# Compost Tea for the Management of Dollar Spot (*Sclerotinia homoeocarpa*) on Turfgrass

**by**

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**Submitted in partial fulfillment of the requirements**
**for the degree of Master of Science**

**at**

**Dalhousie University**
**Halifax, Nova Scotia**

**In co-operation with**

**Nova Scotia Agricultural College**
**Truro, Nova Scotia**

**August 2012**

DALHOUSIE UNIVERSITY

NOVA SCOTIA AGRICULTURAL COLLEGE

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Dated: August 7, 2012

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AND

NOVA SCOTIA AGRICULTURAL COLLEGE

DATE: August 7, 2012

AUTHOR: Stephen Kelloway

TITLE: Compost Tea for the Management of Dollar Spot (*Sclerotinia homoeocarpa*) on Turfgrass

DEPARTMENT OR SCHOOL: Department of Environmental Sciences

DEGREE: M.Sc. CONVOCATION: October YEAR: 2012



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Signature of Author

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**ABSTRACT**

Turfgrasses are unique in their capability of tolerating foot traffic and physical wear, while still remaining functional and aesthetically pleasing. Fungal disease represents one of the most common limiting factors in managing turf for economical purposes. Dollar spot (*Sclerotinia homoeocarpa* F.T. Bennett) represent one of the most common and persistent fungal diseases of turf grasses here in the Maritime Provinces. The results demonstrated the potential of compost tea (CT) to negate the harmful effects of the fungal toxic metabolite produced during a dollar spot infection, as well the teas showed to increase the activity of defense enzymes as compared to the control. The field study showed the potential for mink compost tea (M-CT) to control disease but its efficacy was site specific and quite variable. Finally the teas were determined to be quite consistent in composition and four secondary metabolites were determined to be present in the teas.

## List of Abbreviations and Symbols Used

| | |
|---|---|
| % | percent |
| °C | degrees Celsius |
| µl | microliters |
| µM | micromolar |
| µmol $m^{-2}s^{-1}$ | micro-moles per square meter per second |
| $^{1}H$ | proton NMR |
| ANOVA | analysis of variance |
| ATRF | Atlantic Turfgrass Research Foundation |
| C:N | carbon to nitrogen |
| CAT | catalase activity |
| C-CT | cow compost tea |
| CEC | cation exchange capacity |
| CFU | colony forming units |
| Ch-CT | chicken compost tea |
| cm | centimeter |
| CT | compost tea |
| Cu | copper |
| Cv | cultivar |
| $D_2O$ | heavy water |
| DSS | 4,4-dimethyl-4-silapentane-1-sulfonic acid |
| EDTA | ethylenediaminetetraacetic acid |
| EtOAC | ethyl acetate |
| Fe | iron |
| FID | free induction decay NMR |
| G | grams |
| GLM | general linear model |
| $H_2O$ | water |
| Hr | hour |
| HSD | honestly significant difference |
| $kg/m^2$ | kilograms per meter squared |
| L | liters |
| M | molar |
| M | meter |
| m² | meters squared |
| M-CT | mink compost tea |
| MeOH | methanol |
| mg | milligrams |
| $mgml^{-1}$ | milligrams per millilitre |
| MHz | megahertz |
| min | minute |
| ml | millilitres |
| mm | millimetre |

| | |
|---|---|
| mmhos | measure of soil conductivity |
| Mn | manganese |
| MS | murashige and skoog salt |
| NaOCl | sodium hypochlorite |
| nm | nanometre |
| nMol | nanomoles |
| NMR | nuclear magnetic resonance |
| NS | Nova Scotia |
| NSAC | Nova Scotia Agricultural College |
| PAL | phenylalanine ammonia lyase activity |
| PCA | principal component analysis |
| PDA | potato dextrose agar |
| PLS-DA | particle least square discriminant analysis |
| POD | peroxidase activity |
| ppm | parts per million |
| PPO | polyphenol oxidase activity |
| psi | pound per square inch |
| PVP | polyvinylpyrrolidone |
| rpm | revolutions per minute |
| S | second |
| Sat. | saturation |
| SE | standard Error |
| SH-Toxin | *sclerotinia homoeocarpa* toxin |
| spp. | species |
| V/V | volume to volume |
| Zn | zinc |
| ΔOD | change in absorbance |

**Acknowledgements**

I would like to take the time to express my sincere thanks to my supervisor, Dr. Balakrishnan Prithiviraj, for his dedication, support and guidance throughout this project. I would also like to thanks my committee members, Dr. Jeff Norrie (Acadian Seaplants Limited) and Dr. Nathan Boyd (Nova Scotia Agricultural College) for this support and guidance during this project. My special thanks to Dr. Kevin Sibley for his enthusiasm and support during the early stages of this project.

I am thankful to all the members of the Marine Bio-products research Laboratory (MBRL) at the Nova Scotia Agricultural College for their continued support, assistance and encouragement throughout this project. In particular, I would like to thank Mullaivannan Manoharan for his hard work and dedication during the past few years.

I would also like to acknowledge the Natural Science and Engineering Research Council of Canada (NSERC), Atlantic Turfgrass Research Foundation (ATRF), Canadian Turfgrass Research Foundation (CTRF) and Nova Scotia Department of Agriculture for their financial contributions to this project.

I would like to thank Shane Sutherland and the staff at Mountain Golf and Country Club, Valley, Nova Scotia; Brian Gouthro and the staff at New Ashburn, Fall River, Nova Scotia and Jason Fillion and the staff at Old Ashburn, Halifax, Nova Scotia for their guidance and accommodation during the field trials.

Finally I would like to thank my wife, Jennifer for all her encouragement and support throughout the entire project.

## Chapter 1.0 Introduction

The turfgrass industry of Atlantic Canada represents a fast growing, and profitable market. For example, it has been estimated that golf and associated business activities accounts for $11.3 billion of Canada's Gross Domestic Product (NAGA, 2009). Therefore, with the potential for significant economic development from this market, turfgrass is becoming an important component in the Canadian horticultural industry. Turfgrasses represent a very unique group of grass species that have the ability to withstand foot traffic and retain the desired aesthetic looks. There are a number of biotic and abiotic factors that affect the health of turfgrass. Among the biotic factors, dollar spot caused by *Sclerotinia homoeocarpa* F.T. Bennett, is the most important (Walsh *et al*. 1999). Dollar spot disease is a common, persistent, and destructive fungal disease affecting both cool and warm season turfgrass in most areas of the world (Smith *et al.*, 1989; Smiley *et al*., 1992). The disease in the northern regions of North America is primarily associated with bentgrass, (*Agrostis spp.*) on putting greens (Smith *et al.*, 1989). It is however, also known to infect and severely damage various other species including, *Poa spp., and Lolium spp*., managed as fairways, sports fields and residential lawns. Due to the persistent nature of the disease in managed turfgrass, intensive control measures are often needed (Smiley *et al*. 2005). It has been stated that dollar spot is the most chronic and problematic disease of golf course turf throughout the United States (Kaminski and Fidanza, 2009), and North America (Couch, 1995). More money is spent to control this disease than any other turfgrass diseases on golf courses (Goodman and Burpee,1991; Atlantic Turfgrass Research Foundation Board members, 2009, *personal communication*).

The Atlantic Turfgrass Research Foundation (ATRF) is interested in research related to non-chemical or environmentally friendly management options for dollar spot due in part to public pressure to reduce chemical usage. As well, there has been a recent ban on pesticide use within the Atlantic Provinces banning the sale of pesticides for residential and municipal maintained turfgrass applications. Golf courses are currently exempt from this ban but the future is uncertain. Research has suggested the use of compost soil amendments may reduce the severity and incidence of a wide variety of turfgrass diseases (Block, 1997; Nelson and Boehm, 2002a; Roulston, 2006). However the use of compost can pose some issues in terms of application, smell, and transportation. Compost tea (CT) may offer an easy alternative and researchers suggest CT can also have disease suppressive qualities in turfgrass as a result of microbial activity in the tea, while others suggest it stimulates microbial activity in the soil which in turn suppresses fungal growth (Kone *et al.,* 2009; Siddiqui *et al.,* 2009; Ryan *et al.,* 2005). It has also been postulated that the composition of CT might induce disease resistance in turfgrass by stimulating physiological changes in the plants (Weltzein and Ketterer, 1986; Zhang *et al.*1998). However, very little is actually known about the effect of CT and the physiological mechanisms of action within the plant.

**1.1.0 Literature Review**

**1.1.1 Turfgrass**

Cool season turfgrass species that a temperature optimum ranging from 20 to 25 $^{o}$C (Beard, 1973). The most common species of cool season grass grown in Atlantic Canada include Kentucky Bluegrass, (*Poa pratensis*) Creeping Bentgrass, (*Agrostis palustris*) and Perennial Ryegrass, (*Lolium perenne*). Kentucky bluegrass is a highly variable rhizomatous species that has

a close mowing tolerance and a varying level of fungal disease resistance depending on the cultivar (Turgeon, 2005). Well drained, moist, slightly acidic to neutral soils are the best for growing this species of grass. There are cultivars of bluegrass with differing levels of dollar spot resistance, such as the cultivar Blue Chip Plus II. Perennial ryegrass is a well suited species to the cool season climate. It is a bunch-type grass and depending on environmental conditions can grow as an annual or a short lived perennial. It is adapted to a wide range of soil conditions but does well in neutral to slightly acidic moist soils. It is often blended with Kentucky bluegrass and used in mixtures because of its fast establishment. Creeping bentgrass is a fine textured, stoloniferous grass. There are many different cultivars that have varying levels of fungal disease resistance. Penncross is a commonly used cultivar with some fungal diseases resistance, but it commonly gets infected with dollar spot in Atlantic Canada. This species grows well in moist soil that is acidic to slightly acidic and does well as closely mowed turf on golf greens and tees.

### 1.1.2 Dollar Spot Disease

#### 1.1.2.1 Ecology

Dollar spot disease caused by the fungal pathogen *Sclerotinia homoeocarpa* F.T., is a common, persistent, and destructive fungal organism that affects cool and warm season turfgrass in most areas of the world (Smith *et al.*, 1989; Smiley *et al*., 1992). The disease is primarily associated with bentgrass on putting greens in northern regions of North America (Smith *et al.*, 1989). It is however, also known to infect and severely damage other species including *Poa spp., and Lolium spp*., managed as fairways, sports fields and residential lawns. Due to the persistent nature of the disease in managed turfgrass, intensive control measures

are often needed (Smiley *et al*. 2005). Kaminski and Fidanza (2009) stated that dollar spot is the most chronic and problematic disease of golf course turf throughout the United States.

*Sclerotinia homoeocarpa* isolates can be identified by a characteristic mat of fluffy white mycelium (Figure 1.1), (Smiley *et al.* 1992). Microscopic examination of the mycelium shows a filamentous morphology that is dense in culture (Figure 1.2). The isolates of the fungus can vary in color from white to shades of gray, brown, or yellow as the culture ages (Walsh *et al.* 1999). The fungus however does not produce true sclerotia as compared to other members of this genus, therefore the proper taxonomic classification is still undetermined.

Figure 1.1. Fluffy white mycelial mat of *Sclerotinia homoeocarpa* fungus.

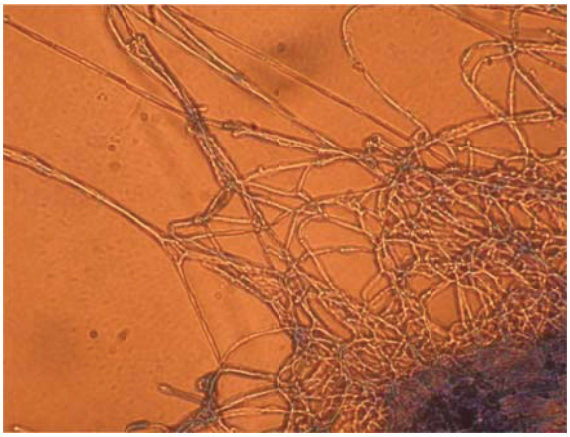

Figure 1.2. Filamentous mycelium of *Sclerotinia homoeocarpa* fungus.

The fungus survives unfavourable conditions as a persistent mycelial infection in plant debris, often being linked to the presence of thatch (Smith *et al.*, 1989; Smiley *et al.,*2005). The fungus will also survive unfavourable growth conditions as mycelia within infected plants and as stromata on foliar surfaces (Smiley *et al*., 1992; Smiley *et al.,*2005). Favourable conditions for the growth of the fungus often include warm, humid weather accompanied by cool nights

resulting in heavy dews. The disease is most severe in dry soils even though free water on the surface of the grass blades is needed. Ambient temperatures ranging from 15-25$^{o}$C for 24 hours or longer and low nitrogen fertility of the soil in combination with the surface wetness of the blades promotes rapid growth of the fungus (Smith *et al.*, 1989; Smiley *et al*., 1992; Smiley *et al.,*2005).

**1.1.2.2 Pathogenesis**

When weather conditions become favourable for fungal growth, mycelia will grow out of infected tissue or stromata and colonize adjacent grass blades (Smiley *et al.,* 1992). During conditions of warm humid days followed by cool nights with heavy dew, mycelium spread into the humid air. When these aerial mycelium contact a moist leaf surface of an adjacent grass blade it will penetrate the blade and cause an infection. The fungus can also enter through stomates, cut leaf tips, and other wounds on the blades of grass (Smiley *et al.,* 2005). Once the fungus infects a leaf, the leaf will develop a lesion that first looks chlorotic (Figure 1.3a), then water-soaked (Figure 1.3b), and finally bleached (Figure 1.3c). The lesions are characteristically bounded by darker margins, are often hour glass shaped, and spread across the entire leaf surface from edge to edge. Individual leaves can have single lesions, many small lesions, or the entire leaf may be completely blighted.

Once infected, the disease morphology varies with the texture and cut height of the turfgrass. On a fine textured closely mown turf, the spots first turn brown and then become a bleached or straw color, growing to about 5 cm in diameter (Figure 1.3d). If the disease is left unchecked, the spots will become sunken and may coalesce to affect a large area (Smith *et al.*, 1989). The small, circular, sunken patch is the characteristic symptom on closely mown turf

and is where the name “Dollar spot” is derived due to the size of the spots resembling the size of a silver dollar (Smiley *et* al., 1992; Walsh *et* al., 1999). On coarse textured higher mown turf, the spots are much larger and irregular in appearance and may reach 15 cm is diameter; often coalescing to form very large areas of diseased turfgrass. The fungus has also been associated with production of a root damaging toxin, produced by the fungus during an infection (Malca and Endo, 1965, Smiley *et al.,* 2005).


Figure 1.3. Developmental stages of dollar spot disease on turfgrass. A) Initial chlorotic stage B) Water soaked stage C) Bleached stage D) Dollar spot infection on close mown turf.

#### 1.1.2.3 Dollar Spot Management

Dollar Spot is managed with chemical fungicides including boscalid, chlorothanonil, fernarimol, iprodione, mancozeb, propiconazole, thiophanate, triadimefon, thiram, and vinclozolin (McCarty, 2005). Fungicide treatments last between 10 to 28 days depending on the chemical. As a result, multiple applications are usually required throughout the growing season during periods of disease susceptibility. Resistance of *S. homoeocarpa* to chemicals used for its management has developed, and has become problematic for the benzimidazole class of fungicides and the sterol biosynthesis inhibitors (McCarty, 2005).

Cultural practises that make the leaf surface less favourable for the fungus include thoroughly irrigating the turf to deep root-zone level, as infrequently as possible without causing moisture stress between irrigations as well as avoiding late afternoon or evening watering (Williams *et al.*1996; Smiley *et al.,* 2005). Good air circulation around the turfgrass, as well as regular mowing at recommended heights, will also help prevent infections. The addition of nitrogen coupled with the removal of clippings help remove the inoculum present and help in the management of the disease, the disease has often been reported as being most severe on weak, slow growing turfgrass therefore good cultural practises can aid in the management of the fungus. Maintaining adequate nitrogen fertility during times of dollar spot infection and collection of clippings after mowing may also help to suppress the disease. Applications of nitrogen will help in the growth of the grass and will promote tiller and leaf production, and this helps the grass overcome the disease (Williams *et al.*1996). Breeding of turfgrass for dollar spot resistance is another approach to manage this disease. However, there has been only marginal

success in developing turfgrass varieties that are highly resistant to this disease (Bonos *et al.*2004).

Among the more promising alternative to chemical management methods is the use of compost amendments (Nelson and Boehm, 2002a) and CT's (Schuerell and Mahaffee, 2002; Litterick *et al.,* 2004). This research and other studies have shown the potential for compost amendments to reduce the severity and incidence of a wide variety of turfgrass diseases, including dollar spot, particularly when applied as a topdressing, winter cover, or root-zone amendment, or as a surface applied CT (Nelson and Craft, 1992; Block, 1997; Roulston, 2006). Utilization of compost amendments and teas for turfgrass disease control, however, has been limited because of inconsistent performance that has been attributed to an overall lack of understanding of the disease suppression mechanisms (Nelson and Boehm, 2002b; Scheuerell, 2003; Hsiang and Tian, 2007).

### 1.1.3 Composts and Compost Teas

#### 1.1.3.1 Consistency of Source

CT's are liquid suspensions of composted materials that are prepared using either of two general methods: aerated or non-aerated digestion (Schuerell and Mahaffee, 2002; Litterick *et al.,* 2004). The literature suggests that various preparations of tea, altered by differences in methods of aeration, feedstock, or various added carbon source, can produce teas that act in different ways against pathogens (Scheurell 2003). However, very little is known about the specific mechanisms by which CT's control plant diseases. Therefore, there is a need to develop a CT from a known feedstock that shows consistent composition. Further, it is

necessary to develop a tea that is consistent every time it is prepared and can therefore be reproduced with confidence and used effectively in the turfgrass industry.

#### 1.1.3.2 Physio-Chemical Profiles of Compost Teas

Studies using compost as soil amendments have focused on the influence of the seasonal dynamics of plant-available Mn, Fe, Cu, and Zn in soil after a single application to bermudagrass (Provin et al., 2007). Bioactive compounds such as polyphenols, phenylalanine ammonia-lyase, superoxide dismutase, and catalase, are important indicators which help in identifying physiological changes in plants. Many of these compounds exhibit a range of biological activities, such as antioxidant, antifungal, antibacterial, and antiviral properties (Boudhrioua et al., 2009). The microorganisms present in CT's are associated with a large number of bioactive compounds such as plant growth hormones, as well as many chemical compounds such as phenols, tannins (Shrestha *et al.*2011). Microorganisms can help in the extraction of nitrogen from compost feedstocks to liberate a number of plant nutrients.

