EVALUATING THE INFLUENCE OF FUNGICIDES ON THE NEMATOPHAGOUS FUNGUS *ARTHROBOTRYS OLIGOSPORA* IN PUTTING GREEN SOILS

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ABBREVIATIONS

AG	Anastomosis Group
a.i.	active ingredient
AMU	Atomic Mass Units
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
ATP	Adenosine Tri-Phosphate
DAP	Days After Planting
d.i.	day interval
dia	diameter
DMI	Demethylation Inhibitor Fungicides
EC_{50}	Effective concentration at which spore germination is inhibited by 50%
EPA	Environmental Protection Agency
GC	Gas Chromatography
GC/MS	Gas Chromatography Mass Spectrometry
HP	Hewlett Packard
HPLC	High Pressure Liquid Chromatography
MDL	Minimum Detection Limit
MS	Mass Spectrometry
PDA	Potato Dextrose Agar
ppm	parts per million
RSD	Relative Standard Deviation
SIM	Selected Ion Monitoring

CHAPTER I

INTRODUCTION

Turfgrasses. Turfgrasses are grown on over 12 million hectares in the United States. The turfgrass industry in 1993 was valued at $45 billion, with a projected growth to $90 billion by the end of 2000 (Beard and Green, 1994). Golf courses account for approximately five percent of the total U.S. turf acreage and eighteen percent of the total annual turf industry expenditures. Oklahoma has approximately 250 golf courses throughout the state, covering 9,800 hectares. Creeping bentgrass [*Agrostis palustris* Hudson], used on golf course putting greens, is planted on nearly 182 hectares in Oklahoma. In 1996, the golfing industry contributed $219.3 million to the Oklahoma economy, making it an important component of the state's economy (Duxbury *et al.* 1998).

The United States is generally separated into four different turfgrass climatic zones; cool humid, warm humid, cool arid, and warm arid (Christians, 1998). Turfgrasses adapted to these various zones are commonly known as cool- and warm-season grasses. Cool-season (C-3) grasses are better adapted to the cooler regions of the country, while warm-season (C-4) grasses are better adapted to the warmer regions of the south. The primary physiological difference between cool- and warm-season grasses is the initial production of carbohydrates (Jones, 1985).

A turfgrass transition zone extends throughout the central part of the country and includes parts of each of the other four zones. Oklahoma is located within the turfgrass transition zone. Although the transition zone is considered to be a difficult region to

manage turfgrasses, various cool- and warm-season grasses can be successfully grown in this region. However, geographic location and turf use dictate which species are selected. For example, bermudagrass [*Cynodon dactylon* L. or *C. dactylon* x *C. transvaalensis* Burtt-Davis] is primarily used for residential and commercial lawns, golf course fairways and tees; while tall fescue [*Festuca arundinacea* Schreb.] is primarily used at locations with mature tree canopies in the central and northern parts of the transition zone. In contrast, creeping bentgrass is used exclusively on golf course putting greens throughout the zone.

Creeping bentgrass is grown on putting greens in temperate regions of the United States and can be manipulated to provide a high-quality putting surface. It has a stoloniferous growth habit, rapid injury recovery, and excellent cold temperature tolerance. Bentgrass also maintains a dense canopy at various mowing heights and can tolerate heights as low as 3.12 mm. To minimize wear and other stresses, creeping bentgrass is irrigated daily during warm periods, fertilized frequently, and grown on a well-drained, sand-based root zone. Due to it's characteristics, an increase in use on putting greens in the warmer climates of the United States has occurred over the last 30 years.

Bentgrass tolerance to warm temperatures, shade, and wear is moderate for a cool-season grass. Thin turf areas may occur in the presence of adverse environmental conditions. High temperatures and rapid air movement across the canopy can dehydrate leaf tissue and can lead to plant death. Disease severity is often greater for creeping bentgrass located in partial to fully shaded areas (Koh *et al.* 2002). This is attributed to the persistence of moisture on leaf surfaces as a result of decreased solar radiation and air

movement within the canopy. In response, breeding programs are currently attempting to improve the color, texture, playing surface, and heat tolerance of creeping bentgrass.

Currently the most widely used cultivars of creeping bentgrass in Oklahoma are SR1020 (Seed Research of Oregon Inc.; Corvallis, OR) and Penncross (Tee-2-Green Corp.; Canby, OR) (Walker *et al.* 2002). SR1020 has a more upright growing habit and a more desirable playing surface, is resistant to *Pythium* spp., and is moderately susceptible to dollar spot. In contrast, Penncross, one of the first bentgrass cultivars developed for use in warmer climates, is more resistant to common turfgrass diseases, such as dollar spot, and has a less dense, more vertical growth habit (Christians, 1998).

Bentgrass Diseases Caused by Fungi. Many pathogens cause diseases of turfgrass, including the fungi, plant-parasitic nematodes, bacteria, and viruses. Bacteria and viruses cause few economically important diseases of turfgrass. In southern latitudes of the United States, nematodes are often responsible for significant turfgrass management problems and turf decline (DiEdwards, 1963; Dunn and Noling, 1997; Lucas *et al.* 1974; Smiley *et al.* 1992; and Todd and Tisserat, 1990). However, fungi cause the most numerous, devastating, and economically important turfgrass diseases.

Fungi are eukaryotic, heterotrophic organisms that obtain nutrition through absorption, and have a cell wall composed of chitin. Fungi can engage in beneficial as well as harmful associations with plants. Beneficial fungi are often involved in intimate symbiotic relationships with plant roots and assist plants in moisture and nutrient acquisition. Other fungi cause plant disease by colonizing plant tissues and utilizing the host as a nutrient source. Three important fungal diseases of creeping bentgrass in the southern United States are dollar spot, *Pythium* blight, and brown patch.

Sclerotinia homoeocarpa, the causal agent of dollar spot, is a common disease of creeping bentgrass putting greens. More resources are devoted to the management of dollar spot than on any other disease of bentgrass turf (Vargas, 1981). The fungus over-winters as mycelia within infected plant tissue or as stroma on tissue surfaces. Environmental conditions conducive for disease are warm (15-30 °C), humid days with cool nights, which result in heavy dew formation on greens. The affected turf has small, circular, sunken, straw colored areas that may coalesce into large, irregularly shaped patches. More severe symptoms are associated with low nitrogen fertility and dry soils (Smiley *et al.* 1992).

Diseases caused by *Pythium* species are some of the most devastating to affect turfgrasses. Symptoms on bentgrass are most severe during periods (>14 hours) when temperatures are 30-35 °C and relative humidity is greater than ninety percent. On putting greens, the affected areas may appear as circular, water soaked patches that spread rapidly and are often characterized as having a bronze to orange color (Smiley *et al.* 1992). Turf fertilized at high nitrogen rates is especially susceptible to *Pythium* blight, and the disease may become more severe when soils are low in pH (Couch, 1991). Root and crown rots of bentgrass are also caused by *Pythium* species; however, much less is understood about these diseases.

Brown patch [*Rhizoctonia solani* Kühn] is a common disease of most turfgrasses. Fungi from two anastamosis groups (AG) of *R. solani* cause diseases of turfgrasses. *Rhizoctonia solani* AG 1-1 causes disease of cool-season grasses, whereas of *R. solani* AG 2-2 causes disease of warm-season grasses (Shen *et al.* 1991). *Rhizoctonia* spp. are facultative saprophytes and can survive in the soil as sclerotia or on dead tissue (Smiley *et*

al. 1992). When conditions are conducive for disease, the fungus grows rapidly in thatch and canopy layers where hyphae infect leaves and sheaths. Crowns and leaf sheaths of infected plants may appear water-soaked; while brown leaf lesions with a red margin may be observed on infected leaves. Plants often die from brown patch when exposed to high temperatures or drying winds. Balanced inputs of fertilizer and removal of thatch can reduce disease severity (Smiley *et al.* 1992).

Fungicides Used for the Control of Bentgrass Diseases. More fungicides are applied to bentgrass based on surface area than to any other agronomic commodity (Vargas, 1981). Golf course superintendents frequently apply preventative and curative fungicides to greens to manage turfgrass diseases. A survey of Oklahoma golf course's (Duxbury *et al.* 2000) reported that approximately $865,000 was spent on fungicides in 1996. Applications generally start in mid-April and continue through summer into early fall for the management of numerous diseases and terrestrial algae. Fungicides can be grouped according to when they are applied, i.e. pre- or post-infection. Contact fungicides are applied prior to infection. Systemic fungicides are applied prior to or after infection occurs where the material is translocated throughout plant tissues.

Contact or protectant fungicides are non-systemic and are considered to be fungistatic. Some prevent hyphal growth while most prevent spore germination or fungal penetration of plant tissues. Turfgrasses grow from meristematic tissue located in the crown, and new tissue is constantly produced while frequent mowing removes older, fungicide treated tissue. Therefore, contact fungicides require repeated applications, as often as every 7-10 days, when environmental conditions are favorable for disease. Common contact fungicides used for management of bentgrass diseases include

chlorothalonil, mancozeb, and anilazine. A recent survey of the Virginia turfgrass industry reported that 37% of all fungicide applications were chlorothalonil or mixtures of chlorothalonil and other fungicides (Anonymous, 2000).

Systemic chemistries are fungicidal and cause the death of hyphae or spores. A common class of systemic fungicides are the sterol-inhibitors, which are translocated acropetally and can be applied directly to plants or drenched into soils with large quantities of water. Sterol inhibitors act on fungi by inhibiting C_{14}-demethylase in the sterol synthesis pathway resulting in cell death. Fungicides with this mode of action are called demethylation inhibiting (DMI) fungicides. Common sterol-inhibiting fungicides include propoconizole, fenarimol, and myclobutanil. Demethylation inhibiting fungicides will adequately suppress disease when applied on 14 to 28 day intervals.

Strobulurins are systemic fungicides that are derived from various species of wood decaying fungi, including *Strobilurus tenacellus* and *Oudemansiella mucida*. Strobulurins prevent fungi from generating energy in the form of adenosine triphosphate (ATP) by blocking electron transfer within mitochondria. Strobulurins have proven to be highly effective against a wide range of turfgrass diseases and can be used preventatively or curatively. Due to the fungicidal properties of strobulurins, applications can be applied on 28-day intervals and still provide acceptable disease suppression. Current strobulurin chemistries include azoxystrobin, pyraclostrobin, and trifloxystrobin.

Fate of Fungicides Applied to Bentgrass Putting Greens. Fungicides can have a variety of different fates in the environment. When applied to established turf, leaf surfaces intercept the majority of the fungicide. Eventually, many fungicides are bound and subsequently degrade in the soil. For example, chlorothalonil has a low binding rate

to organic matter and moderate mobility in sand, which can permit movement in soil water. It is not degraded by sunlight and is moderately persistent in aerobic soils, with a half-life greater than 4 months. However, greater soil moisture and temperature can increase chlorothalonil degradation rates (Anonymous, 1987). In contrast, the potential for persistence in soil and mobility in water of some fungicides, such as myclobutanil, have raised questions about their fate in the environment (Anonymous, 1993).

Bentgrass Diseases Caused by Nematodes. One group of important turfgrass pests are the plant-parasitic nematodes. Nematodes are ubiquitous, aquatic, vermiform animals belonging to the phylum *Nematoda*. Most are soilborne, and only a small percentage of species are capable of causing plant disease. Plant-parasitic nematodes cause damage to plant roots by inserting a protractible, spear-like mouthpart, the stylet, into cells and ingesting cell contents. Affected plants tend to lack vigor, exhibit symptoms of nutrient deficiency, and are generally less dense. In addition, probing or movement of the nematode through plant tissues causes extensive damage to roots and permits the entry of facultative parasites. Nematodes are obligate pathogens and rarely cause the death of host plants. However, when populations are high, feeding can reduce plant growth and occasionally cause plant death. Nematodes feed on all turfgrasses and populations are highest in late summer to mid-autumn when soil temperatures are between 20 and 30 °C. A visible reduction in turfgrass quality may not occur until this time when populations exceed tolerance thresholds (Smiley *et al.* 1992).

The constant presence of a host, frequent irrigation, and high sand content of the plant root zone preferred for golf course putting greens can encourage the accumulation of high populations of plant-parasitic nematodes (Wick, 1997). Plant-parasitic nematodes

commonly found in Oklahoma bentgrass putting greens are ring (*Criconemella* spp.), spiral (*Helicotylenchus* spp.), stunt (*Tylenchorhynchus* spp.) and stubby-root (*Paratrichodorus* spp.) (Walker *et al.* 2002). However, the geographical location of a golf course is not a contributing factor in determining the nematode species present in a green (Safford and Riedel, 1976).

Nematode Life-Cycle. The nematode life-cycle consists of six different stages, which usually requires 3 to 4 weeks to complete (Dropkin, 1980). Female nematodes may lay eggs separately or in masses either inside plant roots or in the soil. Eggs are the primary survival stage of nematodes and can permit persistence of a population in the absence of a host or during unfavorable environmental conditions (Agrios, 1997). All nematodes have four juvenile (J_1, J_2, J_3, and J_4) stages, which are distinguished by a molt of the cuticle and an enlargement of the nematode.

