Fortification of Potassium Silicate with Compost Tea and Seaweed Extract for the Management of Dollar Spot (*S. homoeocarpa*) of Turfgrass

by

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A child said, what is the grass?

…Or I guess it is the handkerchief of the Lord…Or I guess the grass is itself a child… the

produced babe of the vegetation…Or I guess it is a uniform hieroglyphic,

And it means, sprouting alike in broad zones and narrow zones,

Growing among black folks as among white,

Kanuck, Tuckahoe, Congressman, Cuff, I give them the same, I receive them the same

And now it seems to me the beautiful uncut hair of graves.

Walt Whitman (1819-1892)

I dedicate this thesis to my parents Manoharan and Gounaselvy

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ABSTRACT

The effect of potassium silicate fortified with cow manure compost tea and seaweed extract on dollar spot (*Sclerotinia homoeocarpa*) was studied in creeping bentgrass (*Agrostis stolonifera* L.) and perennial ryegrass (*Lolium perene* L.). Application of potassium silicate fortified compost tea increased the silicon content in bentgrass and ryegrass at 5 days after treatment, but there was only a minimal effect on silicon content in the grass at 15 days after treatment. However, the fortified treatments did not affect the susceptibility of grass to dollar spot disease in the greenhouse experiments. Fortified treatments did not have a significant effect on the chlorophyll content, total phenol content and phenylalanine ammonia lyase activity in creeping bentgrass. Similarly, the treatment did not affect area of the lesion and mycelial growth of the fungus. Although the synergistic effect of silica fortification was not investigated in detail, preliminary results of the study indicate potassium silicate fortified with compost tea or seaweed extract was not different from that of non-fortified treatments in reducing dollar spot disease in the greenhouse trial.

LIST OF ABBREVIATIONS USED

%	percent
μl	microliters
μM	micromolar
$\mu mol\ m^{-2}s^{-1}$	micro-moles per square meter per second
μg	microgram
ANOVA	analysis of variance
CAT	catalase activity
CBG	creeping bentgrass
CMCT	cow manure compost tea
cm	centimeter
Cv	cultivar
DAI	days after infection
DAYS AFTER TREATMENTS	days after treatment
DS	dollar spot
EDTA	ethylene diamine tetra acetic acid
g	grams
GDP	gross domestic product
H_2O	water
H	hour
ISR	induced systemic resistance
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
$mg\ ml^{-1}$	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
OD	optical density
PAL	phenylalanine ammonia lyase activity
PDA	potato dextrose agar

PDB	potato dextrose bath
POD	peroxidase activity
ppm	parts per million
PPO	polyphenol oxidase activity
psi	pound per square inch
PVP	polyvinylpyrrolidone
rpm	revolutions per minute
S	second
SE	standard error
SWE	seaweed extract
spp.	more than one species (i.e. plural)
UV	ultra violet
v/v	volume to volume
ΔOD	change in absorbance

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CHAPTER 1

INTRODUCTION

Turfgrass is an important entity of man-made landscapes in different parts of the world. The utilization of turfgrass in gardens dates back to the early Persian gardens in the early 300 BC (Turgeon, 2008). The turf industry has intensively developed over the past few decades as a result of growing populations and increased urbanization. Turfgrass has become an integral component of North American landscapes; there are an estimated 20 million hectares of turfgrass in the United States (Johnson *et al*. 2009). Over 200,000 hectares are currently managed as green space on golf courses in Canada (NAGA, 2009). The benefits of turfgrass are functional and recreational in nature. Environmental services provided by turfgrasses include reduction in runoff during storm events, reduced soil erosion, dust pollution, heat dissipation ground cover and prevention of moisture loss. There are growing number of businesses, industry and research that are exclusively focussed on the turf industry. Golf industry is an important part of the turf industry. It is estimated that golfing as a sport and recreation contributes 11.3 billion to the gross domestic product (GDP) of Canada (NAGA, 2009). Maintenance and operation costs of golf course's tees, fairways and greens contribute a significant portion of this economic output. Apart from golf, turfgrass is used in athletic fields, residential lawns, parks, sod farms as well as industrial and institutional property (Nelson and Boehm, 2002a).

Turfgrass requires intensive maintenance regimes that include the use of a number of chemical inputs including herbicides, fungicides, insecticides and fertilizers (Walsh *et al*. 1999). One of the unique requirements of turfgrasses is that the grass species must tolerate foot traffic and physical wear yet remain aesthetically and functionally pleasing. Turfgrasses undergo various biotic and abiotic stresses. Fungal diseases are one of the major limitations to

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maintaining good turf. This is especially true in highly-managed golf courses where turf is maintained at ultra-low mowing heights and exposed to high foot-traffic compaction, and low nutrient inputs (Nelson, 2003).

Dollar spot, caused by the fungus *Sclerotinia homoeocarpa* F.T. Bennet, is one of the major diseases affecting turfgrass (Walsh *et al*. 1999; Bonos *et al*. 2003). It is prevalent in closely mowed greens, fairways, as well as poorly managed residential lawns and athletic fields. There is high incidence of dollar spot disease in eastern parts of North America due to the prevalent environmental conditions (high humidity, warm days and cold nights). Costs of protecting turfgrass from dollar spot fungus increased over the years with the inability of potential products to manage this problem. Fungicides have been used to manage dollar spot; however, their use is restricted in many areas due to environmental and health concerns. There are several potential alternatives that have been studied such as potassium silicate (soluble silicon source), compost tea (water derivative of composts), and seaweed extract (water soluble extract made by processing seaweed). These products when used individually are proven to be beneficial to plants under biotic and abiotic stresses.   Our research will examine the combination of these potential alternatives for enhanced disease suppression.

Silicon is the second most abundant element on Earth and has proven to suppress some of the important fungal and bacterial diseases in monocot and dicot plants (Schmidt *et al*. 1999; Deliopoulos *et al*. 2010). Silicon is associated with imparting beneficial effects on mechanical and physiological properties of plants. Silicon-mediated disease suppression has been studied in both cool and warm season grasses (Nanayakkara *et al*. 2008). The mechanism of suppression is a topic of intense research. One of the problems with silicon is its solubility and absorption rate in plants. Methods that enhance solubility could potentially improve plant absorption and

ultimately disease suppression. Compost tea is one potential alternative for the control dollar spot (Boulter *et al*. 2002; Nelson and Boehm, 2002a). Compost tea is a rich source of nutrients, microbial population and metabolites. However, the mechanism(s) of disease suppression by compost tea is not well understood. Induction of systemic resistance (ISR) is considered to be one of the modes of action. Seaweed extracts (SWE) have been evaluated for turfgrass management, and is used in turfgrass industry as a biostimulants. It is reported to contain phytohormones and osmoprotectants that improved physiological health of creeping bentgrass during environmental stress (Zhang *et al*. 2003). Application of seaweed extract increase both visual quality and physiological health of the turf.   SWE also improves turf quality during heat stress and cold tolerance (Munshaw *et al*. 2006; Zhang *et al*. 2010). The effect of SWE on turfgrass fungal pathogens has not been intensively explored.

The goal of this research was to evaluate the potential of combined applications of potassium silicate with compost tea or seaweed extract study their effect on the physiological health of turfgrass and the ability to reduce dollar spot disease severity.

