

The influence of fungicides on *Arthrobotrys oligospora* in simulated putting green soil

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Summary

Plant-parasitic nematodes are destructive pests in bentgrass putting greens. Few chemical or non-chemical approaches for nematode management exist. Studies were conducted to determine: the *in vitro* tolerance of the nematophagous fungus *Arthrobotrys oligospora*, to the fungicides chlorothalonil and myclobutanil used to manage diseases on putting greens; the concentration of fungicides obtained from simulated putting green soil; and the ability of the fungus to reduce populations of the ring nematode, *Criconebella ornata*. Both fungicides reduced *in vitro* hyphal growth and germination of conidia above 10 mg kg⁻¹. Soil concentrations of chlorothalonil were less than 5 mg kg⁻¹ and concentrations of myclobutanil were below detection limits. Nematode populations were not affected by *A. oligospora* in simulated greens but nematode populations were lowest in pots inoculated with *A. oligospora* and receiving fungicide treatments. Results of these studies indicate that applications of chlorothalonil and myclobutanil used to manage fungal diseases of bentgrass may not adversely affect *A. oligospora*; however, the fungus may not reduce nematode populations below desired thresholds.

Key words: *Agrostis palustris*, bentgrass, chlorothalonil, myclobutanil, putting green, *Arthrobotrys oligospora*

Introduction

Golf course putting greens are typically constructed of sand or a sand/sphagnum mixture (Anon., 1993a) on which creeping bentgrass (*Agrostis palustris* Hudson) is grown. This system is utilised to provide a high quality and consistent putting surface and can be maintained throughout the year. Although putting greens comprise a small portion of the golf course they are the most intensely managed part of the course. This is in part due to the susceptibility of *A. palustris* cultivars to various pests including insects, fungal diseases and nematodes.

The high sand content of putting greens can encourage many genera of plant-parasitic nematodes to become established at populations that may damage bentgrass. The plant-parasitic nematodes *Helicotylenchus*, *Criconebella*, *Hoplolaimus*, and *Tylenchorhynchus* spp. were found in 55%, 52%, 47%, and 38%, respectively, of 81 greens from 20 Kansas golf courses (Todd & Tisserat, 1990). Mean nematode populations in the study were 425, 900, 81, and 242 in 100 cm³ soil for *Helicotylenchus*, *Criconebella*, *Hoplolaimus* and *Tylenchorhynchus*, respectively. Similar results were found in a study conducted in Oklahoma in which *Criconebella*,

Tylenchorhynchus, *Paratrichodorus* and *Helicotylenchus* were present in 59%, 48%, 57%, and 32%, respectively of 99 samples collected from putting greens (Walker *et al.*, 2002). Mean nematode populations in this study were 968, 460, 89 and 727 per 100 cm³ soil for *Criconebella*, *Tylenchorhynchus*, *Paratrichodorus* and *Helicotylenchus*, respectively.

Nematodes can reduce plant vigour, stand density and, in the presence of high populations, cause plant death (DiEdwards, 1963; Winchester & Burt, 1964; Johnson & Powell, 1968; Johnson, 1970; Lucas *et al.*, 1974; Murdoch *et al.*, 1978; Todd & Tisserat, 1990; Dunn & Noling, 1997). The damaged areas often require more management inputs to maintain an acceptable putting surface and to prevent undesirable algae formation or weed encroachment. As damage to turf becomes more severe, the green may fail to provide an acceptable putting surface at which point management practices which reduce nematode populations are desired. Most petroleum-derived nematicides are no longer available on the market due to the potential for these compounds to contaminate drinking water. Currently, only a few nematicides are available for nematode management, of which only a single product, fenamiphos, is labelled for use in the USA on existing greens. Fenamiphos is scheduled to be removed from the

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market by 2007 due to environmental concerns and risks to the applicator. Unfortunately, new or alternative nematicides for use on turfgrasses have not proven to be very effective (Dunn, 1999).

