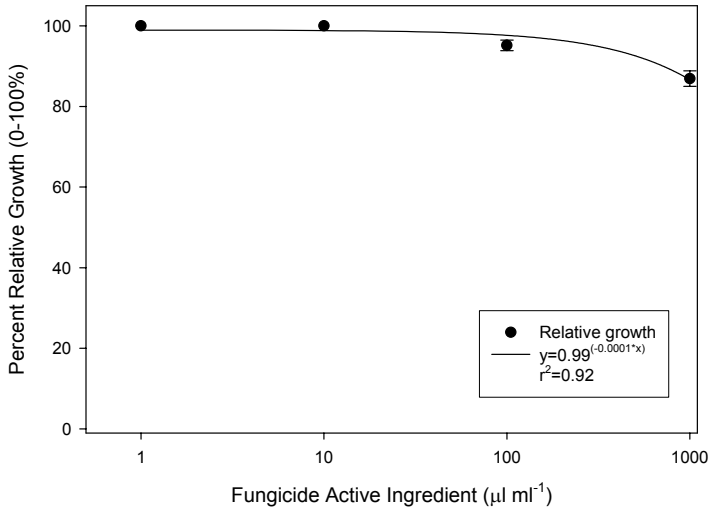

Figure 2.42. Dosage-response curve for *S. homeocarpa* isolate with least resistance to thiophanate-methyl at location WS.

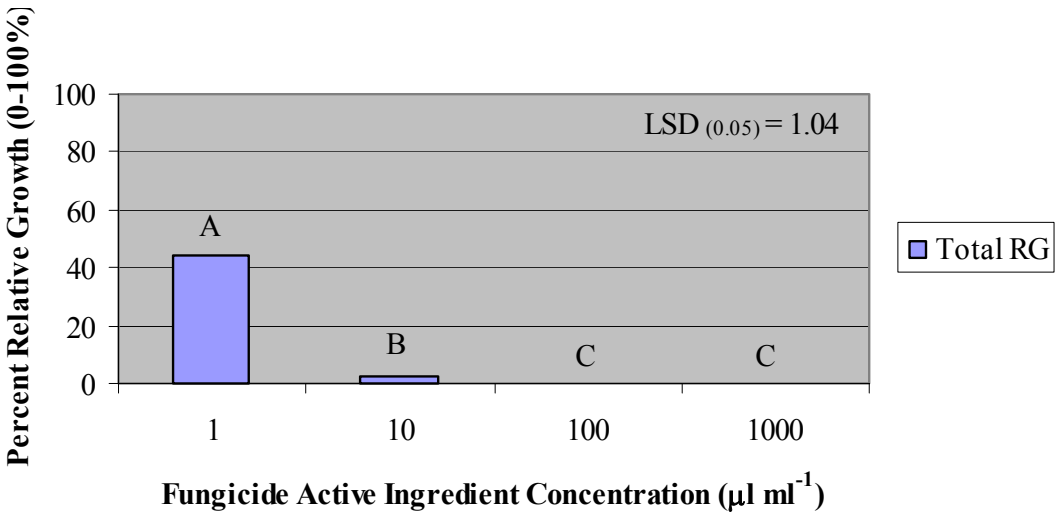

Figure 2.43. Relative growth (RG) interaction for propiconazole concentration by total *S. homeocarpa* growth for location WS.

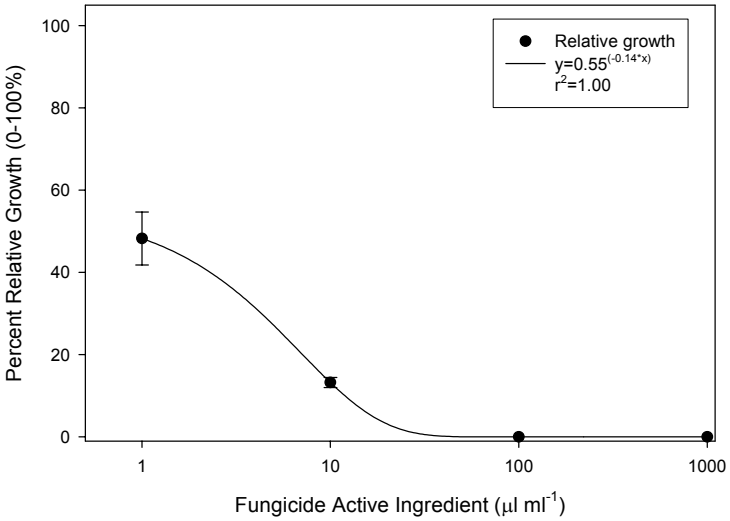

Figure 2.44. Dosage-response curve for *S. homeocarpa* isolate with greatest resistance to propiconazole at location WS.

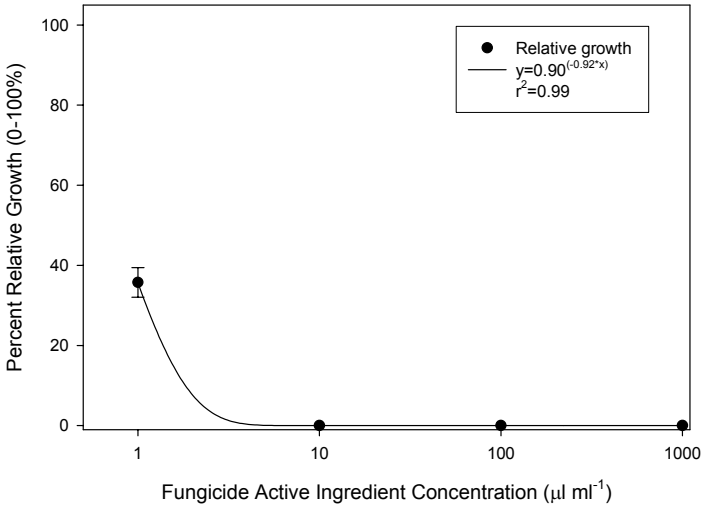

Figure 2.45. Dosage-response curve for *S. homeocarpa* isolate with least resistance to propiconazole at location WS.

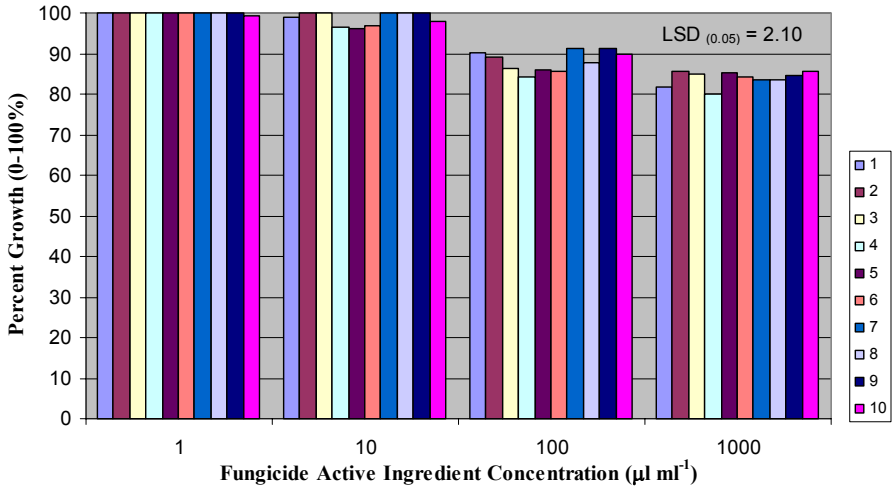

Figure 2.46. Relative growth interaction for thiophanate-methyl concentration by *S. homeocarpa* isolate for location WW.

