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Identification and Management of Moss and Phytopathogenic Algae Common on Creeping Bentgrass Putting Greens

Steven Michael Borst
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Steven Michael Borst entitled "Identification and Management of Moss and Phytopathogenic Algae Common on Creeping Bentgrass Putting Greens." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Plant Sciences.

J. Scott McElroy, Major Professor

We have read this thesis and recommend its acceptance:

John C. Sorochan, Steven W. Wilhelm

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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

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IDENTIFICATION AND MANAGEMENT OF MOSS AND PHYTOPATHOGENIC ALGAE COMMON ON CREEPING BENTGRASS PUTTING GREENS

A Thesis
Presented for the
Masters of Science
Degree
The University of Tennessee, Knoxville

Steven Michael Borst
August 2008

DEDICATION

I dedicate this thesis to my wife Golda, my parents Gina and Steve, my brothers Justin and Stewart and the rest of my loving family. They mean the world to me and I cannot thank them enough for all of their love and support.

"Besides pride, loyalty, discipline, heart,

and mind, confidence is the key to all the locks."

-- Joe Paterno

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ABSTRACT

Taxonomic traits were utilized to identify problematic moss species common to golf course putting greens. Three predominant species of moss were identified on two golf course putting greens located in East Tennessee. *Bryum argenteum*, *Amblystegium serpens* and *Entodon seductrix* were identified on creeping bentgrass putting greens. Green house studies were initiated to investigate all three moss species control with carfentrazone and mancozeb. Utilizing digital image analysis investigations concluded carfentrazone controlled all three moss species greater than mancozeb. Sequential carfentrazone applications controlled all three moss species greater than single applications. Moss recovery and regrowth was observed with carfentrazone.

Field studies were initiated to evaluate *Bryum argenteum* control utilizing mancozeb, carfentrazone, and cultural practices. Cultural practices improved carfentrazone long term efficacy. Carfentrazone controlled *Bryum argenteum* greater than mancozeb. Similar *Bryum argenteum* control was observed with cultural practices alone and carfentrazone alone. *Bryum argenteum* recovery was observed with carfentrazone alone treatments. Mancozeb and non-treated plots increased in *Bryum argenteum* populations.

A common problematic species of cyanobacteria was identified on three golf courses all located near Knoxville, TN. Isolates were identified genetically and compared to other similar isolates. The Tennessee cyanobacteria isolate had a 94 % match to a *Phormidium murryi*, a filamentous mat forming cyanobacteria. The isolate was then subjected to a ten day *In vitro* screen determining copper and zinc toxicity levels. Both copper and zinc killed the Tennessee cyanobacteria isolates at 3.2 micromole concentrations. Both zinc and copper at 0.6 micromole concentrations increased the Tennessee cyanobacteria isolates growth when compared to the non-treated.

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Literature Review

Introduction

Moss and algae are persistent problematic weeds in creeping bentgrass (*Agrostis stolonifera*) golf course putting greens (Burnell et al. 2004; Danneberger and Taylor 1996; Elliot 1998; Happ 1998; Hummel 1986, 1994; Maddox et al. 2001; Maddox et al. 1999; Nelson 2007; Snow 1984). Maintaining competitive golf course putting greens can impose stress on a turf stand making it weak and a desirable habitat for phytopathogenic algae and moss encroachment. The demand for faster, more competitive putting green surfaces has added additional abiotic stresses to naturally occurring biological pressure. Previous research has indicated that reduced mowing heights, low fertility, water status, and the discontinued use of mercury-based fungicides can be attributed to the increased encroachment of each organism (Baldwin and Whitton 1992; Boeshch and Mikowski 2005; Burnell et al. 2004; Hummel 1994). Both organisms can disrupt putting greens playing surfaces, drainage, and aesthetics. Phytopathogenic algae and mosses are organisms that have not been considered common weed problem in the past. Increases in appearance and problems associated with these organisms however, has created a demand for new and improved control strategies as well as a better understanding of their role in a golf course putting green microenvironment.

Moss encroachment

Moss infestations on creeping bentgrass (*Agrostis stoloniferia* L.) putting greens are increasing problem (Burnell et al. 2004; Danneberger and Taylor 1996; Happ 1998; Hummel

1986, 1994; Nelson 2007; Snow 1984). Mosses are able to reproduce sexually via spores or asexually from displaced fragments (Nelson 2007; Boeshch and Mikowski 2005; Yelverton 2005). Mosses lack vascular systems and can absorb water and minerals throughout the entire plant (Boeshch and Mikowski 2005). They lack root systems and utilize rhizoids to adhere to substrates (Shaw and Goffinet 2000). Several different moss species have been identified on golf course putting greens; *Byrum lisae*, *Amblystegium trichopodium*, *Brachytheciium spp*., and *Bryum argenteum* (Happ, 1998; Yelverton 2005). *Bryum argenteum* is the most common species found on golf course putting greens and has been deemed the most problematic. (Burnell et al. 2004; Danneberger and Taylor 1996; Happ 1998; Yelverton 2005). *Bryum argenteum* also known as silvery-thread moss is a cosmopolitan moss found on many continents including Antarctica (Boeshch and Mikowski 2005; Skotnicki 1998). The *Bryum* family of mosses has adapted to survive in full sun, long periods of drought and cold environments making them a versatile weed on golf course putting greens (Weber and McAvoy 2003). Moss encroachment on putting greens can reduce turfgrass vigor, create a non-uniform putting surface and disrupt the visually aesthetics of a golf course putting green (Burnel et al. 2004; Happ 1998)

Moss control

Mosses lack phloem and xylem therefore, cannot translocate foliar-absorbed nutrients throughout the entire plant (Boeshch and Mikowski 2005; Cook et al. 2002). This lack of a vascular system makes the translocation of systemic herbicides a potential problem (Yelverton 2005). Golf course superintendents have tried numerous products aside from labeled chemicals for the control of *Bryum argenteum*. Products investigated include chlorothalonil fungicide,

ferrous and ferric sulfates, sodium carbonates such as baking soda, sodium carbonate peroxyhytests and Dawn® dish detergent. Recently carfentrazone, a protoporphyrinogen oxidase inhibitor, utilized for the control of a wide range of broadleaf weeds was labeled for the control of *Bryum argenteum* on creeping bentgrass putting greens (Anonymous 2005; Senseman 2007).

Previous research indicated that the discontinued use of mercury-based fungicides can be attributed to the increased moss encroachment (Boeshch and Mikowski 2005; Burnell et al. 2004; Hummel 1994). The sequence of metal toxicity for *Bryum argenteum* has been identified as; Hg>Cu>Pb>Ni>Cd>Zn>Mg (Weber and McAvoy, 2003). With the loss mercury, other metal ions have been researched for moss control, specifically copper. Mancozeb plus copper hydroxide (mancozeb) is a coordination product of zinc ion and manganese ethylenebisdithiocarbamate. Copper hydroxide makes up 46.1% (30% metal equivalent) of the total formulation (Anonymous 2006). The highest recommended labeled rate of 12.2 kg/ha of product weekly would apply copper at an equivalent of 5.6 kg cu/ha (Anonymous 2005). This high rate of copper accumulating in a golf course putting green could potentially induce iron deficiency in turfgrass plants (Boeshch and Mikowski 2005; Landschoot et al. 2004). Along with chemical control, cultural practices such as aerification, light and frequent topdressing, and the removal of thatch could also potentially aide in control (Happ 1998). Limited knowledge of species diversity and refereed publications evaluating chemical and potential cultural control has postponed the advancement towards achieving moss control on golf course putting greens

Phytopathogenic algae encroachment

Blue-green algae (cyanobacteria) and Eukaryotic algae that have been attributed to turfgrass decline can be a persistent weed and pathogen commonly found on golf course putting greens (Baldwin and Whitton 1992; Elliot 1998; Maddox et al. 2001; Maddox et al. 1999). Both of these groups have been grouped together as "Algae" when referenced in some trade journals and books (Nus, 1994; Turgeon and Vargas 2006). However; problems associated with turfgrass decline such as subsurface black layer, surface slime mats, filamentous binding of soil particles and Yellow spot disease have been attributed to cyanobacteria (Baldwin and Whitton 1992; Elliot 1998; Hodges and Campbell1998, 1997; Tredway et al. 2006). Though other algae and microfauna could potentially be aiding, all the characteristics of the problems associated with putting greens and turf decline are characteristics of filamentous cyanobacteria encroachment. Specific cyanobacteria species attributed to turf decline that have been identified as *Phormidium* and *Oscillatoria* (Tredway et al. 2006). However, because of the diversity in soils other cyanobacteria species may be involved with encroachment problems. Cyanobacteria are attributed to four problems with golf course putting greens; surface slime mats, subsurface black layer, reduced water infiltration and Yellow spot disease (Baldwin and Whitton 1992; Elliot 1998; Hodges and Campbell1998, 1997; Tredway et al. 2006).

Surface slime mats have been described generally as scum or crust layers that range in color from green, brown, or black (Baldwin and Whitton, 1992). These mats disrupt the playing surface and create a soil medium unsuitable for bentgrass growth. Surface mats are a result of excreted extracellular polymeric substances that are composed of polysaccharides utilized for the creation of capsules and sheaths to protect individual cells (Decho 1990; Stal 1995). When these

slime mats dry they can creates a crust layer that is impermeable to water, limiting bentgrass growth (Turgeon and Vargas 2006). Filamentous cyanobacteria, through mats and fibrous growth, can clog soil pores impede water infiltration and cause anaerobic conditions making the sand medium susceptible to subsurface black layer (Baldwin and Whitton 1992; Elliot 1998; Hodges and Campbell1998, 1997; Stal 1995). Hodges and Campbell (1997) concluded that subsurface black layer was a direct result of an interaction between cyanobacteria and a nitrogen-fixing bacteria *Desulfovibrio desulfuricans*.

Phytopathogenic algae control

Various fungicide/algaecides are labeled for the control of phytopathogenic algae on golf course putting greens. Mancozeb and chlorothalonil are both labeled for phytopathogenic algae on creeping bentgrass putting greens. According to Dernoeden and Shmitt, (1992), chlorothalonil controls phytopathogenic algae effectively at a variety of formulations. Elliot (1998) also concluded that there was no cyanobacteria control difference between chlorothalonil formulations with or without zinc or mancozeb formulations with or without copper. Research also indicated that chlorothalonil and mancozeb were effective as a preventative but not as a curative treatment (Elliot 1998). Both chemistries have a variety of formulations that include micronutrients such as copper, zinc, and maganese for added efficacy.

Copper and zinc are vital micronutrients which play important roles in many algae enzyme systems (Franklin et al. 2002). Copper in trace amounts participates in biological reactions as an enzymatic cofactor and electron carrier in the photosynthetic process (Andrade et al. 2004; Franklin et al. 2002). The copper protein plastocyanin is involved in the electron

transport of PSI in photosynthesis (Carrow et al. 2001). Zinc in low amounts is utilized to maintain the stability of cell membranes (Stauber and Florense 1990). Zinc can be utilized as a structural component to many different enzymes that act as antioxidants that detoxify free oxygen radicals produce during photosynthesis (Carrow et al. 2001) At higher concentrations however, both copper and zinc inhibit algal growth and can be toxic (Franklin et al. 2002; Cavet et al. 2003; Stauber and Florence 1990, 1987). Copper sulfate is utilized as a common algaecide in aquatic environments such as catch basins and golf course water retention ponds (Carson 2001).