One possible replacement for nematicides could be the incorporation of antagonists into the soil environment of putting greens. Studies with nematophagous fungi have indicated reductions in plant-parasitic nematodes when examined in a few cropping systems. For example, *Arthrobotrys dactyloides* Drechsler suppressed *Meloidogyne javanica* (Trebb) Chitwood juveniles in greenhouse studies on tomato (Stirling & Smith, 1998; Stirling *et al.*, 1998*a,b*). In addition, *in vitro* experiments 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* Travassos, a gastrointestinal parasite of cattle, indicated that *A. oligospora* was effective at reducing populations of nematodes (Gomes *et al.*, 2000). Since *Arthrobotrys* spp. are common inhabitants of soils worldwide (Stirling, 1991) they may be ideally suited for persistent survival in the green environment at populations which can suppress plant-parasitic nematodes.

Incorporation of fungal biological antagonists in putting greens may be most efficacious when nematode populations are at their lowest and microbial antagonistics of the biocontrol agents may not be very active. This optimum period occurs in early spring when soil temperatures are cool in contrast to late summer when soil temperatures range between 20°C and 30°C and nematode populations are usually greatest. In an unreported preliminary study, plots were established on a golf course putting green located at a public course in Tulsa, Oklahoma. The plots were inoculated in the spring at a rate of 5 and 10 conidia of *A. oligospora* per g soil using a syringe. Nematode populations were determined on a monthly basis following inoculation. No reduction in nematode populations was observed. During spring months, the widespread and damaging fungal disease, dollar spot, caused by *Sclerotinia homoeocarpa* Bennett, occurs on bentgrass greens. This disease is aggressively managed through the use of numerous fungicide applications because non-pesticide based control measures are often ineffective at suppressing the disease. Smitley & Rothwell (2003) have suggested that the widespread use of the fungicide chlorothalonil on Michigan golf courses may not adversely effect *Paenibacillus* sp., a fungal antagonist of *Ateanius spretulus* (Coleoptera: Scarabaeidae); however, the impact of fungicides on *A. oligospora* is not known and might account for the absence of fungal activity observed in field plots.

The objectives of this study were to evaluate the tolerance of *A. oligospora* to two fungicides

commonly used on putting greens, the concentration of these fungicides in simulated putting green soils, and the potential of *A. oligospora* to suppress the common ring nematode *Criconemella ornata* at practical infestation rates.

Materials and Methods

In vitro tolerance assay

To determine the effects of the fungicides chlorothalonil (Daconil Ultrex 82.5WDG, Syngenta Crop Protection, Greensboro, NC) and myclobutanil (Eagle 40WP Dow Agrosociences LLC, Indianapolis, IN) on *Arthrobotrys oligospora* Fresenius ATCC 16234 (American Type Culture Collection, Manassas, VA), an *in vitro* Petri plate assay method was used. Fresh hyphal plugs (1-cm-diameter) obtained from the leading edge of 10-day-old *A. oligospora* cultures were placed at the centres of plates containing water agar (Moorehead & Co. Inc., Van Nuys, CA) and potato dextrose agar (9.25 g L⁻¹ water) (Sigma Chemical, St. Louis, MO) (quarter strength potato dextrose agar). Agar was amended with fungicides after autoclaving to provide final concentrations of 1, 10 or 20 mg kg⁻¹ a.i. chlorothalonil or myclobutanil. Non-amended quarter strength potato dextrose 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 and averaged. Plates containing all concentrations of both fungicides were arranged in a randomised complete block design (seven plates total) with ten replications of each block. This experiment was terminated after seven days and was repeated once.