CHAPTER 2

LITERATURE REVIEW

2.1 Creeping Bentgrass and Perennial Ryegrass

Creeping bentgrass is economically important turfgrass species, most widely in used on golf course putting greens, fairways and tees in the temperate climates of North America. Creeping bentgrass (*Agrostis stolonifera* L.) is characterized by its fine texture, dense growth and its ability to tolerate ultra-low mowing conditions (Belanger *et al.* 2004; Bonos *et al.* 2006). However, this species is susceptible to a number of diseases including brown patch (*Rhizoctoinia solani* Kuhn.), dead spot (*Ophiosphaerella Agrostis* Dernoeden), take-all patch (*Gaeumannomyces graminis* var. *avenae* (E.M. Turner) Dennis), antracnose (*Colletotrichum cereal* (ces) G.W.Wils), grey snow mould (*Typhula incarnata* Lasch and *T. Ishikariensis* S.Imai), *Michrodochium* patch and Pythium blight (*Pythium spp.*); dollar spot (*Sclerotinia homoeocarpa* F. T. Bennet). Dollar spot is considered as the most important and persistent disease of turfgrass in North America and around the world (Bonos *et al.* 2006; Cho *et al.* 2011). Creeping bentgrass cultivars are susceptible to the dollar spot fungus and there are only a limited number of cultivars that are moderately resistant (Belanger *et al*. 2004; Uriarte *et al*. 2004; Zhang *et al*. 2006). Creeping bentgrass cultivar 'penncross' is the most commonly cultivated in the Maritimes (eastern part of Canada and north -eastern sea board of United States); unfortunately this cultivar is susceptible to dollar spot.

Perennial ryegrass, a cool-season turfgrass widely used in the golf course fairways, soccer fields and residential properties. Perennial ryegrass (*Lolium perene* L.) is best adapted for Maritimes climate represented by moderate winters and mild summers (Bonos *et al*. 2006). It is preferred for its rapid germination, easy establishment with upright, bunchy growth habit and

tolerance to close mowing (Nanayakkara *et al.* 2008). In the southern United States, perennial ryegrass is used to over-seed dormant bermuda grass (*Cynodon dactylon* (L) Pers.) tees and fairways in golf courses in the fall, for its ability to tolerate cold temperatures. With increase in use of perennial ryegrass throughout the world, concurrent increase in incidence of diseases such as grey leaf spot (*Pyricularia grisea* Sacc.), dollar spot, brown patch, brown blight (*Drechslera siccans* Drechsler), grey snow mould, and red thread (*Laetisaria fuciformis* (McAlpine)Burds.) are reported in this species (Bonos *et al.* 2006).

2.2 Dollar Spot Disease

Dollar spot disease is caused by the fungus *Sclerotinia homoeocarpa* F.T. Bennet; it poses a serious threat to the bowling greens, home lawns and most importantly to the turf industry (Walsh *et al.* 1999; Bonos *et al.* 2003). A major portion of fungicides used on turfgrass is to manage this disease (Belanger *et al.* 2004; Hisang *et al.* 2007). High incidence of dollar spot is reported in eastern parts of North America largely due to the prevalence of favorable environmental conditions (high humidity, warm days and cold nights). This disease has extended period of activity from early June till late October in the Maritimes and Southern Ontario. During this period, dollar spot disease develops rapidly with the increasing summer temperatures between 15°C and 27°C or higher; intense disease pressure is noticed when the humidity is more than 85%. Low nitrogen, poor fertility and prevalence of abiotic stresses may also act as additional contributing factors to the establishment of this disease. It is comparatively not as big of a problem in western Canada and the pacific and north western United States. Dollar spot causes negative impact on the aesthetics and playability on the closely mowed greens and fairways. These impacts are not tolerated in high-maintenance turfgrass fields**.**

Fig. 1 Dollar spot developmental symptoms as seen on creeping bentgrass field or golf greens. A) Cob-web like mycelia scattered in the beginning stages on green B) Closer view of developing mycelia C) Mycelia leading to straw colored lesions or necrotic grass D) Symptoms seen on heavily infested golf green. On pictures A, B and C golf ball is placed as a scale.

2.2.1 Pathogenesis

The pathogenesis or development of dollar spot disease begins when mycelia from an infected grass contacts a healthy grass (Walsh *et al.* 1999). The main mode of entry may be through the opening on a freshly cut grass, stomatal opening or even direct penetration. Local spread of the pathogen occurs when an infected and healthy leaves are in close proximity

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whereas in large areas, through physical displacement of infected grass clippings by equipments or foot transfer.

Dollar spot disease symptoms start with transparent, white cob-web-like mycelia (Fig. 1 A, B), this can be distinctly observed in the morning dew when favorable conditions occur or a new disease establishment beside the older spots (Walsh *et al*. 1999). It is often mistaken as a spider web on grass. Further, the infection develops into a circular, sunken, straw-colored lesion about a size of a silver dollar, hence the name (Fig.1 C). When the disease develops the spots coalesce into a patch of dead grass as seen on Fig.1 D.

There is controversy surrounding the taxonomic placement of this fungus as dollar spot does not produce sclerotia (compact-dense mycelia of fungi formed under unfavorable conditions) but overwinters as plate-like, black pigmented stromata (cushion-like plate of solid mycelium) on dollar spot lesions from the previous infection. Conidia and ascospores are not observed in nature, therefore neither sexual nor asexual spores are considered important for this disease (Zhou and Boland, 1998, Walsh *et al*. 1999; Belanger *et al.* 2004). The fungus is reported to secrete a root damaging toxin during an infection Kelloway (2012).

2.2.2 Management of Dollar Spot Disease

Dollar spot disease is managed by amending cultural practices such as increasing nitrogen fertilizer application, avoiding drought stress, removing morning dew or guttation fluid but mainly with fungicides (Zhou and Boland, 1998; Jo *et al*. 2006; Jo *et al*. 2008; Putman *et al*. 2010). In spite of the effectiveness of fungicide to manage this disease, there is discouragement and increasing concern among the public regarding the use of fungicides (Zhou and Boland, 1998). Fungicides can have a negative impact on the environment. Repeated applications lead to accumulation of fungicides in the soil (Boulter *et al*. 2002). Fungicides may adversely affect the

soil micro flora, water, non-target pests, and also negatively impact beneficial micro flora such as endophytes (an endosymbiont, often a fungus or bacteria that lives inside a plant). Multiple applications of fungicides are required to maintain satisfactory turfgrass quality during the growing period (Ok *et al.* 2011). Fungicides are applied at 7 to 28 days intervals in the growing season to manage this disease (Hisang *et al.* 2007). The need for reduced use of fungicide is particularly important because the public and members of the public that use turfgrass for sports are often exposed for a prolonged period of time to fungicide treated turf (Zhou and Boland, 1998). They also estimated that the annual costs of fungicide application often exceeds $170 per ha in southern Ontario and in the United States in 1998. Belanger *et al.* (2004) estimated that each year one hundred million dollars worth of fungicides are applied to golf courses much of it for dollar spot management.

There is another emerging and alarming impact of the over-use of fungicides; it has led to the development of resistance by *S. homoeocarpa*. There are fewer active ingredients registered for dollar spot management in Canada when compared to the United States (Hisang *et al.* 2007). Increase in resistance in dollar spot to fungicides would lead to reduced availability of fungicides. Lack of awareness of this problem would result in continuous chemical application with ineffective control. With increasing reports on the levels of fungicide resistance and tightened environmental scrutiny of existing fungicides; dollar spot management is left with fewer chemical options (Jo *et al.* 2008). Fungicide effectiveness against *S. homoeocarpa* infection was reduced after 7 to 10 days of application, marginal suppression after 14 days and no suppression after 21 days when investigated by Latin (2006). Continued application of these fungicides could result in the development of cross (i.e. resistance to more than one fungicide within the same chemical group) and multiple-resistance (i.e. resistant to different fungicide

classes) (Jo *et al*. 2008). Indiscriminate use of fungicides has led to the development of resistance against a number of products such as iprodione, propiconazole, and thiophanate methyl in different parts of the United States (Putman *et al.* 2010). Ok *et al*. (2011) reported occurrence of resistance to demethylation inhibitors (DMI) and chemically related Type-2 plant growth regulators (PGR). Hisang *et al*. (2007) states that continued use of DMI fungicides would result in development of resistance to sensitive and resistant isolates of dollar spot fungus in southern Ontario. All these reports clearly indicate the need for environmentally- friendly strategies for dollar spot management.