Elliot (1998) reported that both chlorothalonil and mancozeb applied were effective as a preventative but not as a curative treatments. Both copper and zinc are important for sustaining healthy turfgrass (Carrow et al. 2001). Numerous fungicide/algaecide applications to golf course putting greens can be expensive. Appropriate micronutrient levels could be maintained to achieve healthy turf and potentially aide with cyanobacteria control. This potentially could lower the number of required fungicide applications and lower costs for turfgrass managers. For this to be achieved toxic levels for golf course green specific cyanobacteria needs to be assessed.

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I. Response of Three Moss Species Common in Creeping Bentgrass Putting Greens to Carfentrazone and Mancozeb

Abstract

Carfentrazone and mancozeb based fungicides (mancozeb; containing a combination of zinc ion, manganese ethylenebisdithiocarbamate, and copper hydroxide) are utilized to control *Bryum argenteum* in creeping bentgrass (*Agrostis stolonifera* L.) putting greens. *Bryum argenteum* is the primary moss species infesting putting greens; however, two other species, *Entodon seductrix*, and *Amblystegium serpens*, have also been identified infesting golf course putting greens but little is known about control of these species. Greenhouse studies were initiated to evaluate *Bryum argenteum*, *Entodon seductrix* and *Amblystegium serpens* response to applied carfentrazone and mancozeb. Carfentrazone (0.06 and 0.12 kg/ha) and mancozeb (0.9 and 1.8 kg/ha) were applied singly [0 days after initial treatment (DAIT)] and sequentially (0 and14 DAIT) to greenhouse grown plugs of the three moss species. Carfentrazone controlled *Bryum argenteum* greater than *Entodon seductrix* and *Amblystegium serpens*. Carfentrazone applied sequentially at 0.12 kg/ha controlled *Bryum argenteum* greater than single applications. Mancozeb did not control any of the three moss species. All treatments however, observed a green cover reduction relative to the non-treated as analyzed with digital image analysis (DIA). Carfentrazone applied at 0.12 kg/ha sequentially reduced *Bryum argenteum* and *Amblystegium serpens* percent green cover greater than all other treatments. Variation in moss control on putting greens between different golf courses could potentially be attributed to differences in moss species. Mancozeb and carfentrazone did not control *Entodon seductrix* or reduce green color.

Introduction

Moss encroachment on creeping bentgrass (*Agrostis stoloniferia* L.) putting greens are increasing problem (Burnell et al. 2004; Danneberger and Taylor 1996; Happ 1998; Hummel 1986, 1994; Nelson 2007; Snow 1984). Increased stressed for a more competitive playing surface, reduced fertility, and restriction of mercury-based pesticides are all attributed to the increase in infestation (Burnell et al. 2004; Hummel 1994). *Bryum argenteum* (Hedw.), known as silvery-thread moss, is a common species found on creeping bentgrass putting greens; however, other species such as *Byrum lisae*, *Amblystegium trichopodium* and *Brachythecium* spp. have also been identified (Happ 1998). *Bryum argenteum* can be found in many environments such as concrete surfaces, tree roots, compacted soils, and cool damp soils, indicating they are adaptable to many environments (Boeshch and Mikowski 2005). Mosses are a member of Phylum Bryophyta and are able to reproduce sexually via spores or asexually from displaced fragments (Nelson 2007; Boeshch and Mikowski 2005; Yelverton 2005). They lack vascular systems and can absorb water and minerals throughout the entire plant (Boeshch and Mikowski 2005).

Carfentrazone was recently labeled for the control *Bryum argenteum* on creeping bentrgrass putting greens (Anonymous 2005). It has quickly become the industry standard for *Bryum argenteum* control in creeping bentgrass putting greens. Numerous other non-peer reviewed trade journals have also concluded that carfentrazone provides *Bryum argenteum* control over a broad temperature spectra (Anonymous 2005; Aylward 2007; Boeshch and Mikowski 2005; Nelson 2007; Settle et al. 2006; Yelverton 2005). Despite all of these reports,

no peer reviewed publications are currently available evaluating carfentrazone control of *Bryum argenteum*.

Mancozeb based fungicides are also labeled for control of silvery-thread moss on creeping bentgrass putting greens. Mancozeb is a combination product of zinc ion, copper hydroxide, and manganese ethylenebisdithiocarbamate. The main chemical component of mancozeb is copper hydroxide which makes up 46.1% (30% metal equivalent; Anonymous 2006). Landschoot et al. (2004) investigated mancozeb and concluded that summer treatments did not control *Bryum argenteum*; however, fall applications of 15.3 kg/ha applied biweekly with 5 applications provided 100% moss control. Cook et al. (2002) concluded that five applications of mancozeb applied at 4.85 to 7.27 kg Cu/ha biweekly controlled *Bryum argenteum* for up to two years. The highest recommended labeled rate of 12.2 kg/ha of product weekly would apply copper at an equivalent of 5.6 kg Cu/ha or mancozeb combination products of zinc ion, manganese, and ethylenebisdithiocarbamate at1.8 kg/ha (Anonymous 2005). These rates of copper accumulating in the soil medium however, could potentially induce iron deficiency in turfgrass plants (Boeshch and Mikowski 2005; Landschoot et al. 2004). Landschoot et al. (2004) observed creeping bentgrass injury with multiple applications of mancozeb and attributed it to copper-induced iron chlorosis.

Numerous other products and homemade concoctions have been tested for *Bryum argenteum* control. Such products include chlorothalonil fungicide, ferrous and ferric sulfates, sodium carbonates such as baking soda, sodium carbonate peroxyhytests and Dawn® dish detergent (Boeshch and Mikowski 2005; Burnell et al. 2004; Landschoot et al. 2004; Turgeon and Vargas 2006). These treatments are highly variable and often ineffective compared to

carfentrazone and mancozeb. In addition, little is known regarding the response of other moss species to these control agents. Research was conducted to evaluate the response of three moss species collected from golf course putting greens in Tennessee to carfentrazone and mancozeb.

Materials and Methods

General Information. A replicated greenhouse study was conducted at the University of Tennessee, Knoxville, to evaluated carfentrazone and mancozeb control of *Bryum argenteum*, *Amblystegium serpens*, and *Entodon seductrix*. Moss species were collected from two sites: Gettysview Country Club in Knoxville, TN, and Gatlinburg Country Club in Pigeon Forge, TN. Moss samples were taxonomically identified at the University of Tennessee Herbarium. Separate species were then vegetatively propagated in 10 cm diameter pots diameter pots (0.5L volume), and grown in 80:20, sand to reedsedge[1] peat mix. Plants were overhead irrigated twice daily and greenhouse temperatures were maintained at an average temperature of 24 ºC (±3°).

Research was conducted in a 3 by 9 factorial design and replicated four times. Factors included three moss species (*Amblystegium serpens, Bryum argenteum, Entodon seductrix* by nine herbicide treatments. Treatments included: Carfentrazone[2] at 0.06 and 0.12 kg/ha singly (0 days after initial treatment (DAIT) and sequentially (0 and 14 DAIT); Mancozeb[3] was applied at 0.9 and 1.8 kg/ha singly (0 DAIT) and sequentially (0 and 14 DAIT). Applications were applied

[1] Reedsedge Peat Dakota Peat & Equipment PO Box 14088 Grand Forks, ND 58202

[2] Quicksilver® herbicide. FMC Corporation Agricultural Product Group, Philidelphia, PA 19103

[3] Junction® SePRO Corporation 1550 N Meridian Street, Suite 600 Carmel, IN 46032

with a CO_2 pressurized back pack sprayer calibrated at 280 L/ha with 8002 XR flat fan nozzles[4]

Data Collection. Digital images were taken on each moss visual evaluation date (14, 21, 28 DAIT) of each experimental unit utilizing a digital camera[5]. Pictures were taken 30 cm above each pot and a red cover with a 10 cm diameter hole was utilized to allow only moss green color to be analyzed and to exclude other outside image contaminants. Digital images were downloaded to a personal computer. Each image was standardized to (600 x 800 pixels) and analyzed with SigmaScan Pro[6]. SigmaScan Pro quantifies standard red, green, blue (RGB) chroma pixel values each on a scale of 0- 255 (Karcher and Richardson 2003). SigmaScan coverts RGB values to hue, saturation, and brightness providing a quantitative color digital image analysis for each. Only green color was being analyzed therefore hue angles and percent saturation were adjusted to capture this specific data (Hue angle 30-121°; Saturation 0-99%).

Hue is defined as an angle on a continuous circular scaled from 0 to 360° (0° = red, 60° = yellow, 120° = green, 240° = blue 300° = magenta) (Karcher and Richardson 2003). Saturation [(0% gray) to (100% fully saturated) color] is a measurement of the purity of the color. Utilizing hue, saturation, and determining the amount of green color pixels, percent green cover can be assessed for a given moss sample (green scanned pixels/total pixels = % green cover) (Richardson et al. 2001). Optimum he and saturation values have been evaluated and applied for specific turfgrass cultivars (Karcher and Richardson 2003). However, this evaluation has not been applied to moss therefore, all DIA ratings will be compared to the non-treated check.

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[4] 21 PSI

[5] Cannon Power Shot A510 Canon Inc., Tokyo, Japan

[6] SigmaScan Pro v. 5.0, SPSS, Inc.,Chicago, IL

Moss species were evaluated on a 0 (no moss control, no burned tissue) to 100 (total moss control, total burned tissue) percent scale and was evaluated 14, 21, and 28 (DAIT). Visual color was the main component for these visual ratings. This is a common scale utilized in turfgrass weed control research however, these data are subjective and human bias is difficult to remove (Karcher and Richardson 2003). We therefore, utilized image analysis and visual ratings as dual methods to evaluate moss response to the treatments.

All data were subject to ANOVA ($P = 0.05$). Data were analyzed according to the factorial arrangement (three moss species by nine treatments). Analysis was conducted separately for each observation date (14, 21, 28 DAIT). Moss species by treatment by run interaction was utilized as the error by which all means were separated and sorted. Each moss species was analyzed separately by date of observations. There was no treatment by run interaction therefore data was pooled and both studies were combined for analysis. Means were separated and sorted by species and date. Fisher's LSD ($P=0.05$) was applied for mean comparisons.