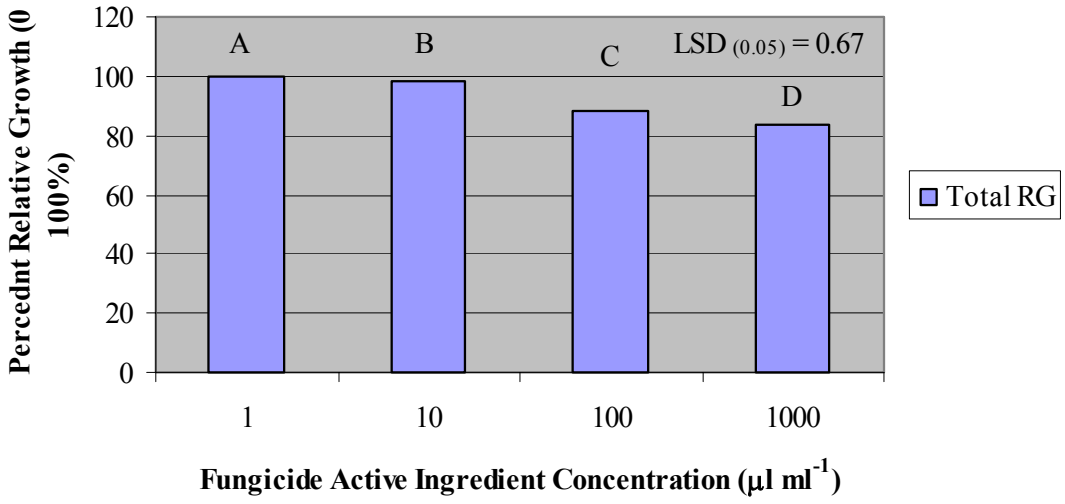

Figure 2.47. Relative growth (RG) interaction for thiophanate-methyl concentration by total *S. homeocarpa* growth for location WW.

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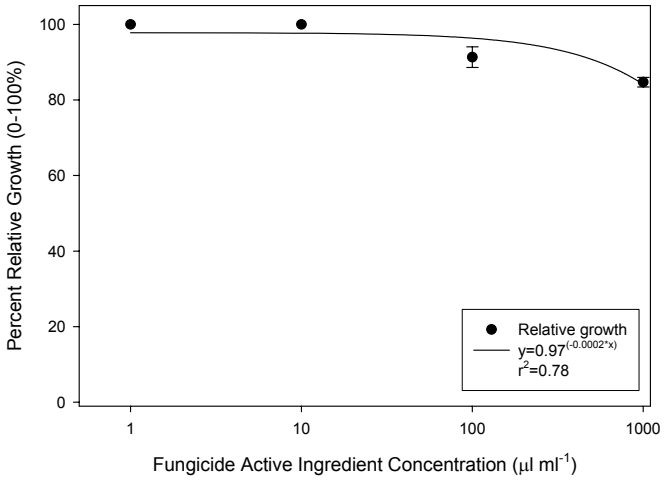

Figure 2.48. Dosage-response curve for *S. homeocarpa* isolate with greatest resistance to thiophanate-methyl at location WW.

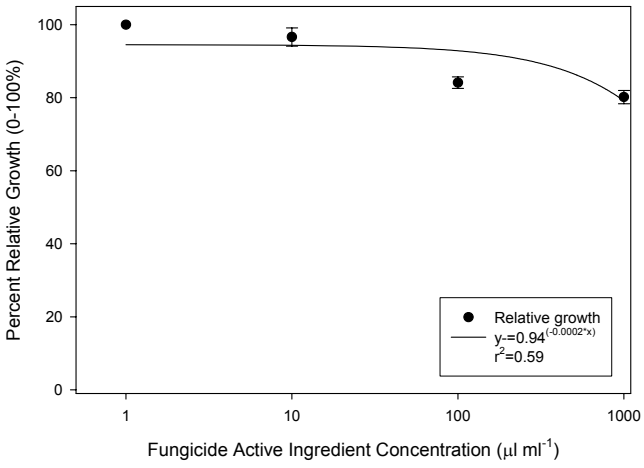

Figure 2.49. Dosage-response curve for *S. homeocarpa* isolate with least resistance to thiophanate-methyl at location WW.

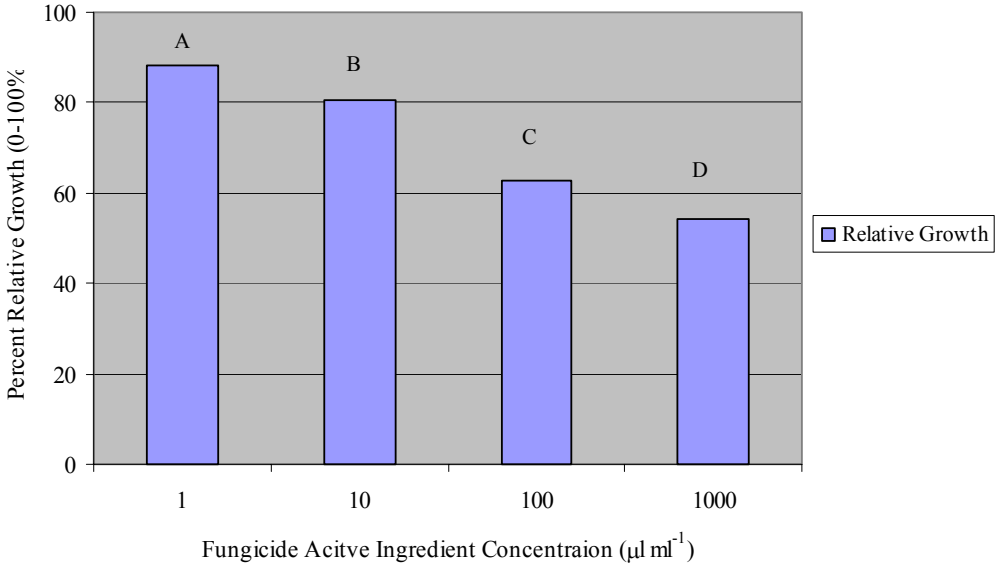

Figure 2.50. Relative growth (RG) interaction for thiophanate-methyl concentration by total *S. homeocarpa* growth for location MIB.

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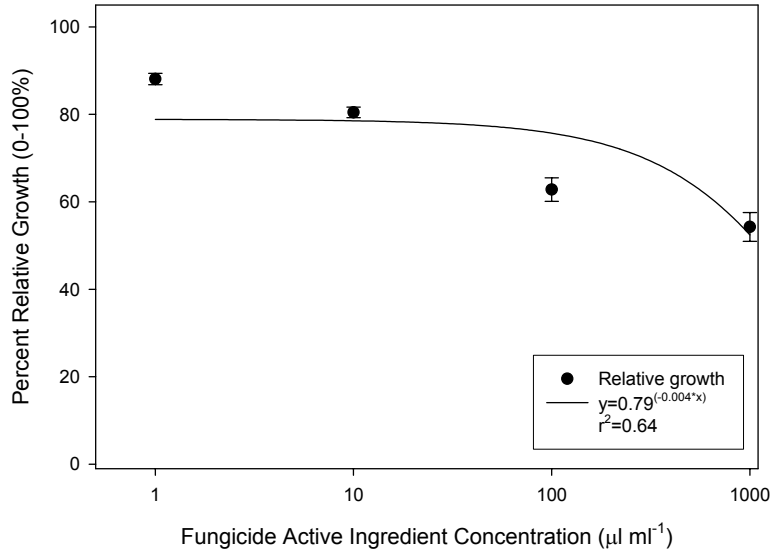

Figure 2.51. Dosage-response curve for *S. homeocarpa* isolate with resistance to thiophanate-methyl at location MIB.

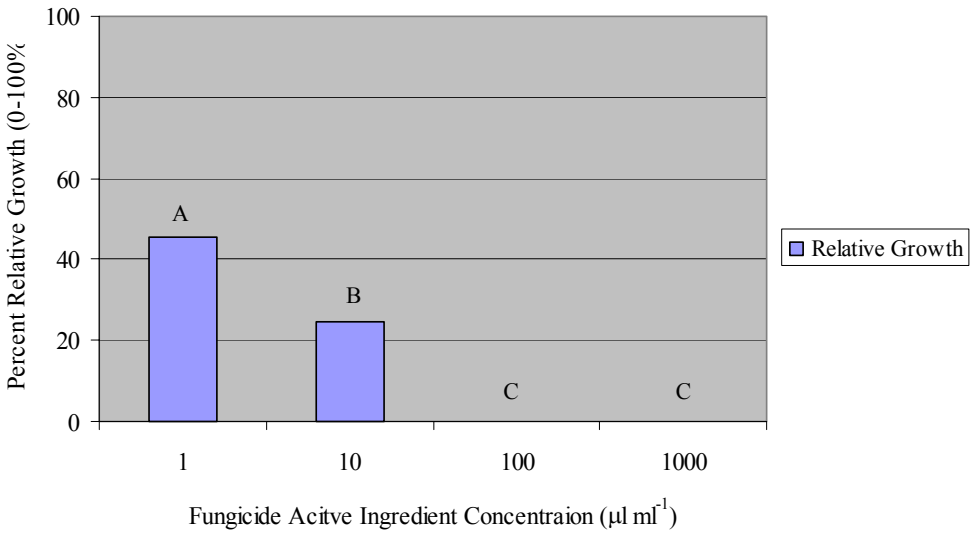

Figure 2.52. Relative growth (RG) interaction for propiconazole concentration by total *S. homeocarpa* growth for location MIDMI.