### 1.1.4 Disease Resistance

#### 1.1.4.1 Compost Teas Impart Disease Resistance

Composted soil amendments have been successful in reducing the severity and incidence of a wide variety of turfgrass diseases (Block, 1997; Nelson and Boehm, 2002a). The disease suppressive qualities of compost generally are attributed to microbes within the compost, in addition to stimulating beneficial indigenous microbes in the soil which can suppress pathogens (Ryan *et al.,* 2005; Siddiqui *et al.,* 2009; Kone *et al.,* 2010). Microbes such as *Rhizobacteria, Trichoderma, Pseudomonas spp.* can all be associated with CT's and play major roles in disease suppressive qualities (Shrestha *et al.*2011). Microbes in compost, as well as CT

are most often associated with disease control through competition with other pathogenic organisms on the leaf surface or in the soil (Ryan *et al.,* 2005; Siddiqui *et al.,* 2009; Kone *et al.,* 2010). Biological control of soil pathogens is known to be related to suppression through secondary metabolites produced by antagonists microorganisms (Dukare *et al.*2011). Additionally the composition of CT can potentially be conducive to induction of disease resistance in turfgrasses, through stimulation of innate physiological pathways within the plants (Siddiqui *et al.,* 2009). However, very little is actually known about the effect of CT treatments and the resultant physiological mechanisms altered within the plant. CT has been described as being capable of increasing rooting depth and health as well as improving plant growth, all attributes that would increase the control of plant pathogens and dollar spot in turfgrass (Ingham, 2005).

### 1.1.5 General Hypothesis

Compost teas prepared from cow, mink, and chicken waste will impart resistance in turfgrass against the fungal pathogen *Sclerotina homoeocarpa* F.T. Bennett.

### 1.1.6 Objectives

**The overall objective of this study is to investigate the physio-chemical effects of compost tea derived from agricultural animal waste on inducing resistance to Dollar Spot in turfgrass species commonly used in Atlantic Canada. Specific objectives of this study are to:**

- Determine whether the incidence and severity of dollar spot is diminished in turfgrass by the application of compost tea prepared from cow, mink, and chicken waste feedstocks.
- Determine if compost tea prepared from cow, mink, and chicken waste feedstocks impart resistance to dollar spot in Creeping bentgrass, by effecting physiological changes in the turfgrass.
- Develop a research based understanding of compost tea's physio-chemical mode of action within Creeping bentgrass, that will lead to recommendations for effective compost tea usage by turfgrass managers in Atlantic Canada.

## Chapter 2.0 Materials and Methods

### 2.1.0 Seeds and Chemicals

Seeds of creeping bentgrass (*Agrostis stolonifera* cv. Penncross) were purchased from Halifax Seeds Co. (Halifax, NS, Canada). All the other chemicals and reagents were of analytical grade and were purchased from Sigma Aldrich (Oakville, ON, Canada), unless otherwise stated.

### 2.2.0 Compost Production

Three mature composts were used in the different experiments. Maturity of the compost was determined for Mink and Chicken compost based on nutrient analysis and continuous monitoring of temperature throughout the composting process. The cow compost, was commercially available (ASB Greenworld Ltd. NB. Canada), and was purchased locally at a garden center. Mink and chicken composts were prepared using feedstocks and hay collected from the NSAC farm, Bible Hill, NS. Canada. Feedstocks were analyzed for C:N ratios as well as moisture content. Compost piles were mixed in proportions that resulted in 25:1, C:N ratios and 65% moisture levels for the composting process. Mink compost consisted of a combination of fine wood shavings, hay and manure, and chicken compost contained manure and hay only. The composting process took place between August 27$^{th}$, 2010 and November 3$^{rd}$, 2010, piles were mixed, constructed and matured at the NSAC composting facility. Piles were turned once monthly and moisture was added as needed. The composting process was completed when the core temperature in the piles fell below 30°C and piles were then moved outside, covered and left to overwinter until the following spring. Each compost pile was monitored to make sure pathogen kill temperatures (>55°C for >5 days), were reached (Boulter *et al*. 2002). Analysis of completed compost is presented in Table 2.1.

**Table 2.1.** Elemental analysis of mature composts.

| Analysis Performed | Cow Compost | Mink Compost | Chicken Compost |
|---|---|---|---|
| **Dry Matter %** | 32.177 | 21.590 | 42.570 |
| **Nitrogen %** | 0.455 | 0.602 | 1.196 |
| **Calcium %** | 0.342 | 1.000 | 4.507 |
| **Phosphorus %** | 0.135 | 0.539 | 0.778 |
| **$P_2O_5$ %** | 0.309 | 1.234 | 1.782 |
| **Potassium %** | 0.183 | 0.256 | 2.045 |
| **$K_2O$ %** | 0.221 | 0.310 | 2.474 |
| **Magnesium %** | 0.087 | 0.129 | 0.370 |
| **Sodium %** | 0.044 | 0.038 | 0.180 |
| **Iron %** | 0.070 | 0.086 | 0.115 |
| **Manganese %** | 0.016 | 0.014 | 0.038 |
| **Copper %** | 0.001 | 0.001 | 0.004 |
| **Zinc %** | 0.007 | 0.011 | 0.021 |
| **Boron %** | 0.001 | 0.001 | 0.002 |

### 2.3.0 SH-Toxin Experiment

#### 2.3.1 SH-Toxin Extraction

SH-toxin extraction was carried out using a procedure similar to that described by Herath *et al*. (2009). *S. homoeocarpa* was isolated from surface sterilized infected creeping bentgrass leaf tissue, exhibiting dollar spot symptoms, from Bible Hill, NS, Canada. Cultures were then sub-cultured monthly on potato dextrose agar (PDA) (Difco, BBL, Franklin Lakes, NJ, USA). Several mycelia plugs of *S. homoeocarpa* (72-96h post subculture), were used to

inoculate 125µl of potato dextrose broth (Difco). A total of 10 flasks were inoculated and then incubated at 27°C, with constant agitation (100rpm) on an orbital shaker for a period of 15 days. Mycelial fragments were separated from the supernatant, through filtration through a double layer of cheesecloth, followed by vacuum filtration using Whatman #1 filter paper (VWR, Mississauga, ON, Canada). Soluble toxic metabolites were extracted from the filtered culture media using three ethyl acetate (EtOAc) washes. The EtOAc extract was then evaporated to dryness and the crude extract was dissolved in methanol (MeOH) and re-evaporated in $N_2$ gas to reduce oxidation. The resulting dried dark residue was weighed and dissolved in a corresponding volume of MeOH producing a solution of 100 mg $ml^{-1}$ toxin. The isolated *S.homoeocarpa* toxin (SH-toxin), was then screened by introducing filter paper embedded with toxin to the root tips of actively growing (*A. stolonifera*) seedlings and assaying plants for root tip darkening and root curving as previously described by Malca and Endo. (1965) (Figure 2.1)

Figure 2.1. Curling, root tip darkening and thickening phenotype observed 18 days after plants have been exposed to SH-toxin in the assay.

### 2.3.2 Compost Tea Production

Cow compost (C-CT) was produced from commercially available composted cow manure (ASB Greenworld Ltd. NB. Canada). Compost analysis is presented in Table 2.1. The tea was brewed in uncovered 5L plastic containers using 1:5 compost to non-chlorinated water ratio supplemented with 0.2 % (v/v) molasses and supplied with constant aeration for 24h. Analysis of tea is presented in Table 2.2. The tea was filtered through a double layer of cheesecloth and sterilized by autoclaving for 15min, at 121$^{o}$C and 15psi.

**Table 2.2.** Elemental and physio-chemical analysis of compost tea.

| Analysis Performed | Cow Compost Tea (C-CT) | Mink Compost Tea (M-CT) | Chicken Compost Tea (Ch-CT) |
|---|---|---|---|
| **pH** | 4.82 | 5.73 | 7.43 |
| **Conductivity (mmhos)** | 0.87 | 2.03 | 5.49 |
| **Elemental Components:** | | | |
| **Nitrate-N (ppm)** | 0.25 | 0.55 | 3.40 |
| **Phosphorus (ppm)** | 44.09 | 127.36 | 165.13 |
| **Potassium (ppm)** | 191.66 | 534.42 | 1685.80 |
| **Calcium (ppm)** | 23.20 | 78.46 | 90.30 |
| **Magnesium (ppm)** | 12.98 | 71.44 | 82.99 |
| **Boron (ppm)** | 0.19 | 0.37 | 0.98 |
| **Iron (ppm)** | 0.11 | 1.80 | 9.57 |
| **Manganese (ppm)** | 0.30 | 0.75 | 2.38 |
| **Copper (ppm)** | 0.05 | 0.11 | 0.88 |
| **Zinc (ppm)** | 0.14 | 0.46 | 1.74 |

### 2.3.3 Plant Material and Treatments

*A. stolonifera* cv. Penncross seeds were surface sterilized using 2.5% (v/v) NaOCl, with 6 subsequent rinses with sterilized $H_2O$. Seeds were sown onto sterilized filter paper within a 100ml glass jar, at a rate of 10 seeds per jar. Jars contained 4ml of sterile solution consisting of half-strength MS media, (Murashige and Skoog salt) (Sigma, Oakville, ON. Canada), and either 20% or 40% CT. SH-toxin was applied at a rate of 12 µl per jar and 12 µl of sterile $H_2O$ served as a control. Jars were then sealed with Magenta B Caps (Sigma, Oakville, ON. Canada), and were grown at 22$^{o}$C with a 16h light and 8h dark cycle with light intensity of 100µmol $m^{-2}$ $s^{-1}$.

### 2.3.4 Measurements

Eighteen days after seeding the number of germinated seeds were counted and percentage of germination was calculated for each individual jar. Germinated plants were then collected and scanned (Epson Expression 10000 XL) (Epson Canada Ltd., Markham, ON. Canada) and the length of the root and blade were measured using Image J Software (Research Services Branch, NIH). The average root and blade length was calculated for plants in each individual jar.

### 2.3.5 Statistical Analysis

The experiment was setup as a completely randomised design with three replicates per treatment. Each experiment was repeated three times. Data was analysed with a one-way ANOVA using Proc GLM procedure with Statistical Analysis Software (SAS)( SAS Institute, Cary, NC, USA) at $P$= 0.05.The Tukey's honestly significant difference (HSD) post hoc test was performed in cases of significance ($P$= 0.05) to further separate the means per treatment.

### 2.4.0 Enzyme Analysis following Compost tea treatment and *S. homoeocarpa* Infection

#### 2.4.1 Plant Material and Treatments

*A. stolonifera* cv. Penncross were grown according to a procedure described by Cortes-Barco *et al*. 2010 with modifications. MK5 Caisson boxes (Caisson Labs, North Logan, UT. USA), were filled with 100g of dry 80:20 topdressing sand (Shaw Resources, NS. Canada) to Pro-Mix BX (Premier Horticulture INC. PA. USA) mixture supplemented with 16-32-6 (N-P-K), starter fertilizer (Nu-gro Golf, Brantford, ON. Canada), following the recommended rate of 1.5kg/100m². Forty milliliters of distilled water was then added to the boxes which were then capped and autoclaved twice for 15min, at 121$^{o}$C and 15psi. Approximately 200g of seed was sown in each box and boxes were capped and grown for 15 days at 22$^{o}$C with a 16h light and 8h dark cycle with light intensity of 100µmol $m^{-2}$ $s^{-1}$. Once grass had grown the sand was directly injected with 10ml of 100% C-CT, 100% M-CT, 100% Ch-CT, 100 µM (2R, 3R)- butanediol (Sigma, Oakville, ON. Canada), or distilled water as control with a pipette.

#### 2.4.2 Compost Tea Production

CT was produced from mink compost (M-CT), chicken compost (Ch-CT) as well as commercially available composted cow manure (C-CT) (ASB Greenworld Ltd. NB. Canada). Compost analysis is presented in Table 2.1. Tea was brewed in uncovered 5L plastic containers, using 1:5 compost to non-chlorinated water ratio supplemented with 0.2 % (v/v) molasses and supplied with constant aeration for 24h. The tea was filtered through a fine mesh screen 1.2mm x 1.2mm, analysis of tea is presented in Table 2.2.

### 2.4.3 Inoculum Preparation

*Sclerotinia. homoeocarpa* was isolated from surface sterilized infected creeping bentgrass leaf tissue, exhibiting dollar spot symptoms, from Bible Hill, NS, Canada. Cultures were then sub-cultured monthly on potato dextrose agar (PDA) (Difco, BBL, Franklin Lakes, NJ, USA). The inoculum was prepared using a procedure described by Boulter *et al*. (2002) with some modifications. Sub-cultured *S.homoeocarpa* plates were grown for a period of 6 days at 22°C, then 20 plugs 5mm in diameter were cut from the boarders of the culture and inoculated directly into 1000ml Erlenmeyer flasks which contained 250ml sterile millet seed, and 100ml of sterile $H_2O$. The sterile millet seed was prepared as follows: millet seed was placed in flasks and autoclaved for 15min, at 121$^{o}$C and 15psi to ensure sterilization of seeds. Flasks were then incubated at 24°C for 21days to ensure full colonization of the seed by the fungus. Seed was then dried in a laminar flow hood for 48 hours cleaned with a 2mm sieve and stored in sterile bags at 4°C until needed.

### 2.4.4 Inoculation Methods

Approximately 10 days after the application of each treatment the boxes were inoculated with 4 seeds from the inoculum described in section 2.4.3. One seed was placed in each corner of the box which was then tightly capped again to allow for fungal growth.

### 2.4.5 Tissue Collection and Storage

Approximately 200g of creeping bentgrass tissue was cut with scissors, collected and placed in a 1.5ml micro-centrifuge tube (VWR, Mississauga, ON, Canada), flash frozen with liquid nitrogen and stored at -80°C until time of extraction.

### 2.4.6 Extraction of Crude Enzyme

The enzyme extraction of creeping bentgrass tissue was carried out using a procedure described by Sarkar *et al*. (2009) with some modifications. Stored creeping bentgrass tissue was thawed on ice and fully macerated in the 1.5ml micro-centrifuge tubes using a micro-pestle (VWR, Mississauga, ON, Canada) and 1ml of cold enzyme extraction buffer. Extraction buffer was composed of 0.1 M $KH_2PO_4/K_2HPO_4$ buffer (pH 7.5), containing 0.5% polyvinylpyrrolidone (PVP), and 3mM EDTA. The extracted sample was centrifuged at 12,000 *g* for 10min at 4°C, the supernatant was then transferred to a new 1.5 ml tube and centrifuged a second time at 12,000 *g* for 10min at 4°C and then stored on ice. This supernatant was then used for all enzyme and biochemical analyses.

### 2.4.7 Determination of Total Protein

The supernatant was collected as a crude enzyme extract. Protein concentration was measured using the Coomassie Plus – The Better Bradford™ Assay Kit (Pierce, Rockford, IL, USA). Two hundred micro-liters of the Bradford reagent was added to 35µL of $H_2O$ and 5µl of crude enzyme extract and the absorbance was read at 595 nm using a BioTek Power XS2 microplate reader (VT, USA) with Gen5™ software. The amount of protein per sample was calculated using bovine serum albumin standard curve (125–2000 µg/mL). The standard, as well as the samples were run in triplicate in the same 96 well plate for each replication.

### 2.4.8 Estimation of Phenylalanine Ammonia Lyase Activity

Enzyme extraction was conducted following the protocol described in section 2.4.6. The phenylalanine ammonia lyase (PAL) assay was based on the methods described by Rahman and Punja (2005), and Indiragandhi *et al*. (2008), with modifications. The 250µL reaction mixture

contained 200µL of 15mM L-phenlalanine, in 0.1 M Tris-HCL buffer at pH 8.8, 35µL of $H_2O$ and 5µl of crude enzyme extract. The mixture was incubated at 37 °C for 60 min and 10µl of 5M HCL was added to stop the reaction. The absorbance of the solution was determined at 290 nm against the blank using a BioTek Power XS2 microplate reader (VT, USA) with Gen5™ software. The concentration of cinnamic acid was calculated using a cinnamic acid standard curve (10–500 nMol). The standard, as well as the samples were run in triplicate in the same 96 well plate for each replication. PAL activity was defined as nMol of cinnamic acid/h/mg protein.

**2.4.9 Estimation of Polyphenol Oxidase Activity**

Enzyme extraction was conducted following the protocol described in section 2.4.6. The polyphenol oxidase (PPo) assay was based on methods described by Wang *et al*. (2005), with modifications. The 240µL reaction mixture contained 200µL 0.5M catechol, in 0.1 M potassium phosphate buffer at pH 6.5, 35µL of $H_2O$ and 5µl of crude enzyme extract, incubated at 24 °C for 2 min. The absorbance of the solution was determined at 398 nm against the blank using a BioTek Power XS2 microplate reader (VT, USA) with Gen5™ software. All samples were run in triplicate in the same 96 well plate for each replication. PPo activity was defined as $\Delta OD_{398}$ /min/mg protein.

**2.4.10 Estimation of Catalase Activity**

Enzyme extraction was conducted following the protocol described in section 2.4.6. The catalase, (CAT) assay was based on the methods described by Sarkar *et al*. (2009), with modifications. The 205µL reaction mixture contained 200µL 0.059 M hydrogen peroxide, in 0.05 M potassium phosphate buffer at pH 7.0, 4µL of $H_2O$, and 1µl of crude enzyme extract. The absorbance of the solution was measured at 240 nm every 20 s for a period of 2-3 min using a

BioTek Power XS2 microplate reader (VT, USA) with Gen5™ software. All samples were run in triplicate in the same 96 well plate for each replication. The change in absorbance $\Delta OD_{240}$ /min from the initial linear portion of the curve was calculated, and one unit of catalase activity was defined as the amount needed to alleviate one micromole of hydrogen peroxide. Catalase activity was expressed as:

$$\text{Units/mg protein} = \frac{(\Delta OD_{240}/\text{min}) \times 1000}{43.6 \times \text{mg protein / ml of reaction mixture}}$$

**2.4.11 Estimation of Peroxidase Activity**

Enzyme extraction was conducted following the protocol described in section 2.4.6. The peroxidase activity (POD) assay was based on the methods described by Rahman and Punja (2005), with modifications. The 205µL reaction mixture contained 200µL 0.05 M guaicol solution, in 25 mM sodium acetate buffer at pH 5.0 and 8.8 mM hydrogen peroxide, 4µL of $H_2O$ and 1µl of crude enzyme extract. The absorbance of the solution was measured at 470 nm every 15 s for a period of 2 min using a BioTek Power XS2 microplate reader (VT, USA) with Gen5™ software. All samples were run in triplicate in the same 96 well plate for each replication. The change in absorbance $\Delta OD_{470}$ /min from the initial linear portion of the curve was calculated. POD activity was defined as $\Delta OD_{470}$ /min/mg protein.

**2.4.12 Statistical Analysis**

The average of each sample triplicate was then calculated for each individual assay. The experiment was conducted in a completely randomised design with three replicates per treatment, and each experiment was repeated twice. Data was then analysed with a one-way ANOVA using Proc GLM procedure with Statistical Analysis Software (SAS)( SAS Institute, Cary,

NC, USA) at *P*= 0.05 to determine significant difference between treatments. The Tukey's honestly significant difference (HSD) post hoc test was performed in cases of significance (*P*= 0.05) to further separate the means per treatment.