Results and Discussion

Moss Control. Treatment by run interaction was not significant ($P>0.05$); therefore, data was pooled and both studies were combined for analysis. The moss species by treatment interaction was significant ($P<0.05$); therefore, data were separated by species and treatment. Sequential carfentrazone applications controlled all three moss species greater than single applications (Table 1). *Bryum argenteum* was controlled *#,* greater than any other moss species. Carfentrazone controlled both *Amblystegium serpens* and *Entodon seductrix* less than 17%

throughout the entire study. Carfentrazone sequentially applied at 0.06 and 0.12 kg/ha 21 DAIT controlled *Bryum argenteum* 78 and 86% respectively; however, at 28 DAIT visual control was reduced to 33 and 39% respectively. Similar regrowth and recovery was observed with the initial carfentrazone applications. At 14 DAIT carfentrazone control was between 26 and 36 % with all treatments. Single carfentrazone applications decreased visual control from 26% 14 DAIT to 18 and 19% 28 DAIT. Sequential carfentrazone applications at 0.12 kg/ha 21 DAIT controlled *Amblystegium serpens* 17%. *Entodon seductrix* visual control was not greater than 15 % with any treatment. A previous non-peer reviewed report indicated that multiple applications of carfentrazone are required to continually suppress and eventually control *Bryum argenteum* (Alyward 2007). In our research, regrowth was observed with single applications over the course of the 14, 21, 28 DAIT. This could potentially be due to the burning of surface moss tissue and residual tissue sustaining the regrowth. Moss species do not contain vascular systems and translocation of systemic pesticides does not progress (Yelverton 2005). Carfentrazone did not control any moss species greater than 86% and regrowth was observed with *Bryum argenteum*.

Mancozeb controlled all moss species minimally. Mancozeb applied at 0.9 and 1.8 kg/ha controlled *Bryum argenteum* 18 and 14 % respectively, 21 DAIT. All moss control with mancozeb was not statistically different from non-treated plots with both *Amblystegium serpens* and *Entodon seductrix*. Landschoot et al. (2004) also observed minimal control of *Bryum argenteum* with mancozeb during summer months; however, five fall applications of 15.28 kg/ha controlled moss 100% in the spring. Only two mancozeb applications were made during this experiment because research has suggested that more treatments can possibly injure creeping

bentgrass due to copper induced iron chlorosis (Boeshch and Mikowski 2005; Landchoot et al. 2004). We wanted to investigate a program that could achieve effective control with limited copper accumulation. The highest recommended labeled rate would apply copper at an equivalent of 5.6 kg cu/ha or 12.2 kg/ha of product weekly (Anonymous 2005).

Digital image analysis. Chemical applications made to moss species affected percent green cover greater than visual ratings. Carfentrazone sequential treatments reduced percent green cover greater than all other treatments (Table 2). Carfentrazone sequential applications decreased *Bryum argenteum* green cover percentages greater than sequential applications. Carfentrazone at 0.12 kg/ha reduced *Amblystegium serpens* green cover 80% relative to the non-treated 21 DAIT. This was different from visual control data and could potentially be due to a change in the plants chlorophyll production. Carfentrazone inhibits a key enzyme in plant chlorophyll production (Aylward 2007; Senseman 2007). This reduction in chlorophyll can disrupt the moss natural green color effectively changing hue angle but not visual injury. Different species observed different color change. Carfentrazone changes *Amblystegium serpens* yellowish green color lighter and *Bryum argenteum* changes to a black or brown color. This disruption in color could potentially be the reasoning for the change in percent green coverage; however this would not affect the percent moss coverage observed in visual ratings. Carfentrazone at 0.12 kg/ha applied sequentially reduced *Bryum argenteum* green cover -69 % 28 DAIT. Single carfentrazone applications at 0.06 and 0.12 kg/ha reduced *Bryum argenteum*, -8 and -26%, 28 DAIT, respectively.

In general, carfentrazone at 0.12 kg/ha sequentially, reduced hue angles greater than any other treatment for all three moss species; however, hue angles increased from 21 to 28 DAIT

(Table 3). Hue angles had similar increasing and decreasing control patterns observed with percent green cover on all three moss species. As noted previously, increases in hue angles denotes recovery and regrowth of moss species and decreases in hue angles denotes injury and control. All treatments 28 DAIT had greater hue angles than 14 DAIT. This could potentially be from moss regrowth and recovery from applied treatments. All treatments at 28 DAIT were not statistically different from the non-treated indicating total moss recovery and regrowth. All mancozeb treatments hue angles did not differ from non-treated checks for any date or rate. Sequential applications of carfentrazone reduced *Entodon seductrix* hue values greater than single applications.

Carfentrazone sequential treatments reduce saturation percentages greater than single treatments indicating a decrease in green color purity (Table 4). As noted previously, increases in saturation denote growth and recovery and a decrease in saturation denotes injury and control. Carfentrazone treatment timing applied to *Bryum argenteum* had a greater impact on saturation percentage than different rates. No difference was observed between rates of carfentrazone when applied to *Bryum argenteum*. Carfentrazone sequential applications reduced *Entodon seductrix* saturation percentages at 28 DAIT greater than 14 and 21 DAIT. Carfentrazone sequential treatment saturation percentages were lower than mancozeb treatments. Initial carfentrazone application hue angles were lower than mancozeb applications. Carfentrazone applied at 0.12 kg/ha sequentially decreased hue angles greater than all other treatments 28 DAIT (Table 3). Hue angles of single carfentrazone applications were greater than sequential applications. Carfentrazone controlled *Bryum argenteum* greater than *Amblystegium serpens* and *Entodon seductrix*. Mancozeb saturation percentages remained consistent from 14 to 28 DAIT.

Carfentrazone controlled all three moss species greater than mancozeb. Sequential applications of carfentrazone are required to reduce moss regrowth. Single carfentrazone applications can potentially increase growth rates of *Bryum argenteum*. Carfentrazone applications made to *Amblystegium serpens* and *Entodon seductrix* stabilized growth rates however, they did not decrease populations. Sequential applications of carfentrazone applied at 0.12 kg/ha were the most effective treatments for all three moss species. Little injury was observed with mancozeb treatments. Different rates did not affect mancozeb efficacy. Regrowth was observed with all treatments. Diversity in moss species could potentially be attributed to erratic carfentrazone efficacy and was most evident with *Entodon seductrix*. Multiple applications of carfentrazone accompanied with cultural practices should be the focus of future research concerning moss infestation on golf course putting greens.

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Appendix A.

Table 1. Percent visual control of *Amblystegium serpens*, *Bryum argenteum*, and *Entodon seductrix* with carfentrazone and mancozeb at three rating dates.[a]

			Amblystegium serpens			*Bryum argenteum*			*Entodon seductrix*		
Treatment[b]	Rate	Timing	14 DAIT	21 DAIT	28 DAIT	14 DAIT	21 DAIT	28 DAIT	14 DAIT	21 DAIT	28 DAIT
	kg/ha	DAIT	%(SE)								
Carfentrazone	0.06	0	6 (2.2)	1 (0.8)	7 (4)	26 (12.6)	24 (13.6)	18 (12.5)	3 (1.9)	6 (3.9)	14 (8.1)
Carfentrazone	0.12	0	14 (7.1)	6 (3.1)	1 (0.6)	26 (9.7)	22 (13.9)	19 (12.5)	6 (3.2)	5 (2.3)	8 (4.4)
Carfentrazone	0.06	0 fb 14	9 (3.6)	6 (2.2)	9 (5)	31 (9.9)	78 (6.6)	33 (13.5)	6 (1.8)	4 (1.8)	14 (5.6)
Carfentrazone	0.12	0 fb 14	14 (7.2)	17 (3.1)	14 (3.9)	36 (8.7)	86 (4.6)	39 (11.7)	1 (0.8)	5 (2.5)	15 (8.0)
Mancozeb	0.9	0	6 (4.3)	1 (0.6)	1 (0.6)	10 (8.0)	11 (7.7)	8 (6.8)	2 (1.9)	0 (0)	2 (1.3)
Mancozeb	1.8	0	11 (7.1)	1 (0.6)	1 (0.6)	7 (4.9)	8 (7.4)	8 (7.4)	4 (2.6)	0 (0)	1 (0.6)
Mancozeb	0.9	0 fb 14	5 (2.5)	0 (0)	0 (0)	3 (2.5)	18 (8.3)	11 (7.0)	2 (1.3)	3 (2.1)	4 (1.6)
Mancozeb	1.8	0 fb 14	4 (2.6)	1 (0.6)	1 (0.8)	8 (7.4)	14 (7.5)	4 (3.7)	3 (1.6)	1 (1.3)	7 (4.2)
Non-Treated			0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
LSD (P= 0.05)			13	4	8	23	26	25	6	5	11

[a] Abbreviations: DAIT, days after initial treatment; fb, followed by; SE, standard error.
[b] Treatments were applied at 0 and 14 DAIT.

Table 2. Percent green cover reduction for *Amblystegium serpens*, *Bryum argenteum* and *Entodon seductrix* relative to the non-treated check.[a]

			Amblystegium serpens			*Bryum argenteum*			*Entodon seductrix*		
Treatment[b]	Rate	Timing	14 DAIT	21 DAIT	28 DAIT	14 DAIT	21 DAIT	28 DAIT	14 DAIT	21 DAIT	28 DAIT
	kg/ha	DAIT	%(SE)[c]								
Carfentrazone	0.06	0	-58 (12)	-55 (10)	-35 (13)	-12 (31)	-12 (23)	-8 (19)	-62 (12)	-59 (11)	-54 (11)
Carfentrazone	0.12	0	-65 (9)	-49 (16)	-20 (19)	-65 (11)	-25 (22)	-26 (17)	-43 (19)	-42 (16)	-33 (17)
Carfentrazone	0.06	0 fb 14	-68 (5)	-73 (8)	-55 (11)	-63 (13)	-77 (8)	-47 (12)	-29 (22)	-48 (19)	-51 (15)
Carfentrazone	0.12	0 fb 14	-71 (8)	-80 (4)	-69 (7)	-90 (4)	-85 (6)	-69 (8)	-57 (9)	-61 (15)	-68 (11)
Mancozeb	0.9	0	-18 (39)	-13 (22)	-8 (20)	-19 (13)	-13 (16)	-6 (15)	-28 (18)	-34 (14)	-30 (16)
Mancozeb	1.8	0	-4 (34)	-6 (26)	-12 (20)	-4 (19)	-14 (14)	-9 (16)	-18 (26)	-15 (20)	-13 (18)
Mancozeb	0.9	0 fb 14	-6 (18)	-36 (14)	-26 (12)	-3 (23)	-35 (14)	-27 (18)	-28 (17)	-33 (25)	-35 (18)
Mancozeb	1.8	0 fb 14	-23 (17)	-40 (11)	-32 (11)	-12 (16)	-32 (11)	-24 (12)	-43 (15)	-42 (15)	-38 (14)
LSD (*P*= 0.05)			58	40	37	49	42	39	51	48	42

[a] Abbreviations: DAIT, days after initial treatment; fb, followed by; SE, standard error.
[b] Treatments were applied at 0 and 14 DAIT.
[c] Negative values indicate a decrease in green cover compared to the non-treated whereas positive values indicate an increase in green cover.