Typically, the second-stage juveniles (J_2) hatch from eggs and migrate toward plant roots. Nematodes feed and progress through a series of three more molts (M_2, M_3, and M_4), until the sexually mature adults are present. Feeding typically occurs at all stages; however, the adult females are the most destructive due to the large quantities of plant nutrients required to support egg production. Sexually mature female nematodes are capable of laying up to 600 eggs depending on species (Agrios, 1997).

Chemical Management of Nematodes. Since the 1950's, nematicides have been used primarily to manage plant-parasitic nematodes (Thorne, 1961). However, most nematicides are no longer available due to the presence of these compounds in sub-surface drinking water. Currently, only a few nematicides are available for nematode management, of which only a single product, fenamiphos, is labeled for use on existing

greens. Fenamiphos is a synthetic, organophosphorus compound which is highly toxic to animals. Fenamiphos has been under scrutiny by the Environmental Protection Agency (EPA) and is scheduled to be removed from the market by 2007. With the loss of this effective nematicide, other methods for nematode management in putting greens are needed.

Biological Control of Nematodes. The biological component of a soil ecosystem is an important factor in limiting the ability of plant pathogens to cause disease. Biological antagonists of nematodes include bacteria, algae, predacious nematodes, protozoans, insects, mites, and nematophagous fungi (Striling, 1991). Nematophagous fungi occur in all classes of the fungi, except the Ascomycetes. Each has evolved different mechanisms with which to parasatize nematodes (Drechsler, 1933). Some have simple thalli, while others posses elaborate mycelial systems. In addition to the elaborate adhesive nets and constriction rings of *Arthrobotrys* spp., other sophisticated mechanisms have been documented. These include adhesive branches of *Dactylella* spp. (Drechsler, 1933), complex spores of *Haptoglossa* spp. that are capable of injecting infective particles into a passing nematode, and the zoospores of *Catenaria* spp. that are capable of following nematode exudates (Boosalis and Manakau, 1970).

Arthrobotrys oligospora. *Arthrobotrys oligospora* Fresenius was first observed growing on organic debris in 1852 (Fresenius, 1852). The fungus, a common soil inhabitant throughout the world (Dreschler, 1941), consists of hyaline hyphae which can differentiate to form long (>200 μm) conidiophores. These conidiophores give rise to hyaline conidia, which are constricted at the septum and their distal cell can be twice as large as the proximal cell (Haard, 1968). Aerial hyphae of *Arthrobotrys* spp. can fuse

with older hyphae to form a series of ring shaped structures (Woronin, 1870). These structures were observed actively trapping nematodes on agar plates (Zopf, 1888), documenting the first case of the predator/prey relationship between a fungus and an animal (Barron, 1977). The trapping structures of *A. oligospora* are comprised of an elaborate hyphal network that ramifies through the soil (Barron, 1977) and captures nematodes as they migrate through the soil environment. An increase in hyphal trap formation has been observed on low nutrient agar, suggesting that *A. oligospora* resorts to the formation of trapping nematodes in nutrient deficient environments (Dreschler, 1941).

Arthrobotrys oligospora utilizes a specific lectin within the wall of the hyphal netting to chemically bond a carbohydrate (n-acetyl-d-galactosamine) found in the nematode epidermis (Rosenzweig and Ackroyd, 1983). This chemical bond, referred to as a lectin/carbohydrate bond, is difficult for the nematode to break (Nordbring-Hertz and Mass, 1979). Once bonding takes place, the fungus penetrates the nematodes body and secretes a toxin into the nematode (Olthof and Estey, 1963). Assimilation of the nematode takes approximately twelve hours; however, the factors involved in fungal penetration and nematode digestion are poorly understood (Nordbring-Hertz and Stalhammar-Carlemalm, 1978).

The effect of fungicides on plant pathogenic fungi is well documented (Doney and Vincelli, 1999; Gleason and Newton, 1999). However, the frequent use of fungicides to manage diseases present on golf course putting greens may have adverse effects of *A. oligospora* if it were to be used to suppress plant-parasitic nematodes. The objectives of this study were to determine i.) the concentration of fungicides in a bentgrass putting green, ii.) the *in vitro* effects of fungicides on the nematophagous fungus *A. oligospora*,

and iii.) the efficacy of *A. oligospora* to suppress plant-parasitic nematodes in simulated golf course putting green soils.

LITERATURE CITED

Agrios, G.N. 1997. Plant Pathology. 4th ed., Academic Press, Inc., San Diego, California.

Anonymous. 1987. United States Environmental Protection Agency. Chlorothalonil Health Advisory. Draft Report. Office of Drinking Water, Washington D.C. pp. 6-36.

Anonymous. 1993. Agriculture Canada. Decision Document: Myclobutanil. E93-01. www.hc-sc.gc.ca/pmra-arla/english/pdf/rdd/rdd_e9301-e.pdf. 37p.

Anonymous. 2000. 1998 Virginia turfgrass pesticide use survey: A report covering golf courses and sod producers. Virginia Agricultural Statistics Service. 9 p.

Barron, G.L. 1977. The Nematode-Destroying Fungi. Canadian Biological Publication Ltd. Guelph, Ontario, Canada.

Beard, J.B. and R.L. Green. 1994. The role of turfgrasses in environmental protection and their benefits to humans. Journal Environmental Quality 23: 452-460.

Boosalis, M.G. and R. Manakau. 1970. Parasitism and predation of soil microorganisms Pages 123-125 in: Ecology of Soil-Borne Pathogens. K.F. Baker and W.C. Snyder, eds. University of California Press, Los Angeles, California.

Christians, N.E. 1998. Fundamentals of Turfgrass Management. Ann Arbor Press Inc., Chelsea, Michigan.

Couch, H.B. 1991. Diseases of Turfgrasses. Robert E. Kreiger Publishing Co., Huntington, New York.

DiEdwards, W.A. 1963. Pathogenicity and host-parasitic relationships of nematodes on turf in Florida. Florida Agricultural Experiment Station Annual Report 109. University of Florida, Gainesville, FL., U.S.A.

Doney, J. and P. Vincelli. 1999. Fugicides for control of dollar spot of creeping bentgrass. Page 382 in: Fungicide and Nematicide Tests. Vol. 54. R. Raids, ed. American Phytopathological Society Press, St. Paul, Minnesota.

Drechsler, C. 1933. Morphological diversity among fungi capturing and destroying nematodes. Jour. Wash. Acad. Sci. 23: 138-141.

Drechsler, C. 1941. Predacious fungi. Biol. Rev. Cambridge Phil. Soc. 16: 256-290.

Dropkin, V.H. 1980. Introduction to Plant Nematology. John Wiley and Sons, New York, New York.

Stirling, G.R. 1991. Biological Control of Plant Parasitic Nematodes. CAB International, Wallingford Oxen, United Kingdom.

Thorne, G. 1961. Principles of Nematology. McGraw-Hill Book Co. Inc., New York, New York.

Todd, T.C. and N.A. Tisserat. 1990. Occurrence, spatial distribution, and pathogenicity of some phytoparasitic nematodes on creeping bentgrass putting greens in Kansas. Plant Disease 74(9): 660-663.

Vargas, J.M. 1981. Management of turfgrass diseases. Burgess Publishing Co. Minneapolis, Minnesota.

Walker, N.R., C.L. Goad, H. Zhang, and D.L. Martin. 2002. Factors associated with populations of plant parasitic nematodes in bentgrass putting greens in Oklahoma. Plant Disease 86:764-768.

Wick, R.L. 1997. Cool-Season Turf: Diagnosing and Managing Nematodes. In: Grounds Maintenance. Pp 20-25. E. Liskey, ed.

Woronin, M. 1870. Sphaeria lemneae, Sordaria coprophilia, *Arthrobotrys oligospora*. Pages 325-360. Bettr. Morph. Physiol. Pize. III. Ges. 7. A. de Bary and M. Woronin, eds. Adhandl. Senckenbergische naturf.

Zopf, W. 1888. Zur Kenntnis der Infektions-Krankheiten neiderer Theire und Pflanzen. Nova Acta Lep. Carol 52: 314-376.

CHAPTER II

Quantification of fungicides from creeping bentgrass putting green soils and their *in-vitro* effects on *Arthrobotrys oligospora*

ABSTRACT

Few management options are available for the suppression of soilborne plant-parasitic nematodes in established golf course putting greens. The exploitation of nematophagous fungi to suppress nematodes may be inhibited by fungicides which are used to manage turfgrass diseases. Studies were conducted to determine fungicide concentrations from a research putting green, as well as to evaluate the *in vitro* tolerance of *A. oligospora* to each fungicide. Concentrations of chlorothalonil ranged from 0.9-19.3 ppm, and concentrations of myclobutanil ranged from 2.8-213 ppm. Hyphal growth of *A. oligospora* was limited at chlorothalonil concentrations in excess of 10 ppm, and ceased at myclobutanil concentrations in excess of 1 ppm. Conidia did not germinate in the presence of chlorothalonil concentrations in excess of 1 ppm and myclobutanil concentrations in excess of 5 ppm. These studies suggest that the continued application of chlorothalonil and myclobutanil to manage fungal pathogens of bentgrass may inhibit the ability of *A. oligospora* to survive in golf course putting greens.

INTRODUCTION

The Oklahoma golf course industry, with expenditures of more than $200 million in 1996 (Duxbury *et al.* 1998), is responsible for generating a significant portion of the state's economy. Unlike annual row crops, no yield value is associated with turfgrasses when used on golf courses; however, the quality and consistency of the turfgrass is very important. The most intensely managed part of the course are the putting greens.

Creeping bentgrass (*Agrostis palustris* Hudson) is the predominant turfgrass used on Oklahoma putting greens. To achieve a high quality putting surface, creeping bentgrass is grown on a sand or sand-sphagnum rooting media (USGA Greens Section Staff, 1993) and requires daily mowing, frequent irrigation, fertilization, and applications of various pesticides. These include pre- and post emergent herbicides to manage undesired weed species, insecticides to manage insect pests, and fungicide applications to manage numerous fungal pathogens of bentgrass. Golf course putting greens receive more fungicides, based on surface area, than any other agronomic commodity (Vargas, 1981).

In addition to diseases, high populations of plant-parasitic nematodes may be present in established putting greens. Nematodes are microscopic roundworms, in the phylum *Nematoda*. They are aquatic organisms found in nearly all terrestrial and aquatic ecosystems. Currently, there are over 100,000 species of identified nematodes. Only a small portion of nematodes have developed as plant parasites. These nematodes are capable of feeding on all plant parts; however, they are predominately soilborne and feed on plant roots. Nematodes feed by inserting a hollow, modified mouthpart, the stylet, into host cells and removing cellular contents. Feeding causes root dysfunction including

abnormal growth and reduced water and mineral acquisition from soil. Nematode feeding disrupts the protective epidermis of root cell's, releasing the cells contents into the soil environment and allowing colonization of roots by additional microbes.

Plant-parasitic nematodes are often overlooked as serious pests of bentgrass, even though they are capable of causing severe damage to putting greens in southern states (DiEdwards, 1963; Dunn and Noling, 1997; Lucas *et al.* 1974; Lucas, 1982; Murdoch *et al.* 1978; Smiley *et al.* 1992; and Todd and Tisserat, 1990). Damage is most severe when nematode populations increase as soil temperatures reach 20 °C, at which time putting green use is frequent, plants are heat stressed, and environmental conditions are conducive for disease (Smiley *et al.* 1992). A recent survey of Oklahoma golf course putting greens found that 92 % of greens contained nematodes from eight different genera. Many of those greens contained populations considered damaging to plants (Walker *et al.* 2002).

Management of plant-parasitic nematodes in established putting greens is limited to a highly toxic, organophosphorous compound, fenamiphos (Nemacur). This pesticide inhibits acetylcholinesterase (Cobertt *et al.* 1984) which causes the accumulation of acetylcholine and causes nematode paralysis (Schumacher *et al.* 1986). Due to the potential harmful effects of fenamiphos to the applicator and the environment, it is often not considered, even when nematode populations are known to be high. As chemical management of nematodes becomes more restricted, safer management approaches are desired.

Due to the intensity of greens management, a potential replacement for chemical nematicides for plant-parasitic nematodes may be biological antagonists. Biological

antagonists of nematodes include, but are not limited to, predatory organisms such as nematodes, bacteria, and fungi. Various organisms such as predatory nematodes and rotifers, injest nematodes. However, this occurs at a very low frequency and is not a feasible control option for putting greens. *Bacillus penetrans*, an actinomycete, has suppressed nematode populations in agricultural crops (Freitas *et al.* 2000). However, the host range of *B. penetrans* is very specific and may not be effective against all of the nematode species found in putting greens.

Nematophagous fungi possess non-specific trapping mechanisms that may be effective in suppressing many different species of nematodes found in golf course putting greens. *Arthrobotrys* spp. have been used effectively to suppress plant and animal parasitic nematodes in other agricultural systems (Stirling *et al.* 1998a; Stirling *et al.* 1998b; Stirling and Smith, 1998; and Gomes *et al.* 2000). Due to the global distribution and ability of *Arthrobotrys* spp. to colonize soil these organisms could potentially be used to manage nematodes in putting greens. However, little is known of the effects of commercial turfgrass fungicides on *Arthrobotrys* spp. The objectives of this study were to determine i.) the concentrations of the fungicides chlorothalonil and myclobutanil in putting green soil, and ii.) the *in vitro* tolerance of *A. oligospora* to each of these fungicides.