A similar assay was used to determine the effects of the fungicides on *A. oligospora* conidia. Conidia were flood harvested from 10-day-old PDA plates and separated from mycelium using 425–600 µm glass beads (Sigma Chemical, St. Louis, MO) placed in a conical centrifuge tube and vortexed for 1 min to remove conidia from the conidiophores. Mycelium was separated from the suspension using a 400 µm sieve and conidia were quantified using a haemocytometer. A 100 µL suspension containing 100 *A. oligospora* conidia was transferred on to plates containing 0.001, 0.01, 0.1, 1.0 or 10 mg kg⁻¹ of chlorothalonil or 0.1, 1.0, 3.0, 5.0, 7.0, or 10 mg kg⁻¹ myclobutanil in quarter strength potato dextrose agar. Non-amended quarter strength potato dextrose agar was used as a control. Plates were incubated at 17 ± 2°C and fungal colonies were counted after 3 days using a compound microscope. Plates containing all concentrations of both fungicides were arranged in a randomised complete block design (12 plates total) with 10 replications of each block and the experiment was repeated once. The effective concentrations at which hyphal growth and spore

germination were inhibited by 50% (EC_{50}) were determined by plotting the percent mortality, derived from a probit table vs fungicide concentration.

Greenhouse studies

Individual greenhouse studies were conducted to determine the concentrations of chlorothalonil and myclobutanil in simulated putting green soil, to determine if low populations of *A. oligospora* would affect populations of *C. ornata*, and to determine the effect of fungicides on *A. oligospora*. Creeping bentgrass (*A. palustris* cv. SR1020, Seed Research of Oregon Inc., Corvallis, OR) was planted at a rate of 25 seeds per cm^2 in plastic pots (10.5 cm \times 10.5 cm \times 9.5 cm high) containing 1100 g of autoclaved sand. Square plastic baffles (8.0 cm \times 8.0 cm \times 1.0 cm high) were placed in the bottom of each pot. A 10 cm \times 10 cm piece of geotextile fabric (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 during the autumn and spring of 2002 and 2003, respectively. Plants were irrigated with a slow drip emitter that was placed into the side of the pot 4 cm below the top of the pot. Turf was fertilised with water-soluble fertiliser (Peters Professional All Purpose Plant Food, St. Louis, MO) at a rate of 14.8 kg N ha^{-1} per month and maintained at a height < 1 cm.

Arthrobotrys oligospora inoculum was obtained from colonies grown on potato dextrose agar as previously described. Conidia were quantified using a haemocytometer. Fifty days after planting, pots were inoculated with 10 mL water containing *A. oligospora* conidia to a final concentration of 20 cfu per g soil. Non-inoculated pots used as controls received 10 mL of water.

Criconebella ornata inoculum 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 flotation (Ayoub, 1980). Nematodes were quantified using a compound microscope. Fifty days after planting, pots were inoculated with 10 mL water containing 100 adult *C. ornata*. Non-inoculated pots were used as controls and received 10 mL of water.

Ninety days after planting, pots receiving fungicide applications were treated with chlorothalonil at 93.15 g a.i. per 100 m^2 and myclobutanil at 7.32 g a.i. per 100 m^2 . Three applications of chlorothalonil or myclobutanil were conducted on a 14- or 21-day interval, respectively. Fungicides were applied using a CO_2 pressurised (1.24×10^5 Pa) wheelbarrow plot sprayer calibrated to deliver 887 L ha^{-2} . Pots treated with fungicides were watered overhead for 1 min for 3 consecutive

days after each fungicide application.

Non-fungicide treated pots were used as controls for fungicide observations. Pots (eight in total) were arranged in a randomised complete block design. Studies with both fungicides were conducted at the same time and placed on the same greenhouse bench. To avoid cross contamination, the studies were separated by 30 cm. The experiment was repeated once with five replications of each block used in the first trial and 10 replications of each block in the second trial.

The study was terminated 130 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 one to five, where one equals thin turf and five equals thick, healthy turf. A 1-cm-diameter soil core was removed from each pot and roots were separated from the soil and rated for discoloration using a colour scale from one to seven, where one equals brown roots and seven equals white roots (PPG Architectural Finishes, Inc. color code 315). To determine nematode populations, a 550 g soil sample was extracted by sieving and centrifugal flotation (Ayoub, 1980) and populations were determined using a compound microscope.