Table 3. Hue angle for *Amblystegium serpens*, *Bryum argenteum* and *Entodon seductrix*.[a]

			Amblystegium serpens			*Bryum argenteum*			*Entodon seductrix*		
Treatment[b]	Rate	Timing	14 DAIT	21 DAIT	28 DAIT	14 DAIT	21 DAIT	28 DAIT	14 DAIT	21 DAIT	28 DAIT
	kg/ha	DAIT	Hue Angle (SE)[c]								
Carfentrazone	0.06	0	63 (1.6)	64 (1.3)	71 (1.7)	64 (1.9)	75 (3.3)	82 (3.1)	62 (2.9)	60 (1.0)	63 (1.0)
Carfentrazone	0.12	0	61 (2.1)	65 (2.8)	72 (2.5)	63 (2.2)	72 (3.5)	79 (4.2)	58 (0.9)	60 (1.3)	63 (1.3)
Carfentrazone	0.06	0 fb 14	62 (2.3)	60 (1.6)	67 (3.0)	63 (1.6)	59 (1.2)	73 (3.1)	60 (1.3)	58 (0.7)	60 (1.2)
Carfentrazone	0.12	0 fb 14	60 (1.2)	59 (1.7)	63 (2.5)	61 (0.9)	60 (1.8)	70 (3.5)	59 (1.3)	58 (1.0)	60 (1.3)
Mancozeb	0.9	0	68 (2.0)	69 (1.2)	74 (1.4)	73 (1.8)	73 (2.5)	80 (2.6)	60 (1.3)	63 (1.2)	66 (0.7)
Mancozeb	1.8	0	66 (2.3)	67 (1.9)	73 (2.2)	74 (1.7)	75 (2.9)	80 (2.9)	61 (1.2)	63 (0.8)	66 (1.3)
Mancozeb	0.9	0 fb 14	70 (1.8)	66 (1.1)	71 (1.4)	76 (2.5)	67 (2.2)	76 (3.5)	62 (0.8)	60 (0.9)	64 (1.5)
Mancozeb	1.8	0 fb 14	69 (2.5)	68 (1.5)	73 (1.7)	74 (2.5)	70 (2.7)	78 (2.9)	60 (1.0)	61 (0.8)	64 (1.0)
Non-Treated			70 (2.5)	70 (2.1)	75 (2.1)	79 (1.4)	78 (1.6)	82 (1.0)	64 (1.9)	63 (1.6)	65(1.2)
LSD (*P*= 0.05)			5	4	5	5	7	8	4	3	3

[a] Abbreviations: DAIT, days after initial treatment; fb, followed by; SE, standard error.
[b] Treatments were applied at 0 and 14 DAIT.
[c] Higher hue angle values indicates an increase in green color whereas a lower value indicates a decrease (0-360°).

Table 4. Percent color saturation for *Amblystegium serpens*, *Bryum argenteum*, and *Entodon seductrix* each individual moss species.[a]

Treatment[b]	Rate	Timing	*Amblystegium serpens* 14DAIT	21DAIT	28DAIT	*Bryum argenteum* 14DAIT	21DAIT	28DAIT	*Entodon seductrix* 14 DAIT	21DAIT	28DAIT
	kg/ha	DAIT	Saturation(SE)[c]								
Carfentrazone	0.06	0	44 (4.3)	37 (3.5)	40 (3.9)	34 (2.2)	41 (3.6)	43 (3.6)	46 (3.9)	41 (3.9)	39 (2.9)
Carfentrazone	0.12	0	40 (4.4)	33 (2.7)	39 (3.7)	29 (1.9)	37 (4.5)	39 (4.0)	48 (2.5)	43 (2.8)	42 (2.7)
Carfentrazone	0.06	0 fb 14	41 (5.4)	33 (4.7)	37 (4.4)	30 (1.5)	24 (2.3)	32 (3.2)	43 (2.0)	40 (4.0)	35 (2.9)
Carfentrazone	0.12	0 fb 14	36 (3.5)	27 (2.7)	30 (1.2)	27 (1.2)	22 (1.6)	29 (2.4)	49 (3.0)	40 (4.3)	36 (2.6)
Mancozeb	0.9	0	46 (3.6)	46 (5.0)	47 (4.5)	37 (1.5)	40 (4.1)	42 (3.3)	45 (1.0)	47 (3.0)	45 (3.3)
Mancozeb	1.8	0	45 (4.6)	43 (5.3)	44 (4.6)	39 (2.1)	44 (4.1)	44 (4.3)	45 (2.0)	50 (3.8)	48 (3.7)
Mancozeb	0.9	0 fb 14	45 (2.9)	39 (3.3)	42 (2.5)	41 (1.9)	37 (4.8)	40 (4.8)	47 (1.2)	48 (3.3)	47 (2.8)
Mancozeb	1.8	0 fb 14	44 (3.2)	41 (3.2)	42 (1.9)	43 (2.0)	41 (4.2)	43 (3.5)	47 (2.7)	47 (2.4)	46 (2.5)
Non-Treated			47 (2.4)	43 (3.9)	43 (3.3)	44 (1.8)	44 (3.2)	46 (2.6)	50 (2.8)	52 (3.4)	50 (3.0)
LSD (P = 0.05)			8	9	9	5	8	8	7	6	6

[a] Abbreviations: DAIT, days after initial treatment; fb, followed by; SE, standard error.
[b] Treatments were applied at 0 and 14 DAIT.
[c] Higher saturation percentages indicates a darker green color whereas a lower percentage indicates a lighter green color (0-100%).

II. Evaluation of Silvery-Thread Moss (*Bryum argenteum* Hedw.) Control in Creeping Bentgrass (*Agrostis stolonifera* L.) Putting Greens with Mancozeb and Carfentrazone applied with Cultural Practices

Abstract

Carfentrazone is a broadleaf weed control herbicide that is also utilized for control of silvery-thread-moss (*Bryum argenteum*) in creeping bentgrass (*Agrostis stolonifera* L.) putting greens. Regrowth and *Bryum argenteum* recovery has been observed with carfentrazone treatments. Common cultural practices such as increased nitrogen (N) rates and sand topdressing (TD) could be utilized to increase carfentrazone efficacy. Mancozeb, a fungicide containing a combination of zinc ion, manganese ethylenebisdithiocarbamate, and copper hydroxide is also labeled for *Bryum argenteum* control. Field studies were initiated to evaluate mancozeb, carfentrazone, and cultural practices for *Bryum argenteum* control. Studies were initiated in 2006 at the Honors Course (HC), Ootlewah, TN and in 2007 at The Crossings Golf Club (CC), Jonesborough, TN. Treatments included carfentrazone alone at 0.12 kg/ha, carfentrazone followed by (fb) (TD) at 3.92 Mg/ha, carfentrazone fb (N) at 12.2 kg/ha, carfentrazone fb N plus TD, mancozeb alone at 1.8 kg/ha, N alone, TD alone, N plus TD. Carfentrazone applied alone and with cultural practices controlled *Bryum argenteum* greater than mancozeb. Mancozeb did not control *Bryum argenteum* greater than 13% throughout the study and actually increased *Bryum argenteum* populations 35% at study completion. All initial applications of carfentrazone controlled *Bryum argenteum* greater than 68 %, 3 weeks after initial treatments (WAIT). Carfentrazone applied alone decreased in visual *Bryum argenteum* control as time progressed. Carfentrazone applied with cultural practices controlled *Bryum argenteum* greater than carfentrazone alone 11 WAIT. All treatments except for mancozeb and the non-treated decreased in *Bryum argenteum* populations. N and TD applied alone and in combination controlled *Bryum argenteum* greater than mancozeb. N, TD and N fb TD decreased *Bryum*

argenteum populations 22, 36, 23%, respectively. Carfentrazone fb TD alone, N alone and combination with both decreased *Bryum argenteum* populations 73, 39, and 66% respectively. These data indicate that carfentrazone, increased nitrogen, and sand topdressing control *Bryum argenteum* greater than mancozeb. Also, cultural practices alone provide equal long term *Bryum argenteum* control as carfentrazone alone.

Introduction

Bryum argenteum encroachment on creeping bentgrass (*Agrostis stolonifera* L.) putting greens has become an increasing problem for turfgrass managers in the U.S. (Burnell et al. 2004; Danneberger and Taylor 1996; Happ 1998; Hummel 1986, 1994; Nelson 2007; Snow 1984). A demand for a faster more competitive playing surface has forced turf managers to reduce fertility and mowing heights adding increased stress to their putting greens (Alyward 2007; Burnell et al. 2004; Danneberger and Taylor 1996; Happ 1998; Yelverton 2005). Golf course putting greens require constant attention and a strong cultural management program that includes aerification and continual topdressing can give a competitive edge to the turf against moss encroachment (Happ 1998). However, cultural practices such as topdressing can influence the playing surface, take time away from other maintenance tasks and therefore is sometimes disregarded. Allowing the thatch layer to grow in a soil medium by reducing cultural practices can disrupt water infiltration and air movement through the profile making the environment more favorable to moss encroachment (Happ 1998). These stresses and the restriction of mercury-based fungicides have been attributed to the increase in *Bryum argenteum* infestations observed recently on golf course putting greens (Nelson 2007; Yelverton 2005).

Several moss species have been identified in a golf course setting; *Byrum lisae*, *Amblystegium trichopodium*, *Brachytheciium spp*., *Amblystegium serpens*, and *Bryum argenteum* (Happ, 1998; Yelverton 2005). The most problematic of these *Bryum argenteum* species found on creeping bentgrass putting greens is *Bryum argenteum* (Burnell et al. 2004; Danneberger and Taylor 1996; Happ 1998; Yelverton 2005). *Bryum argenteum* moss can be found in many environments such as concrete surfaces, tree roots, compacted soils, cool damp soils, as well as

golf course putting greens (Boeshch and Mikowski 2005). Once established on a golf course putting green *Bryum argenteum* can be difficult to control due to its reproductive nature and anatomy.

Bryum argenteum moss is perennial plant that can reproduce sexually via spores and asexually through displaced fragments (Boeshch and Mikowski 2005; Nelson 2007). A common way for *Bryum argenteum* to increase infestation is through tracking of fragments lodged in golf spikes from green to green (Yelverton 2005). *Bryum argenteum* lacks phloem and xylem therefore, cannot translocate foliar-absorbed nutrients throughout the entire plant (Boeshch and Mikowski 2005; Cook et al. 2002). This lack of a vascular system potentially could make the translocation of systemic herbicides difficult (Yelverton 2005).

Golf course superintendents have tried numerous products and homemade concoctions for the control of *Bryum argenteum*. Some of these products have been evaluated and numerous trade journals have reviewed their efficacy. Such products include chlorothalonil fungicide, ferrous and ferric sulfates, sodium carbonates such as baking soda, sodium carbonate peroxyhytests and even Dawn® dish detergent. Control with these products is often insufficient and erratic (Boeshch and Mikowski 2005; Burnell et al. 2004; Landschoot et al. 2004; Turgeon and Vargas 2006). These treatments are highly variable and often ineffective compared to two other chemistries, carfentrazone and mancozeb, that are labeled for the control of *Bryum argenteum*.

In 2005 carfentrazone, a protoporphyrinogen oxidase inhibitor, utilized for the control of a wide range of broadleaf weeds was labeled for the control of *Bryum argenteum* on creeping bentrgrass putting greens (Anonymous 2005; Senseman 2007). It quickly became the industry standard for *Bryum argenteum* control on creeping bentgrass putting greens. Numerous trade

journal reports concluded that carfentrazone provides effective control of *Bryum argenteum* over a broad temperature spectrum (Anonymous 2005; Aylward 2007; Boeshch and Mikowski 2005; Nelson 2007; Settle et al. 2006, Yelverton 2005). Despite all of these reports, no peer reviewed publications are currently available evaluating carfentrazone control of *Bryum argenteum* on creeping bentgrass putting greens.