**2.5.0 NMR Spectroscopy Analysis of Compost teas**

**2.5.1 Sample Preparation**

Freeze dried CT samples were dissolved in $D_2O$ with 1 mM DSS in 1.5 mL vials to make 10 mg/mL solution. The solutions were votexed for 1min, sonicated for 30min, and then centrifuged for 5min at 14,000g. Approximately 45 µL of each CT solution was transferred to 1.7 mm NMR tube.

**2.5.2 NMR Analysis**

NMR spectra were acquired on a Bruker Avance III 600 MHz NMR spectrometer (Bruker Biospin Ltd., Milton, ON, Canada), which has a $^1H$ operating of 600.284 MHz. The $^1H$ NMR chemical shifts in the spectra were referenced to 0.00 ppm with respect to the internal DSS standard. All the data was acquired and processed using TopSpin NMR data acquisition and processing software (Bruker Biospin Ltd., East Milton, ON) to generate proton NMR spectra for comparison.

**2.5.3 NMR Data Processing for Principal Component Analysis**

For global profiling using spectral binning, the FID file of each sample was imported, phase and baseline corrected, and chemical shift calibrated by referencing to DSS. The spectra were then binned into 0.02 ppm buckets from 0-10 ppm, with selected regions excluded for water (4.73-4.82 ppm) and DSS (-0.60-0.66, 1.72-1.80, 2.88-2.94, 4.73-4.82 ppm). For targeted profiling, the processed spectra were loaded into the NMR spectra using Chenomx NMR Suite

(Chenomx Inc., Edmonton, AB), Profiler module, and fit against 304 standard compounds built in the software. Using DSS or DSS-d6 concentration in the sample as reference, the concentrations for metabolites profiled with confidence were obtained through the fitting process. The 4 major components were measured with the chenomx profiler.

### 2.5.4 Multivariate Data Analysis for Principal Component Analysis

The datasets from spectral binning and targeted profiling were imported to SIMCA-P+ (Umetrics, Umea, Sweden) for conducting principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA). The bins or metabolites concentrations were all mean-centered and Pareto-scaled prior to the analysis.

## 2.6.0 Greenhouse Experiment

### 2.6.1 Layout and Design

A greenhouse experiment was conducted in spring 2011, to help strengthen laboratory findings and to study the tea in a more controlled environment before field application. The experiment consisted of a 30 pot design with 5 replicates per treatment, (Figure 2.2).

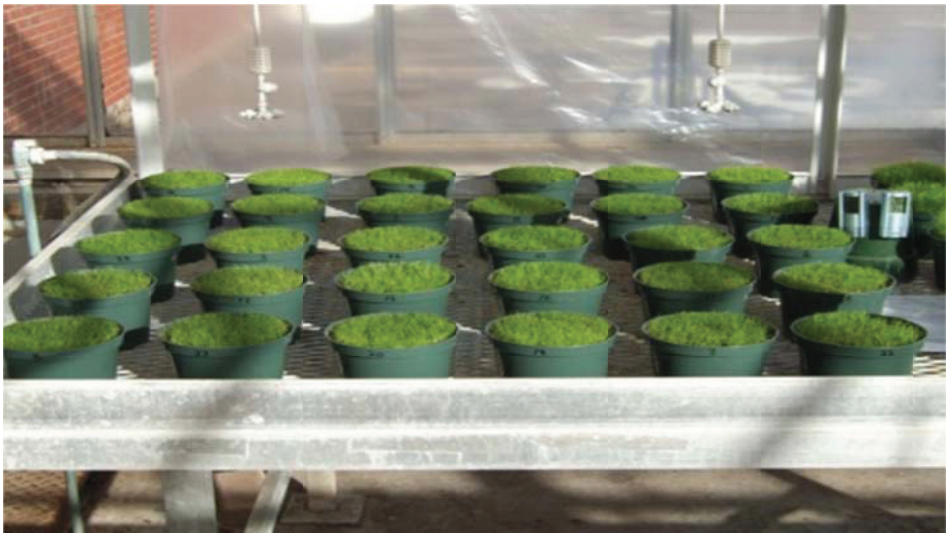

Figure 2.2. Greenhouse experimental setup, 30 pot design.

### 2.6.2 Preparation of Compost Tea

C-CT was produced from commercially available composted cow manure (ASB Greenworld Ltd. NB. Canada), compost analysis is presented in Table 2.1. Tea was brewed in uncovered 5L plastic containers, using a 1:5 compost to non-chlorinated water ratio supplemented with 0.2 % (v/v) molasses and supplied with constant aeration for 24h. The tea was filtered through a fine mesh screen 1.2mm x 1.2mm after brewing and the finished tea was then applied unaltered for 100% treatments or mixed 50% (v/v) with non-chlorinated water for 50% treatments. Analysis of tea is presented in Table 2.2.

### 2.6.3 Plant Material and Treatments

*A. stolonifera* cv. Penncross seeds were sown using a Scotts® Accugreen® 1000 drop spreader (The Scotts Company LLC), onto 15cm plastic pots containing 80:20 sand peat mix as described in section 2.4.1. Seeds were allowed to germinate for 7 days at 25°C under plastic

covers. After germination, covers were removed and pots were watered daily for a 1 month period, supplemented with weekly fertilization of 20-20-20 (N-P-K) all-purpose water soluble fertilizer (Plant-Prod Ultimate, Brantford, ON. Canada). One month old pots were then mowed to a height of 2cm and treatments began. Pots were treated on a 2 day cycle pre–inoculation, with 133ml of 100% cow tea, 50% cow tea or distilled water control as a soil drench and maintained at a mowing height of 2cm prior to each treatment, 4 pre-inoculation applications were applied. The pots were then inoculated and the treatments continued for a period of 9 days post inoculation following the 2day treatment cycle.

**2.6.4 Yield Collection**

Following the 2 day treatment cycle the pots were trimmed and the clippings were collected and weighed to determine yield pre-inoculation. The final post-inoculation yield was taken from a single trimming 9 days post-inoculation.

**2.6.5 Turf Inoculation Procedures**

Pots were inoculated with five disease centers creating a severe infection on each pot. A template was designed and used for inoculation to ensure a similar pattern in each pot (Figure 2.3). Each center was inoculated with 2 seeds from the inoculum described in section 2.4.3. Pots were then randomized and placed in a mist chamber (Figure 2.4), which provided constant humidity 95% and controlled temperature 25°C providing optimal conditions for fungal growth.

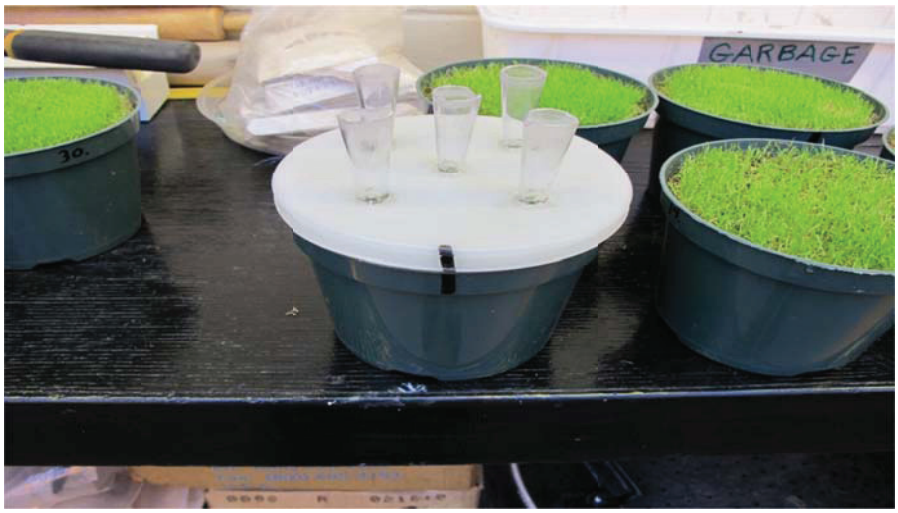


Figure 2.3. Template used to ensure consistent placement of disease on pots for greenhouse experiments.

Figure 2.4. An experimental mist chamber set up at the NSAC to control optimal disease conditions.

### 2.6.6 Disease Severity Ranking

The pots were rated for disease severity, using the following scale: 1 represents a very diseased plant with 100% injury (Figure 2.5); and 9 represents a very healthy disease free plant (Figure 2.6). These ratings were carried out following the trimming of the pots 9 days post inoculation.

### 2.6.7 Turf Quality Ranking

Pots were rated in terms of turf quality on a scale of 1 to 9, 1 represents a very poor quality plant in terms of density and color (Figure 2.7) and 9 represents a very outstanding and healthy plant (Figure 2.6). These ratings were carried out following the trimming of the pots 9 days post inoculation.

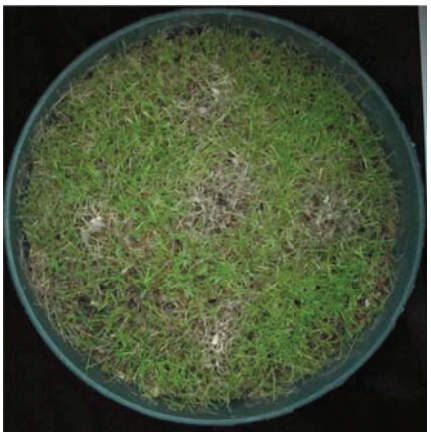

Figure 2.5. Scale 1 ranked plants representing 100% injury.

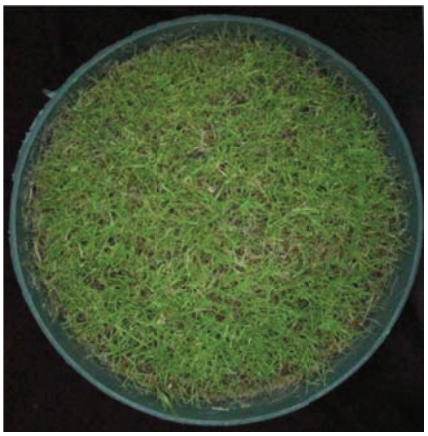

Figure 2.6. Scale 9 ranked plants representing very healthy disease free plant

Figure 2.7. Scale 1 ranked plants representing a very poor quality plant

### 2.6.8 Statistical Analysis

The experiment was setup as a completely randomized design with 5 replicates per treatment, and each experiment was repeated twice. Data was then analysed with a one-way ANOVA using Proc GLM procedure with Statistical Analysis Software (SAS)( SAS Institute, Cary, NC, USA) at *P*= 0.05.The Tukey's honestly significant difference (HSD) post hoc test was performed in cases of significance (*P*= 0.05) to further separate the means per treatment.

### 2.7.0 Field Experiment

### 2.7.1 Layout and Design

A field experiment was conducted spring- summer, and fall 2011, to test the field efficacy of the M-CT. The experiment included three locations all of which are fully operational golf courses. Two sampling sites were selected on a local golf course, (Mountain Golf and Country Club, Valley, NS), four additional sites were selected in the Halifax area, (New Ashburn Golf Club, Fall River, NS) and (Old Ashburn Golf Club, Halifax, NS). The design consisted of a 3x4 randomized design and individual plots were one meter by two meters in size, (Figure 2.8). The plots received 12 consecutive weekly treatments with either50% M-CT, 100% M-CT, or non-chlorinated water as a control. Data was collected regarding the number of visible dollar spots per plot at each location.

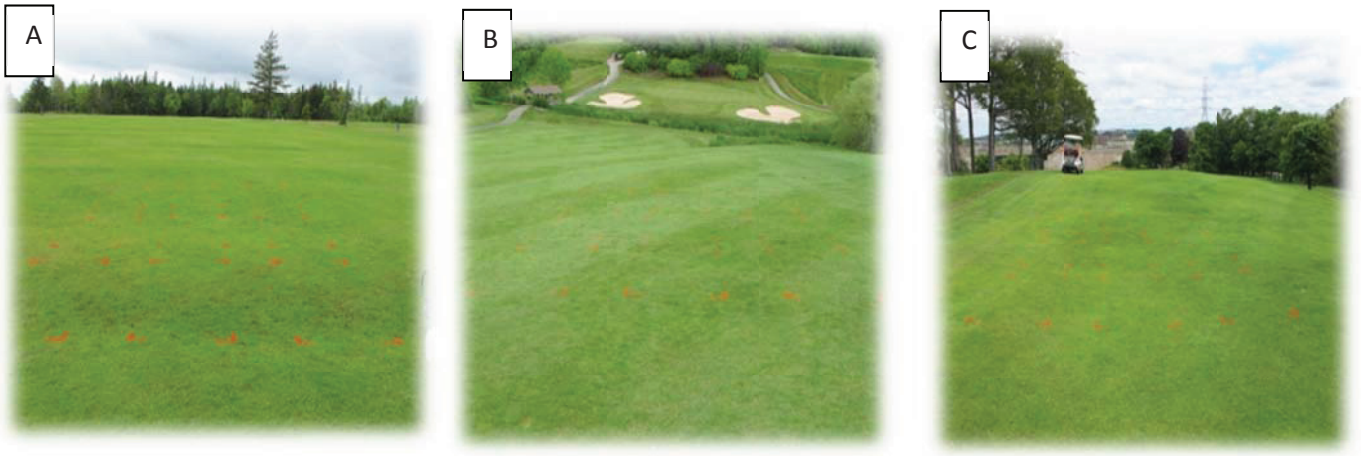


Figure 2.8. Experimental setup for field experiments. **A**) Mountain Golf and Country Club, Valley, NS, **B**) New Ashburn Golf Club, Fall River, NS, **C**) Old Ashburn Golf Club, Halifax, NS.

### 2.7.2 Compost Tea Production

M-CT was produced from mink compost. Compost analysis is presented in Table 2.1. The tea was brewed in covered 75L plastic containers, using 1:5compost to non-chlorinated water ratio supplemented with 0.25 % (v/v) molasses and supplied with constant aeration for 24h. The tea was filtered through a fine mesh screen 1.2mm x 1.2mm after brewing and the finished tea was then applied unaltered for 100% treatments or mixed 50% (v/v) with non-chlorinated water for 50% treatments. Analysis of tea is presented in Table 2.2.

### 2.7.3 Compost Tea Application

M-CT was applied using 6L Gilmour pump sprayers (Gilmour Ltd. Mississauga, Ontario, Canada). Sprayers were calibrated weekly to ensure consistent output. Three sprayers were used and treatments were always applied with the same sprayer. Plots were treated with 600ml of 100%, 50% M-CT, or non-chlorinated water as treatments. Applications were timed to ensure consistent amounts of treatment per plot.

### 2.7.4 Data Collection

Plots were assessed weekly for the presence of dollar spot. The number of spots were counted weekly until plots became greater than 75% diseased. A grid was used to keep track of spots (Figure 2.9), and weekly photos were taken to assess the disease progression. The average number of spots across the 12-week sampling period was then calculated.

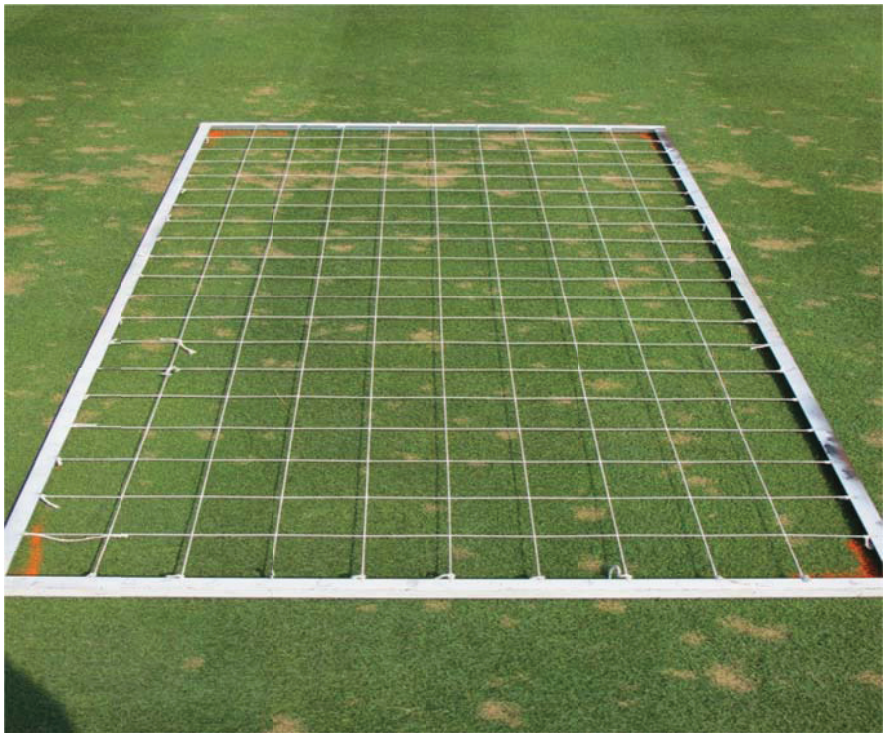

Figure 2.9. 2m x 1m grid used to track the location of dollar spot lesions throughout the summer.

### 2.7.5 Soil Microbe and Soil Analysis

One week following the last treatment soil samples were taken, five 15cm deep soil cores were taken from each plot as well as from a surrounding non-treated area of the Fairway at each experimental location. Samples were then pooled per treatment at each of the 6 locations and sent for standard soil analysis. Some soil was also serial diluted $10^3$, and $10^5$,

plated on selective media and total colony forming units were counted for each treatment per experimental location.

### 2.7.6 Statistical Analysis

The average number of spots across the 12 week sampling period was then analysed with a one-way ANOVA using Proc GLM procedure with Statistical Analysis Software (SAS)( SAS Institute, Cary, NC, USA) at $P$= 0.05 to determine significant difference between the treatments. The Tukey's honestly significant difference (HSD) post hoc test was then performed at $P$= 0.05 to further separate the means per treatment.

## Chapter 3.0 Results

### 3.1.0 SH-Toxin Experiment

### 3.1.1. Compost tea effects on Blade Length

Supplementation with C-CT had no effect across treatments on blade length in plants treated with *S.homoeocarpa* toxin (Figure 3.1). However, at the highest 40% tea treatment length of the blades in the absence of toxin was increased by approximately 20% over the control treatment (Figure 3.1). Treatment of the plants with the toxin reduced the overall blade length of the grass by approximately 25% to that of the untreated control group. Differences were even greater when plants were supplemented with 40% v/v C-CT(Figure 3.1).