2.2.3 Responsible Fungicide Application

With accumulating evidence on the development of fungicide resistance in dollar spot fungus, site specific and judicious application may help in reducing the negative impacts (Horvath *et al*. 2007). Geostatistical analysis was used to determine the spatial structure of dollar spot disease incidence. It can also be used to monitor the development or changes of the disease in the growing season on a putting green for effective and reduced fungicidal use. Optimization of nozzle selection may improve fungicide efficacy (Kaminski, 2009). Five different nozzle types were used that delivered fine to coarse droplets and two fungicides with different mode of action; chlorothalonil (contact) and propicanozole (acropetal penetrant) and was applied alone or tank mixed (in combination). Results of this study did not show interaction between fungicides and nozzle type. Increased dollar spot suppression was observed when tank-mixed fungicides were applied. The study also concluded that the air induction type nozzle may improve the efficacy of the fungicides for disease control and minimize potential drift to the off-target sites. Another field study conducted to assess the influence of irrigation regimes including 1) light but frequent at night 2) deep and infrequent in the morning as well as a combination of chemicals

such as chlorothalonil, paclobutrazol and a wetting agent (McDonald *et al*. 2006). The study was conducted for creeping bentgrass and perennial ryegrass for dollar spot and grey leaf spot, respectively. The results of this study indicated that soil moisture levels were associated with the improved ability for the studied chemicals to control dollar spot. It did not affect grey leaf spot.

2.3 Alternative Control for Dollar Spot Management

2.3.1 Cultural Methods

Dollar spot disease can be reduced by mowing and implementing other cultural practices. Results of a study conducted by (Ellram *et al*. 2007) indicate that in field conditions mowing daily at 0400h was considered most effective to reduce dollar spot when compared to mowing at 1000h or 2200h daily or alternate days. In other words, removing dew at a time which divides the length of continuous leaf wetness in half was effective to control dollar spot. In laboratory experiments lesion size was directly proportional to leaf wetness duration (LWD) on creeping bentgrass. Results also suggested that disruption of moisture 6h after uninterrupted LWD reduced dollar spot diameter.

Another 2 year study compared the combined effects of simulated rainfall (25 to 32 mm), mowing timing (morning – prior to fungicide application to remove dew and afternoon, >24 h following fungicide application and dry canopy) and application of diverse fungicides (such as chlorothalonil, boscalid, iprodione and propiconazole) against dollar spot in creeping bentgrass (Pigati *et al*. 2010). Percentage reduction in dollar spot control with simulated rain *vs*. no rain treatments in 2007 and 2008 respectively was chlorothalonil 67 and 83%, propiconazole 42 and 79%, boscalid 48 and 70% and iprodione 33 and 66%. Over two years, across all fungicide treated plots, the average percent reduction in DS with morning mowing ranged between 54 and 65%, which lead to improved performance of all fungicides.

2.3.2 Breeding and Transgenic Studies

Bonos *et al.* (2006) states that dramatic improvement in the history of disease resistance in cool-season turfgrasses has been accredited to traditional or conventional breeding techniques. However it is likely that functional genomics and molecular techniques plays a significant role in development of disease resistant varieties in the future. A study was conducted in two locations for two years in New Brunswick, NJ, USA to find genetic resistance to dollar spot fungus on 265 clones of creeping bentgrass. Results indicate that resistant clones maintained higher turf density, percentage green turf cover and smaller dollar spot diameter when compared to susceptible clones (Bonos *et al*. 2004). Belanger *et al*. (2004) reported the first interspecific hybrid with creeping bentgrass and colonial bentgrass (*A. capillaris* L.), a related species with resistance to dollar spot. The field studies of such interspecific hybrids showed excellent resistance to dollar spot fungus. The ability of creeping bentgrass to inherit dollar spot resistance was evaluated by Bonos (2006) results indicate that progeny from resistance x resistance crosses had significantly less disease severity than susceptible x resistant or susceptible x susceptible crosses. Bonos (2006) also states that the dollar spot disease is quantitatively inherited. Bonos (2011) validated the quantitative inheritance of dollar spot disease and suggested that there may be several genes interacting in an additive fashion to achieve resistance in creeping bentgrass. Genetically engineered creeping bentgrass with the PR5K gene from *Arabidopsis thaliana* homologous to the PR5 pathogenesis-related protein family delayed the dollar spot symptoms by about 45 days (Guo *et al*. 2003). A pepper esterase (pep EST) gene introduced into creeping bentgrass by *Agrobacterium* – mediated transformation inhibited the growth of fungal pathogens responsible for brown patch (*Rhizoctinia solani* Khun.) and dollar spot (Cho *et al.* 2011). The disease

severity of *R. solani* infected transgenic plants was 10% where as it was 50% for non-transgenic plants.

2.3.3 Biological Control of Dollar Spot Disease

Biocontrol agents are increasingly popular to manage various plant diseases. Powell *et al* (2000) states that use of biocontrol agent *invitro* and in outdoor environments for disease management is ascribed to the production of diffusible metabolites of the biocontrol agent. These metabolites may comprise of enzymes, siderophores (a small high-affinity iron chelating compound secreted by microorganisms), hydrogen cyanide, ethylene and antibiotics. Nelson (1996) reported that biological control is an attractive alternative strategy for controlling turfgrass diseases. A study conducted by Rodriguez and Pfender (1997) at Kansas State University, Manhattan used biological control approaches using spray application of *Pseudomonas fluroscens* Pf-5 reduced mycelia growth of dollar spot on grass clippings and reduced dollar spot disease incidence in creeping bentgrass and Kentucky blue grass. Four strains of *Pseudomonas* were evaluated for their ability to control dollar spot on Kentucky blue grass (Hodges *et al.* 1994). All the strains prevented infection by DS and prevented loss of chlorophyll. *Trichoderma harzianum* strain 1295-22, a commercially available biocontrol agent was studied on creeping bentgrass for the management of diseases such as brown patch, Dollar spot and pythium root rot and blight (Lo *et al.* 1996). All the diseases were significantly reduced in the growth chamber trials. Spray applications of conidial suspensions of the biocontrol agent significantly reduced the three diseases in greenhouse and field experiments. Lo *et al.* (1997) also reported that the population of *Trichoderma* increased 10 to 100 fold in the root zone and suggested that both granular application followed spray applications may lead to efficient management of the disease. Cell extracts of *Pseudomonas aureofaciens* Tx- 1 (ATCC 55670)

were reported to exhibit maximum antifungal activity against dollar spot fungus (Powell *et al*. 2000). The single active compound responsible for activity was identified as phenazine-1 carboxylic acid (PCA). PCA in green house studies managed dollar spot equal to that of fungicides triadimefon and chlorothalonil at equivalent rates. Field studies also demonstrated more efficient dollar spot management than fungicides. Azibenzolar-S-Methyl [(ASM), Actigard, Syngenta Corp, Basel, Switzerland] in combination with 12 commercial biostimulants (applied according to manufacturer's recommendations was studied to manage dollar spot to reduce fungicide use. Thirty eight percent reduction was observed in the dollar spot, but the turf quality was unacceptable during the study (Lee *et al*. 2003). Induced systemic resistance (ISR) was reported to be activated by PC1 a mixture of food grade isoparaffins and (2R,3R)-butanediol a volatile organic compound produced by bacteria (Cortes-Barco *et al*. 2010). Application of these compounds was reported to reduce the dollar spot diseased leaf area from 20-40%. Endophyte *Epichloë festucae* was reported to decreased dollar spot infection on (*Festuca rubra* subsp. r*ubra*) creeping red fescue (Clarke *et al*. 2006).