Soil fungicide concentrations were determined from five pots treated with chlorothalonil or myclobutanil. Five, 1-cm-diameter soil cores were removed from each pot to a depth of 4.5 cm and mixed thoroughly and stored at 4°C prior to extraction. Chlorothalonil was extracted from a 10 g composite soil sample using an EPA protocol modified to remove soil solids (Anon., 1988). A protocol obtained from Dow Agrosiences, LLC (formerly Rohm and Haas, Inc., Philadelphia, PA) was modified to determine myclobutanil concentrations. The fungicide was extracted from a 10 g composite soil sample in methanol. The extract was filtered using 0.2 μm Whatman filter paper (Whatman International Ltd., Maidstone, UK) and rinsed with 5 mL of methanol and mixed with 130 mL of hexane : 2% aq sodium chloride (10 : 3, wt/wt). The aqueous layer was mixed with 200 mL of 2% aq NaCl : methylene chloride (1 : 1). To remove excess water, samples were passed through a funnel containing 20 g of anhydrous sodium sulfate. After the water was removed, the funnel was rinsed with 5 mL methylene chloride and collected in a 250 mL beaker and evaporated to dryness. The sample was rehydrated with 20 mL methanol : toluene (1 : 99, v/v) and transferred to a silica packed column. Twenty mL of methanol : toluene (1 : 99, v/v) were added to the column and discarded. Myclobutanil was eluted from the column using 40 mL of methanol : toluene (2 : 98, v/v). Samples were placed in a double boiler, evaporated to dryness, and the residue dissolved in acetone.

A Model 6890 gas chromatograph equipped with a Model 5073 Mass Spectrometer detector (Hewlett Packard, Los Angeles, CA) was used for fungicide detection and quantification. One μL was manually injected into a splitless injector maintained at 260°C . The column was a DB-5 (J&W Scientific, Folsom, CA) fused, silica capillary column ($30\text{ m} \times 0.25\text{ mm}$ i.d.; $0.25\text{ }\mu\text{m}$ film); the operating temperature was 60°C to 260°C increasing 6°C min^{-1} . Helium was used as a carrier at a velocity of 1 mL min^{-1} (Mogadati *et al.*, 1999). The mass spectrometer was operated in selected ion monitoring mode.

All data were analysed using the general linear model procedure (PROC GLM) of SAS (Version 8; SAS Institute, Cary, NC). Data from trials with the same fungicide were combined to identify significant effects of trials, treatments, or interactions; means were combined when no significant interactions were found ($P < 0.05$).

Results

In vitro tolerance assay

Hyphal growth of *A. oligospora* was reduced for all concentrations of both fungicides (Fig. 1). At equivalent concentrations, myclobutanil was superior to chlorothalonil though at the higher concentrations of 10 and 20 mg kg^{-1} the differences were less marked. A significant reduction in the germination of *A. oligospora* conidia was observed for chlorothalonil of 0.1 mg kg^{-1} and above and totally ceased at concentrations 1.0 mg kg^{-1} and above (Fig. 2). Germination of conidia on plates amended with myclobutanil was significantly reduced at concentrations greater than 1.0 mg kg^{-1} and ceased at concentrations of 5.0 mg kg^{-1} and above. The EC_{50} for *A. oligospora* hyphal growth was 7.7 mg kg^{-1} and 4.02 mg kg^{-1} for chlorothalonil and myclobutanil, respectively. The EC_{50} for *A. oligospora* conidia germination was 0.20 mg kg^{-1} and 2.50 mg kg^{-1} for chlorothalonil and myclobutanil, respectively.

Greenhouse studies

No interaction was present between studies conducted with either fungicide ($P \leq 0.05$), therefore the trials with the same fungicide were combined for analysis. *Arthrobotrys oligospora* did not significantly reduce nematode populations (Table 1). Populations of nematodes from pots inoculated with *A. oligospora* and treated with chlorothalonil were lowest at 202 nematodes per pot while pots treated only with chlorothalonil were greatest at 370 nematodes per pot. Similarly, nematode populations were not affected in pots inoculated with *A. oligospora* and treated with myclobutanil. Nematodes in pots inoculated with *A. oligospora* treated with myclobutanil were lowest at 196

nematodes per pot while pots containing the nematode only were highest with 343 nematodes per pot.