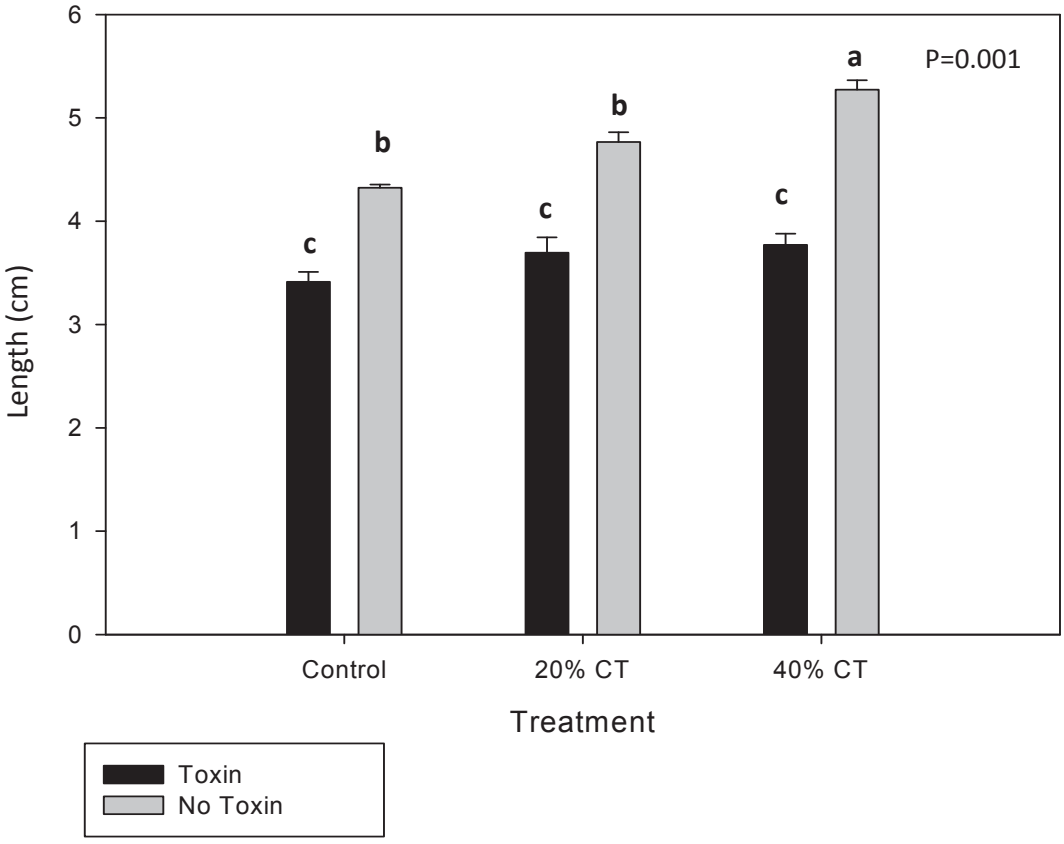


**Figure 3.1.** Leaf Blade Length.
Average blade length (±S.E.), 18 days after planting following treatment with various concentrations of sterilized C-CT, with and without treatment with SH-toxin. Letters indicate significant difference with Tukey's (HSD) at *p=0.05*. There were 3 replications per treatments and the experiment was repeated 3 times.

### 3.1.2. Compost tea effects on Root Length

Application of SH-toxin severely affected root development, with the root apical meristem dying off resulting in root curling. The damage to the root resulted in an increase of approximately 40% in root length in the control treatments (Figure 3.2). This increase in length in the presence of toxin remained similar in both the 20 and 40% C-CT treatments. The C-CT

increased root length compared to control water treatment in both the presence and absence of the toxin (Figure 3.2), with the 40% treatment having the greatest effect on the root systems.

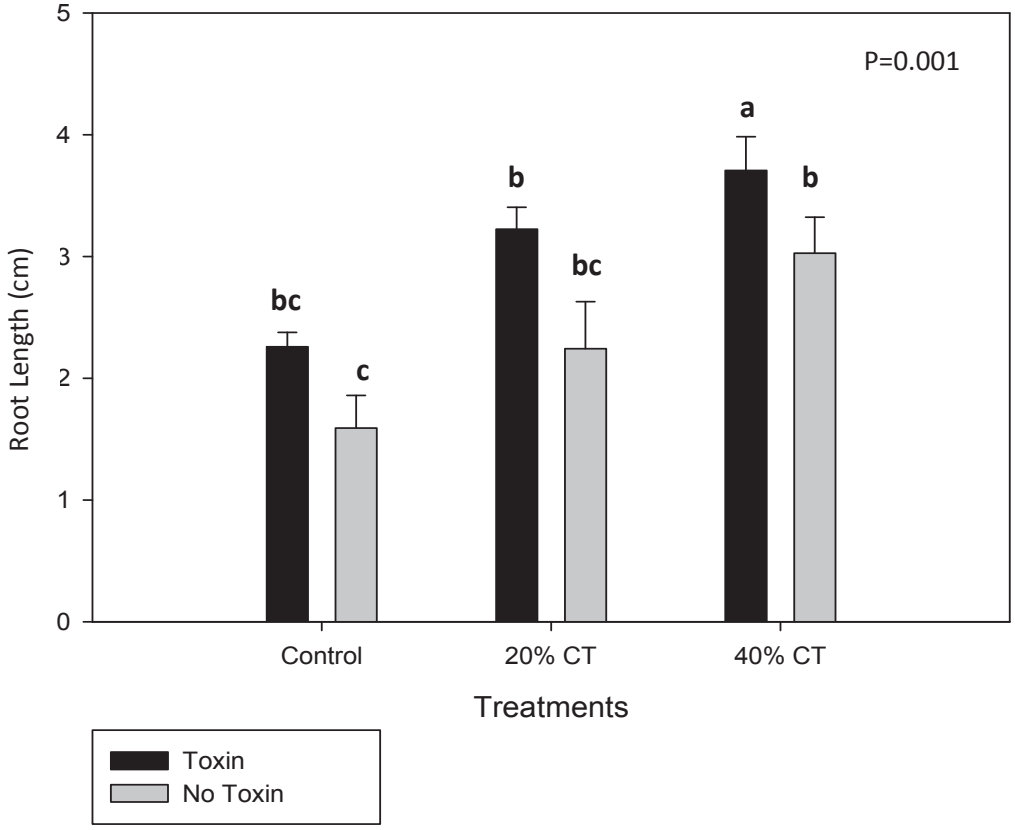


**Figure 3.2.** Root Length.
Average root length (±S.E.), 18 days after planting following treatment with various concentrations of sterilized C-CT, with and without SH-toxin treatment. Letters indicate significant difference, Tukey's (HSD) *p=0.05* , 3 replications per treatments and experiment repeated 3 times.

### 3.1.3. Compost tea effects on Germination

We examined the effect of SH- toxin on the germination of creeping bentgrass seeds, and determined that there was no significant difference in seed germination. Approximately

85% of all seeds germinated in all treatments (Figure 3.3). In addition, the germination rate was unaffected by supplementation of C-CT to the media (Figure 3.3).

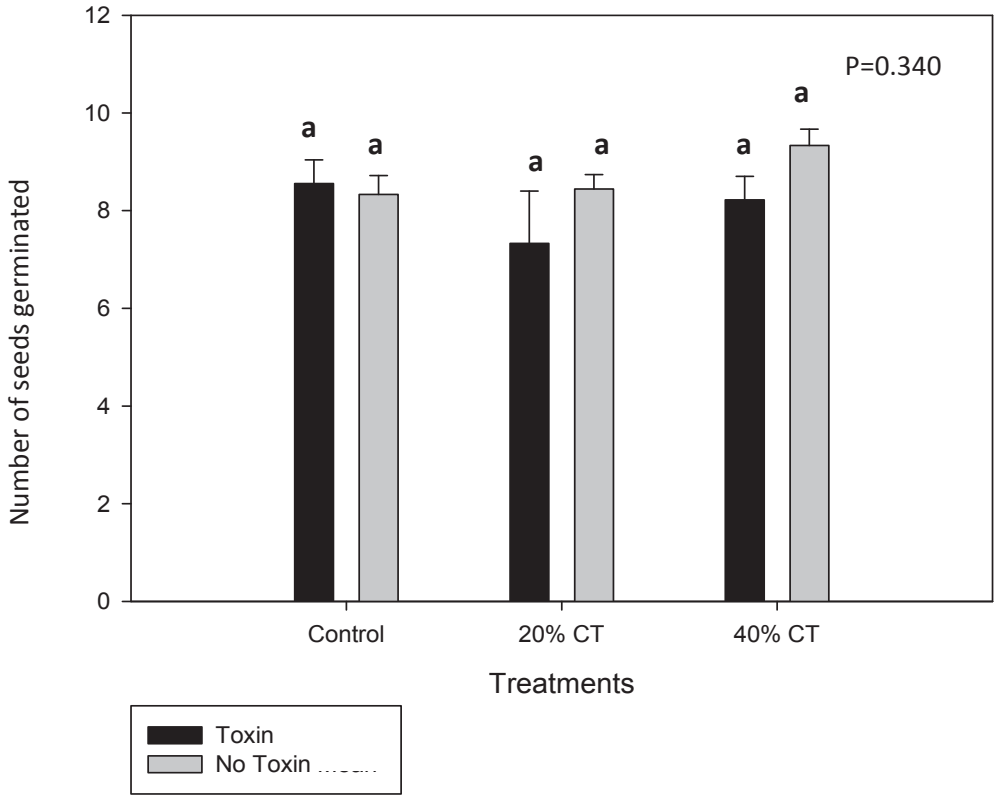


**Figure 3.3.** Germination Rate.
Average germination rate (±S.E.), 18 days after planting following treatment with various concentrations of sterilized C-CT. Letters indicate significant difference, Tukey's (HSD) *p=0.05* , 3 replications per treatments and experiment repeated 3 times.

### 3.2.0 Enzyme Analysis following compost tea treatment and *S. homoeocarpa* infection

### 3.2.1. Catalyse Activity and Polyphenoloxidase Activity

Differences in catalyse activity were not- significant between treatments at day 4. However the CT's induced higher activity when compared to the water control, Ch-CT showing the highest response among CT treatments, and acting very similar to butanediol (Figure 3.4).

An analysis of polyphenoloxidase activity showed a very similar trend to that seen in the catalyse assay with Ch-CT again performing similar to butanediol. Whereas butanediol and Ch-CT were both significantly higher than the control. C-CT and M-CT were not significantly different from the control but C-CT showed significantly less activity as compared to butanediol and Ch-CT (Figure 3.5).

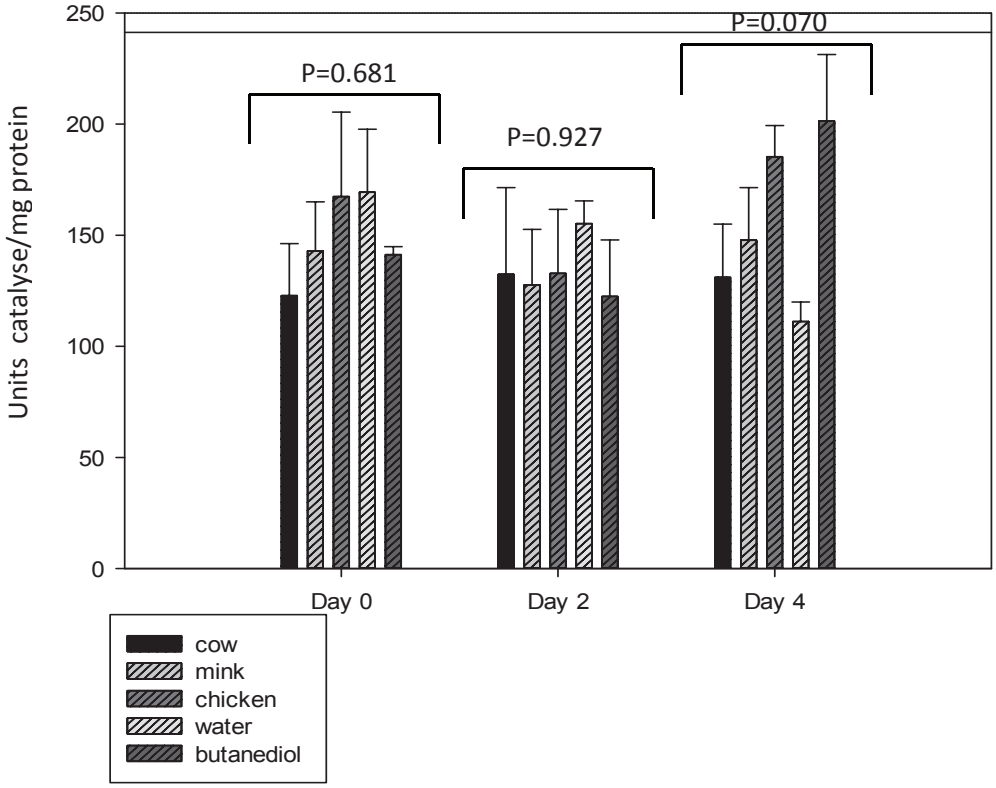


**Figure 3.4.** Catalyse Activity.
Catalyse activity (±S.E.) of creeping bentgrass treated with cow, mink, chicken compost tea, water control and a butanediol control at 0, 2 and 4 days post-infection. Letters indicate significant difference with Tukey's (HSD) at *p=0.05*.

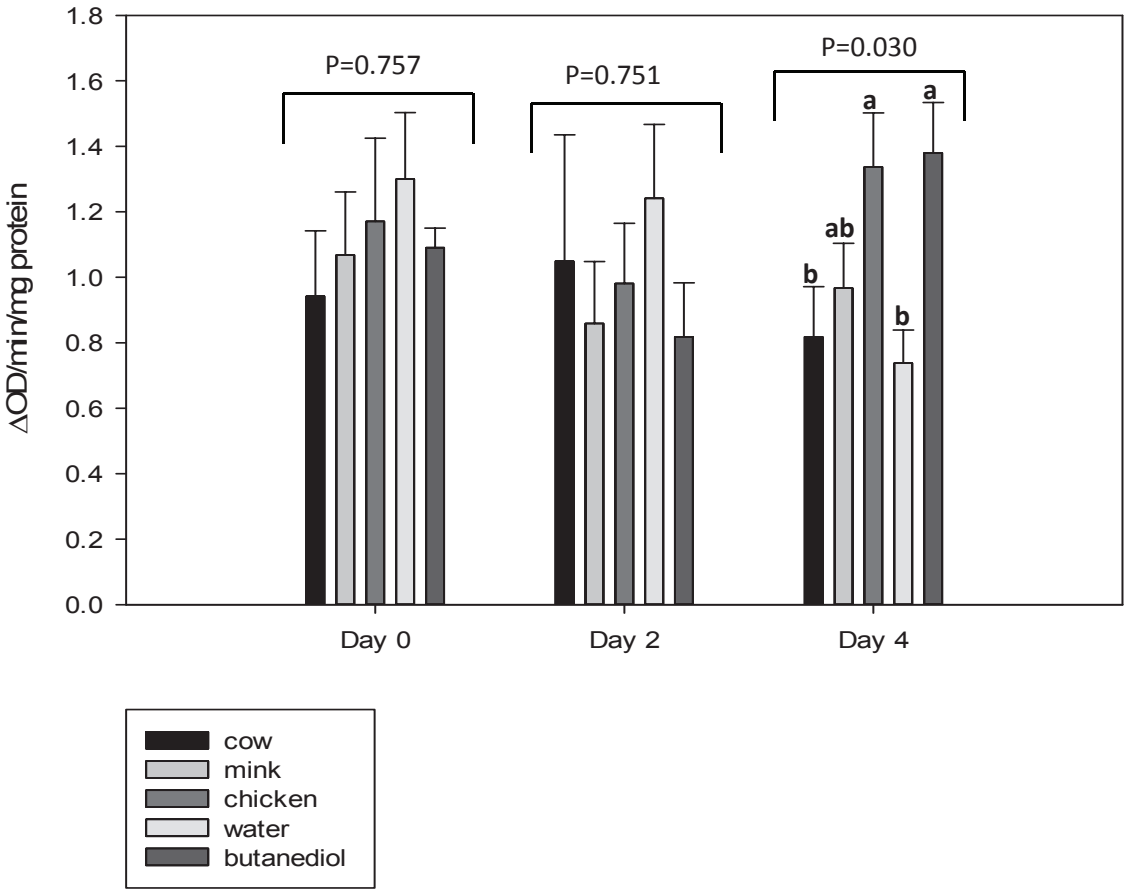


**Figure 3.5.** Polyphenoloxidase Activity.
Polyphenoloxidase activity (±S.E.) of creeping bentgrass treated with cow, mink, chicken compost tea, water control and a butanediol control at 0, 2 and 4 days post-infection. Letters indicate significant difference with Tukey's (HSD) at *p=0.05*.

### 3.2.2. Phenylalanine Ammonia-lyase Activity and Peroxidase Activity

Phenylalanine ammonia-lyase and peroxidase activity showed similar trends to each other. However for the phenylalanine ammonia-lyase assay the teas all trended to perform better than the butanediol with Ch-CT performing significantly better than butanediol, (Figure 3.6). The peroxidase assay showed the cow (C-CT) and mink (M-CT) performing similar to the butanediol however the Ch-CT again showed the highest activity (Figure 3.7). CT treated plants also gave a visual appearance as good as the butanediol treated plants at the final sampling time (Figure 3.8).

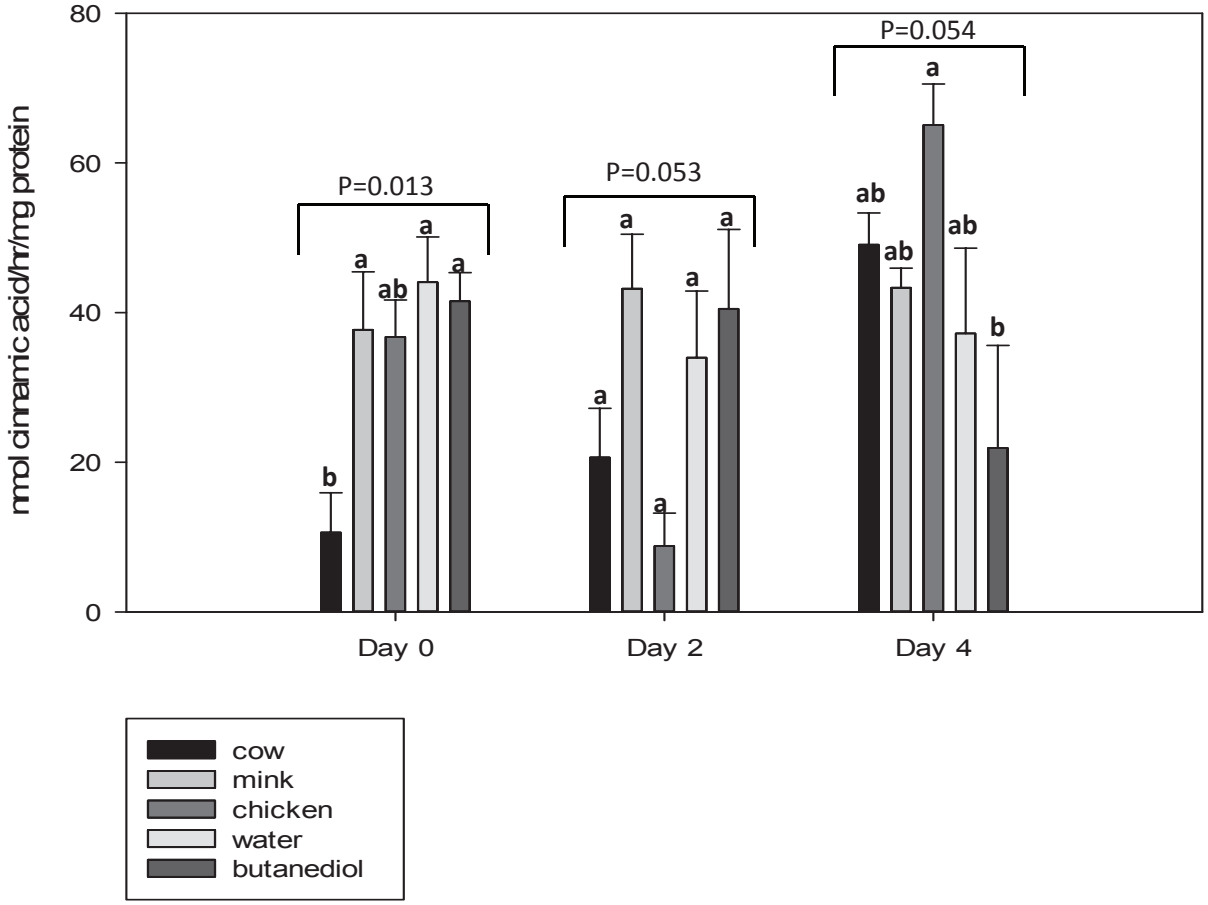


**Figure 3.6.** Phenylalanine ammonia-lyase Activity.
Phenylalanine ammonia-lyase activity (±S.E.) of creeping bentgrass treated with cow, mink, chicken compost tea, water control and a butanediol control at 0, 2 and 4 days post-infection. Letters indicate significant difference with Tukey's (HSD) at *p=0.05*.