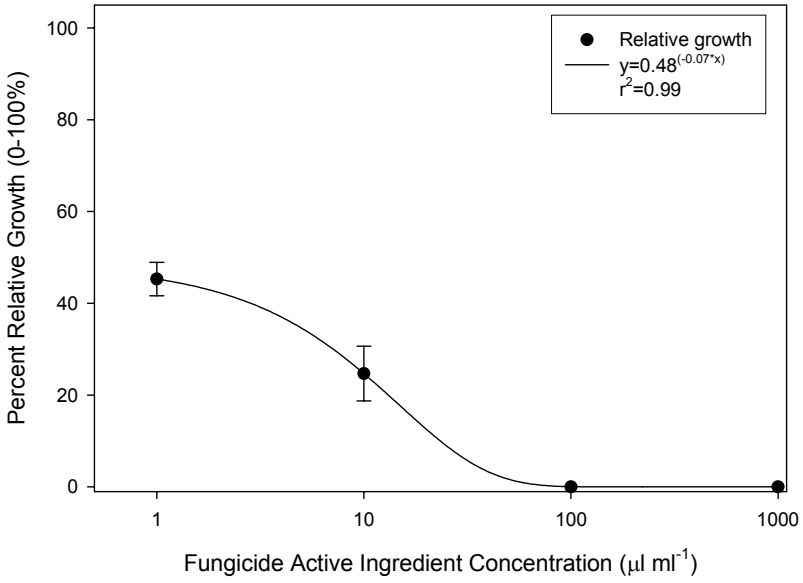

Figure 2.53. Dosage-response curve for *S. homeocarpa* isolate with resistance to propiconazole at location MIDMI.

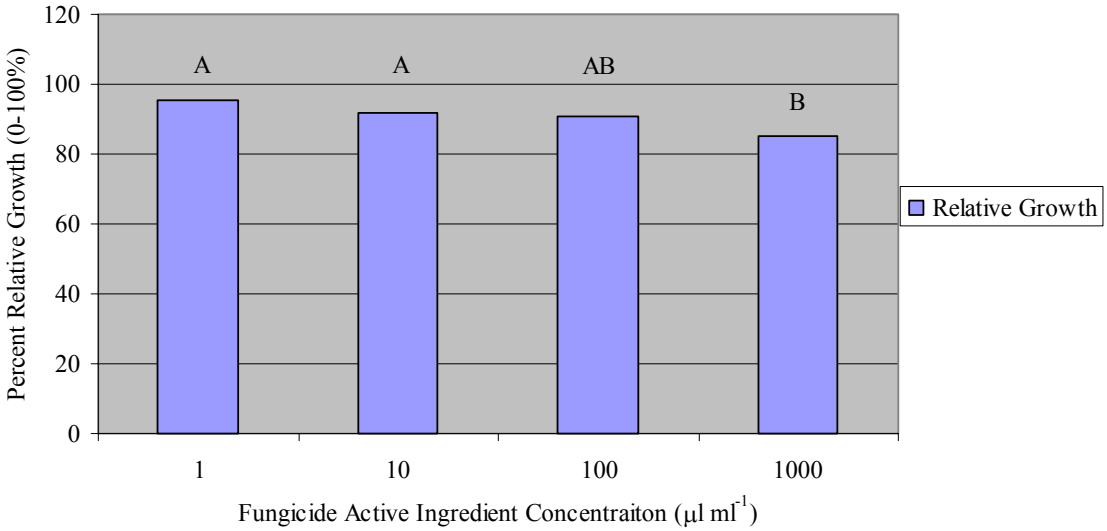

Figure 2.54. Relative growth (RG) interaction for thiophanate-methyl concentration by total *S. homeocarpa* growth for location MIDMI.

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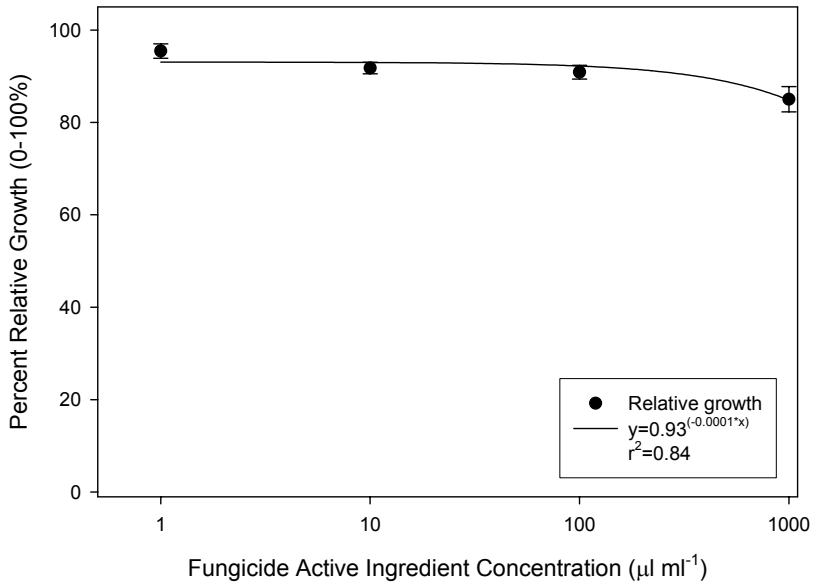

Figure 2.55. Dosage-response curve for *S. homeocarpa* isolate with resistance to thiophanate-methyl at location MIBDMI.

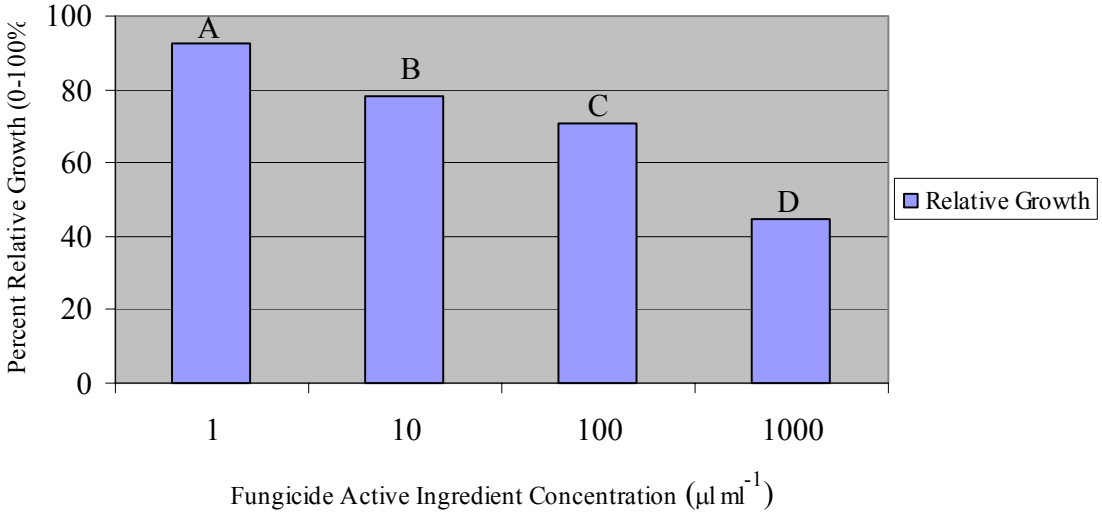

Figure 2.56. Relative growth (RG) interaction for iprodione concentration by total *S. homeocarpa* growth for location MID.