2.4 Silicon

Silicon is the second most abundant element on Earth and has proven to suppress fungal and bacterial diseases in monocot and dicot plants (Deliopoulos *et al*. 2010; Schmidt *et al*. 1999). Silicon-mediated disease suppression has been studied in both cool and warm season grasses (Nanayakkara *et al*. 2008). One of the problems with silicon is its solubility and absorption by plants. Methods that enhance solubility could potentially improve plant absorption and ultimately disease suppression.

Silicon is up taken in to the roots in the form of silicic acid [$Si(OH)_4$]. It is translocated to the leaves in the transpiration stream through xylem and is polymerized to silica gel (Ma and

Yamaji, 2006). The mechanism by which Si confers disease resistance is not well understood (Cherif and Belanger, 1992). The possible resistance mechanisms reported are (a) depositions of Si in leaf surfaces that act as a physical barrier to fungal penetration during and after infection, (b) mediation in activation of antifungal compounds, phytoalexins, phenolic compounds and pathogenesis related proteins, (c) induce defense responses in hosts that are similar to systemic acquired resistance and (d) activation of stress-related genes (Fauteux *et al*. 2005; Cai *et al*. 2009).

Silicate salts of potassium are widely used in green house hydroponic systems (Deliopoulos *et al*. 2010). Silicon has proven to be effective means to protect wheat, barley, rice and cucumber from pathogens. In *Arabidopsis thaliana,* silicon appears to induce defence reactions through the accumulation of phenolic compounds (Ghanmi *et al*. 2004). Silicon treated wheat plants display resistance to bacterial streak through tissue lignifications and accumulation of chitinases and peroxidises (Silva *et al*. 2010). Silicon also plays a major role in the activation of defence enzyme in cucumber (Yu *et al*. 2010).

Application of silicate at 100 and 200 ppm reduces mortality and increases yield and fruit quality in crown and root rot (*Pythium ultimum)* infected long English cucumber (Cherif and Belanger, 1992). Silicon reduces the severity of dollar spot and brown patch in creeping bentgrass (Uriarte *et al*. 2004). Tissue silicon levels increased in creeping bentgrass treated with calcium silicate (Zhang *et al*. 2006). Reduced fungicide rates with a combination of calcium silicate reduced gray leaf spot in warm season turfgrass (Datnoff *et al*. 2005).

2.5 Compost Tea

Compost tea is a promising alternative to chemical inputs to improve turf health (Nelson and Boehm, 2002b). The water extracts of composts are known as compost tea. Other names

used for compost tea are organic tea, compost extracts, steepages and slurries (Litterick *et al.* 2004; Sturz *et al.* 2006). Compost extracts are rich source of nutrients, microbial population and metabolites (Boulter *et al.* 2000). Therefore, the disease suppressive nature of compost extracts may be directly proportional but not restricted only to the population of microorganisms present. Compost tea production can be enriched by addition of nutrients and microorganisms. The re-colonization during curing process after the high temperature stage of composting process can be solved by controlled-inoculation of composts with biocontrol agents in compost tea (Hoitink *et al.* 1997). Little information is available on the fortification of compost tea. Nutrients, molasses, soluble kelp, humic materials and minerals are some of the currently used additives in compost tea (Scheuerell and Mahaffee, 2002).

As compost tea is a derivate of composts, the disease suppressive properties of composts can be related to compost teas. Effective disease suppression can be achieved from composts of consistent quality (Hoitink *et al.* 1997). Possible mechanisms of disease suppression by composts are competition, antibiosis, and hyperparasitism and induced systemic resistance in plants. Composts are rich in organic and inorganic minerals and biocontrol agents that improve plant health. Boulter *et al.* (2002) observed that compost topdressing effectively reduced dollar spot better than fungicide control. Multiple compost application as topdressing can be an appropriate means to manage dollar spot on creeping bentgrass. Cheng *et al.* (2007) reported that composted sewage sludge as a soil amendment at 10-20% improved soil nutrient content in perennial ryegrass turf. Compost inclusion in topdressings after core aeration increased soil water content and turf quality under drought stress in Kentucky bluegrass (Johnson *et al.* 2009). Nelson and Boehm (2002a) reported that compost from animal and other organic wastes suppressed various diseases in turfgrass. Compost extracts activate pathogenesis-related proteins that contribute to

systemic acquired resistance in plants. Zhang *et al*. (1998) observed that compost water extract reduces disease symptoms of bacterial speck in *A. thaliana* compared to peat water extract; GUS (beta-glucuronidase) activity was also induced with spraying compost extract in Arabidopsis. Compost tea has been fortified with biocontrol agents like *Trichoderma sp*., cyanobacteria and *Bacillus sp.* (Siddiqui *et al*. 2008a; Siddiqui *et al*. 2009; Dukare *et al*. 2011). Addition of biocontrol agents such as Trichoderma to compost tea suppressed Choanephora wet rot of okra and increased its morphological and physiological growth (Siddiqui *et al*. 2008b).

Compost water extracts, obtained by soaking compost in water with a ratio of 1:1 (v/v) has produced a number of aerobic biocontrol agents (Hoitink *et al*. 1997). In general, compost extracts are prepared in the ratio of 1: 5/10 ratios of compost and water respectively, filtered after a particular brewing time (Boulter *et al*. 2000). Compost tea is produced through either aerated or non-aerated process (Scheuerell and Mahaffee, 2002). Non-aerated compost tea is not widely used due to risk of spread of animal and human pathogens and also their unpleasant odours.

2.6 Seaweed Extract

Seaweeds are macroalgae found in marine ecosystems. Brown seaweed algae (*Ascophyllum nodosum* L.) have been researched for its use in agriculture (Khan *et al*. 2009). Seaweed extracts promote plant growth and development and improve plant resistance to biotic and abiotic stress. Numerous articles recently published emphasize the prophylactic nature of seaweed extracts in agriculture. *A. nodosum* on creeping bentgrass turf increased leaf cytokinins levels and drought resistance (Zhang, 2004). *A. nodosum* increased root and shoot growth in Arabidopsis by increasing the auxin concentration (Rayorath *et al*. 2008). *Alternaria radicina*, black rot and *Botrytis cinerea,* grey mould of carrot were significantly reduced with *A. nodosum* extract (Jayaraj *et al*. 2008). In this study, seaweed extract performed better than salicylic acid

(SA) by activation of defence related enzymes and defence related proteins. Organic components and cytokinins of seaweed extract enhance heat tolerance in creeping bentgrass (Zhang and Ervin, 2008). Seaweed extract has protected *A. thaliana* and has mediated in expression of freezing response genes during freezing stress (Rayirath *et al*. 2009). Seaweed extract based cytokinins was responsible to improve turf quality and performance during heat stress when investigated (Zhang *et al*. 2010).

Seaweed extracts (SWE) have also been used for turfgrass management. SWE is used in turfgrass industry as a biostimulants; it is reported to contain phytohormones and osmoprotectants that improved physiological health of creeping bentgrass during environmental stress (Zhang *et al*. 2003a). Application of seaweed extract has increased both visual quality and physiological health of the turf, SWE has also improved turf quality during heat stress and cold tolerance in different species of turfgrass (Munshaw *et al*. 2006; Zhang *et al*. 2010). The effect of SWE on turfgrass fungal pathogens has not been explored.