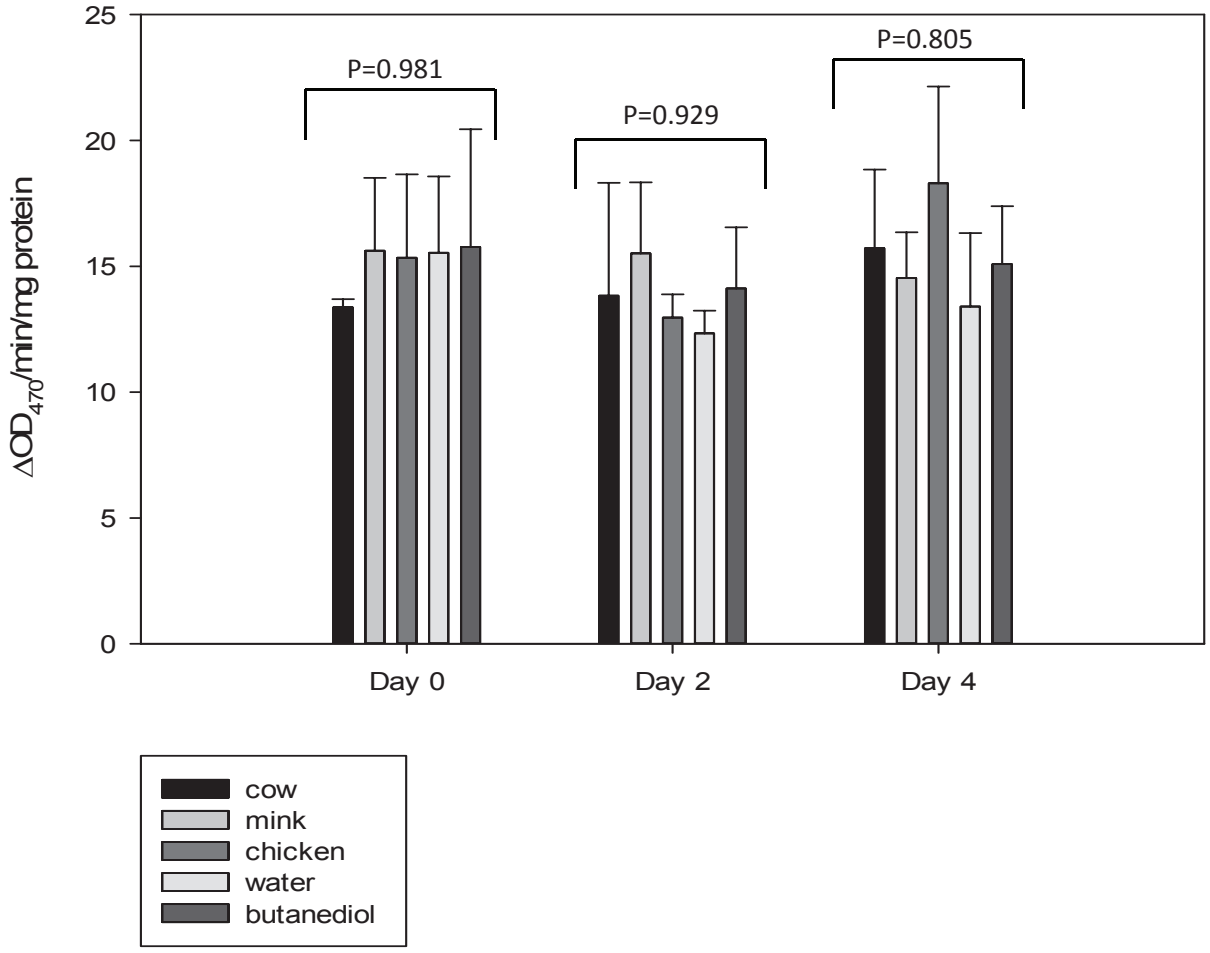


**Figure 3.7.** Peroxidase Activity.
Peroxidase activity (±S.E.) of creeping bentgrass treated with cow, mink, chicken compost tea, water control and a butanediol control at 0, 2 and 4 days post-infection. Letters indicate significant difference with Tukey's (HSD) at *p=0.05*.

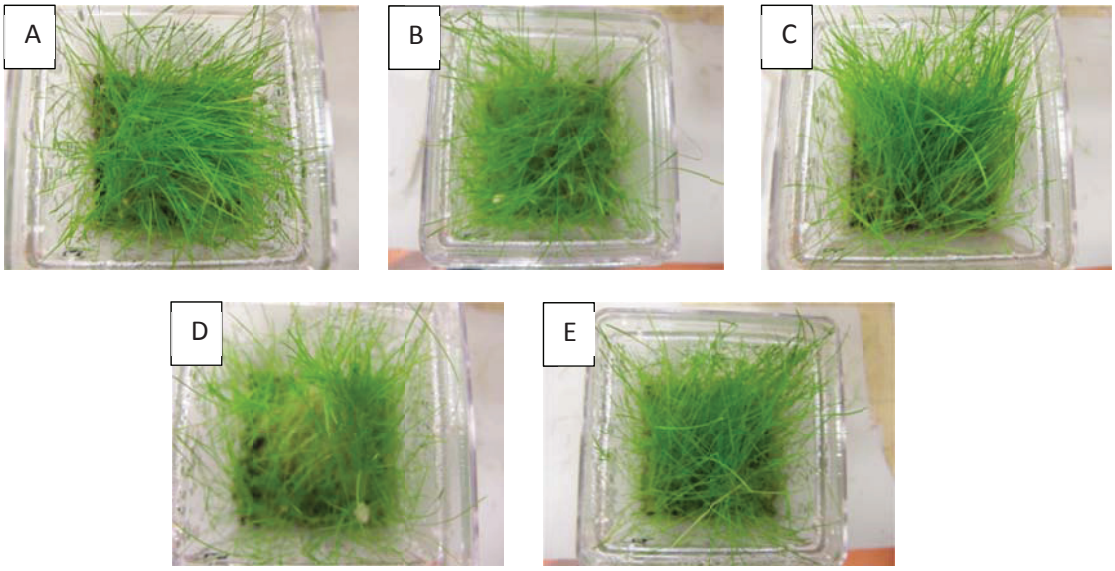


**Figure 3.8.** Comparison of all treatments.
Comparison of A) cow compost tea, B) mink compost tea, C) chicken compost tea, D) water control and E) butanediol control 4 days post-inoculation.

### 3.3.0 NMR Spectrocscopy Analysis of Compost teas

The Nuclear Magnetic Resonance spectroscopy spectra for the 3 CT samples; cow, mink, and chicken was determined to be very consistent per tea over the three separate brewing times, June 17$^{th}$ , July 11$^{th}$, and July 25$^{th}$ (Appendix A-C). The principal component analysis determined 4 major components present in the CT's: acetate, butyrate, formate, and methanol (Appendix D). Analysis determined clear separation between cow and mink teas, however chicken teas showed overlap in profiles with the other two (Appendix E). Cow was determined to contain higher levels of formate, mink had higher amounts of methanol and butyrate, and chicken had relatively more acetate (Appendix D). CT's from the June 17$^{th}$ and July 25$^{th}$ brewing

were similar while the July 11th brewing was different due to higher amounts of methanol (Appendix D).

### 3.4.0 Greenhouse Experiment

#### 3.4.1. Compost tea effects on Yield

In the greenhouse experiments we found that in terms of yield, 100% C-CT gave slightly higher yields than the 50% C-CT and the water control, however the difference was not significant, (Figure 3.9). Pots were then inoculated and the treatments continued for a period of 9 days post-inoculation. No significant difference was found between the infected and non-infected plants, however the control plants tended to have slightly more yield than the C-CT plants, (Figure 3.10).

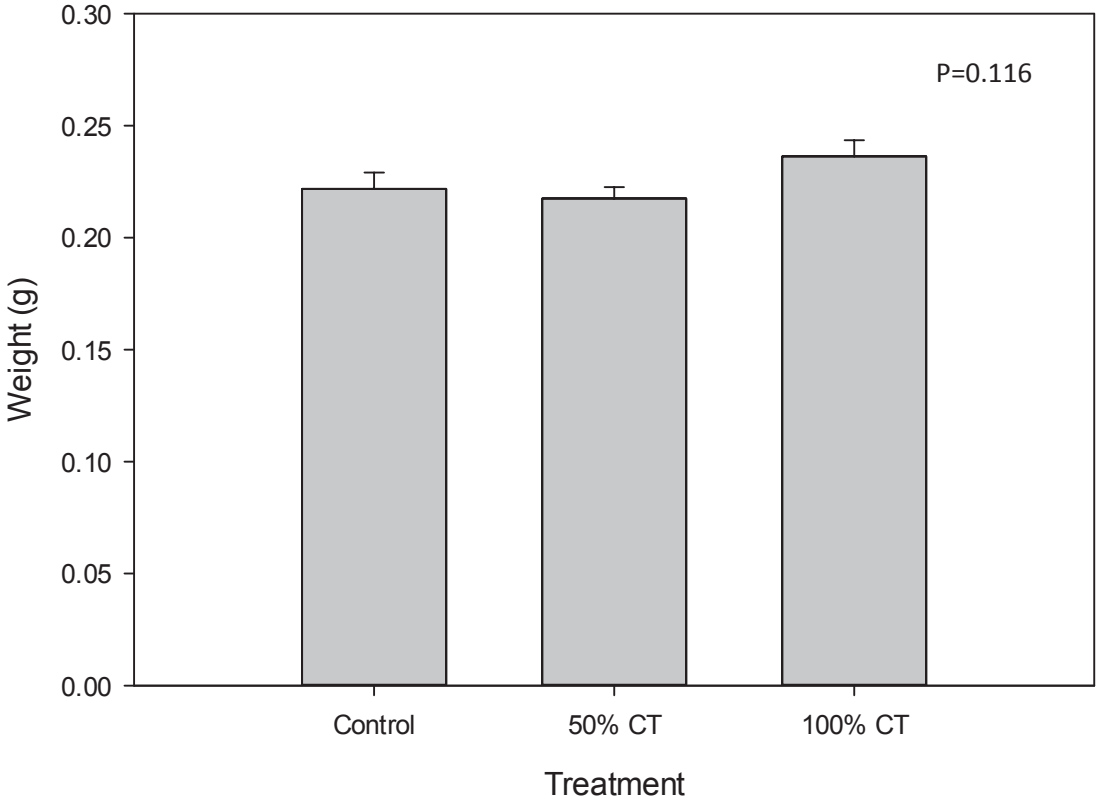


**Figure 3.9.** Average yield pre- inoculation.
Average yield pre- inoculation (±S.E.) following treatment with 2 concentrations of cow compost tea, and a water control on a 2 day cycle for 1 week. 10 replications per treatment and experiment was repeated 2 times.

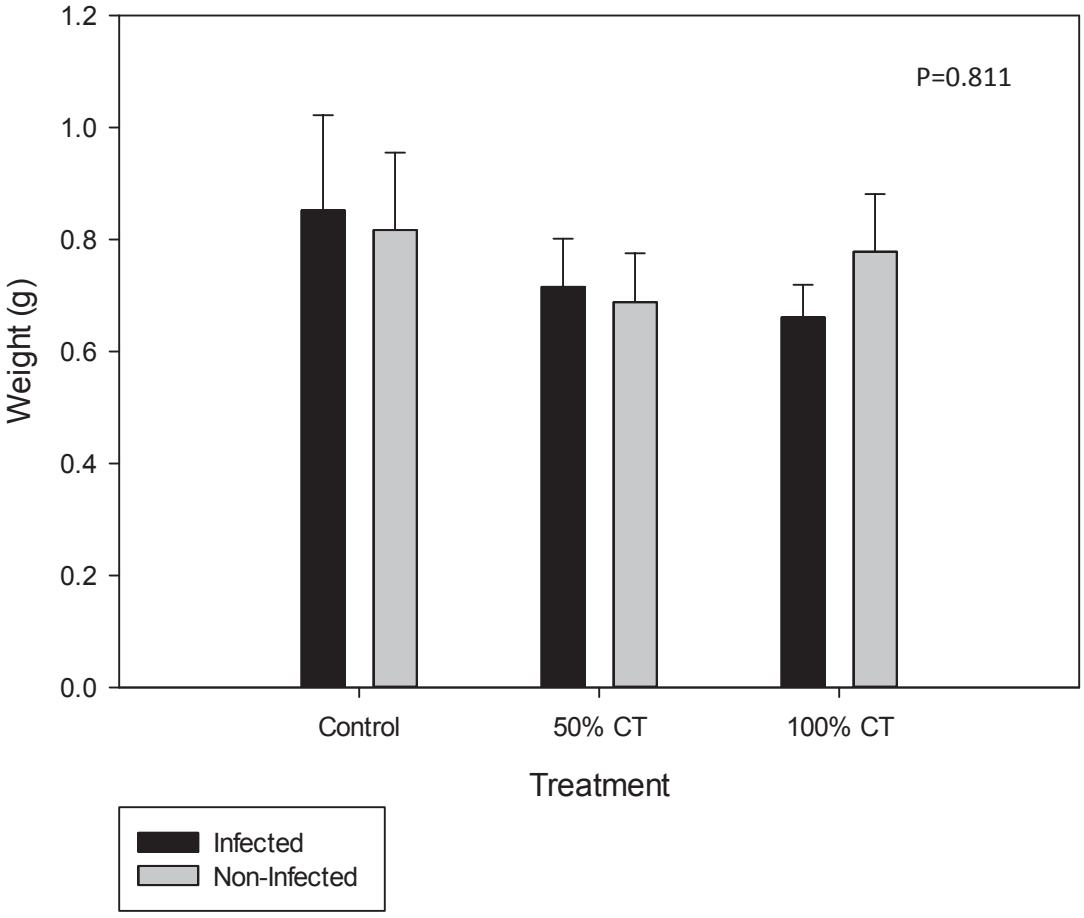


**Figure 3.10.** Average yield 9 days post inoculation.
Average yield 9 days post-inoculation (±S.E.) with continued treatment with 2 concentrations of cow compost tea, and a water control on a 2 day cycle for 9 days. 5 replications per treatment and experiment was repeated 2 times.

### 3.4.2. Turf Quality and Disease Severity

Plants were rated in terms of turf quality on a scale of 1 to 9 where 1 represents a very poor quality score and 9 represents an outstanding and healthy plant. Turf quality for non-infected plants were rated similarly across all treatments, however infected plants gave slightly higher ratings under 50% and control treatments when compared to the 100% treatments (Figure 3.11). Infected plants were also rated in terms of disease severity on a scale from 1 to 9. However on the disease severity scale 1 represents a diseased plant with 100% injury and 9 represents a healthy disease-free plant. There was no significant difference in disease severity among infected treatments, however 100% cow tea was ranked the lowest and the control ranked the highest (Figure 3.12).

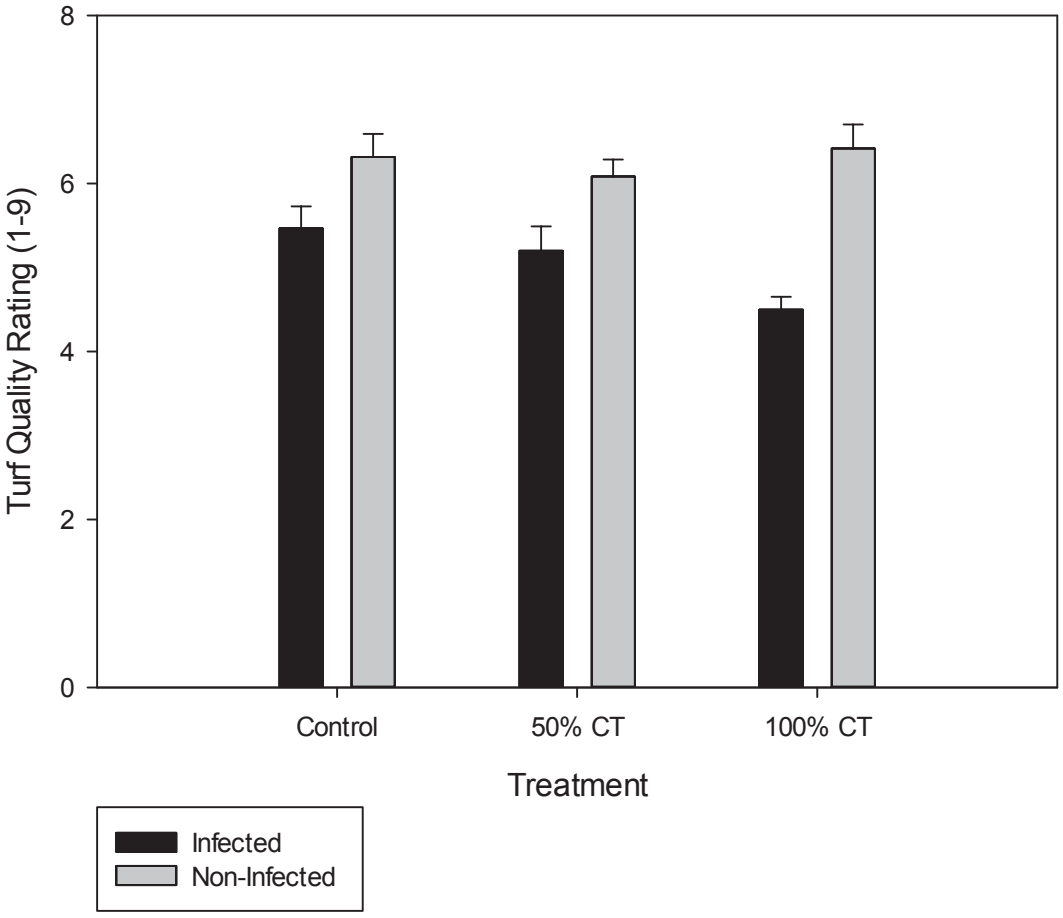


**Figure 3.11.** Turf quality rating 9 days post inoculation.
Turf quality rating 9 days post-inoculation (±S.E.) with continued treatment with 2 concentrations of cow compost tea, and a water control on a 2 day cycle for 9 days. 5 replications per treatment and experiment was repeated 2 times. 1 represents a very poor quality plant and 9 represents a very outstanding and healthy plant.