None of the treatments in the studies conducted with chlorothalonil affected turfgrass quality. Quality was numerically highest for the non-treated control and lowest in pots inoculated with *C. ornata* (Table 2). Leaf clipping weight was not affected by any treatment; however, clipping weight was greatest in pots inoculated with *C. ornata*. Although no

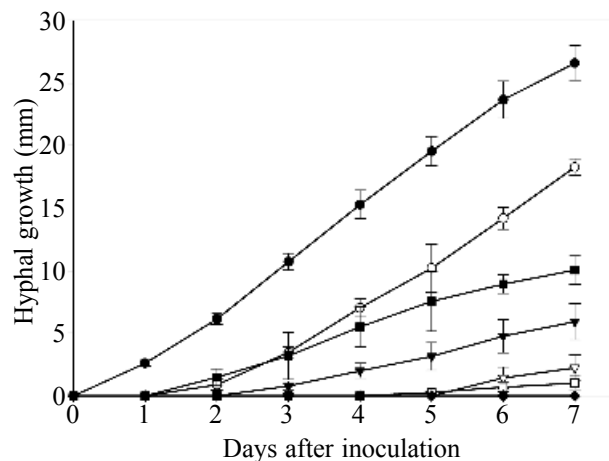


Fig. 1. Growth of *Arthrobotrys oligospora* mycelium on quarter strength potato dextrose agar amended with chlorothalonil or myclobutanil. Each point represents a mean of two trials ($n = 20$) \pm SE. Error bars not shown if smaller than the mean indicator. ●, Non-amended control; ○, 1 mg kg^{-1} chlorothalonil; ▼, 10 mg kg^{-1} chlorothalonil; ▽, 20 mg kg^{-1} chlorothalonil; ■, 1 mg kg^{-1} myclobutanil; □, 10 mg kg^{-1} myclobutanil; ◆, 20 mg kg^{-1} myclobutanil.

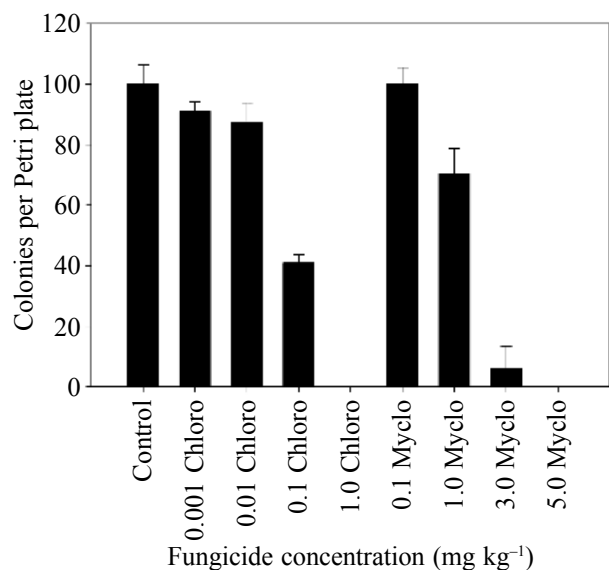


Fig. 2. Germination of *Arthrobotrys oligospora* conidia on quarter strength potato dextrose agar amended with chlorothalonil or myclobutanil. Data are the means of two trials with 10 replications each. Bars represent the standard error.

treatment was observed to affect root colour, roots from non-treated control pots were noted to be the whitest.

A similar trend was observed for studies conducted with myclobutanil. None of the treatments affected turfgrass quality; however in contrast to the chlorothalonil study, quality was numerically highest for pots inoculated with *C. ornata* and lowest in the non-treated control (Table 3). Leaf clipping weight was not affected by any treatment and was greatest in pots treated with myclobutanil and lowest in pots inoculated with *C. ornata*. No treatment was observed to affect root colour; roots from pots inoculated with *C. ornata* were noted to be the darkest.