Mancozeb-based fungicides are also labeled for the control of *Bryum argenteum* on creeping bentgrass putting greens. Mancozeb is a coordination product of zinc ion, manganese ethylenebisdithiocarbamate, and copper hydroxide. The main chemical component of mancozeb is copper hydroxide which makes up 46.1% (30% metal equivalent) (Anonymous 2006). Landschoot et al. (2004) concluded mancozeb summer treatments did not control *Bryum argenteum* but, fall applications of 15.28 kg/ha applied biweekly with 5 applications provided 100% *Bryum argenteum* control. Cook et al. (2002) also found that five applications of mancozeb applied at 4.85 to 7.27 kg Cu/ha biweekly controlled *Bryum argenteum* for up to two years. The highest recommended labeled rate of 12.2 kg/ha of product weekly would apply copper at an equivalent of 5.6 kg Cu/ha (Anonymous 2005). These high rates of copper accumulating in the soil medium however, could potentially induce iron deficiency in turfgrass plants (Boeshch and Mikowski 2005; Landschoot et al. 2004). Landschoot et al. (2004) observed creeping bentgrass injury with multiple applications of mancozeb and attributed it to copper-induced iron chlorosis. Research was initiated to evaluate carfentrazone and mancozeb *Bryum argenteum* control on creeping bentgrass putting greens. In addition, increased fertility and increased sand topdressing were also evaluated, as the lack of these practices is often linked with increased silver-tread *Bryum argenteum* infestation.

Materials and Methods

Field studies were initiated in summer at the Honors Course (HC), Ootlewah, TN 2006 and The Crossings Golf Club (CC), Jonesborough, TN 2007. Studies evaluated the control of *Bryum argenteum* with carfentrazone[7], carfentrazone applied with cultural practices, cultural practices alone, and mancozeb[8]. HC putting greens were built to the U.S. Golf Association specifications, consisting of 85 to 90% sand, > 15% peat, < 1% silt and humic matter, and a pH of 6.7. CC greens were constructed as California greens, only sand mix and a pH of 6.8. Creeping bentgrass putting greens were mowed daily to keep a 3.2 mm cutting height. HC putting greens received 235 kg N/ha annually and CC received 171 kg N/ha.

Research was conducted in a randomized complete block design and replicated three times. Treatments included carfentrazone alone at 0.12 kg/ha, carfentrazone followed by (fb) sand topdressing (TD) at 3.92 Mg/ha, carfentrazone fb nitrogen[9] (N) at 12.2 kg/ha, carfentrazone fb N plus TD, mancozeb alone at 1.8 kg/ha, N alone, TD alone, N plus TD. All carfentrazone treatments were made sequentially, at trial initiation and 2 weeks after initial treatments (WAIT). Cultural measures were applied 4 WAIT or 2 weeks after the last carfentrazone treatments and continued biweekly until 10 WAIT. Mancozeb was applied biweekly for the 10 weeks. Spray applications were applied with a CO_2 pressurized back pack sprayer calibrated at 280 L/ha equipped with 8002 XR flat-fan nozzles. Initial applications were made in the middle of May 2006-07. Granular nitrogen applications were applied over each individual plot by hand and

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[7] Quicksilver® herbicide. FMC Corporation Agricultural Product Group, Philidelphia, PA 19103

[8] Junction® SePRO Corporation 1550 N Meridian Street, Suite 600 Carmel, IN 46032

[9] Fertilizer. 24-4-12 Pro source one granular greens grade fertilizer, Knoxville, TN 37920

sand treatments were brushed into the turf canopy. Overhead irrigation was then utilized to water in granular nitrogen and topdress treatments.

Percent *Bryum argenteum* control was evaluated visually on a 0 (no control) to 100% (complete control) scale and was evaluated 3, 5, 11, 16 WAIT. No bentgrass injury was observed with any treatment, timing, or location (data not shown). A grid system was utilized to evaluate total *Bryum argenteum* population change within the m-2 plots. Grids contained 100 intersecting points, if *Bryum argenteum* persisted within an intersecting point it would be counted, giving an accurate *Bryum argenteum* population percentage, eliminating human bias. Grid counts were made at study initiation and 16 WAIT. Percent *Bryum argenteum* reduction was calculated using the following equation:

Percent *Bryum argenteum* reduction =

$$-[100-((\textit{Bryum argenteum}_2/\textit{Bryum argenteum}_1)*100)],$$

Where $\textit{Bryum argenteum}_2$ is the percent *Bryum argenteum* cover at 16 WAIT and $\textit{Bryum argenteum}_1$ is the percent *Bryum argenteum* cover at trial initiation.

All data were subject to ANOVA (P = 0.05). Data were analyzed according to the randomized complete block design. *Bryum argenteum* was analyzed by date of observations. There was no treatment by location interaction therefore data was pooled and both studies were combined for analysis. Means were separated and sorted by treatment. Fisher's LSD (P=0.05) was applied for mean comparisons.

Results and Discussion

Visual *Bryum argenteum* Control. Treatment by location interaction was not significant (P>0.05); therefore, data was pooled over location. Treatment by date interaction was significant (P<0.05); therefore, data were separated and sorted by treatment and date. All carfentrazone applications controlled *Bryum argenteum* greater than 68% 3WAIT. However, our research observed *Bryum argenteum* regrowth and recovery when carfentrazone was applied alone, control decreased from 68%, 3WAIT to 36%, 11 WAIT. The authors observed that carfentrazone applications burned surface *Bryum argenteum* tissue but would leave a residual *Bryum argenteum* layer that resembled thatch. *Bryum argenteum* differs from higher, plants because its non-vascular system, it lacks phloem and xylem, making the translocation of systemic herbicides improbable (Boeshch and Mikowski 2005; Yelverton 2005). This would indicate the reason for the capability of regrowth and recover after carfentrazone applications. Previous trade journal articles concluded that carfentrazone at the recommended label rate effectively controls *Bryum argenteum* in creeping bentgrass putting greens (Aylward 2007; Nelson 2007; Settle et al. 2006, Yelverton 2005).

Cultural practices improved carfentrazone long-term efficacy. Carfentrazone applied with TD controlled *Bryum argenteum* 76% 16 WAIT. TD is utilized on golf greens to control thatch by diluting the organic matter that is generated by the turf (Turgeon and Vargas 2006). McCarty et al. (2007) observed that TD plots maintained excellent water infiltration and turf quality when compared to non-treated plots. McCarty et al. (2007) also theorized that maintained constant organic matter composition was due to the dilution affect on topdressed plots. It is theorized in our research that TD aided carfentrazone control through dilution of

residual *Bryum argenteum* thatch tissue left behind after carfentrazone applications, improving carfentrazone efficacy. Similarly, N applications improved carfentrazone efficacy as well. Carfentrazone applied with N controlled *Bryum argenteum* 68% 16 WAIT. An increase in *Bryum argenteum* infestations on golf course putting greens are attributed to lower mowing heights, discontinued use of mercury-based fungicides and reduced fertility (Burnell et al. 2004; Cook et al. 2002; Hummel 1994). Allowing dead *Bryum argenteum* patches to remain without promoting bentgrass growth or competition increases the chance of *Bryum argenteum* recovery and regrowth. We theorize that increasing nitrogen rates promotes bentgrass growth enabling the turf stand to compete with necrotic *Bryum argenteum* patches. Carfentrazone fb N plusTD and Carfentrazone fb TD controlled *Bryum argenteum* greater than carfentrazone alone 11 WAIT. Both treatments controlled *Bryum argenteum* 77% 11 WAIT. Aylward (2007) stated that cultural practices are vital to stop *Bryum argenteum* from reoccurring. These data indicate the importance of cultural practice additions to carfentrazone efficacy.

Mancozeb did not control *Bryum argenteum* greater than 13% at any evaluation (Table 5). All carfentrazone-containing treatments controlled *Bryum argenteum* greater than mancozeb at all rating dates. Mancozeb controlled *Bryum argenteum* equivalent to topdressing, nitrogen, or TD+N at all rating dates. Mancozeb, N, TD, and N plus TD controlled *Bryum argenteum* 7, 32, 34 and 29%, respectively 16 WAIT. Mancozeb could potentially be more effective applied during fall conditions rather than spring as was applied in this research. Landschoot et al. (2004) observed minimal *Bryum argenteum* control with mancozeb when applied during summer months; however applications made in fall provided good *Bryum argenteum* control in the previous spring.

Cultural applications alone controlled *Bryum argenteum* greater than applications of mancozeb. These applications were not statistically different from carfentrazone alone 16WAIT.

***Bryum argenteum* population change.** All treatments except mancozeb and non-treated check plots decreased *Bryum argenteum* populations 16 WAIT (Table 6). Carfentrazone alone, Carfentrazone fb TD, carfentrazone fb N, Carfentrazone fb TD plus N reduced *Bryum argenteum* populations 39, 73, 39, and 67%, respectively. Cultural practices reduced *Bryum argenteum* populations similar to carfentrazone alone applications. N, TD, and N plus TD reduced *Bryum argenteum* populations 22, 36, 23%, respectively. According to Alyward et al. (2007) fertilization is one of the most-vital cultural practices for long-term *Bryum argenteum* suppression. However, TD decreased *Bryum argenteum* populations numerically greater than nitrogen applications when each was applied with or without carfentrazone. It is hypothesized that removal or dilution of *Bryum argenteum* thatch layer through TD reduced regrowth and recovery. N applications decreased *Bryum argenteum* populations greater than non treated and mancozeb applications. *Bryum argenteum* is a difficult weed species to evaluate visually on bentgrass putting greens. Changes can be observed in populations with changing environmental conditions. Burnell et al. (2004) observed fluctuation in non-treated plots and attributed it to the ephemeral nature of *Bryum argenteum*. We observed a 53 % population increase with the non-treated plots. Despite mancozeb applications, *Bryum argenteum* populations increased 35 %.

A healthy turf stand is one of the best ways to prevent *Bryum argenteum* invasion (Cook et al. 2002). Previous research has attributed increased encroachment to lower mowing heights, reduced fertility and the elimination of mercury-based fungicides (Burnell et al. 2004; Hummel 1994; Yelverton 2005; Nelson 2007). Any stress applied to the turf, thinning cover and

decreasing plant vigor can add to the risk of *Bryum argenteum* encroachment. These data suggest that carfentrazone applied in conjunction with TD and/or N can increase *Bryum argenteum* control. Mancozeb controlled *Bryum argenteum* ineffectively and actually contributed to an increase in population.

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Appendix B.