Seaweed extracts (SWE) have improved turf quality during heat, drought, stress and cold tolerance in different species of turfgrass (Munshaw *et al*. 2006; Zhang *et al*. 2010). Zhang *et al*. (2003) reported that seaweed extracts are biostimulants which are reported to contain phytohormones and osmoregulants such as cytokinins, auxins, polyamines, and betaines and when applied as monthly field applications positively influence the physiological health of creeping bentgrass (*Agrostis stolonifera L*.) cv. 'penncross' at the Virginia Tech Turfgrass research Center, Blacksburg, Va. The results of this study reported an increase in endogenous antioxidant superoxide dismutase activity, photochemical activity and visual quality of bentgrass in a course of two years (1996-1997) and also observed significant reduction in dollar spot symptoms. The same two natural-products were studied on three cultivars of creeping bentgrass

('Penn G-2', 'L-93', 'Penncross') in greenhouse environment for drought resistance (Zhang, 2004). The results indicated that the turf quality and photochemical efficiency started to decline in 2 weeks for control plants whereas it took 3 weeks when treated with seaweed extracts and humic acid. The combination of seaweed extracts and humic acid resulted in increased root mass and endogenous cytokinins levels. Another study by Zhang and Ervin (2008) reports that organic components of seaweed extracts, especially cytokinins resulted in beneficial effects to creeping bentgrass for enhanced heat tolerance. Results indicated increased concentrations of trans-zeatin riboside concentrations, superoxide dismutase, and lead to enhanced turfgrass quality, photochemical efficiency and root viability compared to control. (Zhang *et al*. 2010) reported that 10μM of seaweed extract based cytokinins (SWEC) may be optimum dosage to improve turfgrass performance and heat stress tolerance from a study with creeping bentgrass exposed to heat stress in a growth chamber.

2.7 Summary

The idea of fortification of composts or compost teas with biocontrol agents and/or nutrients to attain consistent or increased disease suppression is an exciting strategy to improve compost tea efficiency to control fungal diseases (Dukare *et al*. 2011; Hoitink *et al*. 1997; Siddiqui *et al*. 2008a). From previous reports, efficiency of dollar spot management can be achieved when potential disease control products and practices are used in combination. Current disease management literature for plants is focused on strategies that combine products that may provide disease control. This strategy would be an example of how one may mimic nature when a plant is encountered with a pathogen. In nature, there may be more than one factor responsible in the fight against disease establishment. Unfortunately, this phenomenon is not taken advantage of by agriculturalists while they usually tend to find solutions through the use of one product

alone. Secondly, there are presumptions that only certain products can be combined; which undermines biological activity and thereby shunning away from identifying synergies and discovering novel techniques. This project evaluates the use of potassium silicate, compost tea (a liquid derivate of composts) and commercial seaweed extract alone or in combination for dollar spot management.

2.8 General Hypothesis

Potassium silicate when combined with compost tea or seaweed extract will significantly reduce the dollar spot disease severity and improve physiological quality such as tissue silicon content, chlorophyll, phenolics and enzyme activity of creeping bentgrass.

2.8.1 Working Hypotheses

1. Tissue Silicon content is significantly different when potassium silicate is combined with compost tea or seaweed extract for dollar spot management in creeping bentgrass and perennial ryegrass. **Objective** – Determine silicon content and dollar spot disease severity across the applied treatments.

2. Potassium silicate when combined with compost tea or seaweed extract significantly increases physiological health of creeping bentgrass for the management of dollar spot disease. **Objective**- Determine physiological parameters such as chlorophyll content, total phenolics, enzyme activity and disease severity in the *in vitro* experiments.

CHAPTER 3

Effects of Potassium silicate with Compost tea or Seaweed extracts in Creeping Bentgrass and Perennial Ryegrass for Dollar Spot Management

3.1 Introduction

Dollar spot caused by the fungus *Sclerotinia homoeocarpa* F. T. Bennet is an economically important turfgrass disease infecting cool season turfgrasses (Walsh *et al*. 1999). Belanger *et al*. (2004) stated that there are about one hundred million dollars worth of fungicides used to control diseases in turfgrasses; much of it is used for dollar spot management. Dollar spot disease is wide spread in the north eastern part of North America, especially the Maritimes (Bonos *et al*. 2003). This disease is one of the most highly researched of all turfgrass diseases. It has been traditionally managed with frequent applications of high doses of potentially harmful fungicides (Latin, 2006). Several recent reports indicate that dollar spot fungus has developed fungicide resistance (Jo *et al* 2008; Ok *et al* 2011). Public pressure has increased in recent years to decrease fungicide use and alternate management options are needed.

Creeping bentgrass (*Agrostis stolonifera .L*) is one of the predominant, cool season grasses planted in golf greens in the Maritimes and north-eastern United States (Bonos *et al*. 2006). Unfortunately, most of its cultivars are susceptible to dollar spot disease, which can cause huge economic loss for the golf course operators. Perennial ryegrass (*Lolium perenne* L.) is an important cool-season grass species used for its hardy nature in fairways, athletic, fields as well as in home lawns. Dollar spot also infects perennial ryegrass but some varieties are moderately resistant.

Many integrated disease management strategies attempt to reduce reliance on fungicides and rely on less toxic products that have different mode of action. Biostimulants with low

toxicity in the environment have been increased recently in plant protection programs. Products such as potassium silicate, compost tea and seaweed extract have been individually proven to be beneficial under biotic and abiotic stresses in a multitude of plant-pathogen studies. These products are commercially used in the agriculture industry but, they have never been combined and aerated like aerated compost tea to estimate their fortified effects. This type of integration may lead to enhanced products or biostimulants that work synergistically to manage plant biotic and abiotic stresses.

Use of silicon has been beneficial against a number of pathogens. Silicon (Si) is the second most abundant element in the earth crust followed by oxygen (Ma and Yamaji, 2006). About 50-70% of soil mass is comprised of silicon dioxide (SiO_2), Si uptake by plants depends on the ability of the plants to translocate Si or if Si is present in an available form in the rhizosphere. The role of Si in plant physiology has recently been explored (Ma, 2010). Application of silicate salts of sodium, potassium and calcium are used in several production and disease management programs. Si in plants has been reported to alleviate various biotic and abiotic stresses (Ma, 2004; Currie and Perry, 2007; Liang, 2007). Si uptake is *via* roots in the form of silicic acid [Si $(OH)_4$]. It is translocated to the leaves in the transpiration stream *via* xylem and polymerised to silica gel (Ma and Yamaji, 2006). The mechanism by which Si confers disease resistance is not well understood (Cherif and Belanger, 1992). The possible resistance mechanisms are reported to be (a) depositions of Si in leaf surfaces that act as a physical barrier for fungal penetration during and after infection, (b) mediation in activation of antifungal compounds, phytoalexins, phenolic compounds and pathogenesis related proteins, (c) induce defence responses in hosts that are similar to systemic acquired resistance and (d) activation of stress-related genes (Fauteux *et al*. 2005; Cai *et al*. 2009).