METHODS AND MATERIALS

Fungicide application and soil sampling. Field plots were established in 2001 and 2002 at the Oklahoma State University, Department of Entomology and Plant Pathology Research Farm in Stillwater, Oklahoma. Plots (0.9 m x 3.0 m) were

established on a Norge loam-sand soil mix research putting green, containing *Agrostis palustris* Hudson cv. Penncross (Tee-2-Green Corporation, Canby, OR) on 6 Apr 2001 and 25 Mar 2002. The green had a bulk density of 1.55 g cm^{-3}, a pH of 7.1, and organic mater content of 1.3%. Granular fertilizer was broadcasted at a rate of 504 g N/100 m^2 $month^{-1}$. Bentgrass was clipped to a height of <3 mm five times a week.

Fungicide treatments consisted of chlorothalonil (ISK Biosciences, Meantor, OH) and myclobutanil (Dow Agrosciences, Houston, TX) applied at 93.15 g active ingredient (a.i.) per 100 m^2 and 7.32 g a.i. per 100 m^2, respectively. Fungicides were applied using a CO_2 pressurized wheelbarrow plot sprayer calibrated to deliver 887 L ha^{-2}. Applications were conducted on a 14-day interval (d.i.) starting 16 May 2001 and 19 Apr 2002 with the third application in 2002 conducted on a 15 d.i. due to weather conditions. A randomized complete block design with four replications was used for both years. Non-treated plots served as a control. Seven (2.54 cm dia x 7.62 cm deep) soil cores were obtained from each plot 20 days prior to, 10 days after, and 40 days after the first fungicide application. Soil samples were stored at 4 °C prior to analysis.

Reagents. High pressure liquid chromatography (HPLC) grade acetone, methylene chloride, hexanes, methanol, toluene, and sodium chloride were purchased from Fisher Scientific (Springfield, NJ). Anhydrous sodium sulfate and reagent grade acetonitrile were purchased from Spectrum Chemical Manufacturer (New Brunswick, NJ). HPLC grade petroleum ether (Malinkcroft Chemical Mfg., Berlin Germany) and reagent grade sulfuric acid (EM Sciences, Gibbstown, CT) were also used.

Extraction and purification of chlorothalonil from soil. A ten-gram composite soil sample was placed in 50 ml of extraction solvent (10N H_2SO_4:acetone, 5:95 , v/v)

and sealed. The solution was shaken on a wrist action shaker at three hours. To remove solids, the solution was filtered through 0.2 μm Whatman filter paper (Whatman International Ltd., Maidstone, England) and the solids rinsed with 5 ml of extraction solvent.

The extract was combined with 50 ml petroleum ether, 40 ml of extraction solvent, and 20 ml deionized water in a 500 ml bottle, and shaken vigorously by hand for two minutes. Solutions were transferred to a seperatory funnel and the phases allowed to separate. The lower aqueous phase was drained and this process was repeated twice. The ether phase was collected and evaporated to dryness by allowing it to stand in an operating fumehood. Glass columns (9 mm i.d. x 200 mm h) were packed with glass wool (0.5 cm), 2.0 g Florisil (US Silica, Berkeley Springs, WV) and 2.0 g anhydrous sodium sulfate. Florisil was activated at 121 ± 2 °C, for 12 hours in an isothermic oven (Fisher Scientific, Springfield, NJ). Columns were washed with 10 ml hexane which was discarded. Dried samples were dissolved in 10 ml of methylene chloride:hexane, 20:80, v/v (Eluant 1). After the sample migrated through the sodium sulfate layer an additional 15 ml of Eluant 1 was added to each column. A total of 25 ml of Eluant 1 was collected from each column and discarded. Chlorothalonil was eluted from each column using 35 ml of methylene chloride:hexane:acetonitrile, 50:48.5:1.5, v/v/v (Eluant 2) (Anonymous, 1988). The sample was collected in a 50 ml conical pyex tube (Fisher Scientific, Springfield NJ). The conical pyrex tube was placed in a heated ultrasonic bath (Fisher Scientific, Springfield, NJ) and evaporated to dryness in a fumehood. Sample residue within the conical tubes was dissolved in 100 μl acetone and vortexed for 10 seconds (Anonymous, 1988).

Extraction and purification of myclobutanil from soil. A ten-gram composite soil sample was placed in a flask containing 50 ml of methanol and sealed. The sample was shaken for 4 hours on a bench-top rotary shaker. To remove solids, the solution was filtered using 0.2 μm Whatman filter paper (Whatman International Ltd., Maidstone, England) and rinsed with 5 ml of methanol.

The extract was added to 100 ml hexane and 30 ml 2% aqueous sodium chloride (2:98, NaCl:water, wt/wt) in a 500 ml bottle, and shaken vigorously by hand for three minutes. The solution was transferred to a seperatory funnel and the phases were allowed to separate. The lower aqueous phase was collected and transferred to a bottle containing 100 ml 2% aq NaCl and 100 ml methylene chloride. Solutions were shaken vigorously by hand for three minutes and transferred to a seperatory funnel and the phases allowed to seperate. The lower methylene chloride phase was collected in a 250 ml beaker, and the process was repeated twice with 50 and 25 ml methylene chloride, respectfully for the remaining aqueous phase (J. Roth, personal communication).

To remove excess water, samples were passed through a 65 mm dia polystyrene funnel filled with 20 g of anhydrous sodium sulfate. The methylene chloride containing the sample was filtered through the sodium sulfate and evaporated to dryness. Residue was dissolved in 15 ml toluene and added to a column as previously described. Myclobutanil was eluted by adding 20 ml 1:99, methanol:toluene v/v (Eluant A) to the column. Fifteen ml toluene and 20 ml Eluant A were discarded and a 50 ml conical pyrex tube was placed under the column. Myclobutanil was eluted from the column using 40 ml 2:98, methanol:toluene, v/v (Eluant B). Samples were placed in a double boiler and evaporated to dryness by allowing the boilers to operate in an operating fumehood. The

residue within the conical tube was then dissolved in 100 µl acetone and agitated using a mini Vortex shaker for 10 seconds (J. Ross, personal communication).

Soil extraction efficiency. Studies were performed in triplicate to determine the recovery of fungicides from simulated putting greens soils. Sand and sand containing 2% peat moss by weight were amended to provide final concentrations of 0.5 and 5.0 ppm chlorothalonil and 5.0 and 50 ppm myclobutanil. Soils were mixed thoroughly by hand and allowed to stand 2 hours prior to extraction.

Extraction and purification of chlorothalonil from nematodes. One-hundred pant-parasitic nematodes were obtained from both fungicide treated and non-treated plots and placed in separate 20 ml glass vials. Five ml extraction solvent (5:95, 10N H_2SO_4:acetone, v/v) and 0.01 g acid washed glass beads (Sigma Chemical, St. Louis, MO) were added to the sample and the vial was placed in an ultrasonic bath for 2 hours. The sample was added to 10 ml petroleum ether, 2 ml of extraction solvent, 2 ml deionized water, and shaken vigorously by hand for two minutes. The solution was transferred to a seperatory funnel and the phases allowed to separate. The lower aqueous phase was drained and the process was repeated twice. The ether phase was collected and evaporated to dryness by allowing it to stand in an operating fumehood. Glass columns, (5.0 mm i.d. x 10 mm h), were packed with a bed of glass wool, 0.2 g Florisil and 0.1 g anhydrous sodium sulfate. Columns containing activated Florisil (121 ± 2 °C, for 12 hours) were washed with 3 ml hexane that was discarded.

The sample extract was dissolved in 3 ml of 20:80, methylene chloride:hexane, v/v (Eluant 1) and transferred to a prepared column. After the sample extracts migrated through the sodium sulfate layer an additional 3 ml of Eluant 1 was added to the column.

A total of 5 ml Eluant 1 was collected from each column and discarded. A 15 ml glass vial was placed under the column and chlorothalonil was eluted from the column using 10 ml of 50:48.5:1.5 methylene chloride:hexane:acetonitrile, v/v/v (Eluant 2) (Anonymous, 1988). The vial containing column extract was placed in a heated ultrasonic bath (Fisher Scientific, Springfield, NJ) and evaporated to dryness. The residue within the vial was dissolved in 100 µl acetone for analysis.

Extraction and purification of myclobutanil obtained from nematodes.

One-hundred plant-parasitic nematodes were obtained from both treated and non-treated plots and placed in separate 20 ml glass vials. Five ml of methanol and 0.01 g acid washed glass beads were added to the solution and the sample was placed in an ultrasonic bath for 2 hours. The extract was transferred to a 25 ml bottle containing 10 ml hexane and 3 ml 2% aqueous sodium chloride (2:98, NaCl:water, wt/wt). The bottle was shaken vigorously by hand for three minutes, transferred to a 250 ml seperatory funnel, and the phases were allowed to separate. The lower aqueous phase was collected in another 25 ml bottle and an additional 10 ml 2% aq NaCl and 10 ml methylene chloride were added. The bottle was shaken vigorously by hand for three minutes and the solution was transferred to a 250 ml seperatory funnel and the phases allowed to separate. The lower methylene chloride phase was collected in a 50 ml beaker and the aqueous extraction phase was repeated twice with 5 and 3 ml methylene chloride, respectively.

To remove excess water, samples were passed through a 65 mm dia polystyrene funnel filled with 5 grams of anhydrous sodium sulfate. The column extract in methylene chloride was filtered through the funnel and rinsed with an additional 5 ml methylene chloride. The extract was allowed to evaporate to dryness in an operating fumehood.

Glass columns (5.0 mm i.d. x 10 mm h) were packed with a bed of glass wool, 0.2 g Florisil and 0.1 g anhydrous sodium sulfate. Florisil was activated and deactivated as described previously.

The sample extract was dissolved in 2 ml toluene and transferred to prepared columns. After the sample extract migrated through the sodium sulfate layer of the column, an additional 1 ml of toluene was added to the column. The sample was eluted by adding 5 ml 1:99, methanol:toluene, v/v (Eluant A) to the column. A 15 ml glass vial was placed under the column and myclobutanil was eluted using 10 ml 2:98, methanol:toluene, v/v (Eluant B). Samples were placed in a double boiler and evaporated to dryness in an operating fumehood. The residue within the conical tubes was then dissolved in 100 µl acetone. The vials were vortexed for 10 seconds.

Fungicide Quantification. A Hewlett Packard (HP) Model 6890 gas chromatograph (GC) equipped with an HP Model 5073 Mass Spectrometer (MS) detector (Hewlett Packard, Los Angeles, CA) was used for fungicide detection and quantification. One microliter of each sample was manually injected into a splitless injector maintained at 260 °C. The column type was a J&W DB-5 (Folsom, CA) fused silica capillary column (30 m x 0.25 mm i.d.; 0.25 µm film thickness) the operating temperature was 60 to 260 °C increasing 6 °C min^{-1}. Helium was used as a carrier gas at a velocity of 1 ml min^{-1}. The mass spectrometer was operated in selected ion monitoring (SIM) mode. Filament emission current was 12 uA and electron multiplier voltage was 1650 V. Multiplier gain was 105. Scan range was from 87 to 450 amu at 1 s/scan. Transfer line and manifold temperatures were maintained at 260 °C and 220 °C, respectfully (Mogadati *et al.* 1999).

Fungicide analysis. Analytical standards of 98.5% pure 2,4,5,6-tetrachloro-1,3-benzenedicarbonitrile (chlorothalonil) and 98.5% pure RS-2-(4-Chlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)hexanenitrile (myclobutanil), were purchased from Crescent Chemical Company (Islandia, NY). Stock solutions were prepared in 100 ml of acetone. Chlorothalonil concentrations of 0.05, 0.1, 0.5, 1.0 and 5.0 ppm, and myclobutanil concentrations of 5.0, 25, 50, 100, and 200 ppm were analyzed in triplicate by injecting 1 µl of the standard into a Hewlett Packard (HP) gas chromatograph equipped with a mass spectrometer detector (GC/MS). Concentrations were determined using selected ion monitoring (SIM) and averaging chromatograms of each standard.

***Arthrobotrys oligospora* tolerance to fungicides (hyphal growth).** To determine the effect of chlorothalonil and myclobutanil on *Arthrobotrys oligospora* (American Type Culture Collection 16234) (ATCC, Manassas, VA) an *in vitro* petri plate assay method was used. Fresh hyphal plugs (10 mm dia) obtained from the leading edge of ten-day-old *A. oligospora* cultures maintained on water agar (Fisher Scientific, Springfield, NJ) were placed at the centers of plates containing ¼ strength potato dextrose agar (9.25 g L^{-1} water) (Sigma Chemical, St. Louis, MO). Agar was amended to provide a final concentration of 1, 10, or 20 ppm of either chlorothalonil or myclobutanil. Non-amended agar was used as a control. Plates were incubated at 17 ± 2 °C and hyphal growth was measured daily in four directions from the edge of each plug. Plates were arranged in a randomized complete block design with ten replications. This experiment was terminated after ten days and was repeated once.