Low concentrations of chlorothalonil were found in soils obtained from pots and averaged less than 5 mg kg⁻¹. Soil concentrations of myclobutanil in pots were below the minimum detection limit for the extraction techniques and equipment used in this study.

Discussion

The impending removal of the last effective cool-season turfgrass nematicide from the US market will mandate that alternative methods for nematode

Table 1. Populations of *Criconebella ornata* in soils inoculated with *Arthrobotrys oligospora* and treated with chlorothalonil or myclobutanil^a

Treatment	Numbers of <i>C. ornata</i> ^b	
	No Chlorothalonil	No Myclobutanil
<i>C. ornata</i>	271	343
<i>A. oligospora</i> – <i>C. ornata</i>	256	274
	Chlorothalonil	Myclobutanil
<i>C. ornata</i>	370	239
<i>A. oligospora</i> – <i>C. ornata</i>	202	196
SE (df)	100 (59)	50 (59)

^a For details see Materials and Methods

^b Nematode populations represent the entire pot

management be developed. These approaches must be effective and should integrate smoothly into existing turfgrass management programmes. In Oklahoma, the most common and populous nematode found in bentgrass putting greens is *C. ornata* (Walker *et al.*, 2002) and for this reason it was selected for inclusion in these studies. An *Arthrobotrys* sp. was selected since it has been suggested to suppress plant-parasitic nematodes in other annual cropping systems (Stirling & Smith, 1998; Stirling *et al.*, 1998a,b) and may have fewer restrictions to widespread use and commercialisation. The fungus does not preferentially trap specific nematodes and may be effective against the numerous nematode genera commonly present in putting greens. In addition, *A. oligospora* has been isolated worldwide and may be well adapted to survive in diverse environments such as putting greens. In preliminary studies, pots infested with 10 *A. oligospora* cfu per g soil yielded an average of 3.9 cfu per g soil after 80 days (J Woodward, unpublished data). Recovery of the fungus from soils in this study was not attempted because its survival was confirmed in previous greenhouse studies (J Woodward, unpublished data). The inoculum rate of *A. oligospora* used in this study was selected as a rate which may not adversely affect the physical composition of the putting green soil and may be realistically available to turfgrass managers if the organism was commercialised for the turfgrass industry.

The fungicides chlorothalonil and myclobutanil were chosen for evaluation in this study because they are representative of fungicides which are widely used on golf course putting greens. The rates selected represent the highest and lowest permissible rates for chlorothalonil and myclobutanil, respectively and represent the typical rates turfgrass managers use for dollar spot management. As expected, both fungicides had an adverse effect on *A. oligospora* hyphal growth and spore germination *in vitro*. Chlorothalonil, a protectant fungicide with

Table 2. Turfgrass quality and growth of creeping bentgrass affected by *Arthrobotrys oligospora*, *Criconebella ornata*, or treatment with chlorothalonil^a

Treatment	Turfgrass quality	Leaf weight (g)	Root colour
Non-treated control	4.03	0.432	3.67
<i>Arthrobotrys oligospora</i>	3.73	0.488	3.10
<i>Criconebella ornata</i>	3.45	0.588	3.50
<i>A. oligospora</i> – <i>C. ornata</i>	3.85	0.485	3.33
Chlorothalonil	3.96	0.535	3.37
<i>A. oligospora</i> + Chlorothalonil	3.93	0.541	3.40
<i>C. ornata</i> + Chlorothalonil	3.53	0.501	3.46
<i>A. oligospora</i> – <i>C. ornata</i> + Chlorothalonil	3.69	0.519	3.37
SE (df)	0.20 (119)	0.069 (119)	0.17 (119)

^a For details see Materials and Methods

Table 3. *Turfgrass quality and growth of creeping bentgrass affected by Arthrobotrys oligospora, Criconemella ornata, or treatment with myclobutanil^a*