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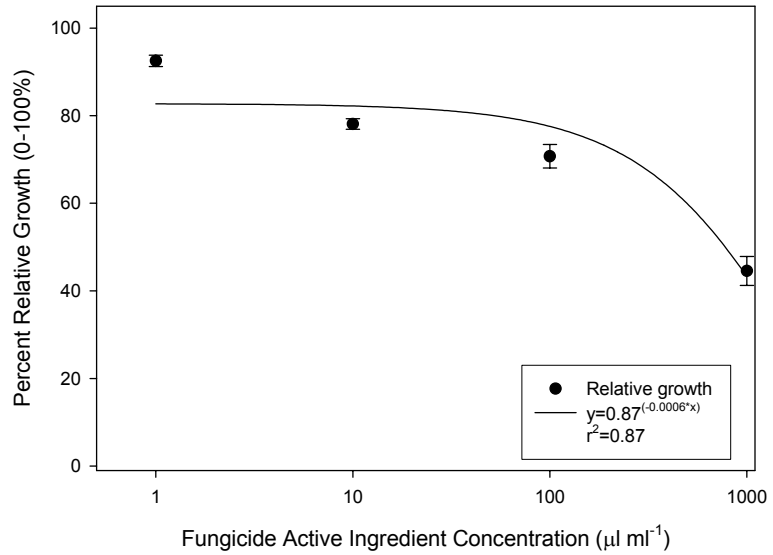

Figure 2.57. Dosage-response curve for *S. homeocarpa* isolate with resistance to iprodione at location MID.

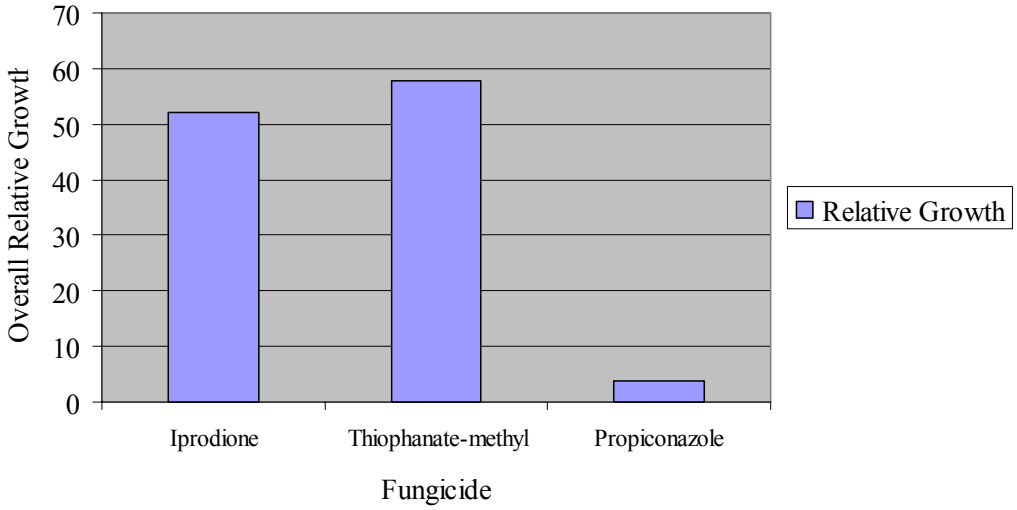

Figure 2.58. Overall relative growth of *S. homeocarpa* for each fungicide.

Figure 3.1. Irregular growth of *S. homeocarpa* on potato dextrose agar amended with 10 μg ml^{-1} chlorothalonil.

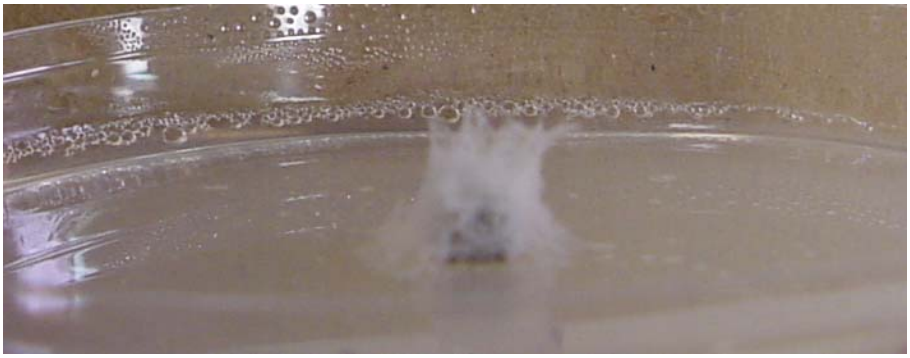

Figure 3.2. Close-up of aerial mycelial growth of *S. homeocarpa* on potato dextrose agar amended with 100 μg ml^{-1} chlorothalonil.

Table 3.1. Apparent relative growth of selected *S. homeocarpa* isolates at various concentrations of chlorothalonil active ingredient.

Isolate	**Apparent Percent Relative Growth**			
	1 µg ml^{-1}	**10 µg ml^{-1}**	**100 µg ml^{-1}**	**1000 µg ml^{-1}**
BM	0	0	0	0
GV	0	0	0	0
WS	28	11	4	0
LG	24	12	0	0
LT1	0	0	0	0
CW1	54	20	16	0
CW2	63	15	0	0
MN	0	0	0	0
MIW	43	28	15	0

Figure 3.3. Suction method to remove excess chlorothalonil-amended potato dextrose broth from mycelial mass.

Table 3.2. EC_{50} and EC_{90} values for all *S. homeocarpa* isolates tested for resistance to chlorothalonil.

Isolate	**EC_{50}[z]**	**EC_{90}**
BM	3	8
GV	3	6
WS	5	8
LG	9	15
LT1	4	7
CW1	4	8
CW2	3	7
MN	7	13
MIW	4	8

[z] = EC_{50} and EC_{90} values given in $\mu g\ ml^{-1}$

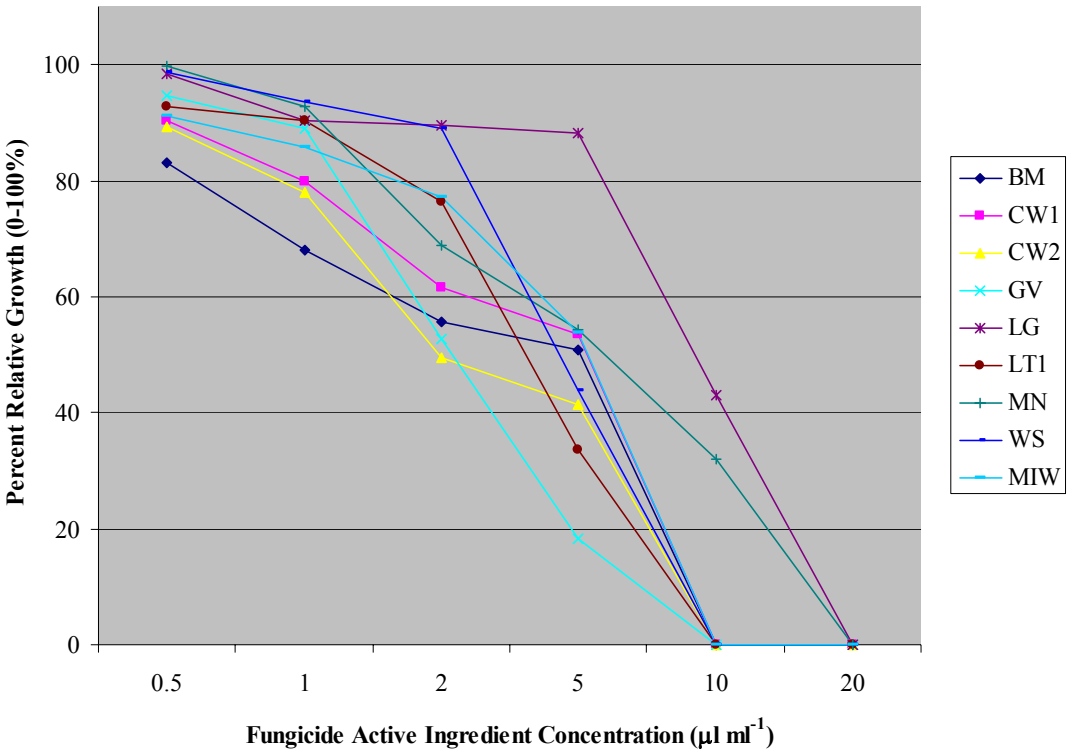

Figure 3.4. Visual comparison of relative growth of all isolates at discrete concentrations of chlorothalonil in potato dextrose broth.