***Arthrobotrys oligospora* tolerance to fungicides (conidia germination).** To determine the effects of the fungicides on *A. oligospora* conidia a similar assay method

was employed. *A oligospora* conidia were obtained from ten-day-old cultures maintained on potato dextrose agar (PDA) (Sigma Chemical, St. Louis, MO) and quantified using a hemacytometer. A 100 µl suspension containing 100 *A. oligospora* conidia was transferred in sterile water onto plates containing 0.001, 0.01, 0.1, 1.0 or 10 ppm of chlorothalonil or 0.1, 1.0, 3.0, 5.0, 7.0, or 10 ppm myclobutanil. Non-amended agar was used as a control. Plates were incubated at 17±2 °C and fungal colonies were counted after three days. Plates were arranged in a randomized complete block design with ten replications and this experiment was repeated once. Effective concentrations (EC_{50}) were determined by graphing concentration versus percent mortality expressed as a Probit value (Finney, 1975). Linear regression was used to determine the EC_{50} of each fungicides. Data were analyzed using analysis of variance (ANOVA). All calculations were conducted with the statistical analysis system SAS (version 8.0, SAS Institute, Cary, NC).

RESULTS

Percent recovery. Extraction efficiency for high and low concentrations of chlorothalonil from sand and sand amended with 2% organic matter ranged from 57.8 to 77.8%. Percent recovery of myclobutanil ranged from 81.3 to 88.6 % depending on the extraction matrix. Relative standard deviations were below 25 % (Table 2.1).

Concentrations of chlorothalonil in soil. Chlorothalonil concentrations in soil in 2001 varied among sampling dates. Soil chlorothalonil concentrations from non-treated plots for sampling dates 1, 2, and 3 were 0.9, 1.5, and 2.2 ppm, respectively (Figure 2.1). Soil concentrations from treated plots were 1.7, 1.1, and 2.1 ppm for the

Added ppm (soil)		Recovery (%) (mean ± SD, n=3)	R.S.D.*(%)
Sand			
Chlorothalonil	0.5	77.8 ± 2.4	18.0
	5.0	61.6 ± 1.8	20.1
Myclobutanil	5.0	88.6 ± 3.2	11.4
	50	86.3 ± 3.1	14.8
Sand + 2% OM			
Chlorothalonil	0.5	66.7 ± 4.1	13.1
	5.0	57.8 ± 4.3	19.0
Myclobutanil	5.0	86.7 ± 5.5	21.5
	50	81.3 ± 5.6	23.6

*R.S.D. Relative Standard Deviation

Table 2.1 Percent recovery of chlorothalonil and myclobutanil at varying concentrations and extraction matrices.

same sampling dates, respectively (Figure 2.1). Chlorothalonil concentrations in soil from 2002 also varied among sampling dates. Chlorothalonil present in non-treated plots for sampling dates 1, 2, and 3 were 9.5, 8.3, and 14.8 ppm, respectively (Figure 2.2). Average soil concentrations from treated plots for the same sampling dates were 14.2, 11.4, and 19.3 ppm, respectively (Figure 2.2).

Concentrations of myclobutanil in soil. Soil concentrations of myclobutanil in 2001 from non-treated plots for sampling dates 1, 2, and 3 were 12.4, 109.0, and 16.9 ppm, respectively (Figure 2.3). Soil concentrations of myclobutanil from treated plots were 62, 8.6, and 28.2 ppm, respectively (Figure 2.3). Myclobutanil concentrations present in non-treated plots in 2002 for sampling dates 1, 2, and 3 were 6.6, 27.0, and 213.0 ppm, respectively (Figure 2.4). Soil concentrations in treated plots for the same sampling dates were 2.8, 122.0, and 149.0 ppm, respectively (Figure 2.4).

Concentrations of fungicides obtained from nematodes. Both chlorothalonil and myclobutanil concentrations obtained from nematodes were below the minimum detection limit for both years. Concentrations were not statistically different (P=0.05) in nematodes obtained from non-treated control plots versus nematodes obtained from plots receiving fungicide treatments.

***In vitro* tolerance (Chlorothalonil).** Hyphal growth of *A. oligospora* was substantially reduced at concentrations above 10 ppm (Figure 2.5). *Arthrobotrys oligospora* conidia in the presence of concentrations exceeding 1 ppm did not germinate (Figure 2.6). The EC_{50} of *A. oligospora* spores to chlorothalonil was 0.2 ppm.

***In vitro* tolerance (Myclobutanil).** Hyphal growth of *A. oligospora* was reduced (P=0.05) at concentrations above 10 ppm (Figure 2.7). *Arthrobotrys oligospora* conidia

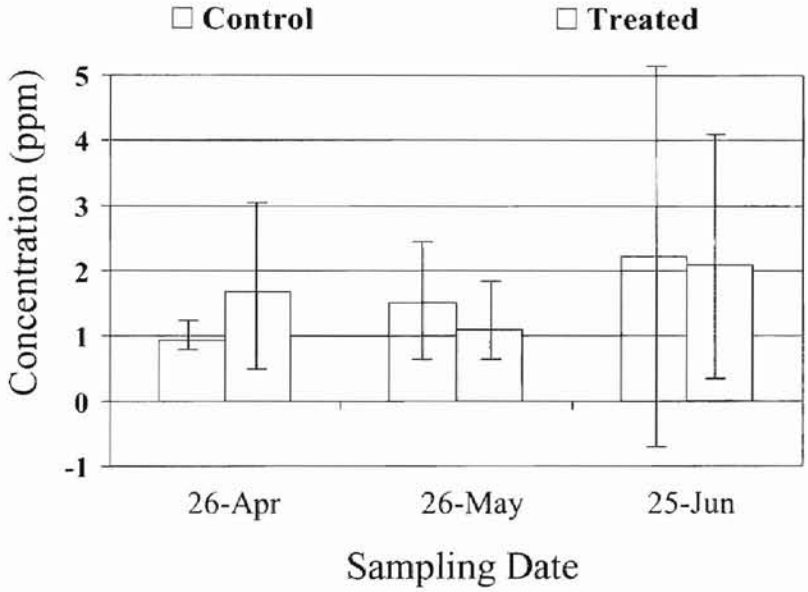

Figure 2.1 Concentrations of chlorothalonil in putting green soil in 2001. Three fungicide applications were conducted on a 14 day interval with the first application applied on 16-May. Data are the means of four replications per sampling date.

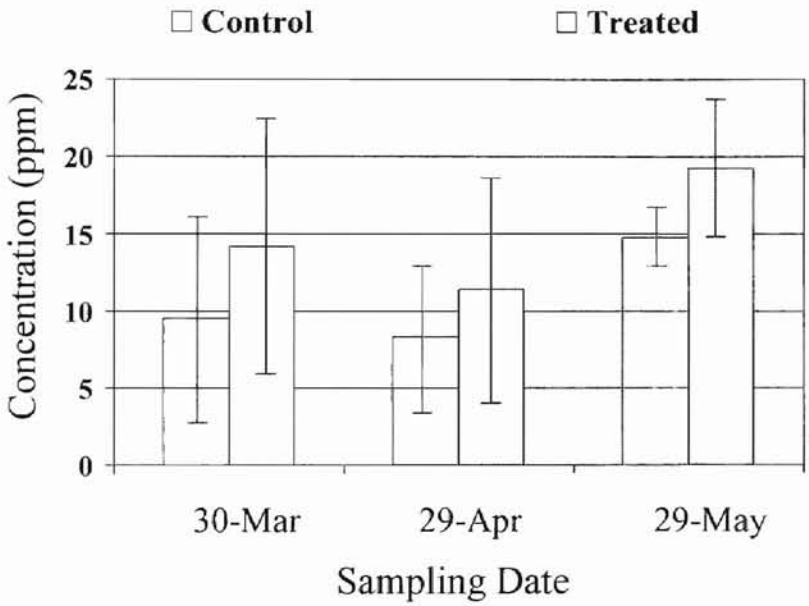

Figure 2.2 Concentrations of chlorothalonil in putting green soil in 2002. Three fungicide applications were conducted on a 14 day interval with the first application applied on 19-Apr. Data are the means of four replications per sampling date.

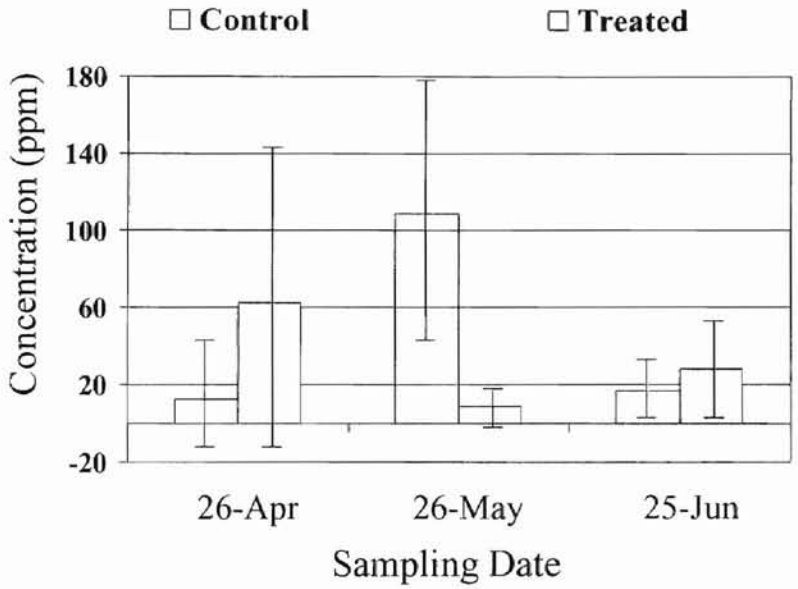

Figure 2.3 Concentrations of myclobutanil in putting green soil in 2001. Three fungicide applications were conducted on a 14 day interval with the first application applied on 16-May. Data are the means of four replications per sampling date.

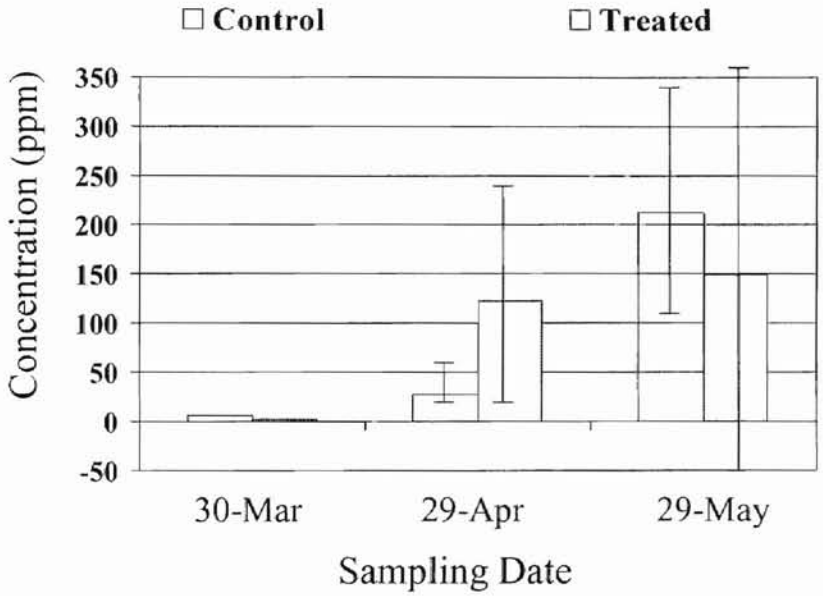

Figure 2.4 Concentrations of myclobutanil in putting green soil in 2002. Three fungicide applications were conducted on a 14 day interval with the first application applied on 19-Apr. Data are the means of four replications per sampling date.

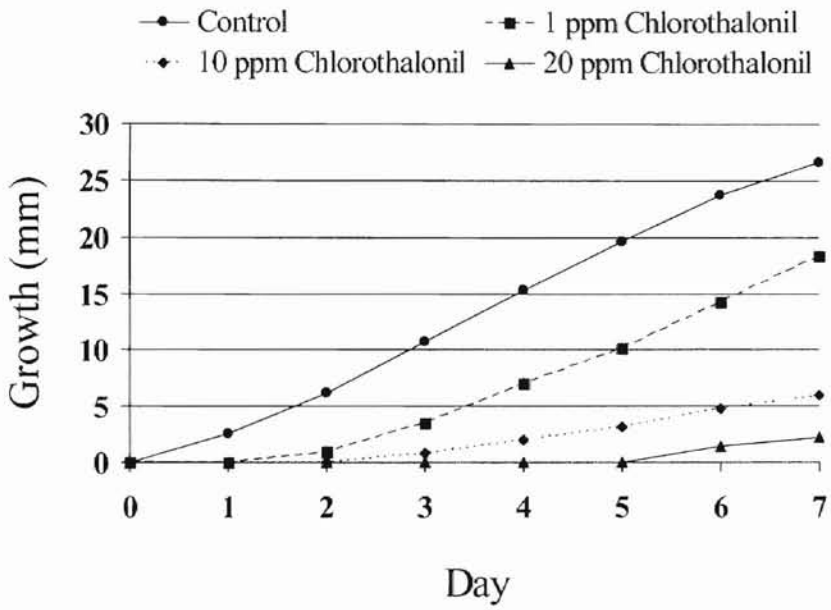

Figure 2.5 *In-vitro* growth of *Arthrobotrys oligospora* mycelium on agar amended with varying concentrations of chlorothalonil. Data are the means of two trials with ten replications each.