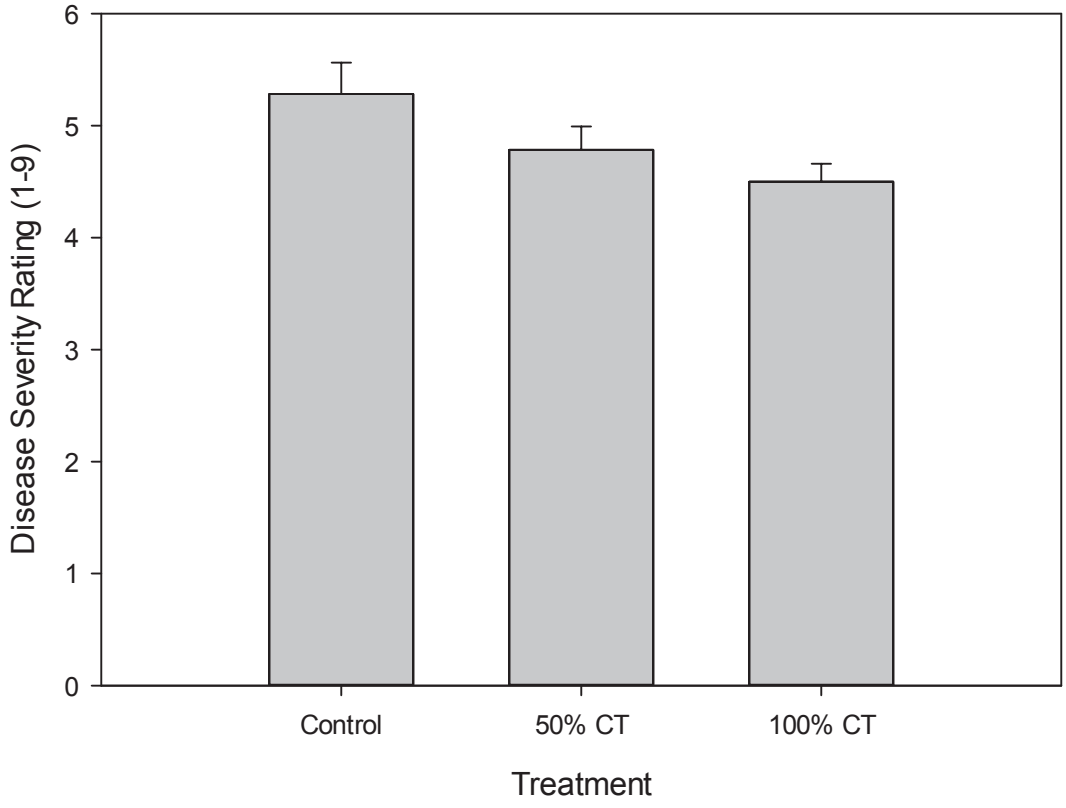


**Figure 3.12.** Disease severity rating 9 days post inoculation.
Disease severity rating 9 days post-inoculation (±S.E.) with continued treatment with 2 concentrations of cow compost tea, and a water control on a 2 day cycle for 9 days. 5 replications per treatment and experiment was repeated 2 times. 1 represents a very diseased plant 100% injury and 9 represents a very healthy disease free plant.

### 3.5.0 Field Experiment

### 3.5.1. Dollar Spot Control

The results displayed pertain to the 2011 field season only two courses from the 2011 season are included in the results, Mountain Golf and Country Club, Valley, NS and New Ashburn Golf Club, Fall River, NS. The third course, Old Ashburn Golf Club, Halifax, NS, had no visible dollar spots over the course of the experiment. M-CT treatment demonstrated significant control of dollar spot compared to the control plots under light disease conditions as

shown in (Figure 3.13), however under severe conditions (Figure 3.14), the 100% M-CT was found to be significantly worse than the other treatments. The second course, Mountain Golf and Country Club, Valley, NS showed no significant difference between treatments at both the severe infection site (Figure 3.15), as well as the light infection site (Figure 3.16).

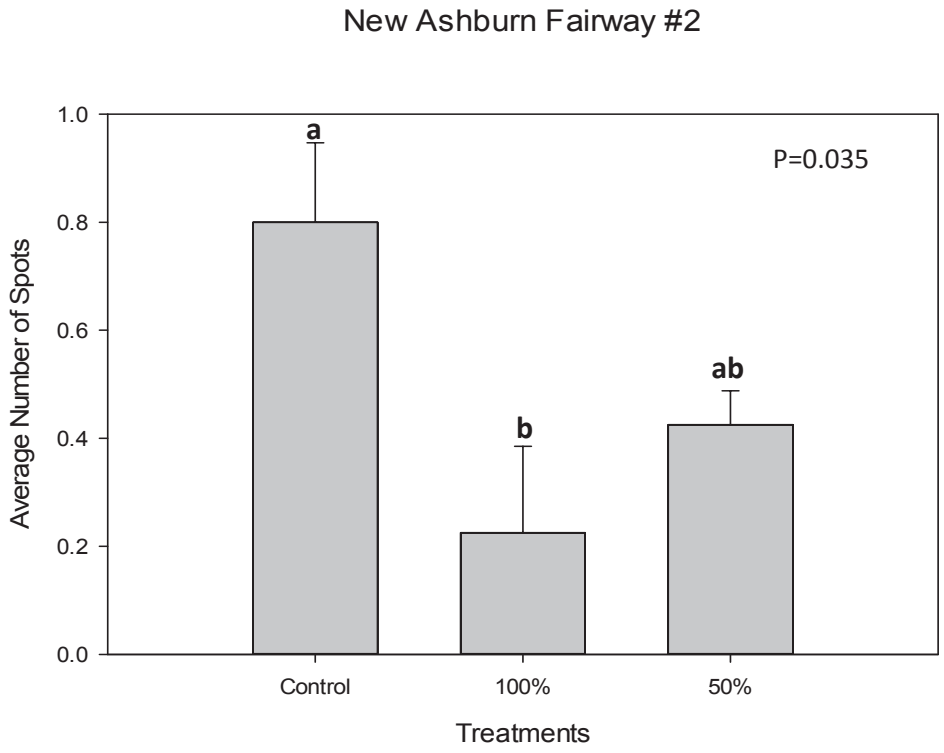


**Figure 3.13.** Average number of dollar spots.
Average number of dollar spots (±S.E.) following weekly treatment with various concentrations of mink compost tea, 4 replications per treatment and 10 sampling periods from June 21st through September 7th 2011. Letters indicate significant difference with Tukey's (HSD) at *p=0.05*.

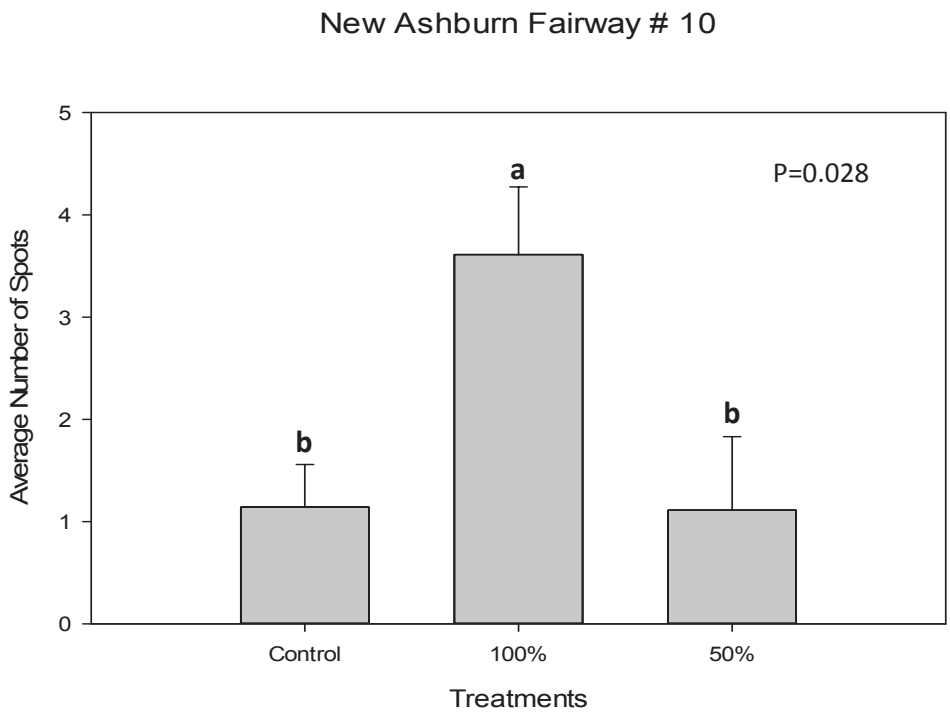


**Figure 3.14.** Average number of dollar spots.
Average number of dollar spots (±S.E.) following weekly treatment with various concentrations of mink compost tea, 4 replications per treatment and 10 sampling periods from June 21st through September 7th 2011. Letters indicate significant difference with Tukey's (HSD) at *p=0.05*.

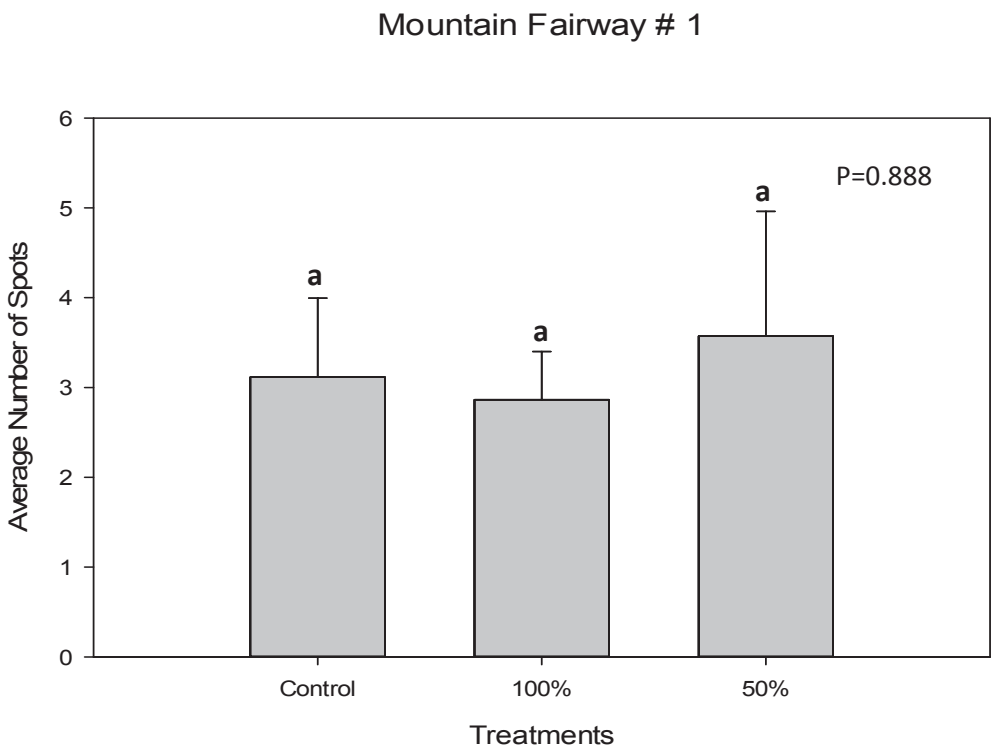


**Figure 3.15.** Average number of dollar spots.
Average number of dollar spots (±S.E.) following weekly treatment with various concentrations of mink compost tea, 4 replications per treatment and 10 sampling periods from June 21st through September 7th 2011. Letters indicate significant difference with Tukey's (HSD) at *p=0.05*.

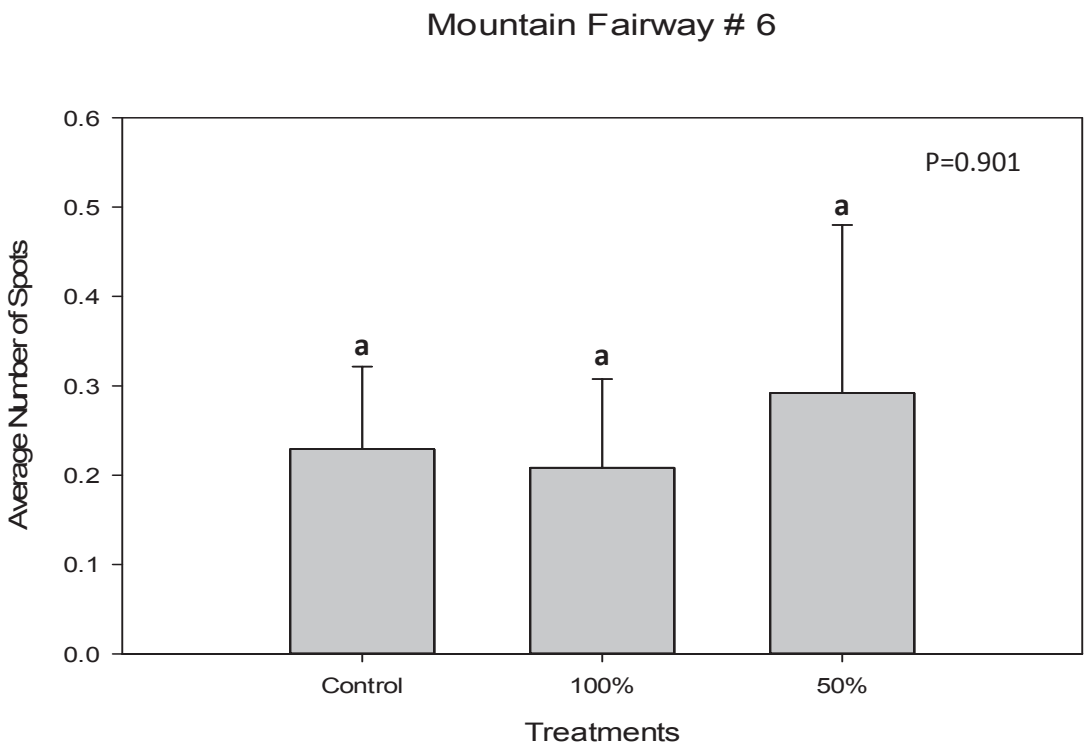


**Figure 3.16.** Average number of dollar spots.
Average number of dollar spots (±S.E.) following weekly treatment with various concentrations of mink compost tea, 4 replications per treatment and 10 sampling periods from June 21st through September 7th 2011. Letters indicate significant difference with Tukey's (HSD) at *p=0.05*.

### 3.5.2. Soil Analysis and Microbe Counts

When soil analysis were performed on plots from Mountain Golf and Country Club Fairway # 1, (Table 3.1), as well as Fairway #6, (Table 3.2), soil nutrient levels were found to be similar across all treatments and the surrounding fairway. The number of soil bacteria showed no increase in population or activity with the addition of M-CT. Similar trends were observed at New Ashburn Golf Club Fairway #2, (Table 3.3), as well as Fairway # 10, (Table 3.4) and Old Ashburn Golf Club practise Fairway 12, (Table 3.5), as well as practise Fairway # 2, (Table 3.6).

**Table 3.1.** Comparison of compost tea treated plots vs. control plots on soil nutrient levels and colony forming units (CFU), on Fairway number one, located at Mountain Golf and Country Club, Valley, NS.

| Analysis Performed | 100% M-CT | 50% M-CT | Water Control | Surrounding Fairway |
|---|---|---|---|---|
| Organic Matter % | 8.20 | 8.50 | 8.20 | 8.10 |
| $P_2O_5$ ppm | 63.00 | 64.50 | 66.50 | 59.00 |
| $K_2O$ ppm | 149.50 | 126.00 | 128.00 | 114.00 |
| Calcium ppm | 1453.50 | 1620.50 | 1485.00 | 1582.00 |
| Magnesium ppm | 274.00 | 299.00 | 266.50 | 292.50 |
| Sodium ppm | 54.50 | 27.00 | 29.50 | 25.00 |
| Sulfur ppm | 17.00 | 19.00 | 19.00 | 20.00 |
| Aluminum ppm | 858.73 | 1018.84 | 964.41 | 943.16 |
| Iron ppm | 272.00 | 310.00 | 295.00 | 281.00 |
| Manganese ppm | 77.00 | 99.00 | 93.00 | 118.00 |
| Copper ppm | 0.91 | 0.97 | 0.95 | 0.97 |
| Zinc ppm | 1.70 | 1.80 | 1.90 | 1.80 |
| Boron ppm | <= 0.50 | <= 0.50 | <= 0.50 | <= 0.50 |
| CEC(meq/100gm) | 13.10 | 14.00 | 13.00 | 13.30 |
| Base Sat. K % | 2.40 | 1.90 | 2.10 | 1.80 |
| Ca % | 55.60 | 57.80 | 57.10 | 59.70 |
| Mg % | 17.50 | 17.80 | 17.10 | 18.40 |
| Na % | 1.80 | 0.80 | 1.00 | 0.80 |
| H % | 22.70 | 21.70 | 22.80 | 19.30 |
| pH | 5.80 | 5.90 | 5.90 | 6.00 |
| CFU- $1.0x10^4$/g | 2.50 | 1.80 | 2.50 | 1.80 |

**Table 3.2.** Comparison of compost tea treated plots vs. control plots on soil nutrient levels and colony forming units (CFU), on Fairway number six, located at Mountain Golf and Country Club, Valley, NS.