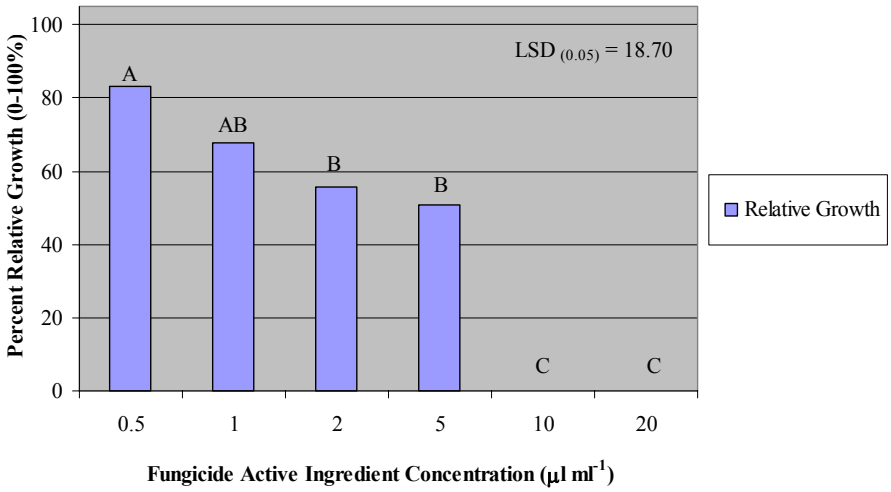

Figure 3.5. Relative growth interaction for chlorothalonil concentration by *S. homeocarpa* isolate BM.

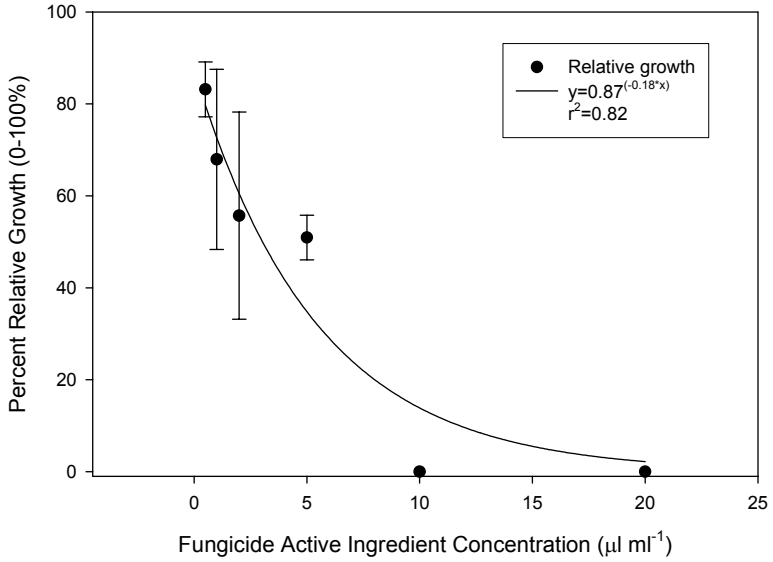

Figure 3.6. Dosage-response curve for *S. homeocarpa* isolate BM to chlorothalonil concentration.

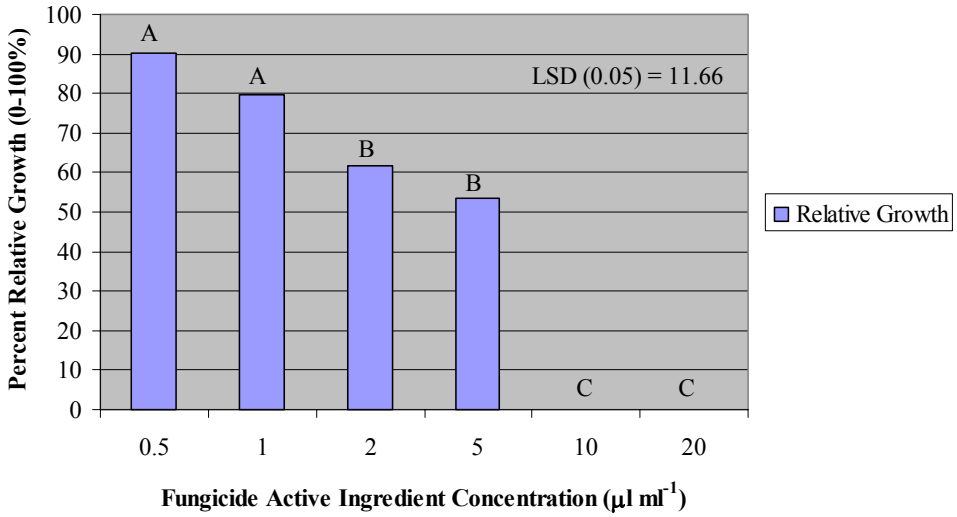

Figure 3.7. Relative growth interaction for chlorothalonil concentration by *S. homeocarpa* isolate CW1.

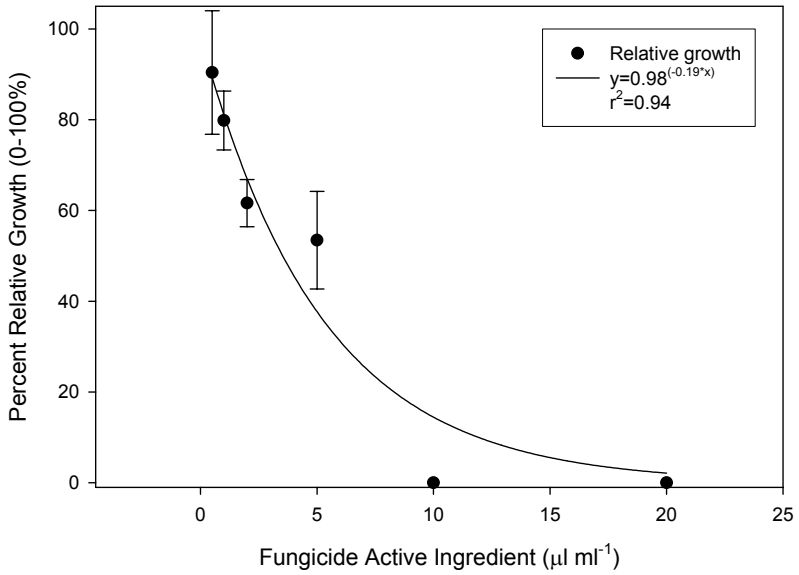

Figure 3.8. Dosage-response curve for *S. homeocarpa* isolate CW1 to chlorothalonil concentration.

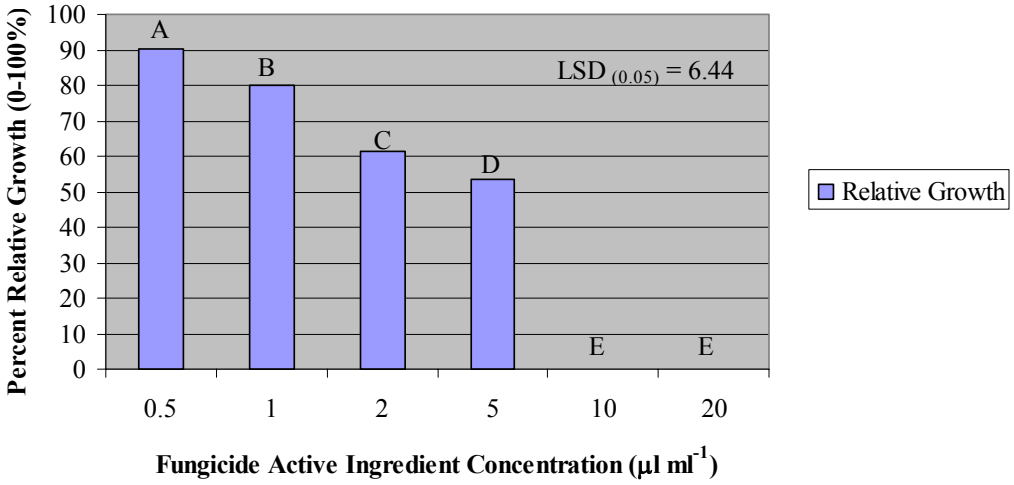

Figure 3.9. Relative growth interaction for chlorothalonil concentration by *S. homeocarpa* isolate CW2.