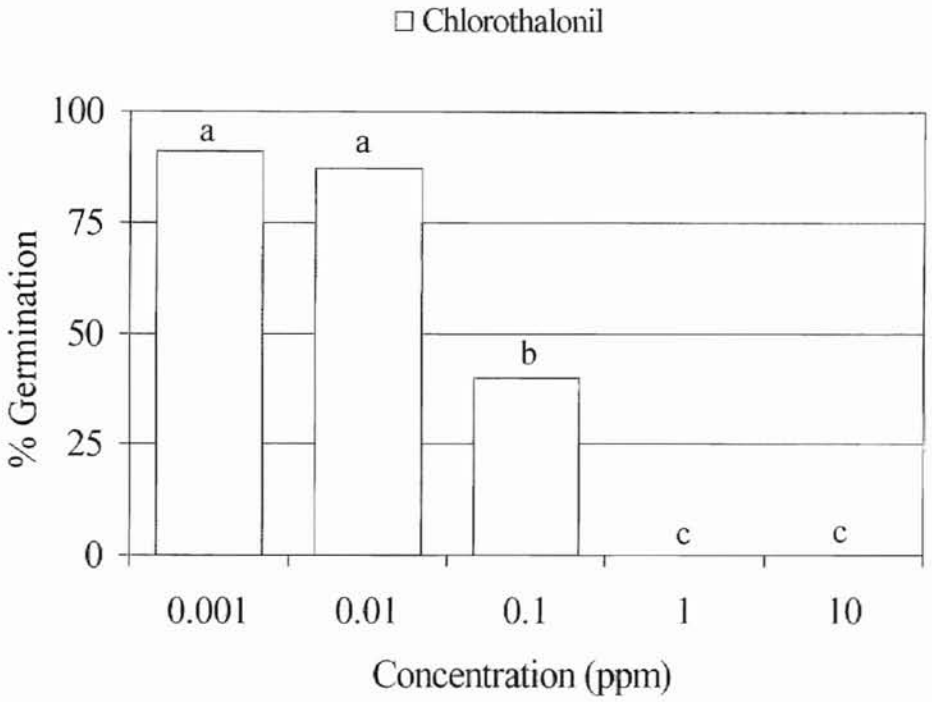

Figure 2.6 *In-vitro* germination of *Arthrobotrys oligospora* conidia on agar amended with varying concentrations of chlorothalonil. Data are the means of two trials with ten replications each. Bars followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's protected least significant difference test.

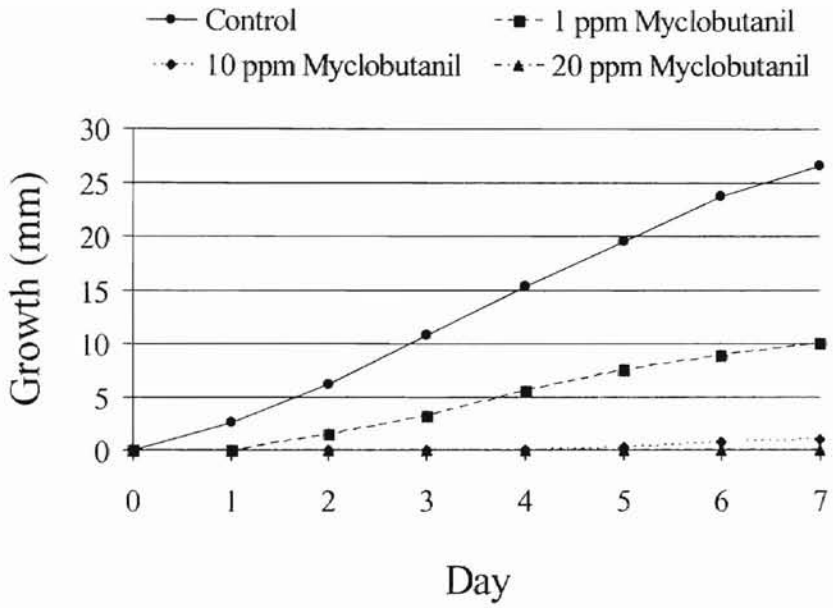

Figure 2.7 *In-vitro* growth of *Arthrobotrys oligospora* mycelium on agar amended with varying concentrations of myclobutanil. Data are the means of two trials with ten replications each.

did not germinate in the presence of concentrations exceeding 1 ppm (Figure 2.8). The EC_{50} of *A. oligospora* spores to myclobutanil was 3.5 ppm.

DISCUSSION

The consistency and quality of turfgrass is of greatest importance on golf courses. From a management perspective, putting greens are the most important area of the course receiving a significant portion of the total resources devoted to maintaining high-quality turfgrasses. As a result, various groups of pesticides are used to manage pests of turfgrasses present in putting greens (Anonymous, 2000; and Duxbury *et al.* 1998). Fungicides, used to control various fungal diseases, are often the most extensively used pesticides. In this study, fungicides suppressed the diseases dollar spot and brown patch (*Rhizoctonia solani*) within treated plots compared to non-treated control plots where damage from disease was unacceptable (data not shown).

Chlorothalonil and myclobutanil were chosen because of their common use throughout the golfing industry. Chlorothalonil is a widely used fungicide in many disease management programs due to its broad range of activity against numerous fungal pathogens and a limited incidence of pathogen resistence. A recent survey of pesticides used by the Virginia turfgrass industry reported that approximately 43,000 kg chlorothalonil (active ingredient) was used to manage fungal diseases of turfgrasses in the state (Anonymous, 2000). Although considerably lower than chlorothalonil, myclobutanil, belongs to the largest class of fungicides, the demethylation inhibiting fungicides. It was selected as a representative of this fungicide class because it was recently approved for use on golf courses.

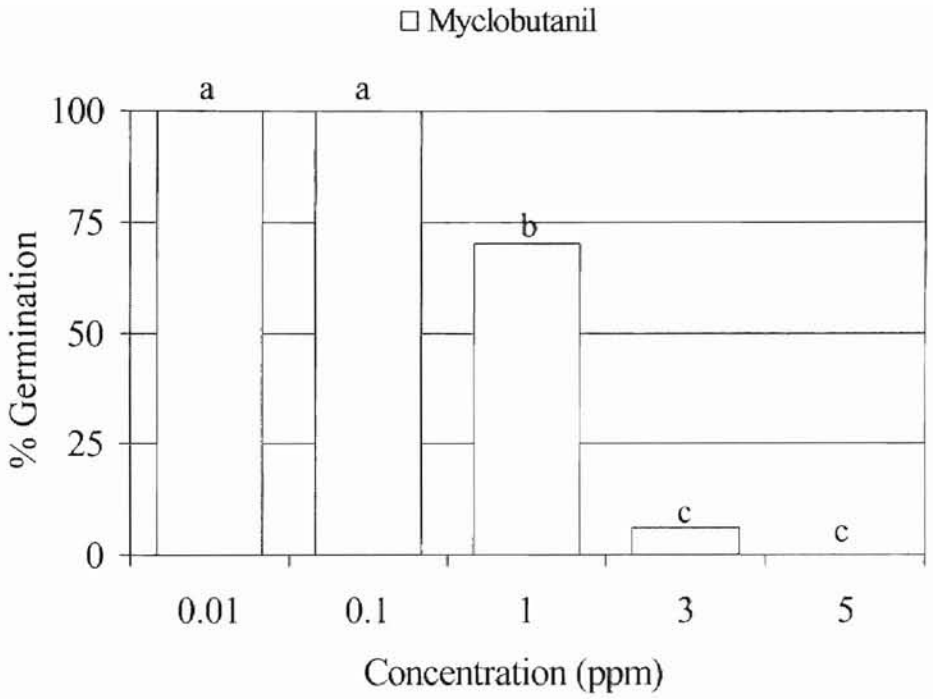

Figure 2.8 *In-vitro* germination of *Arthrobotrys oligospora* conidia on agar amended with varying concentrations of myclobutanil. Data are the means of two trials with ten replications each. Bars followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's protected least significant difference test.

The persistence of fungicides in soils is often variable and affected by binding to soil particles including clay and organic matter or microbial degradation. In previous studies, fungicides were found in the top 10 cm of the soil profile where thatch or organic matter content was greatest (Wu *et al.* 2002). Concentrations of chlorothalonil and myclobutanil in this study varied over years in both treated and non-treated plots. Accumulation of chlorothalonil was observed over time; however, accurate accumulation rates could not be derived due to the variability within non-treated controls.

The concentrations of chlorothalonil from soil was higher in this study than previously reported (Anonymous, 1988). The artificial rooting medium comprised of a sand, sand-sphagnum mixture (USGA Greens Section Staff, 1993) used for greens construction may explain this difference. In addition, chlorothalonil is known to persist in, but not leach from sandy soils (Gamble *et al.* 2000) and has a long half-life (>4 months) (Anonymous, 1987). Although not numerous, it is possible that previous chlorothalonil applications to the research site were detected by the methods used in this study and may explain why it was found in non-treated soils.

Soil concentrations of myclobutanil were found to be higher than expected. Little documentation is available on the concentration or persistence of myclobutanil in soil. However, environmental concerns have been raised about myclobutanil's persistence and mobility in soil (Anonymous, 1993). Results from these studies support those concerns and suggest that myclobutanil may persist in soils containing sand and organic matter.

Fungicide metabolites could also have adverse effects on *A. oligospora* and other nematophagous fungi. A primary degradation product of chlorothalonil (4-hydroxy-2,5,6-trichloroisophthalonitrile), is more acutely fungicidal than its parent

compound (Anonymous, 1987). Metabolites were not quantified in this study because the reagents used to extract metabolites are very hazardous, and chemical standards of metabolites are extremely expensive making *in vitro* studies impractical.

With the future loss of fenamiphos, other management tools for nematodes will be needed. Biological antagonists could potentially be used to manage plant-parasitic nematodes in golf course putting greens. Granular formulations containing *A. dactyloides* or *Verticillium chlamydosporium* have successfully suppressed nematodes present in tomato roots grown in nematode infested soil (Stirling and Smith, 1998; Stirling *et al.* 1998a; and Stirling *et al.* 1998b). Granular formulations containing *Arthrobotrys* may be incorporated into exiting or newly constructed greens to reduce populations of plant-parasitic nematodes.

Nematode antagonists have been isolated from soils throughout the world (Barron, 1977). *Arthrobotrys oligospora* was chosen for this study because of its worldwide distribution, ability to survive in the soil, non-specific trapping mechanism, and ability to form numerous conidia in culture. Fungicides were not observed to be directly associated with nematodes. This may indicate that *A. oligospora* would not be affected by fungicides if it were to parasitize nematodes present in soils which receive fungicide applications. However, *A. oligospora* may not be able to survive in the green soils if numerous fungicide applications were conducted. In addition, other fungicide chemistries not evaluated in this study which are used on bentgrass may also have adverse effects on *A. oligospora*.

For these approaches to work, data are required on the effects of management practices, such as fungicide inputs, on biological antagonists. The use of nematophagous

fungi such as *A. oligospora* may be possible due to the high maintenance of golf course putting greens. Lower nematode populations may increase plant health and subsequently reduce the frequency of fungicide applications to golf course putting greens. In addition, greater plant health may reduce the overall demand for maintenance inputs required to maintain high-quality putting surfaces required on golf course putting greens.

LITERATURE CITED

Anonymous. 1987. United States Environmental Protection Agency. Chlorothalonil Health Advisory. Draft Report. Office of Drinking Water, Washington D.C. pp. 6-36.

Anonymous. 1988. United States Environmental Protection Agency. Chlorothalonil Extraction Protocol. 1401-86-0084-CR-004. Report/SDS-2787.

Anonymous. 1993. Agriculture Canada. Decision Document: Myclobutanil. E93-01. www.hc-sc.gc.ca/pmra-arla/english/pdf/rdd/rdd_e9301-e.pdf. 37p.

Anonymous. 2000. 1998 Virginia turfgrass pesticide use survey: A report covering golf courses and sod producers. Virginia Agricultural Statistics Service. 9 p.

Barron, G.L. 1977. The Nematode-Destroying Fungi. Canadian Biological Publication Ltd. Guelph, Ontario, Canada.

Corbertt, J.R. K. Wright, and A.C. Baillie. 1984. The biochemical mode of action of pesticides. Second Edition. London, Academic Press, 382 p.

DiEdwards, W.A. 1963. Pathogenicity and host-parasitic relationships of nematodes on turf in Florida. Florida Agricultural Experiment Station Annual Report 109. University of Florida, Gainesville, FL., U.S.A.

Dunn, R.A. and J.W. Noling. 1997. Nematode Management Guide. Florida Cooperative Extension Service. SP-54. University of Florida, Gainesville, FL., U.S.A.

Duxbury, D., D. Martin, M. Woods, C. Willoughby, M. Wooten, B. Bloyd, and R. Maloney. 1998. 1996 Oklahoma golf course maintenance cost survey. Oklahoma Agricultural Statistics Service. 16 p.

Finney, D.J. 1975. Statistical methods in biological assay. Griffin & Co. Ltd, London.