| Analysis Performed | 100% M-CT | 50% M-CT | Water Control | Surrounding Fairway |
|---|---|---|---|---|
| Organic Matter % | 8.60 | 8.20 | 8.60 | 8.90 |
| $P_2O_5$ ppm | 184.50 | 180.50 | 159.00 | 165.50 |
| $K_2O$ ppm | 230.50 | 184.50 | 175.50 | 222.00 |
| Calcium ppm | 2195.00 | 1996.00 | 1917.50 | 2053.00 |
| Magnesium ppm | 526.00 | 438.00 | 449.50 | 466.00 |
| Sodium ppm | 21.00 | 22.00 | 18.00 | 22.00 |
| Sulfur ppm | 18.50 | 21.00 | 17.00 | 21.50 |
| Aluminum ppm | 781.03 | 736.49 | 554.37 | 635.89 |
| Iron ppm | 265.00 | 208.00 | 183.00 | 190.00 |
| Manganese ppm | 91.00 | 85.00 | 53.00 | 68.00 |
| Copper ppm | 1.39 | 1.49 | 1.04 | 1.10 |
| Zinc ppm | 2.60 | 2.50 | 1.90 | 2.20 |
| Boron ppm | 0.66 | 0.58 | 0.60 | 0.63 |
| CEC(meq/100gm) | 18.40 | 16.00 | 15.60 | 16.50 |
| Base Sat. K % | 2.70 | 2.50 | 2.40 | 2.90 |
| Ca % | 59.60 | 62.50 | 61.40 | 62.30 |
| Mg % | 23.80 | 22.90 | 24.00 | 23.60 |
| Na % | 0.50 | 0.60 | 0.50 | 0.60 |
| H % | 13.50 | 11.50 | 11.80 | 10.70 |
| pH | 6.30 | 6.00 | 6.30 | 6.40 |
| CFU- $1.0x10^4/g$ | 4.00 | 1.30 | 3.90 | 4.50 |

**Table 3.3.** Comparison of compost tea treated plots vs. control plots on soil nutrient levels and colony forming units (CFU), on Fairway number two, located at New Ashburn Golf Club, Fall River, NS.

| Analysis Performed | 100% M-CT | 50% M-CT | Water Control | Surrounding Fairway |
|---|---|---|---|---|
| Organic Matter % | 5.20 | 5.90 | 5.20 | 5.60 |
| $P_2O_5$ ppm | 555.50 | 508.50 | 492.50 | 568.00 |
| $K_2O$ ppm | 301.50 | 306.00 | 321.00 | 294.00 |
| Calcium ppm | 1694.00 | 1651.50 | 1691.00 | 1553.00 |
| Magnesium ppm | 308.50 | 304.00 | 318.00 | 278.00 |
| Sodium ppm | 32.50 | 36.00 | 38.00 | 36.00 |
| Sulfur ppm | 116.00 | 113.00 | 104.50 | 94.50 |
| Aluminum ppm | 405.09 | 446.27 | 419.67 | 370.96 |
| Iron ppm | 383.00 | 326.00 | 329.00 | 401.00 |
| Manganese ppm | 69.00 | 71.00 | 68.00 | 59.00 |
| Copper ppm | 1.35 | 1.43 | 1.50 | 1.10 |
| Zinc ppm | 28.50 | 26.80 | 28.00 | 25.80 |
| Boron ppm | 3.56 | 3.71 | 3.89 | 3.05 |
| CEC(meq/100gm) | 12.20 | 11.90 | 12.60 | 11.50 |
| Base Sat. K % | 5.20 | 5.40 | 5.40 | 5.40 |
| Ca % | 69.30 | 69.30 | 67.20 | 67.50 |
| Mg % | 21.00 | 21.30 | 21.00 | 20.10 |
| Na % | 1.20 | 1.30 | 1.30 | 1.40 |
| H % | 3.30 | 2.70 | 5.10 | 5.60 |
| pH | 6.40 | 6.40 | 6.40 | 6.30 |
| CFU- $1.0x10^4/g$ | 2.30 | 1.10 | 8.00 | 2.00 |

**Table 3.4.** Comparison of compost tea treated plots vs. control plots on soil nutrient levels and colony forming units (CFU), on Fairway number ten, located at New Ashburn Golf Club, Fall River, NS.

| Analysis Performed | 100% M-CT | 50% M-CT | Water Control | Surrounding Fairway |
|---|---|---|---|---|
| Organic Matter % | 5.30 | 5.10 | 5.20 | 4.90 |
| $P_2O_5$ ppm | 225.00 | 207.00 | 237.00 | 265.00 |
| $K_2O$ ppm | 250.00 | 245.50 | 265.00 | 237.50 |
| Calcium ppm | 924.00 | 916.50 | 944.50 | 886.00 |
| Magnesium ppm | 174.00 | 176.50 | 175.00 | 162.50 |
| Sodium ppm | 38.00 | 30.00 | 50.00 | 30.50 |
| Sulfur ppm | 37.50 | 33.50 | 37.00 | 35.50 |
| Aluminum ppm | 616.74 | 724.83 | 680.76 | 557.83 |
| Iron ppm | 399.00 | 431.00 | 455.00 | 420.00 |
| Manganese ppm | 55.00 | 64.00 | 64.00 | 58.00 |
| Copper ppm | 1.28 | 1.29 | 1.37 | 1.19 |
| Zinc ppm | 14.40 | 13.20 | 13.90 | 15.40 |
| Boron ppm | 0.97 | 0.82 | 0.95 | 1.02 |
| CEC(meq/100gm) | 8.60 | 9.10 | 9.30 | 8.30 |
| Base Sat. K % | 6.20 | 5.70 | 6.10 | 6.10 |
| Ca % | 53.70 | 50.30 | 50.90 | 53.60 |
| Mg % | 16.90 | 16.20 | 15.70 | 16.40 |
| Na % | 1.90 | 1.40 | 2.30 | 1.60 |
| H % | 21.40 | 26.40 | 25.00 | 22.30 |
| pH | 5.50 | 5.40 | 5.60 | 5.50 |
| CFU- $1.0x10^4/g$ | 4.80 | 3.30 | 1.70 | 3.80 |

**Table 3.5.** Comparison of compost tea treated plots vs. control plots on soil nutrient levels and colony forming units (CFU), on practise Fairway number one, located at Old Ashburn Golf Club, Halifax, NS.

| Analysis Performed | 100% M-CT | 50% M-CT | Water Control | Surrounding Fairway |
|---|---|---|---|---|
| Organic Matter % | 10.60 | 9.80 | 9.80 | 10.70 |
| $P_2O_5$ ppm | 251.00 | 239.00 | 283.50 | 217.50 |
| $K_2O$ ppm | 441.50 | 429.50 | 410.00 | 403.50 |
| Calcium ppm | 955.00 | 942.50 | 852.00 | 877.00 |
| Magnesium ppm | 127.00 | 126.50 | 113.00 | 113.50 |
| Sodium ppm | 34.00 | 39.50 | 37.50 | 38.00 |
| Sulfur ppm | 24.50 | 29.50 | 28.00 | 27.00 |
| Aluminum ppm | 1323.18 | 1476.07 | 1454.29 | 1510.97 |
| Iron ppm | 198.00 | 203.00 | 202.00 | 181.00 |
| Manganese ppm | 25.00 | 27.00 | 27.00 | 23.00 |
| Copper ppm | 2.06 | 1.92 | 2.54 | 2.05 |
| Zinc ppm | 26.30 | 37.20 | 37.60 | 35.00 |
| Boron ppm | 0.64 | 0.66 | 0.53 | 0.65 |
| CEC(meq/100gm) | 11.30 | 11.50 | 11.00 | 11.20 |
| Base Sat. K % | 8.30 | 7.90 | 7.90 | 7.60 |
| Ca % | 42.20 | 41.00 | 38.90 | 39.00 |
| Mg % | 9.40 | 9.20 | 8.60 | 8.40 |
| Na % | 1.30 | 1.50 | 1.50 | 1.50 |
| H % | 38.90 | 40.40 | 43.10 | 43.50 |
| pH | 5.30 | 5.30 | 5.20 | 5.30 |
| CFU- $1.0x10^4/g$ | 6.10 | 1.60 | 8.00 | 5.80 |

**Table 3.6.** Comparison of compost tea treated plots vs. control plots on soil nutrient levels and colony forming units (CFU), on practise Fairway number two, located at Old Ashburn Golf Club, Halifax, NS.

| Analysis Performed | 100% M-CT | 50% M-CT | Water Control | Surrounding Fairway |
|---|---|---|---|---|
| Organic Matter % | 10.10 | 10.40 | 10.30 | 10.30 |
| $P_2O_5$ ppm | 171.50 | 216.50 | 153.00 | 167.50 |
| $K_2O$ ppm | 224.50 | 241.50 | 175.50 | 182.00 |
| Calcium ppm | 527.00 | 716.00 | 527.50 | 526.50 |
| Magnesium ppm | 82.00 | 95.00 | 79.50 | 81.50 |
| Sodium ppm | 42.00 | 46.50 | 35.50 | 46.00 |
| Sulfur ppm | 31.00 | 28.00 | 30.50 | 31.00 |
| Aluminum ppm | 1512.93 | 1495.74 | 1463.01 | 1465.89 |
| Iron ppm | 229.00 | 279.00 | 240.00 | 242.00 |
| Manganese ppm | 22.00 | 24.00 | 21.00 | 29.00 |
| Copper ppm | 1.91 | 2.29 | 1.96 | 2.72 |
| Zinc ppm | 10.50 | 15.60 | 11.90 | 11.00 |
| Boron ppm | <= 0.50 | <= 0.50 | <= 0.50 | <= 0.50 |
| CEC(meq/100gm) | 10.10 | 11.60 | 9.60 | 9.70 |
| Base Sat. K % | 4.70 | 4.40 | 3.90 | 4.00 |
| Ca % | 26.20 | 31.00 | 27.50 | 27.30 |
| Mg % | 6.80 | 6.80 | 6.90 | 7.00 |
| Na % | 1.80 | 1.70 | 1.60 | 2.10 |
| H % | 60.50 | 56.00 | 60.10 | 59.60 |
| pH | 5.10 | 5.10 | 5.10 | 5.20 |
| CFU- $1.0x10^4/g$ | 4.80 | 2.50 | 2.30 | 4.70 |

### 3.5.3. Temperature and Precipitation

Temperature and precipitation data for Mountain Golf and Country Club (Table 3.7), show that the month of August when the disease is usually most severe in Atlantic Canada the 2011 field season received 40.5mm more rain while the temperatures remained very similar to the previous year. The second location New Ashburn Golf Club, showed the same trend as the Mountain course (Table 3.8). August was wet while temperatures remained high. However the New Ashburn Golf Club located in Fall River, NS received 26.8mm more precipitation then

Mountain Golf and Country Club in Valley, NS. The Old Ashburn Golf Club in Halifax, NS received the most rain in 2011 during August, and showed the largest difference in rainfall from 2010 to 2011, (Table 3.9). However no dollar spot infection was observed at this location.

**Table 3.7.** Temperature and precipitation data for Mountain Golf and Country Club, Valley NS. Data was obtained from the Debert, NS weather station from the Environment Canada Weather Data web site, (www.climate.weatheroffice.gc.ca).

| Year | Month | Average Temperature (°C) | Precipitation (mm) |
|---|---|---|---|
| 2010 | June | 14.5 | 206.6 |
| 2010 | July | 19.8 | 121.9 |
| 2010 | August | 18.5 | 68.3 |
| 2010 | September | 15.8 | 96.7 |
| **Total Summer Average:** | | **17.2** | **123.4** |
| 2011 | June | 13.5 | 84.7 |
| 2011 | July | 18.1 | 90.4 |
| 2011 | August | 18.3 | 108.8 |
| 2011 | September | 15.1 | 63.4 |
| **Total Summer Average:** | | **16.3** | **86.8** |

**Table 3.8.** Temperature and precipitation data for New Ashburn Golf Club, Fall River, NS. Data was obtained from the Halifax Stanfield International Airport, NS weather station from the Environment Canada Weather Data web site, (www.climate.weatheroffice.gc.ca).

| Year | Month | Average Temperature (°C) | Precipitation (mm) |
|---|---|---|---|
| 2010 | June | 15.2 | 99.6 |
| 2010 | July | 19.8 | 125.2 |
| 2010 | August | 19.5 | 65.3 |
| 2010 | September | 16.3 | 117.5 |
| **Total Summer Average:** | | **17.7** | **101.9** |
| 2011 | June | 13.6 | 144.3 |
| 2011 | July | 18.8 | 94.3 |
| 2011 | August | 18.5 | 135.6 |
| 2011 | September | 16.0 | 43.1 |
| **Total Summer Average:** | | **16.7** | **104.3** |

**Table 3.9.** Temperature and precipitation data for Old Ashburn Golf Club, Halifax, NS. Data was obtained from the Halifax Shearwater RCS, NS weather station from the Environment Canada Weather Data web site, (www.climate.weatheroffice.gc.ca).

| Year | Month | Average Temperature (°C) | Precipitation (mm) |
|---|---|---|---|
| 2010 | June | 14.9 | 91.4 |
| 2010 | July | 19.7 | 104.2 |
| 2010 | August | 19.5 | 41.8 |
| 2010 | September | 17.0 | 53.6 |
| **Total Summer Average:** | | **17.8** | **72.8** |
| 2011 | June | 13.5 | 156.0 |
| 2011 | July | 18.2 | 87.5 |
| 2011 | August | 18.7 | 174.5 |
| 2011 | September | 16.7 | 44.9 |
| **Total Summer Average:** | | **16.8** | **115.7** |

## Chapter 4.0 Discussion

### 4.1.0 SH-Toxin Experiment

In this experiment, we investigated the efficacy of C-CT on reducing the amount of damage caused by SH-toxic metabolites produced by the fungus *S.homoeocarpa* on the creeping bentgrass cultivar pencross. Additionally, we demonstrated that root application of C-CT can also improve root and blade growth in bentgrass. C-CT application increased the overall root and blade length of bentgrass in the absence of the toxic metabolites. In the presence of SH-toxin the blade length is unaffected by application of the C-CT. However, the effect is visible in the roots as described above. C-CT treatment also had no effect on the germination rate of creeping bentgrass.

CT's have been shown to provide disease resistance in a number of scientific studies. Non-aerated CT effectively inhibited mycelium of foliar powdery mildew and gray mold in tomato (Kone *et al.,* 2009), with resistance due to increased microbial activity in the soil. However, factors such as chemical composition of the CT itself cannot be ruled out as a means of fungal disease suppression in tomato. A similar study on *Choanephora cucurbitarum*, the causal agent of wet rot of okra, showed the potential of CT to suppress fungal infections (Siddiqui *et al.,* 2009). This suggests that the suppressive effect may be due to physiologically induced resistance in the plant and further suggests chemicals such as salicylic acid, heavy metals, fungal metabolites, amino acids and metabolic inhibitors, all present in the CT, may also play a key role. Most recent studies on CT, attributes disease suppressive potential as being microbial in nature. Our studies ruled out microbial suppression as a major effect and demonstrated that the effects are at least partially biochemical in nature.

CT is often associated with improved plant health. This research suggests that the C-CT itself is capable of stimulating root and blade growth. This effect can be nutrient related with the C-CT containing nutrients from the original compost source. It could also be an inducible trait of the C-CT due to an accumulation of a metabolite present in the compost from microorganisms active during the brewing procedure. Research would also suggest that the CT helps the plant better tolerate disease outbreaks. The increase in root length and mass, as well as the increase in adventitious roots can help the plant recover quicker after such periods and help the plant regain nutrients lost during high infection periods.

### 4.2.0 Enzyme Analysis following compost tea treatment and *S. homoeocarpa* Infection

This experiment focused on the ability of the CT to induce the activity of enzymes known to play key roles in plant pathogen defence. The experiment tested CT against a negative control in water as well as a positive (butanediol) control. Effects were demonstrated over three separate sampling points. The third time point, day 4, was the most important because it represents plants under an active infection by the *S.homoeocarpa* fungus. It was determined that catalase activity in creeping bentgrass was higher in all three CT's, cow (C-CT), mink (M-CT), and chicken (Ch-CT) as compared to the control. The butanediol control showed the greatest catalase activity however the Ch-CT was very close in terms of activity. A similar trend was observed for polyphenoloxidase activity with the three CT's again showing higher activity than water and Ch-CT showing similar activity to butanediol. In phenylalanine ammonia-lyase activity the CT's performed better than the control, with Ch-CT having the highest activity. However the butanediol was much lower than all the teas, and was significantly lower than Ch-CT . The peroxidase enzyme assay showed a similar trend to that of the

phenylalanine ammonia-lyase activity, however the difference between Ch-CT and butanediol was not significant.

CT has been previously reported to cause an increase in inducible enzymes such as polyphenoloxidase and phenylalanine ammonia lyase in okra (Siddiqui *et al.*, 2009). Enzyme activities such as peroxidases have been reported to be elevated in okra, tomato and onion plants (Haggag and Saber, 2007). Butanediol reduced dollar spot disease, increased growth of plants, and induced systemic resistance in creeping bentgrass (Cortes-Barco *et al.* 2010). We found that our CT-treated plants look similar to butanediol-treated plants 4 days after infection. CT treatments in our research also increased the production of enzymes in the grass. CT increased rooting depth and improved plant health and growth, all attributes that would increase the control of plant pathogens and dollar spot in turfgrass (Ingham, 2005).

The fact that the plants looked significantly better following CT or butanediol treatment indicates that the CT is able to help protect and promote growth in the plant. The induced enzyme activity and increased growth can be attributed to microbial or chemical composition of the CT's acting at the root level. This conclusion can be reached because the sand media was fully sterilized upon application of the CT's directly to the soil. This experiment can also rule out microbial competition on the leaf surface as a means of disease suppression because the CT's were added 10 days prior to soil inoculation of the plants.

### 4.3.0 NMR Spectrocscopy Analysis of Compost teas

Nuclear Magnetic Resonance spectroscopy demonstrated that CT produced from compost with known composition can be produced repeatedly, and remain chemically consistent. The CT's were also found to contain four major components: acetate, butyrate, formate, and methanol. These components remained consistent throughout the different brewing times, with only a slight change in one brew demonstrating more methanol then the other two. The analysis also revealed that C-CT and M-CT were distinctly different in composition of these components, while Ch-CT overlapped with the other two teas.

The four major components identified in the teas are chemicals produced primarily by anaerobic microorganisms. Even though we produced aerobic conditions, the CT become anaerobic under intense exponential growth of the microorganisms by the addition of the molasses sugar source (Ingham, 2005). Shrestha *et al.*(2011), demonstrated that compost produced under aerated conditions using molasses as a food source, can contain very high microbial populations. This increase in the microbial population can lead to an increase in secondary metabolites produced by microbes that may enhance disease protection in plants.

### 4.4.0 Greenhouse and Field Experiments

The greenhouse experiment examined the effect of the CT on disease suppression and turf quality in a controlled setting. Yield, turf quality and disease severity were evaluated following application of C-CT. The CT treated plants showed slight, but non-significant increases in yield. When plants were infected with *S.homoeocarpa*, the control and 50% C-CT applications were slightly better. This trend was also reflected in both disease severity and turf quality ratings. Although not significantly different, it appeared that the infected C-CT-treated plants

performed worse than the control treated plants while the opposite was true for the non-infected plants.

In field experiments, investigated effects of M-CT on suppression of Dollar Spot under normal turfgrass management practises. We found that the efficacy of the M-CT in controlling the fungus was variable across locations with only one location showing significant control. M-CT application did not alter the soil composition in terms of microbes or nutrients within the soil.