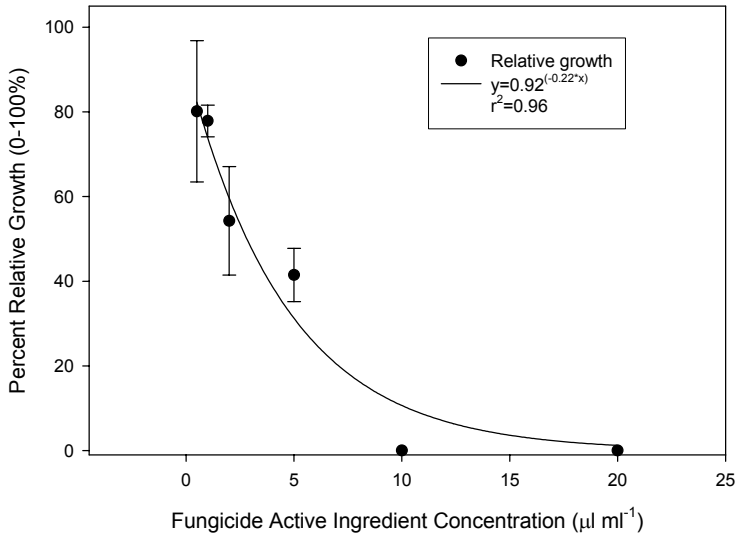

Figure 3.10. Dosage-response curve for *S. homeocarpa* isolate CW2 to chlorothalonil concentration.

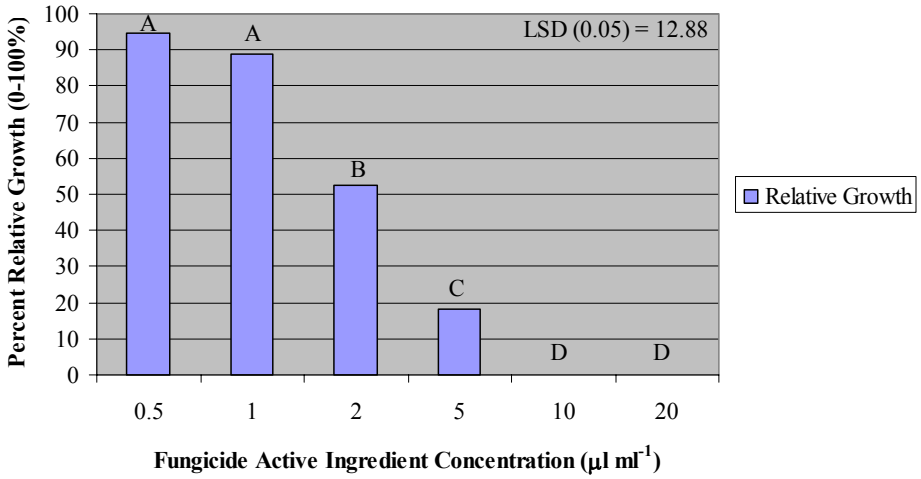

Figure 3.11. Relative growth interaction for chlorothalonil concentration by *S. homeocarpa* isolate GV.

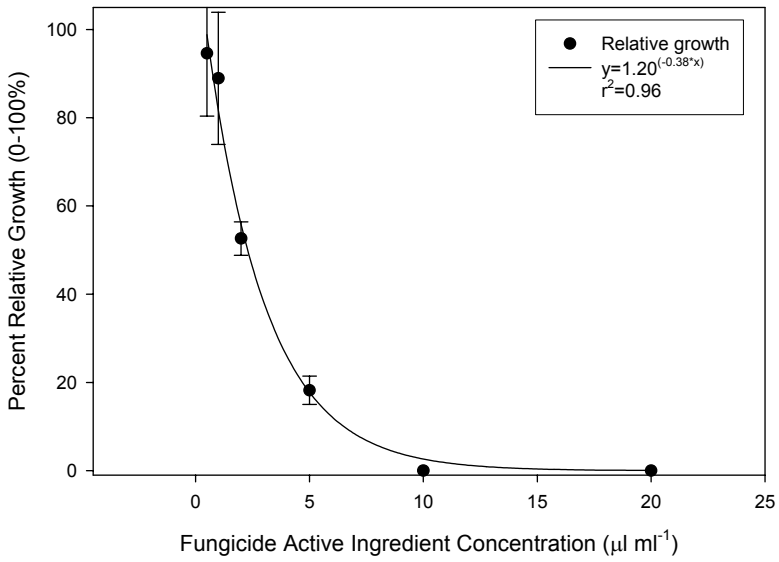

Figure 3.12. Dosage-response curve for *S. homeocarpa* isolate GV to chlorothalonil concentration.

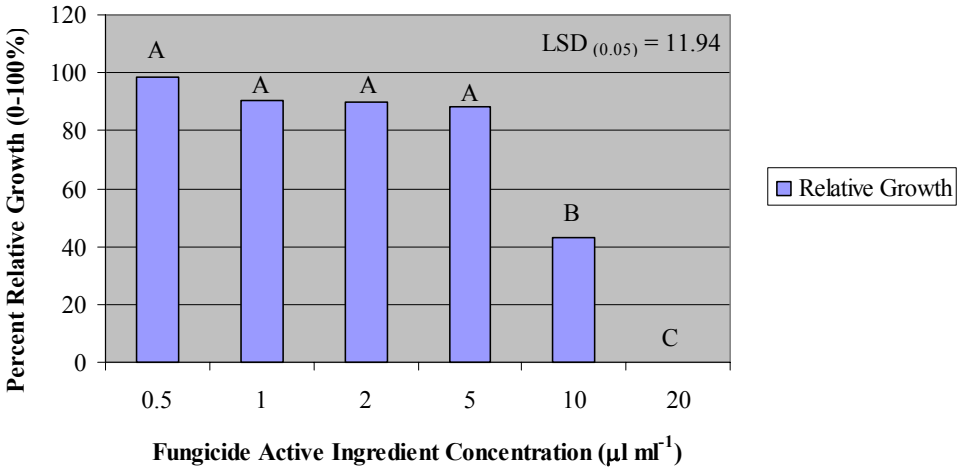

Figure 3.13. Relative growth interaction for chlorothalonil concentration by *S. homeocarpa* isolate LG.

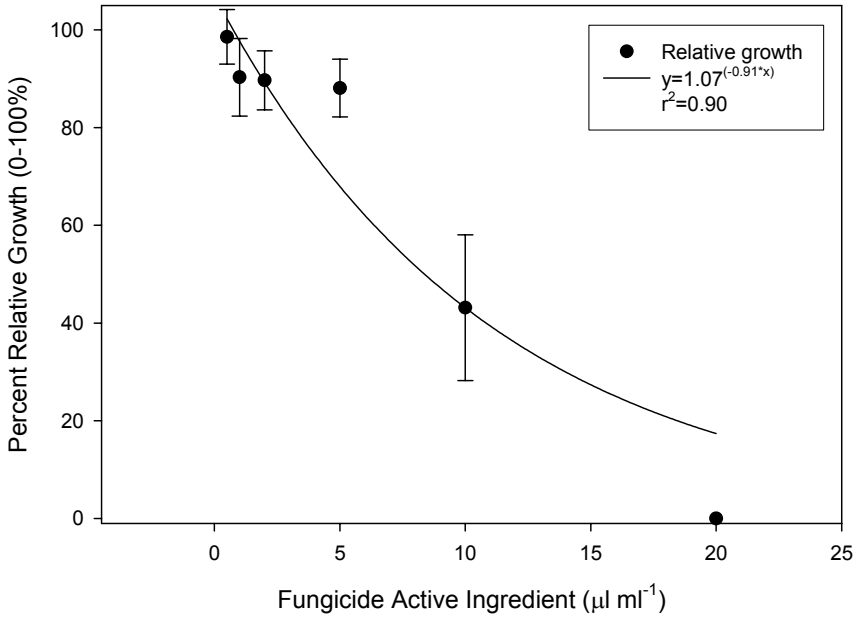

Figure 3.14. Dosage-response curve for *S. homeocarpa* isolate LG to chlorothalonil concentration

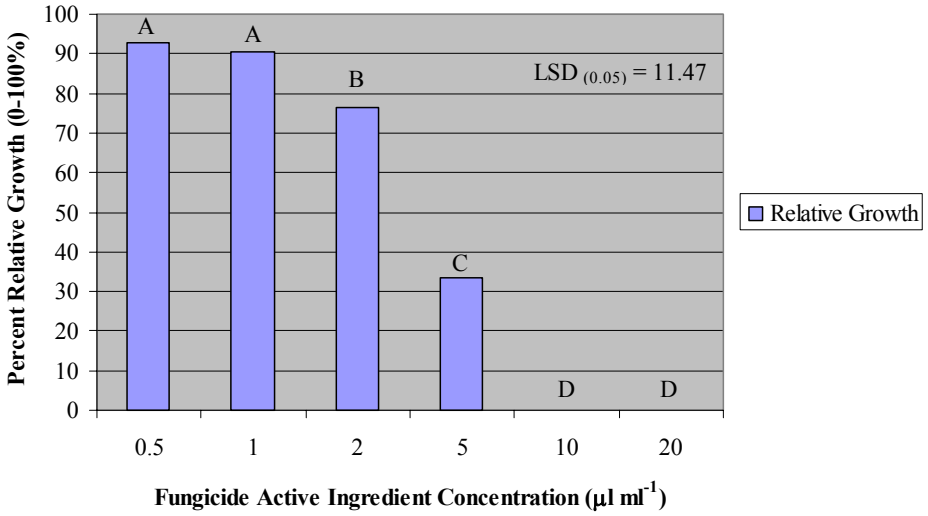

Figure 3.15. Relative growth interaction for chlorothalonil concentration by *S. homeocarpa* isolate LT1.