Freitas, L. G., D.W. Dickson, D.J. Mitchell, and R. McSorley. 2000. Suppression of *Meloidogyne arenaria* by *Pasteuria penetrans* in the field. Nematologia Brasileira, Vol.24, No.2, pp.147-156.

Gamble, D. S., A. G. Bruccoleri, E. Lindsay, C. H. Langford, G. A. Leyes. 2000. Chlorothalonil in a quartz sand soil: wetting effects on sorption capacity and bound residue. Environmental Science and Technology 34:125-129.

Gomes, A.P.S., R.S. Vasconellos, M.C. Ramos, M.P. Guimaraes, A.P. Yatsula, M.C.R. Vieria-Bressen. 2000. *In-vitro* interaction of Brazilian strains of the nematode-trapping fungi *Arthrobotrys* spp. on *Panagrellus* sp. and *Cooperia punctata*. Mem Inst. Oswaldo Cruz. 96:881-884.

Lucas, L.T., C.T. Blake, and K.R. Barker. 1974. Nematodes associated with bentgrass and bermudagrass greens in North Carolina. Plant Disease Reporter 58(9): 822-824.

Lucas. L.T. 1982. Population dynamics of *Belonolaimus longicaudatus* and *Criconemella ornata* and growth response of bermudagrass and overseeded grasses on golf greens following treatment with nematicides. Journal of Nematology 14:358-363.

Mogadati, P., J.B. Louis, and J.D. Rosen. 1999. Multiresidue determination of pesticides in high-organic-content soils by solid phase extraction and gas chromatography/mass spectrometry. Journal of AOAC Interantional 82(3)705-715.

Murdoch, C.L., H. Tashiro, and M.B. Harrison. 1978. Plant parasitic nematodes associated with golf putting green turf in New York. Plant Disease Reporter 62: 85-87.

Schumacher, M., S. Camp, Y. Maulet, M. Newton , K. MacPhee-Quigley, S.S. Taylor, T. Friedmann, P. Taylor. 1986. Primary structure of *Torpedo californica* acetylcholinesterase deduced from its cDNA sequence. Nature 319: 407-9.

Smiley, R.W., P.H. Dernoeden, and C.B. Clarke. 1992. Compendium of Turfgrass Diseases. 2nd ed. American Phytopathological Society Press, St. Paul, Minnesota.

Stirling, G.R., K.A. Licastro, l.M. West, and L.J. Smith. 1998. Development of commercially acceptable formulations of the nematophagous fungus *Verticillium chlamydosporium*. Biological Control 11(3):217-223.

Stirling, G.R., Smith, L.J., K.A. Licastro, and L.M. Eden. 1998a. Control of root-knot nematode with formulations of the nematode-trapping fungus *Arthrobotrys dactyloides*. Biological Control 11(3):224-230.

Stirling, G.R. and L.J. Smith. 1998b. Field tests of formulated products containing either *Verticillium chlamydosporium* or *Arthrobotrys dactyloides* for biological control of root-knot nematodes. Biological Control 11(3):231-239.

Todd, T.C. and N.A. Tisserat. 1990. Occurrence, spatial distribution, and pathogenicity of some phytoparasitic nematodes on creeping bentgrass putting greens in Kansas. Plant Disease 74(9): 660-663.

USGA Greens Section Staff. 1993. USGA recommendations for a method of putting green construction. The 1993 revision. USGA Green Section Record. Mar/Apr pp1-3.

Vargas, J.M., J.R. 1981. Management of turfgrass diseases. Burgess Publishing Co. Minneapolis, Minnesota.

Walker, N.R., C.L. Goad, H. Zhang, and D.L. Martin. 2002. Factors associated with populations of plant parasitic nematodes in bentgrass putting greens in Oklahoma. Plant Disease 86:764-768.

Wu, L., G, Liu, M.V. Yates, R.L. Green, P. Pacheco, J. Gan, and Yates S.R. 2002. Environmental fate of metalaxyl and chlorothalonil applied to a bentgrass putting green under southern California climatic conditions. Pest Management Science 58(4):335-342.

CHAPTER III

Evaluating the efficacy of *Arthrobotrys oligospora* to suppress the ring nematode (*Criconemella ornata*) in simulated golf course putting green soils receiving fungicide applications

ABSTRACT

Greenhouse experiments were conducted to evaluate the effect of the nematophagous fungus, *Arthrobotrys oligospora,* on populations of the ring nematode, *Criconemella ornata*, and to determine the effect of fungicides on *A. oligospora*. Pots containing creeping bentgrass were inoculated with *C. ornata* adults and/or *A. oligospora*. Pots also received applications of the fungicides chlorothalonil or myclobutanil. No significant differences (P=0.05) were observed for turfgrass quality, leaf weight, root color, root length, or root weight. Nematode populations were not affected by *A. oligospora*. However, nematodes populations were lowest in pots inoculated with *A. oligospora* receiving fungicide treatments. Results of these studies indicate that *A. oligospora* may not be effective in suppressing plant-parasitic nematodes in golf course putting greens.

INTRODUCTION

The turfgrass industry in the United States was responsible for generating over $45 billion dollars in 1993 (Beard and Green, 1994) and was projected to double by 2000. Southern states account for approximately 30 percent of the turfgrass industry (Duble, 1996). An integral part of the national turfgrass industry are golf courses, which account for approximately five percent of the total U.S. turf acreage and eighteen percent of the total annual turf industry expenditures. Oklahoma has approximately 250 golf courses within the state covering 9,800 hectares. In 1996, the Oklahoma golfing industry's annual expenditures for maintenance was approximately $219.3 million, and has grown greatly over subsequent years (Duxbury *et al.* 1998).

The average size of Oklahoma golf courses is approximately 50 hectares (Duxbury et. al, 1998), with putting greens occupying two to three hectares of each course. Golf course greens are constructed to provide a high-quality putting surface. The green is comprised of a sand or a sand-sphagnum mixture on which creeping bentgrass (*Agrostis palustris* Hudson) is most commonly grown (USGA Greens Section, 1993).

Creeping bentgrass, the primary turfgrass used on golf course putting greens comprises 97.8 % of the greens in Oklahoma (Duxbury *et al.* 1998). *Agrostis palustris* is used to provide a high-quality putting surface at low mowing heights (< 3.5 mm) and recovers quickly from injury during coll portions of the year. Although putting greens are a relatively small component of the course, they are the most intensely managed part of the course. Putting greens receive numerous applications of fertilizer, insecticides, as well as fungicides to maximize plant health and minimize damage from play.

One reason for the intense management of *A. palustris* is due to its susceptibility

to various plant pathogens. For example, more fungicides, based on surface area, are applied to putting greens than any other agronomic commodity (Vargas, 1981). The frequent fungicide applications are required for the management of diseases such as dollar spot (*Sclerotinia homoeocarpa* Bennett). Nationally, more resources are used to manage *S. homoeocarpa* on greens than any other turfgrass pathogen (Vargas, 1981). In addition to insecticides and fungicides, other pests such as plant-parasitic nematodes are common in established putting greens.

Although nematodes are obligate pathogens that alone rarely causing plant death, they are responsible for crop losses worldwide in excess of $77 billion (Sasser and Freckman, 1987). When nematode populations are high, feeding can reduce plant vigor, plant growth, and stand density (DiEdwards, 1963; Dunn and Noling, 1997; Johnson and Powell, 1968; Johnson, 1970; Lucas *et al.* 1974, Todd and Tisserat, 1990; and Winchester and Burt, 1964). Plant-parasitic nematodes cause damage to plants by inserting a protractible stylet, spear-like mouthpart into plant cells and ingesting the contents. Affected plants tend to lack vigor and exhibit symptoms of nutrient deficiency. In addition, probing or movement of the nematode through plant tissues causes extensive damage to plant roots and permits the entry of facultative parasites. Nematodes feed on all plants including turfgrasses and populations are highest in late summer to mid-autumn when soil temperatures are between 20 and 30 °C. A visible reduction in turfgrass quality may not occur until this time when populations exceed a plant tolerance threshold or when more than a single genus is present (Murdoch *et al.* 1978; Smiley *et al.* 1992).

The environment of a golf course putting green may encourage the accumulation of high populations of plant-parasitic nematodes (Wick, 1997). A recent survey of

Oklahoma putting greens found the ring nematode (*Criconemella ornata*) present in 60% of greens. Approximately fifty percent of greens containing *C. ornata* had populations above a threshold of 500 nematodes per 100 cc soil (Walker *et al.* 2002). Nematode damaged areas often require more management inputs to maintain an acceptable putting surface and to prevent algae formation or weed encroachment. As damage to turf increases and the green fails to provide an acceptable putting surface, nematode management practices may need to be employed.

The application of chemical nematicides has been used for the management of plant-parasitic nematodes since the 1950's (Thorne, 1961). However, most of the older petroleum based nematicides are no longer available due to the presence of these compounds in drinking water. Currently, only a few nematicides are available for nematode management, of which only a single product, fenamiphos, is labeled for use on existing greens. Fenamiphos is an organophosphorus pesticide which is highly toxic and has been under scrutiny by the Environmental Protection Agency (EPA). It is scheduled to be removed from the market by 2007 and no replacement products are expected. With the loss of this effective nematicide, other methods for the management of nematodes in putting greens are needed.

A possible replacement for nematicides could be the incorporation of biological antagonists into the soil environment of golf course putting greens. Biological antagonists of nematodes can include bacteria, predacious nematodes, protozoans, insects, mites, and nematophagous fungi (Striling, 1991). The actenomycete, *Bacillus penetrans*, is an effective biological control agent of nematodes. However, it is species-specific and is difficult to produce in culture. The practical use of protozoan,

insect, and mite predators is limited, and predacious nematodes are often ineffective at reducing nematode populations. However, nematophagous fungi are common soil inhabitants and occur in all classes of the fungi, except for the Ascomycetes, and are common in soils worldwide (Stirling, 1991).

Nematophagous fungi have been used successfully in other cropping systems to reduce plant-parasitic nematodes in other cropping systems. For example, *Arthrobotrys dactyloides* has suppressed *Meloidogyne javanica* juveniles in greenhouse studies on tomato (Stirling and Smith, 1998; Stirling *et al.* 1998a; Stirling *et al.*1998b). In addition, *in vitro* experiments have been conducted to assess the ability of 26 isolates of *Arthrobotrys* spp. to capture the free living nematode *Panagrellus* spp. and the infective juveniles of *Cooperia punctata*, a gastrointestinal parasite of cattle indicating that *A. oligospora* was effective at reducing populations of both groups of nematodes (Gomes *et al.* 2000). The objectives of this study were to evaluate the efficacy of *A. oligospora* to suppress *Criconemella ornata* in a simulated putting green environment and to determine the effects of chlorothalonil and myclobutanil on *A. oligospora*.

MATERIALS AND METHODS

Creeping bentgrass (*A. palustris* cv. SR1020, Seed Research of Oregon Inc., Corvallis, OR) was planted at a rate of 25 seeds cm^2 in plastic pots (10.5 cm x 10.5 cm x 9.5 cm h) containing 1,100 g of autoclaved sand. Baffles (8.0 cm x 8.0 cm 1.0 cm h) were placed in the bottom of each pot. Geotextile fabric (10 cm x 10 cm) (PS Fabrics, Inc., Wadsworth, OH) was placed on top of each baffle. To aid in stand establishment, seeds were germinated by placing pots in standing water. After germination, pots were

transferred to a greenhouse. Plants were irrigated with a slow drip emitter that was placed in the side of the pot 4-cm below the top of the pot. Turf was fertilized with water-soluble fertilizer (Peters, St. Louis, MO) at a rate of 14.8 kg N ha^{-1} month and clipped weekly at < 1 cm.

Inoculum of *Criconemella ornata* was obtained from stock cultures maintained in a greenhouse on perennial ryegrass (*Lolium perenne* L.). Nematode inoculum was extracted from soil by bucket decanting and centrifugal floatation (Ayoub, 1980). Nematodes were quantified using a compound microscope. Fifty days after planting (DAP), pots were inoculated with 10 ml water containing 100 adult *C. ornata*. Non-inoculated pots were used as a control and received 10 ml of water.

Inoculum of *A. oligospora* (American Type Culture Collection 16234) (ATCC, Manassas, VA) was grown on potato dextrose agar (Sigma Chemical Co., St. Louis, MO) for ten days. *Arthrobotrys oligospora* conidia were flood harvested and separated from mycelium using 425-600 µm glass beads (Sigma Chemical Co., St. Louis, MO) placed in a conical centrifuge tube and agitated for one minute on a mini vortex to remove conidia from the conidiaphore. Mycelium was separated from the spores using a 400 µm sieve. Conidia were quantified using a hemacytometer. Fifty days after planting pots were inoculated with 10 ml water containing 22,000 *A. oligospora* conidia. Non-inoculated pots were used as a control and received 10 ml of water.

Ninety DAP, pots receiving fungicide applications were treated with chlorothalonil or myclobutanil applied at 93.15 g active ingredient (a.i.) per 100 m^2 or 7.32 g a.i. per 100 m^2, respectively. Three applications of chlorothalonil or myclobutanil were conducted on a 14 or 21-day interval, respectively. Fungicides were applied using a

CO_2 pressurized (1.24 x 105 Pa) wheelbarrow plot sprayer calibrated to deliver 887 L ha^{-2}. Nontreated pots were used as a control. Pots treated with fungicides were watered overhead for 1 minute for three consecutive days after each fungicide application.