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Si application and accumulation in tissue dry matter helps plants when encountered with fungal pathogens. The importance of silicon has lead to incorporation of silicon into fertilizers. Silicate salts of potassium are widely in used in green house hydroponic systems (Deliopoulos *et al*. 2010). Si has proven to be effective means to protect wheat, barley, rice and cucumber against pathogens. In *Arabidopsis thaliana*, Si appears to be the mediator of induced defence reactions through the accumulation of phenolic compounds (Ghanmi *et al*. 2004). Si treated wheat plants display resistance to bacterial streak through tissue lignifications and accumulation of chitinases and peroxidises (Silva *et al*. 2010). Si plays a major role in the activation of defence enzyme in cucumber (Yu *et al*. 2010). Application of potassium silicate at 100 and 200 ppm reduces mortality and increases yield and fruit quality in crown and root rot (*Pythium ultimum)* infected long English cucumber (Cherif and Belanger, 1992). Si sources have been studied against blast disease in rice (Rodrigues *et al*. 2001), grey leaf spot in perennial ryegrass (Nannyakkara *et al*. 2008), and powdery mildew in wheat (Remus-Borel *et al*. 2005).

Compost tea is another important alternative to chemical inputs to improve turf health (Nelson and Boehm, 2002b). The water extracts of composts are known as compost tea. Other names used for compost tea are organic tea, compost extracts, steepages and slurries (Litterick *et al*. 2004; Sturz *et al*. 2006). Compost extracts are rich source of nutrients, microbial population and metabolites (Boulter *et al*. 2000). Therefore, the disease suppressive nature of compost extracts may be directly proportional but not restricted only to the population of microorganisms present in it. Compost tea production can be enriched by addition of nutrients and microorganisms. The re-colonization during curing process after the high temperature stage of composting process can be solved by controlled-inoculation of composts with biocontrol agents in compost tea (Hoitink *et al*. 1997). Very little research has been done on the fortification of

compost tea. Nutrients, molasses, soluble kelp, humic materials and minerals are some of the currently used additives in compost tea (Scheuerell and Mahaffee, 2002). The challenge of delivering effective and consistent disease control by compost teas may be achieved by combining them to beneficial products such as potassium silicate.

Seaweeds are macro algae that are present in marine ecosystems, *Ascophyllum nodosum* L. brown seaweed algae has been researched for its use in agriculture (Khan *et al*. 2009). Seaweed extracts promote plant growth and development and improve plant resistance to biotic and abiotic stress. Numerous reports have been published describing seaweed extract prophylactic nature in agriculture. Seaweed extracts (SWE) have also been evaluated for turfgrass management. SWE is used in turfgrass industry as a biostimulant; it is reported to contain plant hormones and osmoprotectants that improved physiological health of creeping bentgrass during environmental stress (Zhang *et al*. 2003a). Application of seaweed extract has increased both visual quality and physiological health of the turf, SWE has also improved turf quality during heat stress and cold tolerance in different species of turfgrass (Munshaw *et al*. 2006; Zhang *et al*. 2010). Combining SWE with silicate salts may aid in the synergy and provide enhanced products or biostimulants for plant stress management.

The main objective of this research was to evaluate the differences in tissue silicon accumulation when potassium silicate was applied alone or as combined products with cow manure compost tea (CMCT) or SWE and study its effect on disease severity of dollar spot.

3.2 Materials and Methods

3.2.1 Plant Culture

The plants were grown in the greenhouse of Department of Environmental Sciences Faculty of Agriculture, Dalhousie University under controlled environmental conditions with a temperature range between 18-25 °C. The 4" pots contained potting mixture consisting of USGA (United States Golf Association) Certified sand (Shaw Resources, Nova Scotia, Canada) and peat (Professional PRO- MIX 'BX' MYCORISE ® PRO – Premier Promix, Quebec, Canada) in a 4:1 ratio. Seeds of creeping bentgrass (*Agrostis stolonifera* L. cultivar Penncross) and perennial ryegrass (*Lolium perenne* L. cultivar Top Gun II) were manually sprinkled on the soil surface at a rate of 0.2 g per pot. After seeding, the pots were covered with transparent plastic to ensure uniform germination. Germination was observed after 5 days, pots were fertilized regularly (every week) with Plant Prod Plus (20-20-20 NPK) Plant Products Co. Ltd., Ontario, Canada, at the rate of 1g/L and 50 mL per pot. The pots were individually irrigated as required. Grass was clipped to 1-2 cm above ground for creeping bentgrass, 3 - 4 cm for perennial ryegrass weekly for 30 days until the beginning of treatments.

3.2.2 Experimental Design and Treatments

The experiment was a completely randomised design with 15 treatments (table 3.0) with 4 replicates per treatment contributing to 60 experimental units. For time based sampling purpose, the experiment was conducted for 3 sampling time points therefore each time point had 60 experimental units of the same treatments and replicates. Collectively for each repetition of the experiment, there were 180 experimental units or pots with turfgrass. The experiment was conducted on both creeping bentgrass and perennial ryegrass simultaneously therefore there were

180 experimental units for creeping bentgrass and perennial ryegrass, respectively. The experiment was repeated twice.

Treatments were applied twice with an interval of five days. The three tissue sampling time points were represented as time points (A, B, C) for both creeping bentgrass and perennial ryegrass. 'A' (CBG A or PRG A) represents sampling done five days after second application of treatments. For Tissue Si content estimation, tissue samples were collected by cutting shoot tissues two cm above soil surface. Twenty four hours after sampling the tissue for Tissue Si content, experimental units in 'A' were infected with dollar spot powder (described in 3.2.5). Disease severity was evaluated on the experimental units of 'A', 10 days after inoculation. Disease severity rating was done only on time point 'A' no further sampling was conducted in time point A

Time point B represents a set of experimental units of creeping bent and perennial ryegrass with the same treatments. Treatments were applied at the same when 'A' was treated but was continued in the experiment for 10 more days (CBG B or PRG B). Time point C represents another parallel set of experimental units to 'B' (CBG C or PRG C), but time point C was inoculated with dollar spot powder at the same time as 'A'. At the end of 15 days after treatments, tissues of creeping bentgrass and perennial ryegrass from 'B' and 'C' were sampled to estimate tissue silicon content. While time point A was both sampled for tissues to estimate Tissue Si content and later studied for dollar spot disease severity, sampling in time point B and C was destructive sampling and the pots were discarded after sampling no further studies were done in time point B and C.

The treatments were formulated by combining three concentrations of silicon source KASIL® 6, 26.5% SiO_2 National Silicates, Toronto Canada, (0, 100 and 200 ppm) and five

additives (No additive, 1:10 compost tea (see 3.2.3), 1:5 compost tea, 0.1 % SWE { SWE

Acadian® (powdered alkaline extracts of *Ascophyllum nodosum*; Acadian Seaplants Limited,

Dartmouth, Nova Scotia, Canada)} and 0.3% SWE). The whole experiment had 3 time points for

destructive sampling therefore had 3X the experimental units. Treatments were applied at the

rate of 60mL per pot as a soil drench. The weekly fertilizer application was continued during the

experiments. Treatment # 4 to 9 and 11, 12, 14, 15 were formulated by combining two additives

(described in table 3.0) 24 hours before application and aerated similar to the compost tea

(described in 3.2.4). The other treatments (# 1, 2, 3 and 10, 13) were prepared just before the

application.

Table 3.0 List of Treatments used in the experiment

Sl. #	Treatment	Sl. #	Treatment
1	Control/ dis. water	10	0.1%SWE
2	100 PPM KASIL	11	0.1% SWE+ 100 PPM KASIL
3	200 PPM KASIL	12	0.1% SWE+ 200 PPM KASIL
4	1:10 compost tea	13	0.3% SWE
5	1:10 compost tea+ 100 PPM KASIL	14	0.3% SWE+ 100 PPM KASIL
6	1:10 compost tea+ 200 PPM KASIL	15	0.3% SWE+ 200 PPM KASIL
7	1:5 compost tea		
8	1:5 compost tea+ 100 PPM KASIL		
9	1:5 compost tea+ 200 PPM KASIL		

3.2.3 Treatments and pH

The treatments were formulated by combining two additives based on scientific background information but their chemical nature when combined was unknown. Therefore pH of the treatments was measured (Table 3.1), pH of all the treatments were measured after preparation and before application. From the pH values (table 3.1) it was clear that the alkaline KASIL treatments were below neutral when combined with CMCT (treatments # 5, 6, 8 and 9). When alkaline KASIL and alkaline additive SWE were combined, there was also a slight reduction in pH (treatments # 11, 12, 14 and 15). This indicates that the ions in the additives CMCT and SWE may have an influence on potassium and silicate ions or vice versa.