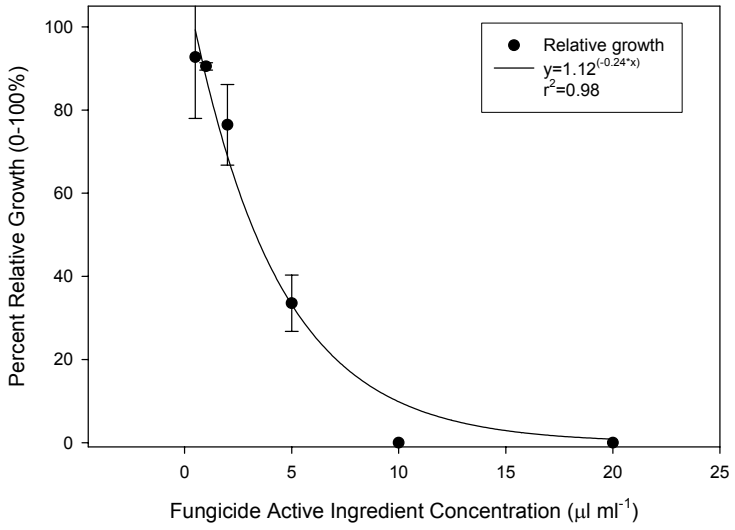

Figure 3.16. Dosage-response curve for *S. homeocarpa* isolate LT1 to chlorothalonil concentration.

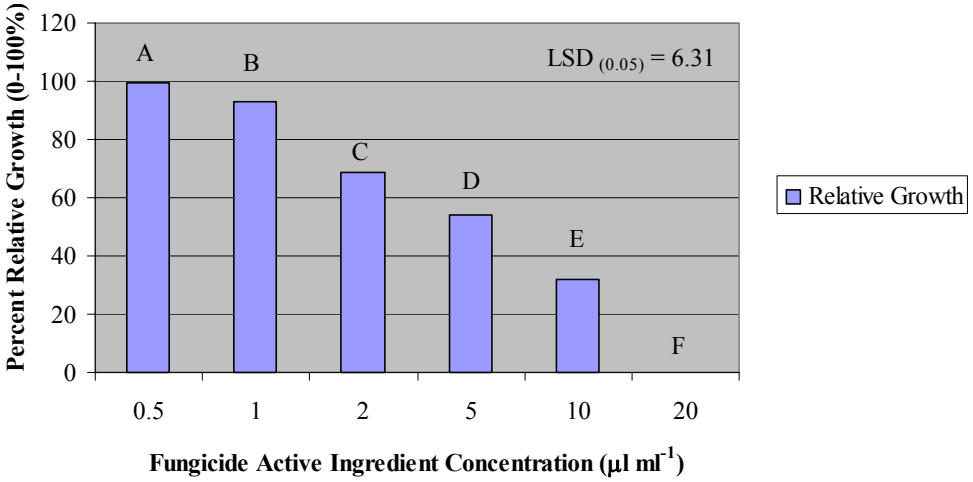

Figure 3.17. Relative growth interaction for chlorothalonil concentration by *S. homeocarpa* isolate MN.

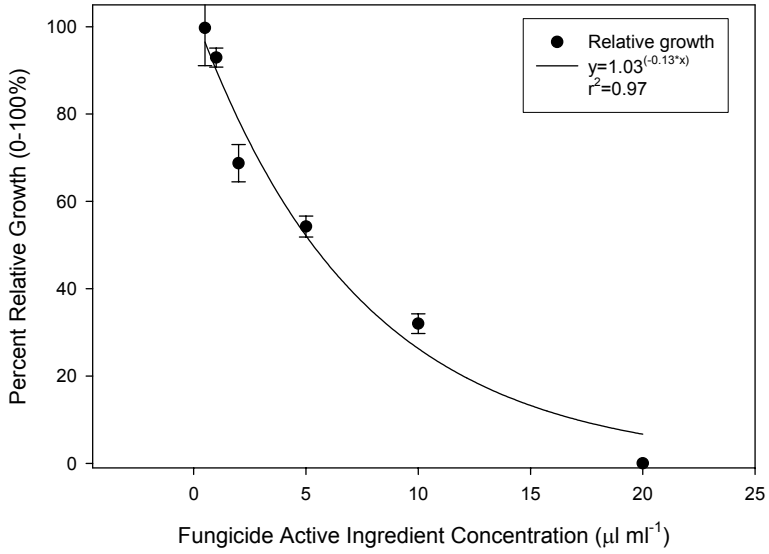

Figure 3.18. Dosage-response curve for *S. homeocarpa* isolate MN to chlorothalonil concentration.

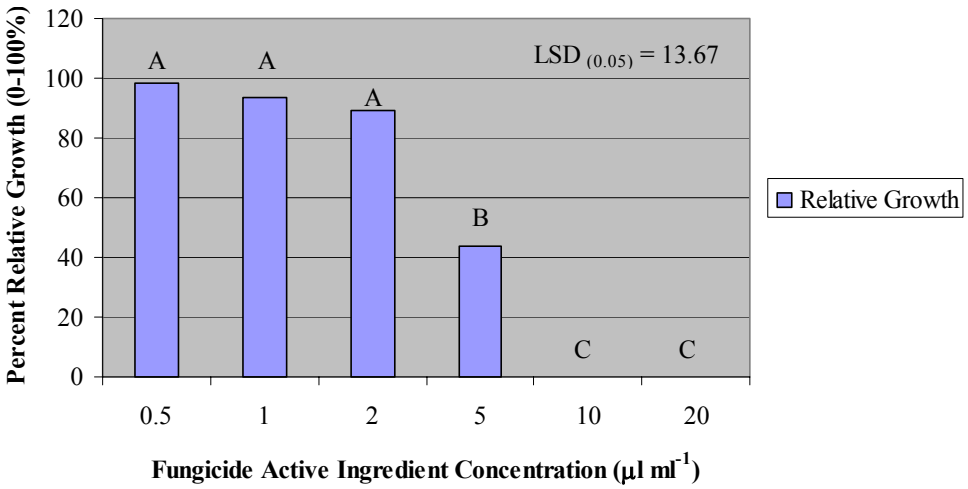

Figure 3.19. Relative growth interaction for chlorothalonil concentration by *S. homeocarpa* isolate WS.

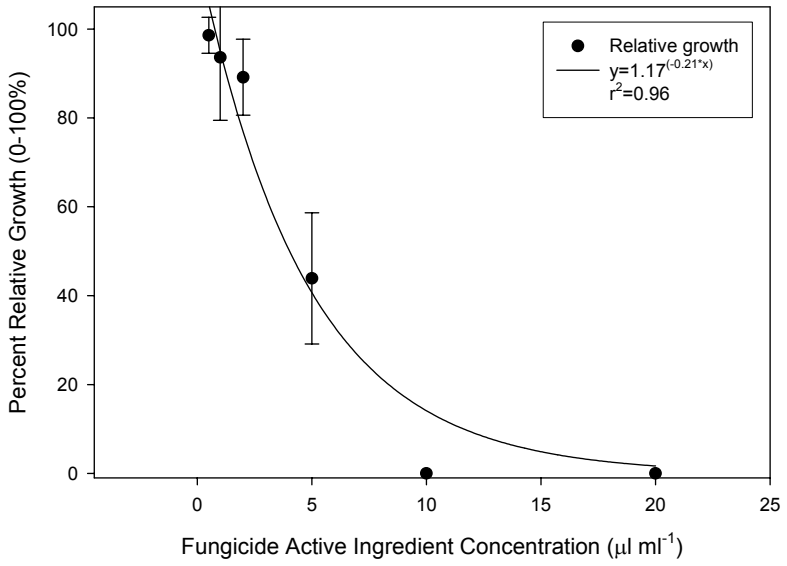

Figure 3.20. Dosage-response curve for *S. homeocarpa* isolate WS to chlorothalonil concentration.