Treatment	Turfgrass quality	Leaf weight (g)	Root colour
Non-treated control	3.07	0.606	3.43
<i>Arthrobotrys oligospora</i>	3.27	0.573	3.63
<i>Criconemella ornata</i>	3.28	0.498	3.37
<i>A. oligospora</i> + <i>C. ornata</i>	3.20	0.607	3.53
Myclobutanil	3.25	0.620	3.57
<i>A. oligospora</i> – Myclobutanil	3.25	0.531	3.40
<i>C. ornata</i> – Myclobutanil	3.15	0.553	3.46
<i>A. oligospora</i> + <i>C. ornata</i> – Myclobutanil	3.27	0.523	3.67
SE (df)	0.13 (119)	0.065 (119)	0.15 (119)

^a For details see Materials and Methods

multiple modes of action, primarily inhibits spore germination and protects plants from fungal infection. The inhibition of fungal germination was apparent at 1 mg kg⁻¹ and could be expected based on the protective action of this fungicide. Myclobutanil, a sterol biosynthesis inhibiting, systemic fungicide, has greater activity against fungal metabolism and subsequent growth. Myclobutanil suppressed hyphal growth at lower concentrations compared to chlorothalonil, but greater concentrations were required to suppress spore germination. Chlorothalonil concentrations obtained from greenhouse soil were low when compared to soil concentrations determined in other studies (Anon., 1987, 1988). In previous, short-term laboratory assays with simulated putting green soil, the recovery efficiency of myclobutanil from soil ranged from 81.3% to 88.6% using the method employed in this study (J Woodward, unpublished data). Although soil concentrations of myclobutanil have not previously been reported, these results support previous reports from laboratory studies demonstrating that myclobutanil may be moderately mobile in sandy soils (Anon., 1993b). The possible leaching of myclobutanil from pots in this study may be consistent with other studies which detected pesticides in leachate water from newly constructed, sand-based greens (Smith & Bridges, 1996) and may explain why it was not detected.

Plants used in this study were maintained at a low mowing height in order to simulate stresses incurred on actual putting greens. Low plant height and related stresses may contribute additively to the damage caused by nematodes and could be masked by taller turf. However, no differences in plant growth were observed in this study. This was not expected because the nematode population selected is representative of early season populations and this is below the threshold for bentgrass. The reduction in nematode populations is most desirable in early spring when populations are low and this would reduce the damage to turfgrass roots over the

growing season. Plant growth information was also of interest to ensure that the inoculation of pots with the fungus would not adversely affect turfgrass quality, and in this case it did not.

Creeping bentgrass, when grown in environments high in humidity and temperature, such as a greenhouse, will often succumb to numerous foliar diseases. In an attempt to exclude foliar disease and the subsequent undesirable use of additional fungicides, a sub-surface watering system was developed in this study. This watering system provided adequate water to the entire pot while avoiding the presence of leaf moisture required by many foliar diseases. This watering approach may be of interest to researchers conducting greenhouse studies with turfgrasses where the use of fungicides is not desired.

No significant reduction in nematode populations was observed in this study; however, populations of *C. ornata* were consistently lower in pots inoculated with *A. oligospora* which received fungicides. The results of this study suggest that despite the *in vitro* findings, the mycelium of *A. oligospora* may not be adversely affected by fungicides used on greens to manage spring diseases. Previous studies have used much higher soil inoculation rates of *A. oligospora* (Stirling & Smith, 1998; Stirling *et al.*, 1998a,b) and the inoculation rate used in this study may have been insufficient in dramatically suppressing nematode populations; however, commercialisation of *A. oligospora* would require a rapid method for inoculation of putting greens at rates which do not alter the performance of the green system. Since the fungus forms numerous asexual conidia in culture, these may be used to repeatedly inoculate putting greens, at low rates, during normal, routine maintenance activities such as core aeration and sanding. The use of conidia may avoid the potential problems associated with using large quantities of mycelium as a soil inoculum which have been used in other studies (Stirling & Smith, 1998; Stirling *et al.*, 1998a,b). Future studies which examine the

effect of repeated applications of *A. oligospora* conidia to greens on nematode populations would ascertain the potential long term benefits of this biologically based approach to nematode suppression.

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