Table 3.1: pH of the treatments used in the experiments before treatment application

Treatments	Application Days after treatmentses			
	Nov 16	Nov 22	Jan 22	Jan 27
1. Control	4.81	5.50	4.47	5.61
2. 100 ppm KASIL	8.61	8.61	8.10	8.66
3. 200 ppm KASIL	8.72	9.02	8.54	9.08
4. 1:10 CMCT	6.30	5.66	6.64	5.70
5. 1:10 CMCT+ 100 ppm KASIL	5.90	5.94	6.17	5.81
6. 1:10 CMCT+ 200 ppm KASIL	6.00	5.87	6.31	5.93
7. 1:5 CMCT	5.75	5.50	6.06	5.55
8. 1:5 CMCT+ 100 ppm KASIL	5.75	5.62	5.85	5.66
9. 1:5 CMCT+ 200 ppm KASIL	5.85	5.60	5.95	5.89
10. 0.1% SWE	9.29	9.29	9.39	9.73
11. 0.1% SWE +100 ppm KASIL	7.50	7.30	7.53	7.53
12. 0.1% SWE + 200 ppm KASIL	7.71	7.76	7.82	7.65
13. 0.3% SWE	10.02	9.97	9.95	10.24
14. 0.3% SWE +100 ppm KASIL	8.53	8.23	8.11	8.13
15. 0.3% SWE +200 ppm KASIL	8.21	8.27	8.16	8.52

3.2.4 Compost Tea

Aerated Compost tea was made using the commercially available composted cow manure from the garden center purchased in summer 2011. Composted cow manure was manufactured by Greenworld Garden Products, NB, Canada. From the label, minimum analysis was total N 0.5%, available phosphoric acid 0.15%, soluble potash 0.35%, organic matter 15% and maximum moisture 65%. Moisture does not stay the same as composts were procured and stored in the greenhouse until use. Compost tea was made by mixing compost and water in the ratio of 1:10 and 1:5 (v/v) respectively, 100 mL of compost was added to 1 L of distilled water for 1:10 CMCT and 200 mL of compost was added to 1 L of distilled water for 1:5 CMCT. Compost teas were aerated continuously for 24 hours using an aquarium pump and 1" diffuser stone. After the brewing period, the compost tea was filtered using approx. 60- mesh screen. This method is also referred as compost extract in literature, but the term 'compost tea' is predominantly used. The major difference between compost tea and compost extract in compost tea according to (Litterick *et al*. 2004) compost is contained in a mesh bag or cheese cloth to contain the bigger sized particles present in the compost.

3.2.5 Inoculum Preparation and Inoculation

Sclerotinia homoeocarpa was obtained from surface sterilized creeping bentgrass tissue with dollar spot symptoms. This isolate was obtained from Mountain golf club, Bible Hill, NS, Canada in 2011. The fungus was sub-cultured and inoculated on healthy creeping bentgrass by placing a mycelial plug under greenhouse conditions to verify Kosh postulates. After the straw-coloured hour-glass-like lesions appeared, the fresh leaf blade with symptoms was surface sterilized and re-cultured on PDA plates. This fungus was sub-cultured on a monthly basis and used in further experiments. The inoculum to infect the pot experiments 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 growing edge of the culture and inoculated directly into 1000ml Erlenmeyer flasks that contained moist 250ml sterile millet seed. The sterile millet seed was prepared by soaking the millet seeds in water and autoclaved twice (with 48 h incubation in between) for 15min, at 121°C and 15 psi to ensure sterilization of seeds. Flasks were then incubated at 24°C for 21 days 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 use. The dry dollar spot disease covered millet seeds were grinded into fine powder. The surfaces of the leaves to be infected were wetted by spraying water using a spray bottle. Dollar spot and millet seed powder was manually sprinkled on the surface of grass evenly in a circular fashion. After inoculation, pots were covered with a transparent plastic, a module made to cover the whole bench that included all treatments in the time point to ensure high humid conditions. The cover was opened and closed during water or fertilizer application.

3.2.6 Silicon Content Estimation in Leaves

Silicon content in all the experiments was determined by modification of autoclave induced digestion method following the procedure of Elliot and Snyder (1991). Samples were oven dried at 70°C for 48 hours. The dried tissue was ground in a micro Wiley mill and the ground material was allowed pass through a 20-mesh screen. One hundred mg of the dried sample was placed in polyethylene tubes (50mL capacity). The sample was wetted with 3.3 mL of 30% H_2O_2 and 3 mL of 50% NaOH, the plastic ware was vortexed and covered with loose – fitting caps. The mixture was autoclaved at 138 KPa for 60 min at 126°C. Subsequently the samples were removed and total volume was equilibrated to 50 mL with deionised water. Silicon

content was determined calorimetrically by taking 100 µL of the digested and mixed with 1 mL of deionised water, 900 µL acetic acid (20% v/v) along with 250 µL of ammonium molybdays after treatmentse solution (100 g L^{-1}, pH 7.0) and 25 µL of reducing agent was added. The mixture was vortexed and after 5 minutes 125 µL of tartaric acid (20% w/v) was added. The reducing agent was made by dissolving 4 g sodium sulphate and 0.8 g 1-amino-2 napthol-4 sulphonic acid and 50 g of sodium bisulphite in 200 mL of water. After incubation for 10 minutes the absorbance was read in a spectrophotometer at 650 nm. A standard curve relating to the amount of Silicon content in the plant material was made. The standard curve was made by dilutions of 1000 ppm Si standard (VWR international) using deionized water.

3.2.7 Disease Assessment and Data Analysis

Disease severity was assessed on pots representing time point 'A' on creeping bentgrass and perennial ryegrass with *S. Homoeocarpa* 10 days after inoculation. Disease parameters taken into account while rating the pots were straw coloured, brownish and dead tissue. Density and turf quality was also considered in the assessment. Individual pots were assessed based on a visual scale index by determining, heavily diseased or pots displaying heavy necrotic symptoms to none or least necrotic symptoms (described in Appendix A). Every experimental unit was rated by Mullaivannan Manoharan and Stephen Kelloway, turfgrass research graduate students in Faculty of Agriculture, Dalhousie University. The disease ratings for individual pots were based on rating system used by Bonos (2006) and Zhang *et al*. (2006). The rating of the disease severity was based on a 1-9 scale: 9 represented 0 to 5%, 8 represented 10% diseased turf, 7 represented approximately 10% diseased turf, 6 represented approximately 30 to 40 % diseased turf, 5 represented 40 to 50 % diseased turf, 4 represented approximately 60 to 70 % diseased

turf, 3 represented approximately 75 to 85% disease symptoms, 2 represented approximately 90% diseased turf, 1 represented 95 to 100% disease symptoms.