Soil fungicide concentrations were determined from five pots treated with chlorothalonil or myclobutanil. Five, 1-cm dia soil cores were removed from each pot to a depth of 4.5 cm and mixed thoroughly. For chlorothalonil, a ten-gram composite soil sample was placed in 50 ml of extraction solvent (5:95, 10N H_2SO_4:acetone, v/v) and sealed. The solution was shaken on a wrist action shaker for at three hours. To remove solids, the solution was filtered through 0.2 μm Whatman filter paper (Whatman International Ltd., Maidstone, England) and the solids rinsed with 5 ml of extraction solvent.

The extract was combined with 50 ml petroleum ether, 40 ml of extraction solvent, and 20 ml deionized water, and shaken vigorously by hand for two minutes. Solutions were transferred to a seperatory funnel and the phases allowed to separate. The lower aqueous phase was drained and this process was repeated twice. The ether phase was collected and evaporated to dryness by allowing it to stand in an operating fumehood overnight. Glass columns (9 mm i.d x 200 mm h) were packed with glass wool (0.5 cm), 2.0 g Florisil (US Silica, Berkeley Springs, WV), and 2.0 g anhydrous sodium sulfate. Florisil was activated at 121 ± 2 °C, for 12 hours in an isothermic oven (Fisher Scientific, Springfield, NJ). Columns were washed with 10 ml hexane which was discarded. Dried samples were dissolved in 10 ml of 20:80, methylene chloride:hexane, v/v (Eluant 1). After the sample migrated through the sodium sulfate layer an additional 15 ml of Eluant 1 was added to each column. A total of 25 ml of Eluant 1 was collected from each

column and discarded. Chlorothalonil was eluted from each column using 35 ml of 50:48.5:1.5, methylene chloride:hexane:acetonitrile, v/v/v (Eluant 2) (Anonymous, 1988). The sample was collected in a 50 ml conical pyex tube (Fisher Scientific, Springfield NJ).

The conical pyrex tube was placed in a heated ultrasonic bath (Fisher Scientific, Springfield, NJ) and evaporated to dryness in a fumehood. Sample residue within the conical tubes was dissolved in 100 µl acetone and vortexed for 10 seconds.

For myclobutanil, a ten-gram composite soil sample was placed in a flask containing 50 ml of methanol and sealed. The sample was shaken for 4 hours on a bench-top rotary shaker. To remove solids, the solution was filtered using 0.2 µm Whatman filter paper (Whatman International Ltd., Maidstone, England) and rinsed with 5 ml of methanol.

The extract was added to 100 ml hexane and 30 ml 2% aqueous sodium chloride, and shaken vigorously by hand for three minutes. The solution was transferred to a seperatory funnel and the phases were allowed to separate. The lower aqueous phase was collected and transferred to a bottle containing 100 ml 2% aq NaCl and 100 ml methylene chloride. Solutions were shaken vigorously by hand for three minutes and transferred to a seperatory funnel and the phases allowed to seperate. The lower methylene chloride phase was collected in a 250 ml beaker, and the process was repeated twice with 50 and 25 ml methylene chloride, respectfully for the remaining aqueous phase.

To remove excess water from the sample, 65 mm dia polystyrene funnels filled with 20 grams of anhydrous sodium sulfate. The methylene chloride containing the sample was filtered through the sodium sulfate and evaporated to dryness. Residue was dissolved in 15 ml toluene and added to a column as previously described. Myclobutanil

was eluted by adding 20 ml 1:99, methanol:toluene v/v (Eluant A) to the column. Fifteen ml toluene and 20 ml Eluant A were discarded, and a 50 ml conical pyrex tube the column was placed under the column. Myclobutanil was eluted from the column using 40 ml 2:98, methanol:toluene, v/v (Eluant B). Samples were placed in a double boiler and evaporated to dryness, by allowing the boilers to operate in an operating fumehood. The residue within the conical tube was then dissolved in 100 µl acetone and vo for 10 seconds (J. Ross, personal communication).

An HP Model 6890 gas chromatograph (GC) equipped with an HP Model 5073 Mass Spectrometer (MS) detector (Hewlett Packard, Los Angeles, CA) was used for fungicide detection and quantification. One µl of each sample was manually injected into a splitless injector maintained at 260 °C. The column was a J&W DB-5 (Folsom, CA) fused silica capillary column (30 m x 0.25 mm internal diameter; 0.25 µm film thickness) the operating temperature was 60 to 260 °C increasing 6 °C min^{-1}. Helium was the carrier gas at a velocity of 1 ml min^{-1}. The mass spectrometer was operated in selected ion monitoring (SIM) mode. Filament emission current was 12 uA and electron multiplier voltage was 1650 V. Multiplier gain was 105. Scan range was from 87 to 450 amu at 1 s/scan. Transfer line and manifold temperatures were maintained at 260 °C and 220 °C, respectively (Mogadati *et al.* 1999).

One hundred and thirty days after planting, leaf clippings were collected from each pot, dried in an oven at 100 °C overnight, and weighed. Turfgrass quality was rated on a scale of 1 to 5, where 1 = thin turf and 5 = thick, healthy turf. Nematodes were extracted from 550 g soil by sieving and centrifugal floatation (Ayoub, 1983) and populations determined using a compound microscope. Two 1-cm dia soil cores were

removed from each pot. Roots were separated from one soil core, rated for discoloration using a color scale from 1 to 7, where 1 = brown roots and 7 = white roots (PPG Architectural Finishes, Inc. color code 315). Roots, from the second soil core, were removed from the crown, dried in an oven overnight at 100 °C, and weighed. Pots were arranged in a randomized complete block design with 5 replications for the first trial and 10 replications for the second trial. Data were analyzed using analysis of variance (ANOVA). All calculations were conducted with the statistical analysis system SAS (version 8.0, SAS Instituite, Cary, NC).

RESULTS

Chlorothalonil did not affect turfgrass quality with quality being numerically highest for the non-treated control and lowest in pots inoculated with *C. ornata* (Table 3.1). Leaf clipping weight was not affected by any treatment; however, clipping weight was highest in pots inoculated with *A. oligospora* and lowest in the non-treated control. Although no treatment was observed to affect root color, plants from the non-treated control had the whitest roots (Table 3.2). Roots were longest in pots treated with chlorothalonil and shortest in pots inoculated with both *C. ornata* and *A. oligospora*, but no treatment affected root length. No treatment differences were observed for root weight with weight found to be greatest for pots inoculated with *A. oligospora* treated with chlorothalonil.

No treatments affected nematode populations (Table 3.3); however, populations of nematodes from pots inoculated with *A. oligospora* treated with chlorothalonil were lowest at 202 nematodes per pot while pots inoculated with *C. ornata* treated with

Table 3.1 Effects of *Criconemella ornata*, *Arthrobotrys oligospora*, and chlorothalonil on greenhouse grown *Agrostis palustris* cv. SR1020 turfgrass quality and leaf mass.

Treatment	Turfgrass Quality[a]	Leaf Mass(g)[b]
Non-treated Control	4.03	0.4322
Arthrobotrys oligospora	3.95	0.5741
Criconemella ornata	3.45	0.5875
A. oligospora + *C. ornata*	3.53	0.5005
Chlorothalonil	3.73	0.4879
A. oligospora + Chlorothalonil	3.93	0.5406
C. ornata + Chlorothalonil	3.85	0.4847
A. oligospora + *C. ornata* + Chlorothalonil	3.92	0.4800
($P = 0.05$)	NS[c]	NS

[a] Turfgrass quality was rated 130 days after planting (DAP) on a scale of 1 to 5, where 1 = thin turf, and 5 = thick, healthy turf.

[b] Leaf clippings were collected from each pot 130 DAP and dried in an oven.

[c] NS = not significant.

Table 3.2 Effects of *Criconemella ornata*, *Arthrobotrys oligospora*, and chlorothalonil on greenhouse grown *Agrostis palustris* cv. SR1020 root color, root length, and root mass.

Treatment	Root Color[a]	Root Length (cm)[b]	Root Mass (g)[c]
Non-treated Control	3.67	4.80	0.0105
Arthrobotrys oligospora	3.43	4.21	0.0120
Criconemella ornata	3.50	4.51	0.0092
A. oligospora + *C. ornata*	3.47	4.01	0.0092
Chlorothalonil	3.10	4.91	0.0172
A. oligospora + Chlorothalonil	3.40	4.71	0.0217
C. ornata + Chlorothalonil	3.33	4.53	0.0131
A. oligospora + *C. ornata* + Chlorothalonil	3.30	4.82	0.0119
($P = 0.05$)	NS[d]	NS	NS

[a] Roots were rated for discoloration on a scale from 1 to 7, where 1 = brown roots and 7 = white roots.

[b] Root length was determined from a 1-cm-dia sample.

[c] Roots from a 1-cm-dia soil core taken from each pot were separated from crown tissue and soil, dried in an oven, and weighed.

[d] NS = not significant.

Table 3.3 Populations of *Criconemella ornata* in pots treated with *Arthrobotrys oligospora* and chlorothalonil on greenhouse grown *Agrostis palustris* cv. SR1020.

Treatment	*C. ornata* Populations[a]
C. ornata	271
A. *oligospora* + *C. ornata*	256
C. ornata + Chlorothalonil	370
A. oligospora + *C. ornata* + Chlorothalonil	202
($P = 0.05$)	NS[b]

[a] Nematodes were extracted from 550 g of soil, populations represent the entire pot.

[b] NS = not significant.

chlorothalonil were highest with 370 nematodes per pot. Soil concentrations of chlorothalonil were at the minimum detection limit (MDL) of the instrument.

Turfgrass quality was not significantly affected by the application of myclobutanil. Quality was numerically greatest in pots inoculated with *C. ornata* (Table 3.4). Leaf clipping weight was not affected by treatment; however, clipping weight was highest in pots inoculated with *A. oligospora*. No treatment effects were observed for root color, roots were longest in pots inoculated with *A. oligospora* treated with myclobutanil (Table 3.5). No treatment effects were observed for root weight, weights were greatest for pots inoculated with *C. ornata*. Nematode populations were not affected by any treatment (Table 3.6); however, populations of nematodes from pots inoculated with *A. oligospora* treated with myclobutanil were lowest at 196 nematodes per pot while pots containing only *C. ornata* were highest with 343 nematodes per pot. Concentrations of myclobutanil were below the minimum detection limit (<MDL) of the instrument.

Table 3.4 Effects of *Criconemella ornata*, *Arthrobotrys oligospora*, and myclobutanil on greenhouse grown *Agrostis palustris* cv. SR1020 turfgrass quality and leaf mass.

Treatment	Turfgrass Quality[a]	Leaf Mass (g)[b]
Non-treated Control	3.07	0.6065
Arthrobotrys oligospora	3.25	0.6577
Criconemella ornata	3.28	0.4976
A. oligospora + *C. ornata*	3.20	0.6066
Myclobutanil	3.27	0.5731
A. oligospora + Myclobutanil	3.25	0.5310
C. ornata + Myclobutanil	3.15	0.5525
A. oligospora + *C. ornata* + Myclobutanil	3.27	0.5230
($P = 0.05$)	NS[c]	NS

[a] Turfgrass quality was rated 130 days after planting (DAP) on a scale of 1 to 5, where 1 = thin turf, and 5 = thick, healthy turf.

[b] Leaf clippings were collected from each pot 130 DAP and dried in an oven.

[c] NS = not significant.

Table 3.5 Effects of *Criconemella ornata*, *Arthrobotrys oligospora*, and myclobutanil on greenhouse grown *Agrostis palustris* cv. SR1020 root color, root length, and root mass.

Treatment	Root Color[a]	Root Length (cm)[b]	Root Mass (g)[c]
Non-treated Control	3.43	4.33	0.0156
Arthrobotrys oligospora	3.57	4.25	0.0194
Criconemella ornata	3.37	4.25	0.0237
A. oligospora + *C. ornata*	3.53	4.24	0.0151
Myclobutanil	3.63	4.16	0.0149
A. oligospora + Myclobutanil	3.40	4.53	0.0143
C. ornata + Myclobutanil	3.47	4.33	0.0153
A. oligospora + *C. ornata* + Myclobutanil	3.67	4.44	0.0125
($P = 0.05$)	NS[d]	NS	NS

[a] Roots were rated for discoloration on a scale from 1 to 7, where 1 = brown roots and 7 = white roots.

[b] Root length was determined from a 1-cm-dia sample.

[c] Roots from a 1-cm-dia soil core taken from each pot were separated from crown tissue and soil, dried in an oven, and weighed.

[d] NS = not significant.

Table 3.6 Populations of *Criconemella ornata* in pots treated with *Arthrobotrys oligospora* and myclobutanil on greenhouse grown *Agrostis palustris* SR1020.

Treatment	*C. ornata* Populations[a]
C. ornata	343
A. *oligospora* + *C. ornata*	274
C. ornata + Myclobutanil	239
A. oligospora + *C. ornata* + Myclobutanil	196
($P = 0.05$)	NS[b]

[a] Nematodes were extracted from 550 g of soil, populations represent the entire pot.