Table 5. Percent visual *Bryum argenteum* control on creeping bentgrass putting greens utilizing carfentrazone, mancozeb and cultural practices.[a]

Treatment[b]	Rate[d]	Visual moss control[c]			
		3 WAIT	5 WAIT	11 WAIT	16 WAIT
		% control			
Carfentrazone	0.12	68 (15.1)	43 (14.4)	36 (13.4)	54 (12.6)
Carfentrazone + TD	0.12 fb 3.92	75 (11.5)	63 (16.6)	77 (11.6)	76 (12.3)
Carfentrazone + N	0.12 fb 12.2	85 (4.5)	54 (16.8)	68 (14.1)	68 (13.8)
Carfentrazone + N + TD	0.12 fb 12.2 + 3.92	78 (9.8)	67 (12.1)	77 (4.8)	78 (5.0)
Mancozeb	1.8	11 (6.9)	13 (9.8)	4 (4.2)	7 (6.7)
N	12.2	0 (0)	3 (3.3)	23 (12.9)	32 (11.5)
TD	3.92	0 (0)	9 (5.2)	25 (15.8)	34 (13.4)
N + TD	12.2 + 3.92	0 (0)	10 (6.8)	20 (13.2)	29 (11.5)
LSD (P= 0.05)		15	25	32	31

[a] Abbreviations: WAIT, weeks after initial treatment; TD, topdressing; N, nitrogen; fb, followed by.
[b] Carfentrazone treatments were applied 0 and 2 WAIT, mancozeb treatments were applied 0, 2, 4, 6, 8, 10 WAIT, and all cultural practices were applied at 4, 6, 8, 10 WAIT.
[c] Percent *Bryum argenteum* visual control was evaluated for each plot at each timing. Numbers were pooled between locations (LSD, $p < 0.05$).
[d] Carfentrazone, mancozeb and nitrogen rates are in kg active ingredient/ha and are in the order they are listed. Topdressing is listed in Mg/ha.

Table 6. Percent *Bryum argenteum* population change from carfentrazone, mancozeb, and cultural practices.[a]

Treatment[b]	Rate[d]	Population change[c] 16 WAIT
		% reduction (SE)
Carfentrazone	0.12	-39 (16.8)
Carfentrazone + TD	0.12 fb 3.92	-73 (8.2)
Carfentrazone + N	0.12 fb 12.2	-39 (26.2)
Carfentrazone + N + TD	0.12 fb 12.2 + 3.92	-66 (5.9)
Mancozeb	1.8	35 (26.8)
N	12.2	-22 (18.1)
TD	3.92	-36 (13.1)
N + TD	12.2 + 3.92	-23 (20.0)
Non-treated		53 (36.9)
LSD (P= 0.05)		43

[a] Abbreviations: WAIT, weeks after initial treatment; TD, topdressing; N, nitrogen; fb, followed by.

[b] Carfentrazone treatments were applied 0 and 2 WAIT, mancozeb treatments were applied 0, 2, 4, 6, 8, 10 WAIT, and all cultural practices were applied at 4, 6, 8, 10 WAIT.

[c] Percent *Bryum argenteum* population change was calculated by comparing moss populations at the beginning and end of each study. Numbers were pooled between locations (LSD, $p < 0.05$).

[d] Carfentrazone, mancozeb and nitrogen rates are in kg active ingredient/ha and are in the order they are listed. Topdressing is listed in Mg/ha.

III. Identification and Control of Cyanobacteria Common to Creeping Bentgrass Putting Greens with Zinc and Copper

Abstract

Zinc and copper-based fungicides are labeled for phytopathogenic algae (cyanobacteria) control on golf course putting greens. Zinc has been added to chlorothalonil and copper to mancozeb for increased fungicide efficacy. Identification of problematic cyanobacteria on golf course putting greens and their tolerance to pesticide micronutrients could potentially help with proper fungicide selection. Laboratory studies were initiated to identify a common problematic cyanobacterium on golf course putting greens and identify its micronutrient toxicities. Gene (16s rDNA) sequencing and taxonomic traits of the Tennessee cyanobacteria isolate (TCI) related to *Phormidium sp*. which are filamentous cyanobacteria. Basic Local Alignment Search Tool (BLAST) identified a 94% match to *Phormidium murrayi*, an arctic filamentous mat forming cyanobacteria, suggesting this isolate is a distant relative and is most likely a Phormidium sp. An *in vitro* response screen utilizing six different concentrations of $ZnSO_4$ (Zn) and $CuSO_4$ (Cu) (0, 0.63, 3.15, 6.3, 9.45, 12.6 µM) was administered to TCI. Micronutrient screens identified that both Zn and Cu at 3.14 µM were lethal 10 days after inoculation (DAI). Both micronutrients at 0.63µM had an increase in growth at 7 and 10 DAI however, Zn increase growth greater than Cu. TCI could potentially have an affinity towards Zn when compared to Cu at the same rate. Pesticide inconsistency could potentially be attributed to species diversity and micronutrient affinities.

Introduction

Blue-green algae (cyanobacteria) can be a persistent weed and pathogen commonly found on golf course putting greens (Elliot 1998; Maddox et al. 2001; Maddox et al. 1999). Cyanobacteria are unique in that they are the only prokaryotic organisms that perform a plant like oxygenic photosynthesis in which PS II and PS I are connected in a series (Stal 1995). Maintaining competitive golf course putting greens can impose high levels of stress on a turf stand making it weak and a desirable habitat for cyanobacteria encroachment. Cyanobacteria are versatile and adaptive enabling them to survive in many environments (Stal 1995). Thin and bare areas, excessive organic matter and high soil moisture, accompanied with humidity and rainfall can compound an encroachment problem (Maddox et al. 2001). Numerous species of algae and cyanobacteria can inhabit a golf course putting green (Baldwin and Whitton 1992; Maddox et al. 2001; Nus 1994).

Four problems have been specifically attributed to cyanobacteria encroachment on golf course putting greens. First is the formation of surface slime mats (Baldwin and Whitton 1992). Cyanobacteria excrete extracellular polymeric substances that are composed of polysaccharides (Decho 1990; Stal 1995). These substances are utilized for the creation capsules and sheaths which are utilized by the cyanobacteria to protect individual cells. Extracellular polysaccharides are known as the slime and mucilage that form this surface slime mats on golf green surfaces (Baldwin and Whitton 1992; Stal 1995). Surface slime mats when hydrated are not favorable for turfgrass growth due to the formation anaerobic conditions and when dehydrated can form a hydrophobic crust layer (Elliot 1998). Surface slime mats and crust layers also disrupt putting green surface playability.

The second problem associated with cyanobacteria encroachment is the reduction of water infiltration into the soil medium. This problem is directly related to the creation of surface slime mats and extracellular polysaccharides production. Extracellular polysaccharides produced by cells along with filamentous cell structure can bind soil and sand particles, and lead to incomplete organic matter mineralization (Elliot 1998; Stal 1995). Elimination of soil pores and increased organic matter can hinder water movement through the putting green profile.

Hindrance of water infiltration can induce subsurface black-layer, the third problem associated with cyanobacteria (Turgeon and Vargas, 2006). Subsurface black layer is a blackened biofilm layer comprised of sulfur-reducing bacteria, cyanobacteria fatty acids, lipids, lipoproteins, proteins, lipoplysaccharides, extracellular polysaccharides, and polysaccharides (Drews and Weckesser 1982; Golecki and Drews 1992; Hodges and Campbell 1997; Hodges and Campbell 1998). When a soil becomes anaerobic it allows for sulfur-reducing bacteria to thrive and cause turf decline (Tredway et al., 2006). This layer can also disrupt water infiltration into a soil medium and can be detrimental to the stand of turf located at the surface. Previous research has indicates that subsurface black-layer in high sand content putting greens is created from an interaction between cyanobacteria and a nitrogen-fixing bacteria *Desulfovibrio desulfuricans* (Hodges and Campbell 1997).

Recent research has identified a fourth potential cyanobacteria associated problem on golf course putting greens. Yellow spot disease has started to become a problem on southeastern and western United States and has been attributed to a (Tredway et al. 2006). Though the disease does not pose a serious killing threat to turf stands, it does pose a problem with aesthetics. Cyanobacteria associated with this disease have been identified as *Phormidium* and

Oscillatoria species (Tredway et al. 2006). Many different species of cyanobacteria can inhabit a microbial mat however; some mats can be dominated by a particular species (Stal 1995). In light of this, specific identification of a particular species would be crucial for cyanobacteria control on golf course putting greens. Individual species can respond differently to chemical treatments (Nus 1994).

Numerous fungicide/algaecide control options have been researched for cyanobacteria control on golf course putting greens. Alleviation of cultural conditions favoring growth is important for long-term control and should be implemented before chemical suppression (Baldwin and Whitton 1992; Baldwin 1989). Mancozeb[10] and chlorothalonil[11] are two chemistries labeled for algae control on golf course putting greens. Chlorothalonil, a non-systemic fungicide, has become an industry standard and research has indicated that it effectively suppresses algae on golf course putting greens (Anonymous 2005; Dernoeden and Shmitt 1992; Elliot 1998). Mancozeb-based fungicides are also labeled for algae control on golf course putting greens. It is a combination product of zinc ion, copper hydroxide, and manganese ethylenebisdithiocarbamate. The main chemical component of mancozeb is copper hydroxide which makes up 46.1% (30% metal equivalent; Anonymous 2006). Elliot (1998) concluded that mancozeb and chlorothalonil will effectively suppress development of blue-green green algae on putting greens. Research also indicated that preventative applications controlled cyanobacteria greater than curative applications. Phytopathogenic algae (filamentous cyanobacteria) encroachment is a persistent problem on golf course putting green. Chlorothalonil plus zinc and

[10] Junction® SePRO Corporation 1550 N Meridian Street, Suite 600 Carmel, IN 46032

[11] Daconil ® Syngenta Crop Protection, Inc., P.O. Box 18300, Greensboro, NC 27419

mancozeb plus copper hydroxide are labeled for the control of cyanobacteria on golf course putting greens. Both chemistries include turfgrass micronutrients for added pesticide efficacy.

Copper and zinc are vital micronutrients which play important roles in many algae enzyme systems (Franklin et al. 2002). At higher concentrations copper and zinc have been shown to inhibit algal growth (Franklin et al. 2002; Stauber and Florence 1987; Stauber and Florence 1990). Copper sulfate is utilized as an algaecide in aquatic environments such as catch basins and golf course water retention ponds (Carson 2001). Individual cyanobacteria species could potentially have different tolerances to copper and zinc. Research was initiated to isolate cyanobacteria found on golf course putting greens, genetically identify each isolate, and evaluate its response to copper and zinc.

Materials and Methods

Identification. Cyanobacteria cultures were taken from Gettysview Country Club, The University of Tennessee golf practice facility, and the East Tennessee Research and Education Center, all located in Knoxville, TN. Isolates were cultured in modified BG-11 growth media (Kerry et al. 1988). Single species were isolated via plate streaking and separation. Single isolates were then transferred to 30 ml of liquid BG-11 liquid media (Kerry et al. 1988; no agar) and contained in 50 ml test tubes. Morphological and taxonomic characteristics outlined in Wehr and Sheath (2003) were utilized to key out and identify species. Single filamentous isolates were also identified via 16S rRNA gene sequencing with specific 16S cyanobacteria primers. 1148 base pair fragments were amplified and the sequence manually curated. The

Basic Local Alignment Search Tool (BLAST, Altschul et al. 1990) was utilized to compare the amplicon to sequences found in the National Center for Biotechnology Information's databases.