Compost soil amendments have been demonstrated to reduce the severity and incidence of a wide variety of turfgrass diseases (Block, 1997; Nelson and Boehm, 2002a; Roulston, 2006). CT's likewise have been shown to control disease in many different crops (Schuerell and Mahaffee, 2002; Litterick *et al.,* 2004). ). Microbes in compost, as well as in CT are most often associated with disease control through competition with other pathogenic organisms on the leaf surface or in the soil (Ryan *et al.,* 2005; Siddiqui *et al.,* 2009; Kone *et al.,* 2010). This study showed that M-CT application did not increase the amount of microbes present in the soil. Utilization of compost amendments and CTs for turfgrass disease control has been limited because of inconsistent performance, usually attributed to an overall lack of understanding of the disease suppression mechanisms prevalent when these materials are applied (Nelson and Boehm, 2002b; Scheuerell, 2003; Hsiang and Tian, 2007).

It can be concluded that CT may offer suppression in specific applications but overall, no control was induced with the treatment., However, much of this difference may be attributed to the movement of the disease into the plots from the surrounding fairway via the use of maintenance equipment. Variation in disease incidence from field locations can be explained by

the lack of control in the amount of disease present in the individual plots. We were unable to artificially infect the field plots therefore we needed to instead rely on the natural infection present in the field. In doing so we could not ensure that each treatment plot was subjected to the same disease pressure. Furthermore we could not prevent movement of the disease into the plots. The fairways for the most part were mowed in a very similar pattern every second day. This created a situation where a number of plots may have been subjected to greater disease pressures than the others. Also since these plots were mowed on a two day cycle, morning dew removal wasn't constant thereby creating longer periods of wetness on plots.

Application time of the CT may also be important in the disease suppression. The CT may be better absorbed into the plant if applied in the evening rather than late morning or early afternoon when our plots were treated. The sun would have been very hot and the treatments would have more time to dry. Moreover, plants would have a reduced respiration rate at this time so stomatal absorption of the tea would likely be much higher following an evening application. Sugars in the CT were also not tested and these may play a role in creating a food source for the fungus. If the CTs still contained high amounts of sugar from the molasses used during brewing, these sugars may dry on the surface of the plant and add to the guttation fluid sugar content creating a healthy food source for the fungus.

Under the greenhouse conditions, CT treated plants demonstrated increased growth before the infection process. Under infection however the disease levels may have been too high to be offset by the CT applications. The pots were inoculated with 4 disease centers while field conditions at most only seen 4 spots on average in a $2m^2$ plot. Once infected, pots were

placed in a mist chamber and kept under 95% relative humidity, which may have affected the plants by weakening them from the stress this condition created.

### 4.5.0 Conclusion

CT demonstrated many positive effects in our in vitro studies. The effect on root growth under SH-toxin stress, and the induction of enzymes related to disease suppression were promising. It is hypothesized that the CT's are most likely functioning through secondary metabolites such as acetate, butyrate, formate and methanol present in the CT's. Future studies that examine these components may reveal some very surprising results in terms of disease suppression through the CT's. The fact that the CT's were produced on a consistent basis and contained the same components is a good sign that a more consistent form of CT can be produced and provide reproducible effects in the field. These results suggest that a CT used in an integrated management regime can help control disease, especially under mild disease severity and potentially serve as an alternative to fungicides under mild infection conditions.

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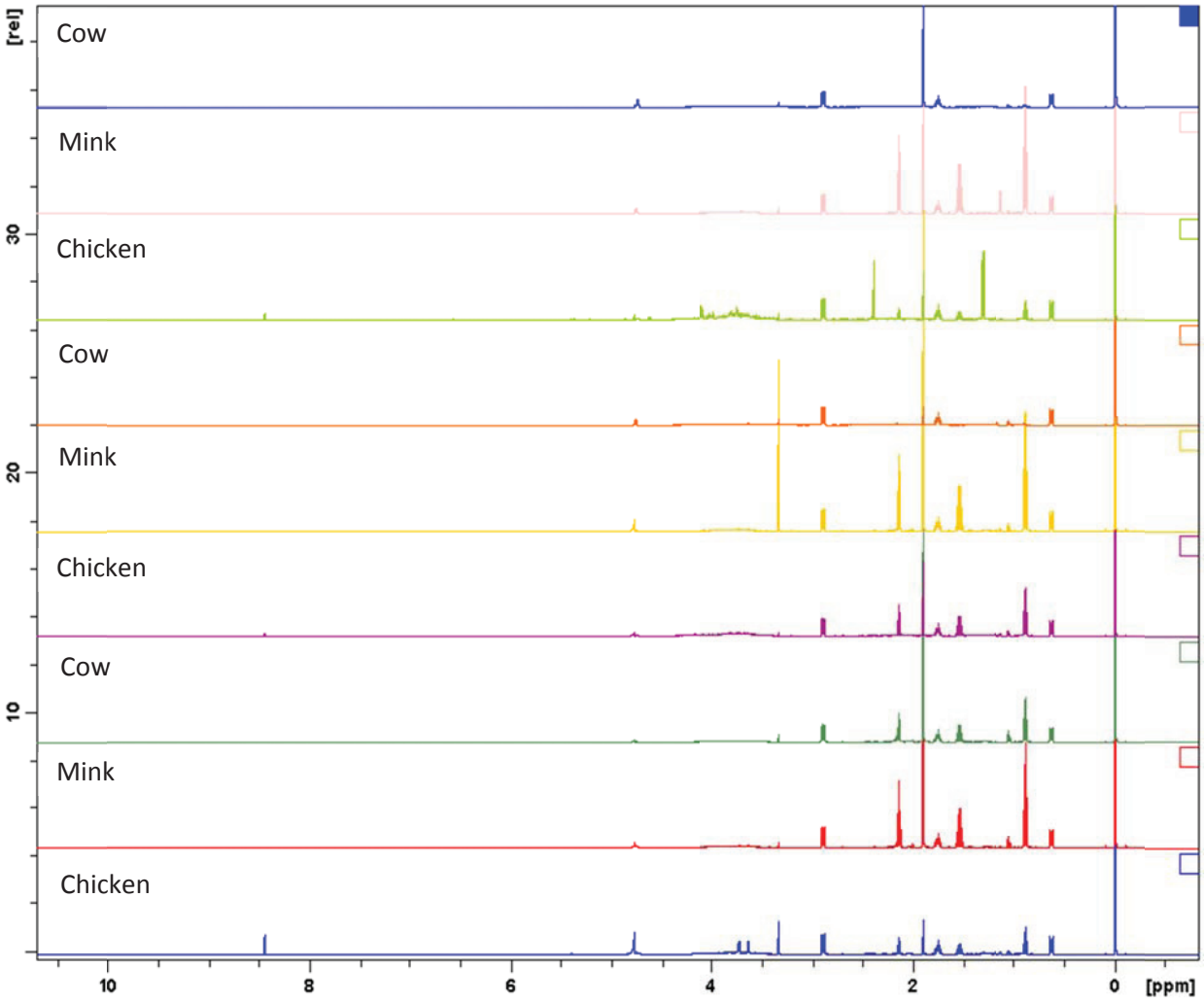
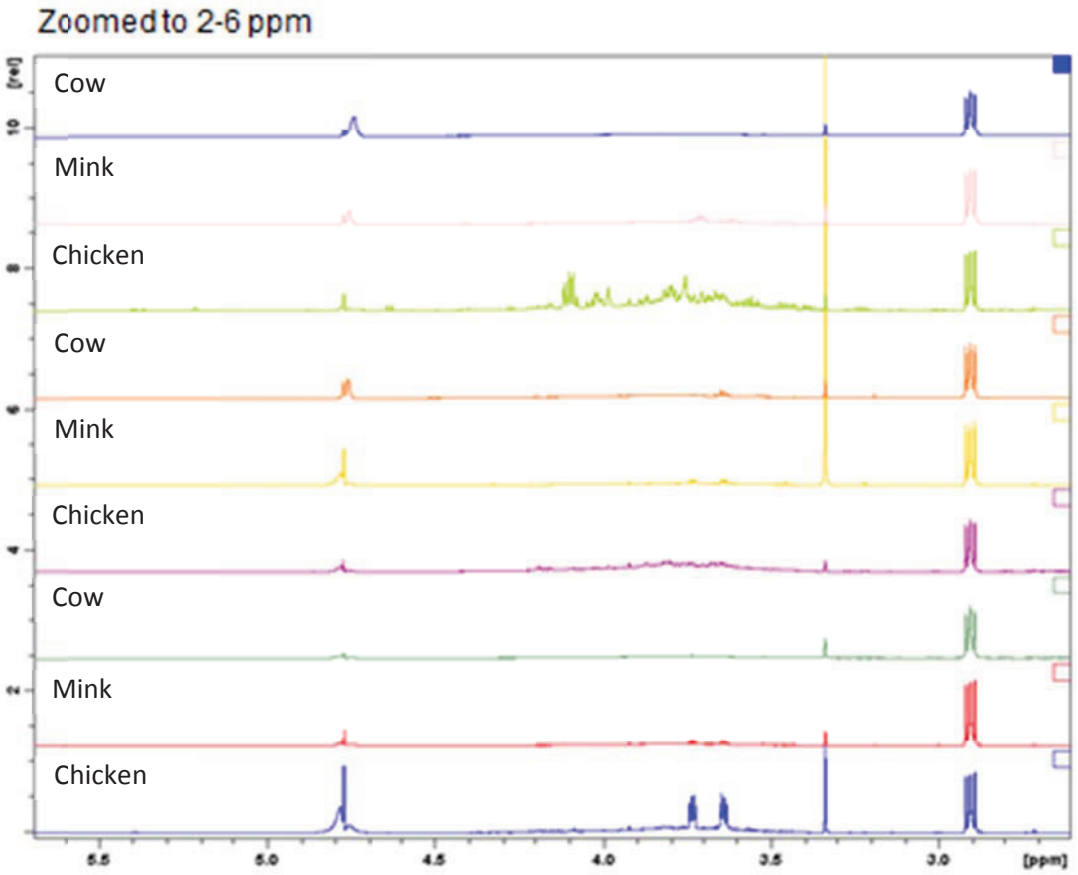
## APPENDIX A



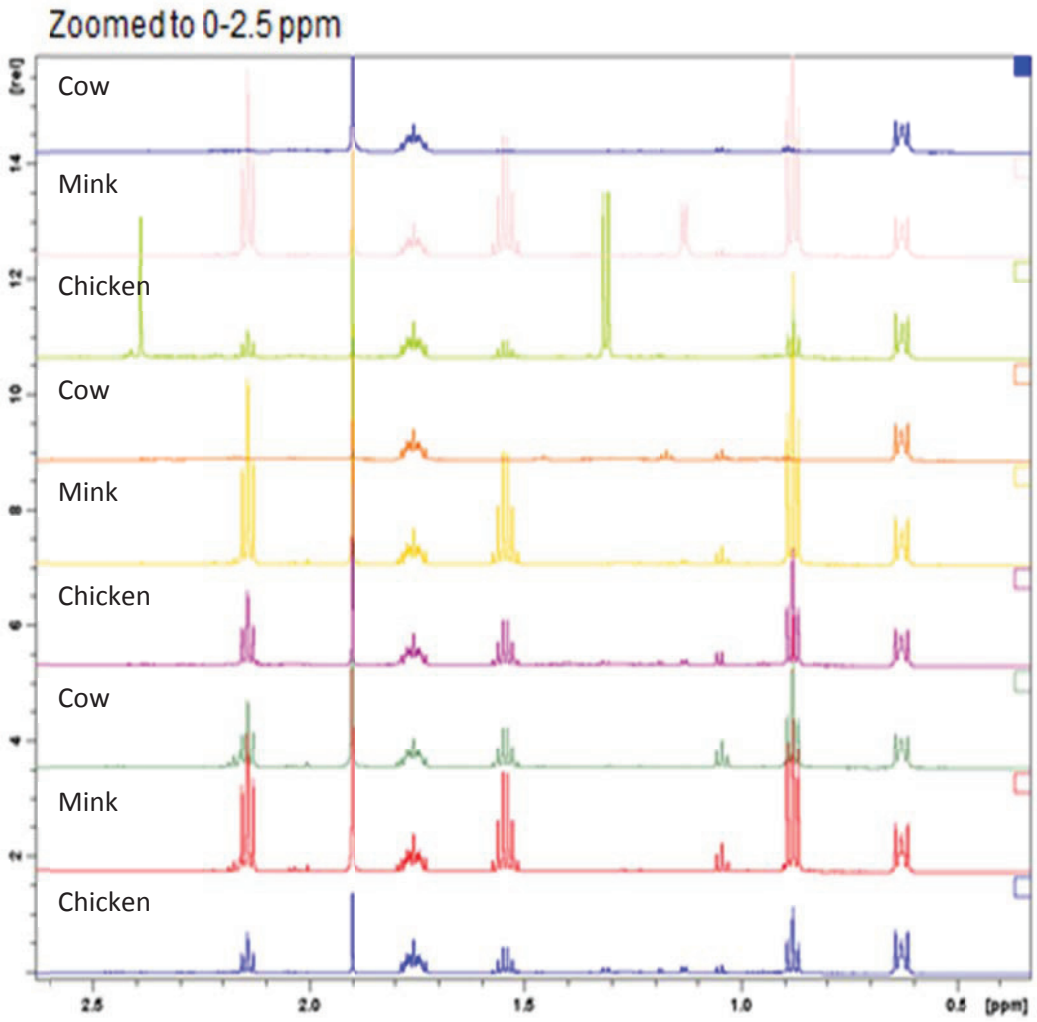

$^{1}$H-NMR analysis of compost tea brewed at three different time points June 17$^{th}$, July 11$^{th}$ , and July 25$^{th}$ .

## APPENDIX B




$^{1}$H-NMR analysis of compost tea brewed at three different time points June 17$^{th}$, July 11$^{th}$ , and July 25$^{th}$ .

## APPENDIX C



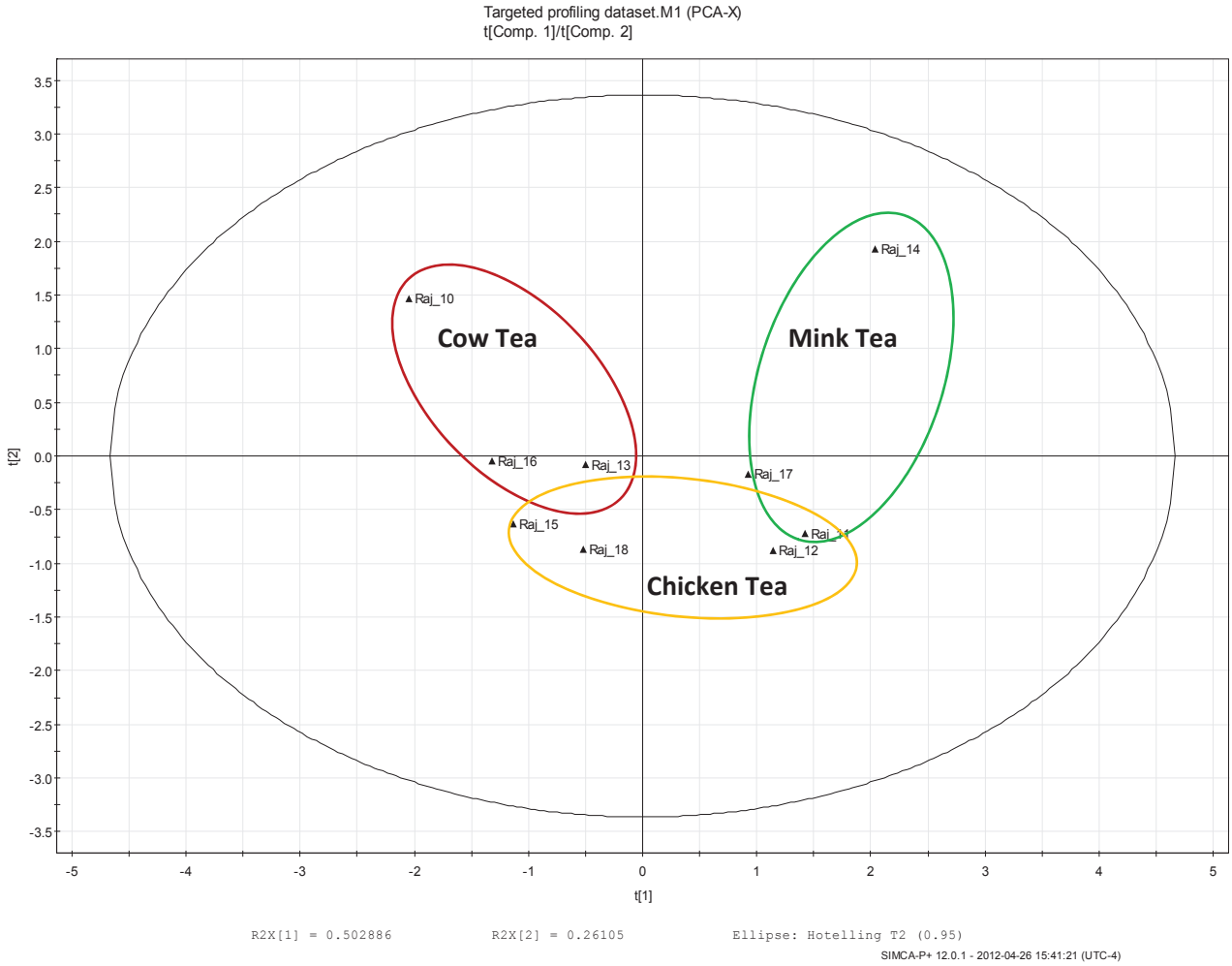

$^{1}$H-NMR analysis of compost tea brewed at three different time points June 17$^{th}$, July 11$^{th}$ , and July 25$^{th}$ .

## APPENDIX D

Quantities of 4 major components measured in the compost tea solution, compost tea was brewed at three different time points June 17th, July 11th , and July 25th .

| **Profiled Data Type** | **Concentrations ( mM )** | | | |
|---|---|---|---|---|
| | Acetate | Butyrate | Formate | **Methanol** |
| Cow Tea June 17th 2011 | 0.3289 | 0.4268 | 0.6902 | **0.3007** |
| Mink Tea June 17th 2011 | 4.1541 | 1.5369 | 0 | **0.0553** |
| Chicken Tea June 17th 2011 | 4.5403 | 0.9376 | 0 | **0.1021** |
| Cow Tea July 11th 2011 | 0.8866 | 0.9802 | 0.1601 | **0.0569** |
| Mink Tea July 11th 2011 | 2.8518 | 1.706 | 0.0084 | **1.3944** |
| Chicken Tea July 11th 2011 | 0.1915 | 0.0071 | 0 | **0.0686** |
| Cow Tea July 25th 2011 | 0.423 | 0.3074 | 0.234 | **0.061** |
| Mink Tea July 25th 2011 | 1.6756 | 2.0683 | 0.0063 | **0.0704** |
| Chicken Tea July 25th 2011 | 1.8795 | 0.0295 | 0 | **0.0627** |

## APPENDIX E




PCA analysis of $^{1}$H-NMR-based targeted profiling of 4 major components (acetate, butyrate, formate, and methanol). Profile indicated clear separation between cow and mink compost teas. Chicken compost tea appeared to have overlaps with the other two.