[b] NS = not significant.

DISCUSSION

Arthrobotrys spp. have been used to suppress plant-parasitic nematodes in other intensely managed cropping systems (Stirling and Smith 1998; Stirling *et al.* 1998a, Stirling *et al.* 1998b). Different isolates of *A. oligospora* have suppressed both free-living and animal-parasitic nematodes *in vitro* (Gomes *et al.* 2000). *Arthrobotrys oligospora* was chosen for this study because of it's non-specific trapping mechanism, worldwide distribution and ability to form numerous asexual conidia in culture.

Plant-parasitic nematodes have been found in golf course putting greens throughout the United States (Davis, 1994; Lucas *et al.* 1974; Safford and Riedel, 1976; Sikora, *et al.* 1972; Tashiro *et al.* 1977; and Walker *et al.* 2002). Studies have demonstrated that high populations of nematodes are responsible for reducing the quality of creeping bentgrass (DiEdward,1963; Johnson and Powell, 1968; Johnson, 1970; Todd and Tisserat, 1990; and Winchester and Burt, 1964). With damage being most severe when multiple genera of nematodes are present (Murdoch *et al.* 1978).

Bentgrass is maintained at a low mowing height which often stresses plants as temperatures increase in summer months. To account for low mowing height, turfgrasses in this study were maintained at a height consistent with that on golf course putting greens. As temperatures increase and environmental conditions become conducive for disease, nematode damage may become severe. In addition, reduced fertilizer uptake and weed encroachment are encountered in these areas, requiring more inputs to maintain a high-quality putting surface.

When grown in the presence of high humidity and temperature such as that found in the greenhouse, fungicides are required to manage diseases of creeping bentgrass. In

an attempt to exclude foliar disease, a sub-surface water delivery system was developed to adequately supply plants with water. In addition, an oscillating fan was used to remove excess leaf moisture. These two measures effectively excluded the growth of foliar diseases thus eliminating fungicide applications for pots not scheduled to receive them.

Chlorothalonil concentrations were very low compared to soil concentrations determined in other studies (Anonymous, 1988; Anonymous, 1987). Myclobutanil concentrations were below the minimum detection limit and were substantially lower than concentrations from putting green soil containing sand and organic matter (J. Woodward, data not shown). This observation suggests that chlorothalonil is capable of binding to sand particles in the absence of organic matter, while myclobutanil is easily leached from soils with little or no organic matter. These observations are consistent with other studies evaluating the leaching of various pesticides in newly constructed, sand-based greens (Smith and Bridges, 1999).

Although no significant differences (P=0.05) were observed in this study, populations of *C. ornata* were consistently lower in pots inoculated with *A. oligospora* that received either fungicide treatment. However, the inoculum rates used in this study were lower than other and may have been insufficient in suppressing nematode populations. This may suggest that *A. oligospora* could be used in concert with a liberal fungicide regime of either chlorothalonil or myclobutanil to manage fungal diseases in greens constructed of sand. However, additional studies must be conducted on the effects of various other fungicides and inoculation methods of *A. oligospora*.

LITERATURE CITED

Anonymous. 1987. United States Environmental Protection Agency. Chlorothalonil Health Advisory. Draft Report. Office of Drinking Water, Washington D.C. pp. 6-36.

Anonymous. 1988. United States Environmental Protection Agency. Chlorothalonil Extraction Protocol. 1401-86-0084-CR-004. Report/SDS-2787.

Ayoub, S.M. 1980. Plant Nematology: An agricultural training aid. Sacromento CA: NemaAid Publications.

Beard, J.B. and R.L. Green. 1994. The role of turfgrasses in environmental protection and their benefits to humans. Journal Environmental Quality 23: 452-460.

Davis, R.F., H.T. Wilkinson, and G.R. Noel. 1994. Vertical distribution of three nematode Genera in a bentgrass putting green in central Illinois. Journal of Nematology 26(4): 518-521.

DiEdwards, W.A. 1963. Pathogenicity and host-parasitic relationships of nematodes on turf in Florida. Florida Agricultural Experiment Station Annual Report 109. University of Florida, Gainesville, FL., U.S.A.

Duble, R. 1996. Turfgrasses, their use and management in the Southern zone. 2nd ed. Texas A&M University Press, College Station TX.

Dunn, R.A., and J.W. Noling. 1997. Nematode Management Guide. Florida Cooperative Extension Service. SP-54. University of Florida, Gainesville, FL., U.S.A.

Duxbury, D., D. Martin, M. Woods, C. Willoughby, M. Wooten, B. Bloyd, and R. Maloney. 1998. 1996 Oklahoma golf course maintenance cost survey. Oklahoma Agricultural Statistics Service. 16 p.

Gomes, A.P.S., R.S. Vasconellos, M.C. Ramos, M.P. Guimaraes, A.P. Yatsula, M.C.R. Vieria-Bressen. 2000. *In-vitro* interaction of Brazilian strains of the nematode-trapping fungi *Arthrobotrys* spp. on *Panagrellus* sp. and *Cooperia punctata*. Mem Inst. Oswaldo Cruz. 96:881-884.

Johnson, A.W., and W.M. Powell. 1968. Pathogenic capabilities of ring nematode, *Crionemoides lobalum*, on various turfgrasses. Plant Disease Reporter 52:109-113.

Johnson, A.W. 1970. Pathogenicity and interaction of three nematode species on six bermudagrasses. Journal of Nematology 2:36-41.

Lucas, L.T., C.T. Blake, and K.R. Barker. 1974. Nematodes associated with bentgrass and bermudagrass greens in North Carolina. Plant Disease Reporter 58(9): 822-824.

Mogadati, P., J.B. Louis, and J.D. Rosen. 1999. Multiresidue determination of pesticides in high-organic-content soils by solid phase extraction and gas chromatography/mass spectrometry. Journal of AOAC Interantional 82(3)705-715.

Murdoch, C.L., H. Tashiro, and M.B. Harrison. 1978. Plant parasitic nematodes associated with golf putting green turf in New York. Plant Disease Reporter 62: 85-87.

Safford, J., and R.M. Riedel. 1976. *Criconemoides* species associated with golf course turf in Ohio. Plant Disease Reporter 60: 405-408.

Sasser, J.N., and D.W. Freckman. 1987. A world perspective on plant nematology: The role of the society. Pp 7-14. In Veech J.A. and DicksonD.W. (ed.) "Vistas on Nematology". Society of Nematologists, Hyattsville, MD.

Sikora, R.A., D.P. Taylor, R.B. Malek, and D.I. Edwards. 1972. Interaction of *Meloidogyne nassi*, *Pratylechuns penatrans*, and *Tylenchorhynchus agri* on creeping bentgrass. Journal of Nematology 4(3): 162-165.

Smiley, R.W., P.H. Dernoeden, and C.B. Clarke. 1992. Compendium of Turfgrass Diseases. 2nd ed. American Phytopathological Society Press, St. Paul, Minnesota.

Smith, A.E. and D.C. Bridges. 1996. Potential movement of certain pesticides following application to golf courses. In: Herbicide metabolites in surface water and groundwater. Developed from the 209th national meeting of the American Chemical Society. Anaheim, CA. April 1995. Pp 165-177.

Stirling, G.R. 1991. Biological Control of Plant Parasitic Nematodes. CAB International, Wallingford Oxen, United Kingdom.

Stirling, G.R., K.A. Licastro, I.M. West, and L.J. Smith. 1998a. Development of commercially acceptable formulations of the nematophagous fungus *Verticillium chlamydosporium*. Biological Control 11(3):217-223.

Stirling, G.R., Smith, L.J., K.A. Licastro, and L.M. Eden. 1998b. Control of root-knot nematode with formulations of the nematode-trapping fungus *Arthrobotrys dactyloides*. Biological Control 11(3):224-230.

Stirling, G.R., and L.J. Smith. 1998. Field tests of formulated products containing either *Verticillium chlamydosporium* or *Arthrobotrys dactyloides* for biological control of root-knot nematodes. Biological Control 11(3):231-239.

Tashiro, H, C.L. Murdoch, and W.J. Apt. 1977. Plant parasitic nematodes associated with golf putting green turf in Hawaii. Plant Disease Reporter 61(11): 919-921.

Todd, T.C., and N.A. Tisserat. 1990. Occurrence, spatial distribution, and pathogenicity of some phytoparasitic nematodes on creeping bentgrass putting greens in Kansas. Plant

Disease 74(9): 660-663.

Thorne, G. 1961. Principles of Nematology. McGraw-Hill Book Co. Inc., New York, New York.

USGA Greens Section Staff. 1993. USGA recommendations for a method of putting green construction. The 1993 revision. USGA Green Section Record. Mar/Apr pp1-3.

Vargas, J.M., J.R. 1981. Management of turfgrass diseases. Burgess Publishing Co. Minneapolis, Minnesota.

Walker, N.R., C.L. Goad, H. Zhang, and D.L. Martin. 2002. Factors associated with populations of plant parasitic nematodes in bentgrass putting greens in Oklahoma. Plant Disease 86:764-768.

Wick, R.L. 1997. Cool-Season Turf: Diagnosing and Managing Nematodes. In: Grounds Maintenance. Pp 20-25. E. Liskey, ed.

Winchester, J.A., and E.O. Burt. 1964. The effect and control of sting nematodes on Ormond bermudagrass. Plant Disease Reporter 48:625-628.

APPENDIX

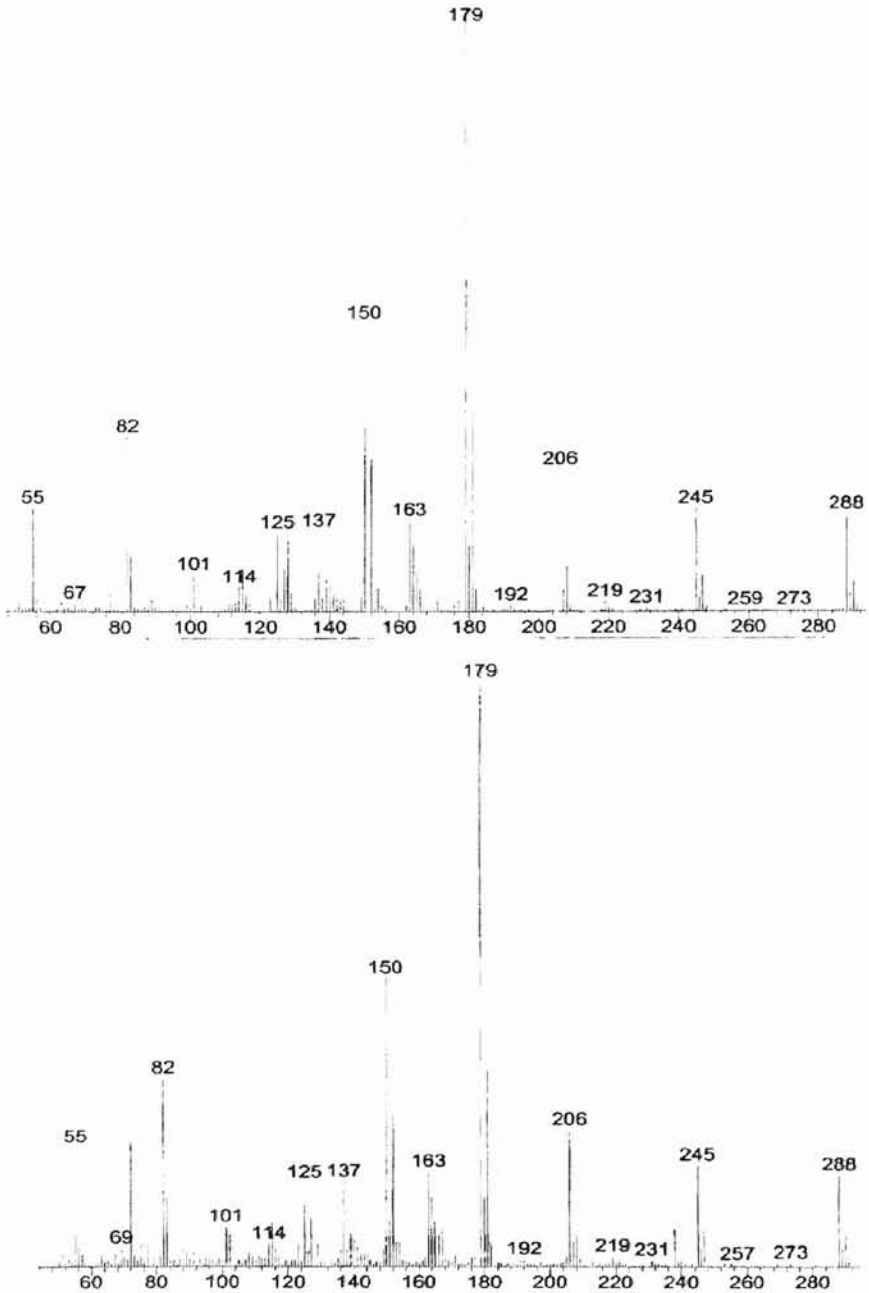

Figure A.1. Mass spectra of myclobutanil standard (top) and field sample (bottom).

VITA

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Master of Science

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