Micronutrient Toxicity Screens. An *in vitro* micronutrient toxicity screen utilizing six different concentrations of $ZnSO_4$ (Zn) and $CuSO_4$ (Cu) (0, 0.63, 3.15, 6.3, 9.45, 12.6 µM) was administered to Tennessee cyanobacteria isolate (TCI). Each concentration was added to 30 ml of modified BG-11 liquid growth media and inoculated with the TCI. Isolates were grown in a growth chamber at 26° C and 1000 µmol per m^{-2} s^{-1} light from a mixture of metal halide and high pressure sodium lamps. Triplicates of each concentration were then harvested at (0, 3, 5, 7, 10) days after inoculation (DAI). Cyanobacteria biomass was collect by parallel filtration. Samples were collected on 0.2 µm polycarbonate filters (Millipore). Algal biomass was then placed in falcon tubes and stored at -80° C until analysis. Twenty-four hours after the final harvest date chlorophyll-*a* was extracted by suspending algal samples in 90% acetone for 24 h at 4° C. Chlorophyll-*a* fluorescence response (F_o) was measured with a Turner Designs TD-700 fluorometer by the nonacidification protocol (Welschmeyer 1994).

All data were subject to ANOVA (P = 0.05) using mixed model methodology (Statistical Analysis Software v. 9.1, Cary, NC). Data were analyzed according to complete randomized design with replication. Means were separated and sorted by micronutrient, concentration, and date. Standard errors were utilized for graphical mean comparison.

Results and Discussion

Identification. All three locations contained similar filamentous cyanobacteria inhabiting golf course putting greens. Morphological characteristics identified TCI closely resembled *Phormidium spp.* cyanobacteria. TCI contained filaments that grew in flat slimy mats, a common characteristic of *Phormidum spp*. (Wehr and Sheath 2003). Other characteristics that TCI had in common with *Phormidium spp.* were fine colorless sheaths, shorter than wide cell orientation and its densely aggregated ropelike tangled growth habit (Wehr and Sheath 2003). However, according to Marquardt and Palinska (2007) identification of particular species based on morphology is highly dubious and impractical. Therefore genetic identification was accompanied with morphological characteristics. Using the gene sequence, BLAST search identified that TCI did not have an exact cultured match in the database. The most similar sequence identified was 97% to uncultured California grassland cyanobacteria. The closest cultured match of 94% was *Phormidium murrayi*, a filamentous mat forming cyanobacteria that was isolated from a meltwater pond on the McMurdo Ice Shelf in Antarctica (Roos and Vincent 1998). The 16 s rRNA sequencing grouped the TCI closely with *Phormidium murrayi* and another Phormidiacean; *Microcoleus glaciei*, also a polar-cultured cyanobacteria (Casamatta et al. 2005; Figure 1). Through morphological traits and gene sequencing it was concluded TCI could be classified and grouped with *Phormidium* sp. Tredway et al. (2006) identified a *Phormidium sp.* taxonomically in North Carolina and determined this isolate was associated with the turf disease yellow spot. At no time however, was yellow spot disease observed at any of the collection locations. In previous research *Phormidium sp*. cyanobacteria has been identified as a

precursor to sub-surface black layer, turf decline and surface slime mat formation (Baldwin and Whitton 1992; Hodges and Campbell 1997; Nus 1994; Tredway et al. 2006).

Micronutrient toxicity screens. Chl- *a* F_o was utilized as a proxy for cyanobacteria biomass. At 7 DAI little variation was observed among Cu concentrations; however, differences in chl- *a* F_o did occur at 10 DAI (Figure 2). Cu at 0.6 μM had a greater chl- *a* F_o than the non-treated 10 DAI. In trace amounts copper is an essential micronutrient for algae, participating in biological reactions as an enzymatic cofactor and electron carrier in the photosynthetic process (Andrade et al. 2004; Franklin et al. 2002). However, at higher concentrations, both copper and zinc inhibit algal growth and can be toxic (Franklin et al. 2002; Cavet et al. 2003 Les and Walker 1983; Stauber and Florence 1990, 1987). The 0.6 μM concentration was a small enough amount to aide in these processes. At 3.2 μM however, the concentration is excessive for TCI and the Cu solution interferes with these processes.

Similar to Cu, little variation was observed among Zn concentrations from 0 to 7 DAI; however, differences in chl- *a* F_o did occur at 10 DAI (Figure 3). Zn at 0.6 μM had greater chl- *a* F_o than the non-treated 10 DAI. Previous research indicates that zinc at low concentrations is essential in maintaining the stability of cell membranes (Stauber and Florense 1990). Zn concentrations of 3.2 μM and greater killed TCI and 0 chl- *a* F_o was noted for all levels 10 DAI. At 0.6 M micronutrient concentration 10 DAI Zn chl- *a* F_o was greater than Cu (Figure 4). This indicates that TCI potentially has an affinity towards Zn compared to the same Cu level. In relation to field applications, these data indicate that lower residual levels of Zn and Cu from field fungicide applications could potentially sustain TCI growth on golf course putting greens. Label rates of mancozeb plus copper hydroxide apply Cu as a 2 molar (M) solution which is

much greater than the toxic level achieved in the laboratory (Anonymous 2005). Laboratory studies indicated that both Cu and Zn toxicity to TCI is 3.2 μM. Laboratory studied suspend the TCI in Zn and Cu solutions compared to broadcast application in the field. Applications made to golf greens contain many more variables when compared to a contained laboratory settings. Uptake by plants, microbial uptake, soil binding, and leaching are potential Cu fates on a golf course putting green.

Successful culture of a cyanobacterium common to three golf course putting greens in Knoxville, TN was achieved. Gene sequence and taxonomic traits of TCI relate to *Phormidium spp*. cyanobacteria. Micronutrient screens identified that both Zn and Cu at 3.2 μM concentration and above were lethal 10 DAI. Zn and Cu at 0.6 μM increased in chl- *a* F_o when compared to the non-treated. Zn solution increased TCI chl- *a* F_o greater than Cu 10 DAI indicating a potential affinity for Zn compared to Cu.

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Appendix C.

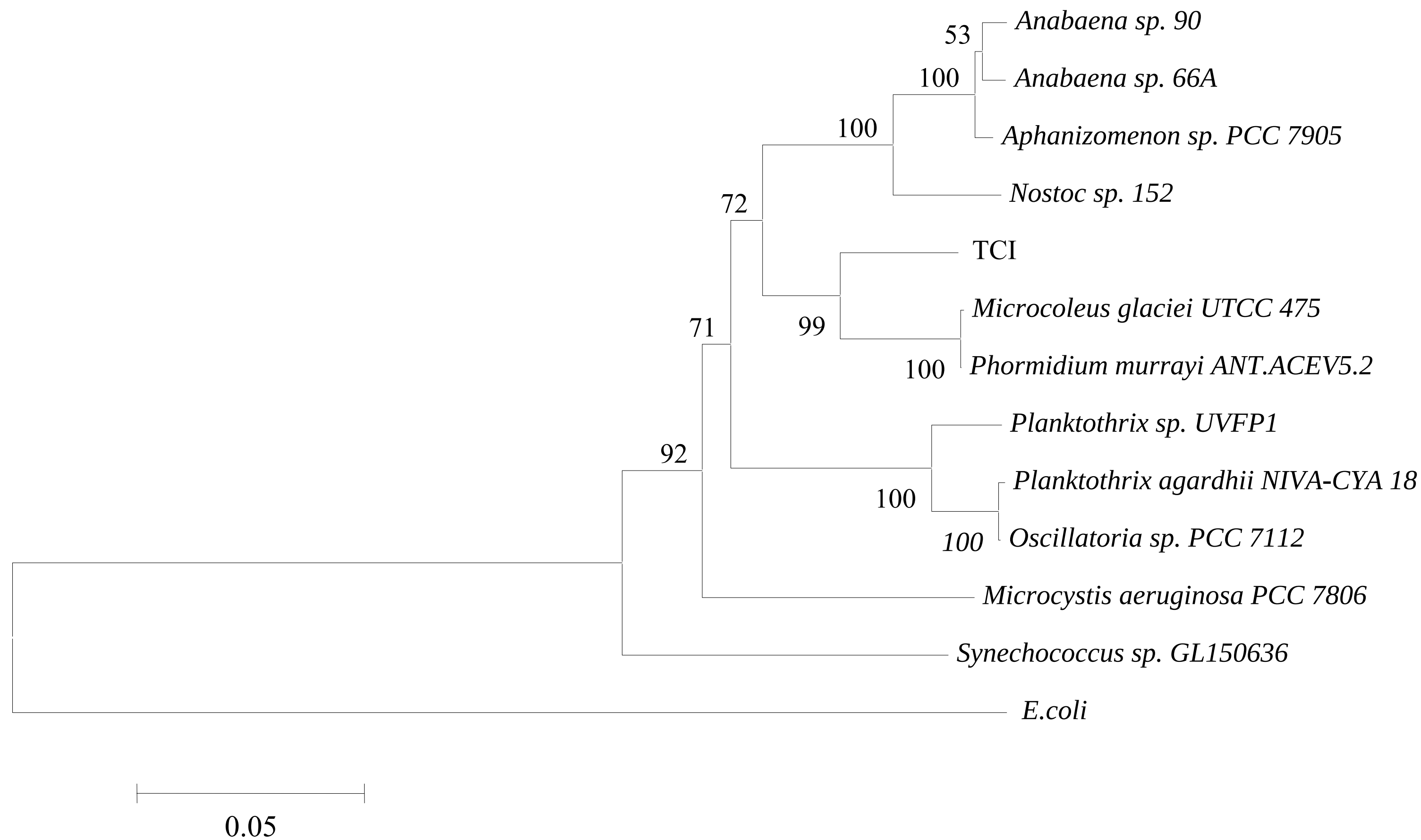

Figure 1. Phylogenetic reconstruction (using the Neighbour-Joining method) displaying the 16 S rRNA sequences to identify relationship between the Tennessee cyanobacteria isolate (TCI) and other cultured algae. Bootstrap values are displayed at the branch nodes as a percentage of 5000 replicates. The scale bar represents substitutions per site.