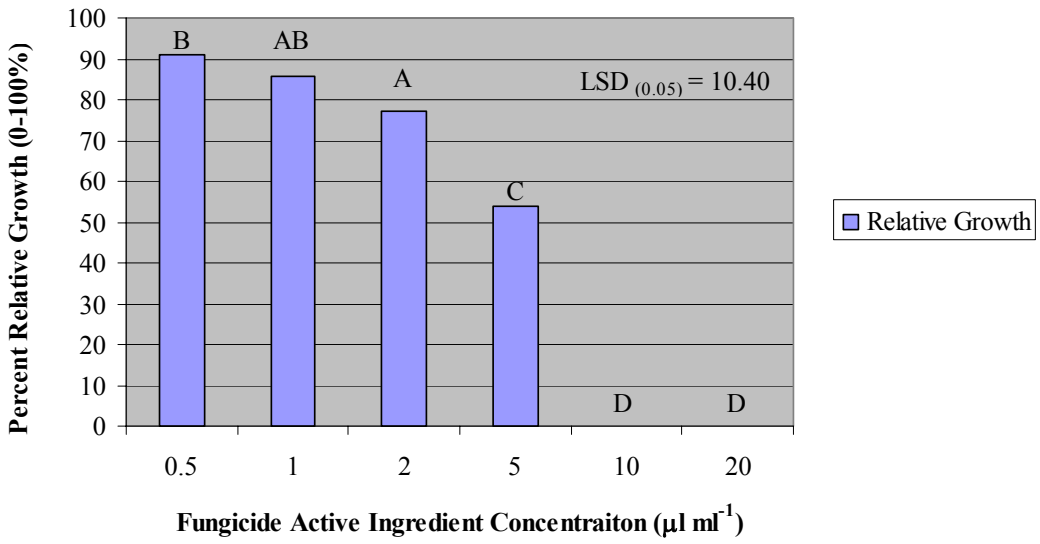

Figure 3.21. Relative growth interaction for chlorothalonil concentration by *S. homeocarpa* isolate MIW.

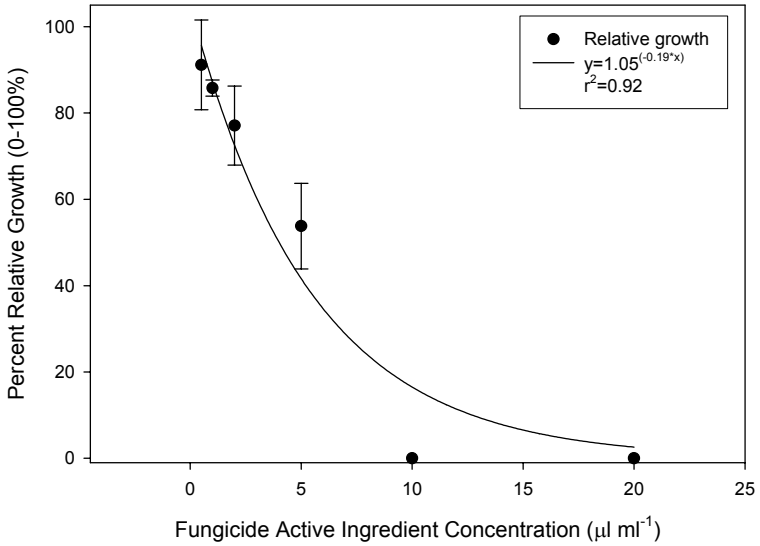

Figure 3.22. Dosage-response curve for *S. homeocarpa* isolate MIW to chlorothalonil concentration.

Table 3.3. Differences between highest and lowest relative growth values at each chlorothalonil concentration.

Active Ingredient Concentration	Highest	Lowest	Difference
0.5 μg ml^{-1}	99%	83%	17%
1 μg ml^{-1}	98%	68%	31%
2 μg ml^{-1}	90%	50%	40%
5 μg ml^{-1}	88%	18%	70%
10 μg ml^{-1}	43%	0%	43%
20 μg ml^{-1}	*	*	*

* = No growth at this concentration

Table 3.4. Chlorothalonil application rates *in situ* for location from which each isolate was collected.

Isolate	Chlorothalonil Rate[y]
BM	3.25
GV	*
WS	3.64
LG	3.64
LT1	*
CW1	3.64
CW2	3.64
MN	ND[z]
MIW	ND

* = No chlorothalonil use reported
[y] = Rates given in oz/1000 ft^2
[z] = No data available for these isolates

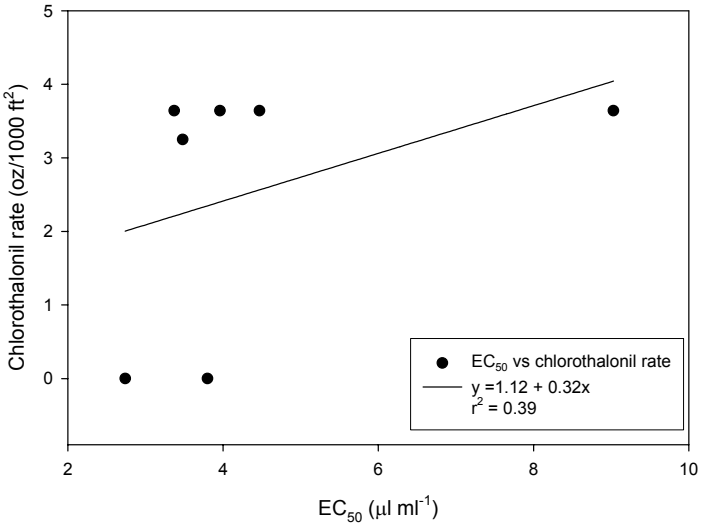

Figure 3.23. Regression correlation for EC_{50} values for *S. homeocarpa* against chlorothalonil rate *in situ*.

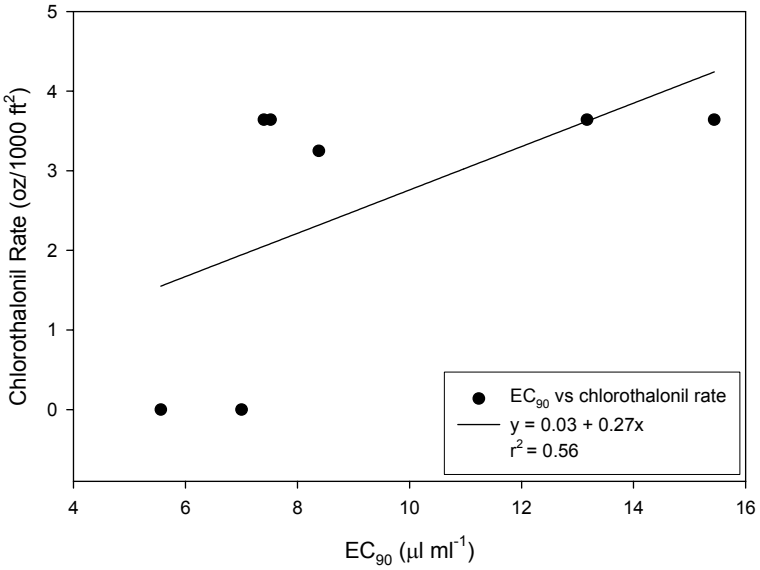

Figure 3.24. Regression correlation for EC_{90} values for *S. homeocarpa* against chlorothalonil rate *in situ*.

VITA

Pamela Baird was born in Detroit, MI. She moved to Knoxville, TN as a child and currently resides there.

She received her Bachelor of Science degree in spring of 2002 in Ornamental Horticulture and Landscape Design, and received a Master of Science degree in Entomology and Plant Pathology in May, 2005 under the joint supervision of Dr. Alan Windham and Dr. John C. Sorochan.

Baird is a member of the American Phytopathological Society, The Tennessee Turfgrass Association, and the Golf Course Superintendents Association of America.