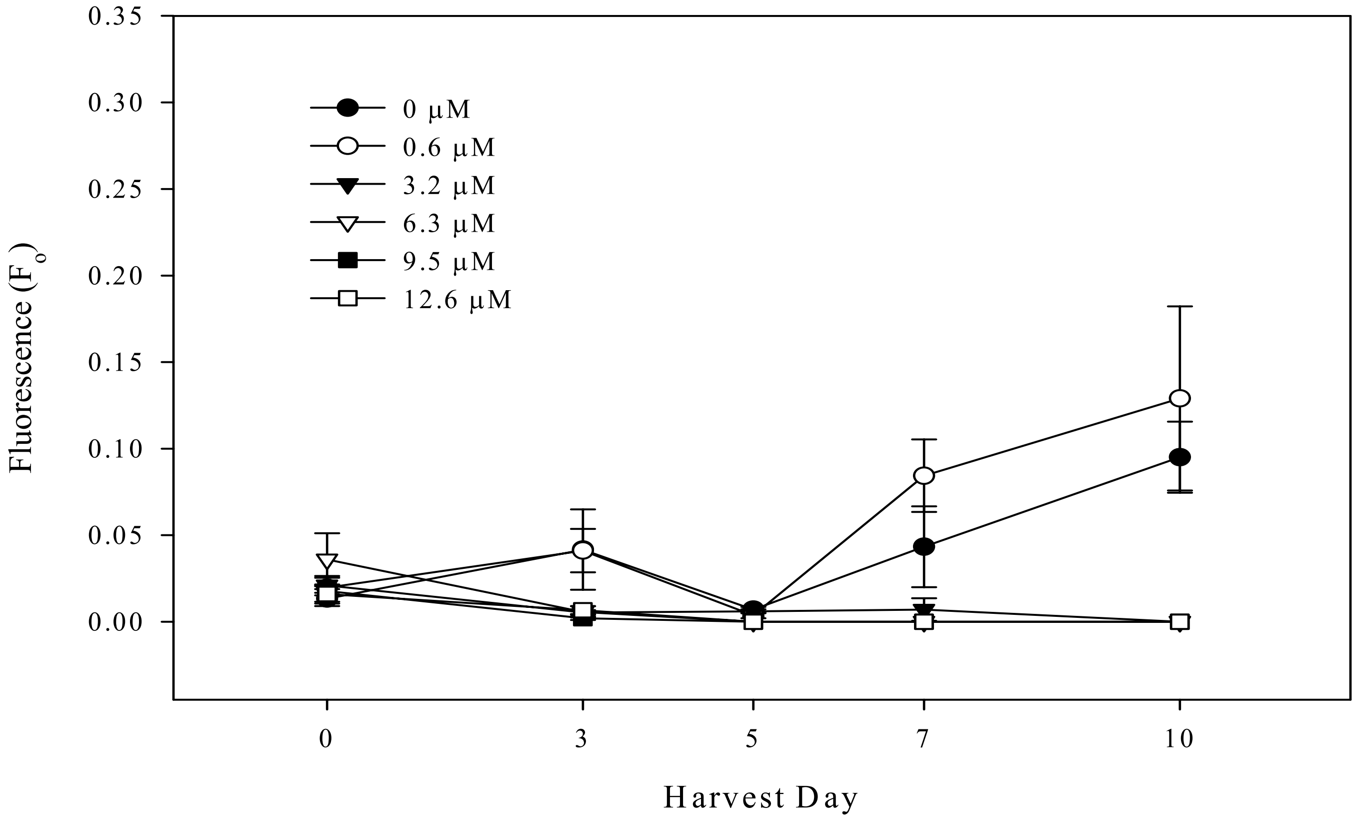

Figure 2. The effect of six different $CuSO_4$ micronutrient levels on TCI Chlorophyll-*a* fluorescence response (F_o) on five different harvest dates.

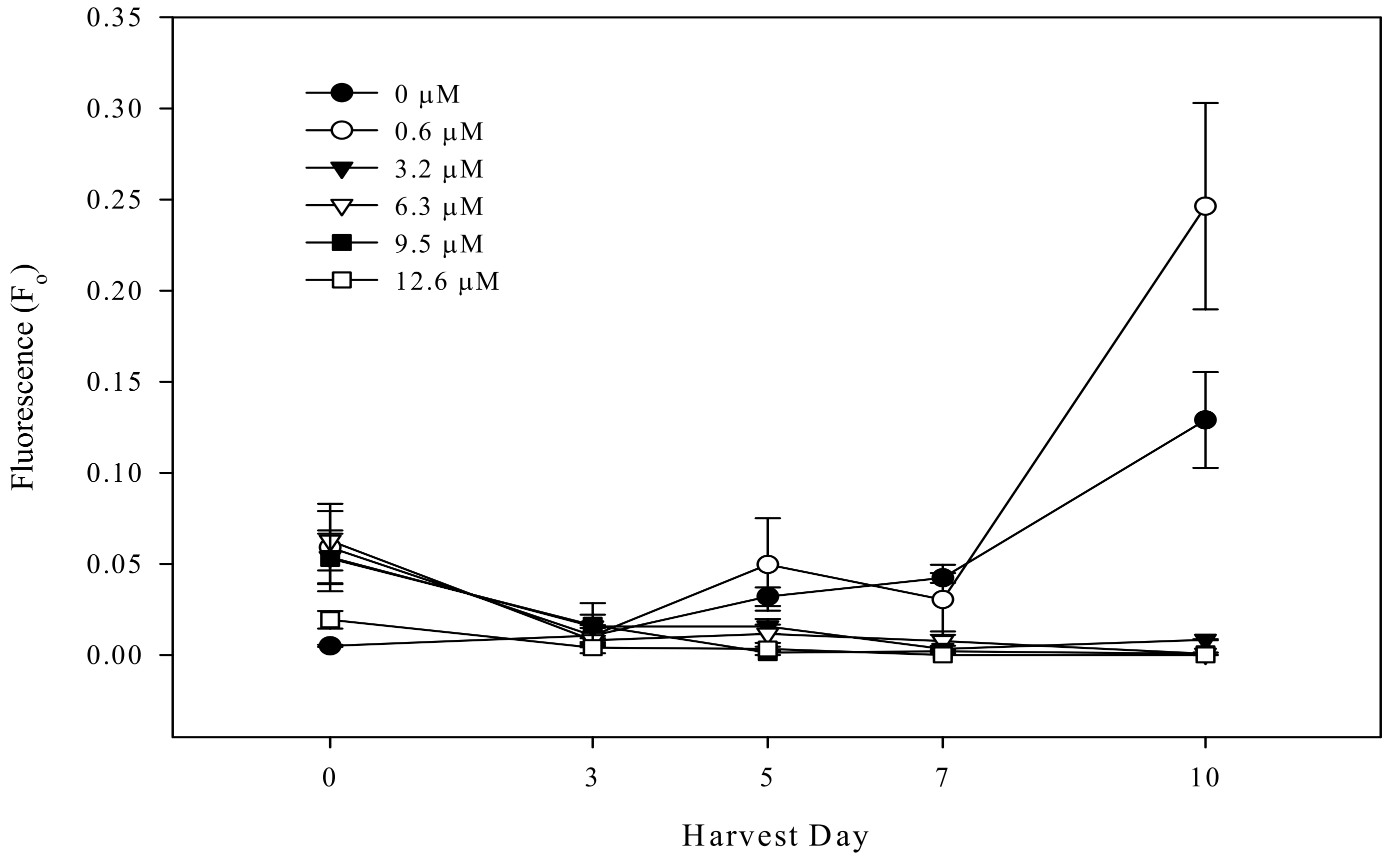

Figure 3. The effect of six different $ZnSO_4$ micronutrient levels on TCI Chlorophyll-*a* fluorescence response (F_o) on five different harvest dates.

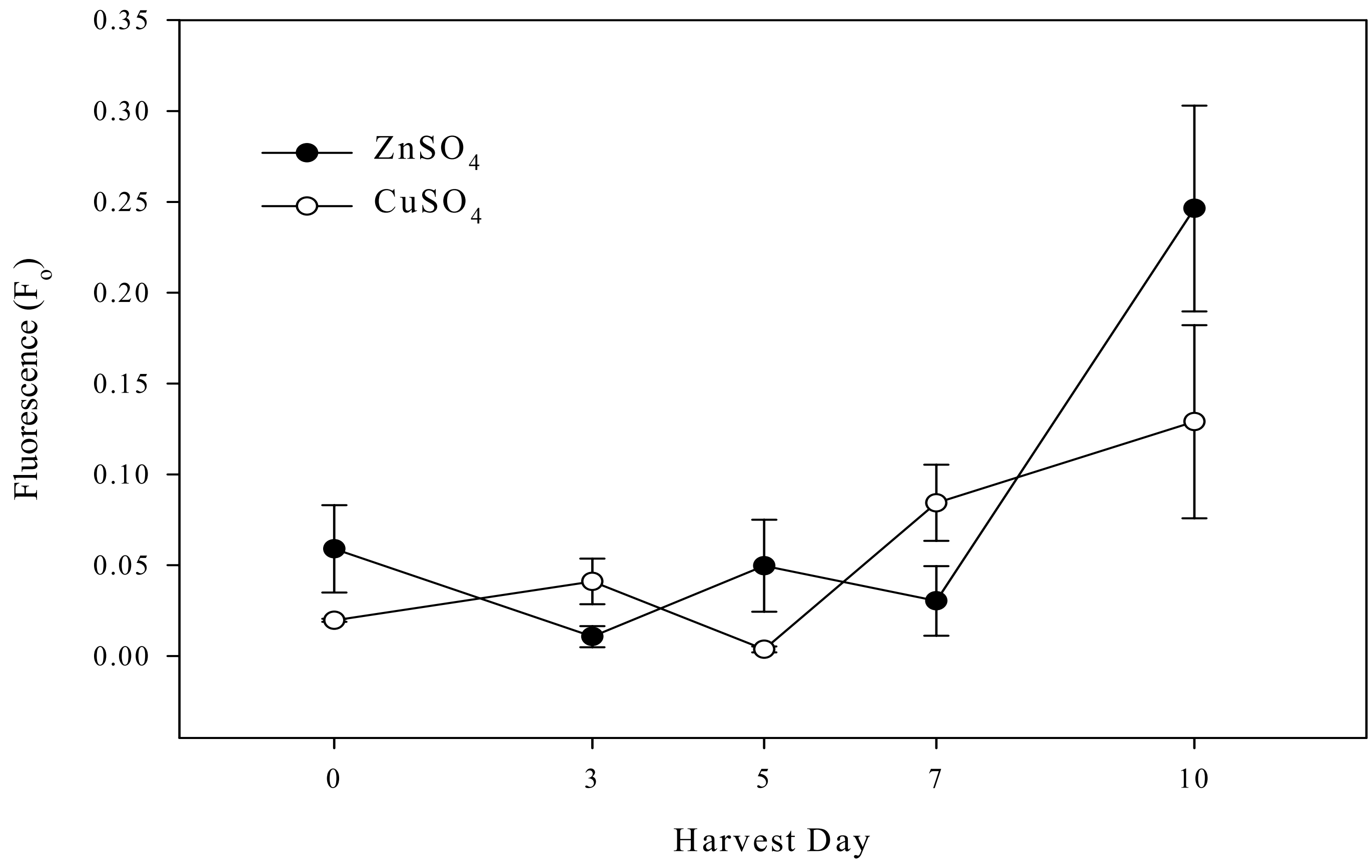

Figure 4. Effect comparison of $CuSO_4$ and $ZnSO_4$ at 0.6 μM concentrations on TCI Chlorophyll-*a* fluorescence response (F_o) at five harvest dates.

Vita

Steven Michael Borst son of Steven Eugene Borst and Gina Porter Borst, married to Golda Johansson Borst was born on the 7th of June 1984, in Huntingdon, Pennsylvania. Steven was raised in Petersburg, a small town in central Pennsylvania. He attended the Juniata Valley High School where he received his diploma in 2002. He then attended the Pennsylvania State University and received a Bachelor of Science degree in Turfgrass Science in 2006. Steven attended the University of Tennessee in June 2006 was appointed a Graduate Research Assistantship and received a Master of Science degree in Plant Sciences August 2008.