As all the sample collection was destructive in nature, Silicon content at each sampling time point was analysed separately. Although the treatments appear as a factorial design, the experiment was analysed as a completely randomised design. The main reason the experiment was analysed in this method is because, it is important to evaluate the collective effect of the combined treatments on the parameters estimated rather than identifying their interaction. The treatment combinations (individual factors or the products) were prepared prior to the application to evaluate the final effect of the combined treatments. Tissue silicon content and disease severity rating was verified for normality, constant variance using MINITAB statistical software and analysed using PROC MIXED procedure of SAS statistical software (SAS Institute, Cary, NC), and significance was determined at $P \leq 0.05$. Two repetitions of experiment were arranged as random variables. Least square means were used for the pair wise comparison of treatments and letter groupings of LS means was generated using a macro "pdmix800.sas" (Saxton, 1998).

3.3. Results

3.3.1 Tissue Silicon Content in Creeping Bentgrass – CBG A – 5 days after treatment application

Fig 3.1 Effect of treatments on Tissue Si content in creeping bentgrass 'A' tissues (samples collected 5 days after treatment). Bars indicate mean ± standard error - P 0.0001. Bars followed by the same letter are not significantly different at 5% level. There was no dollar spot inoculation on these tissues.

Table 3.2 Creeping bentgrass 'A' Tissue Si content – 5 days after treatment application – PROC Mixed – ANOVA TABLE

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatments	14	104	9.30	<.0001
Repetition	1	104	5.16	0.0251

Tissue Si content was significantly different across treatments in creeping bentgrass 'A' (Fig 3.1). From to the letter groupings generated from LS means pair wise comparisons, significantly higher Tissue Si content was in treatment #6 and #9 (200 PPM KASIL with 1:10 and 1:5 CMCT respectively). There was an increasing trend in the Tissue Si content in creeping bentgrass 'A' with the 3 concentrations (0, 100 & 200 ppm) of KASIL applications. When the KASIL was combined with control, 1:5 CMCT and 1: 10 CMCT significantly higher Tissue Si content was found in 200 PPM KASIL than all other treatments. It indicates that creeping bentgrass accumulates more Si in leaf tissue when silicon is applied at higher concentrations 5 days after treatment. When KASIL was combined with 0.1 % and 0.3% SWE, higher Tissue Si content was also measured in 200 PPM KASIL than at lower silicon concentrations but this difference was not significant. From the results, creeping bentgrass tissues 'A', 5 days after treatment had significantly highest silicon content when the highest concentration of KASIL (200 ppm) was combined with CMCT. In this experiment the Si content of CMCT was not measured but it may have contributed to the increase in silicon in the samples compared to when the treatments did not include CMCT. Another reason may be that the organic acids in compost may have increased Si- absorption ability of roots. Although the mechanism was not investigated, the treatment formulation with KASIL and CMCT worked synergistically to increase Si accumulation in bent grass shoot tissues at 5 days after treatments.

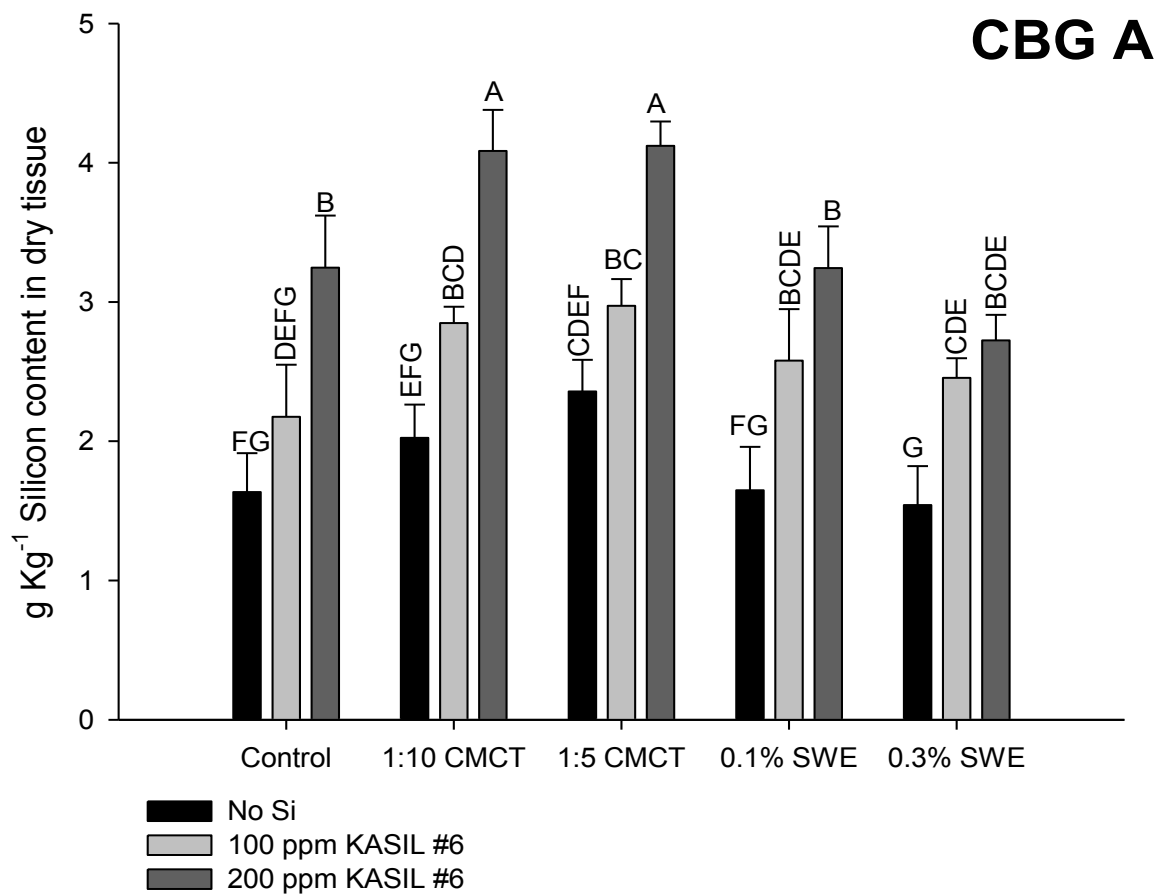**3.3.2 Tissue Silicon Content in Creeping Bentgrass – CBG B - 15 days after treatment application with no inoculation**

Fig 3.2 Effect of treatments on Tissue Si content in creeping bentgrass 'B' tissues (samples collected 15 days after treatments with no dollar spot inoculation). Bars indicate mean ± standard error - P 0.0003. Bars followed by the same letter are not significantly different at 5% level.

Table 3.3 Creeping bentgrass 'B' Tissue Si content – 15 days after treatment application with no dollar spot inoculation PROC Mixed - ANOVA TABLE

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatments	14	95	3.24	0.0003
Repetition	1	95	15.94	0.0001

Tissue Si content was significantly different across treatments in creeping bentgrass 'B' (Fig 3.2). According to the letter groupings generated from LS means pair wise comparisons, highest Tissue Si content was in treatment #6 (200 PPM KASIL with 1:10 CMCT) but not statistically different from the other treatments. There was no clear increasing trend in the Tissue Si content in creeping bentgrass 'B' with the 3 concentrations (0, 100 & 200 ppm) of KASIL applications as there was at 5 days after treatments (Fig 3.1). From the results, creeping bentgrass tissues 'B', 15 days after treatments had significantly increased silicon content when KASIL was combined with CMCT. There was no testing for finding a difference between samples at time point 'A' and samples at 'B' but similar trend was observed even at 15 days after treatments. The mechanism was not investigated, the treatment formulation with KASIL and CMCT worked synergistically to increase Si accumulation in bent grass shoot tissues even at 15 days after treatments.

3.3.3 Tissue Silicon Content in Creeping Bentgrass – CBG C - 15 days after treatment application under 10 days of dollar spot inoculation
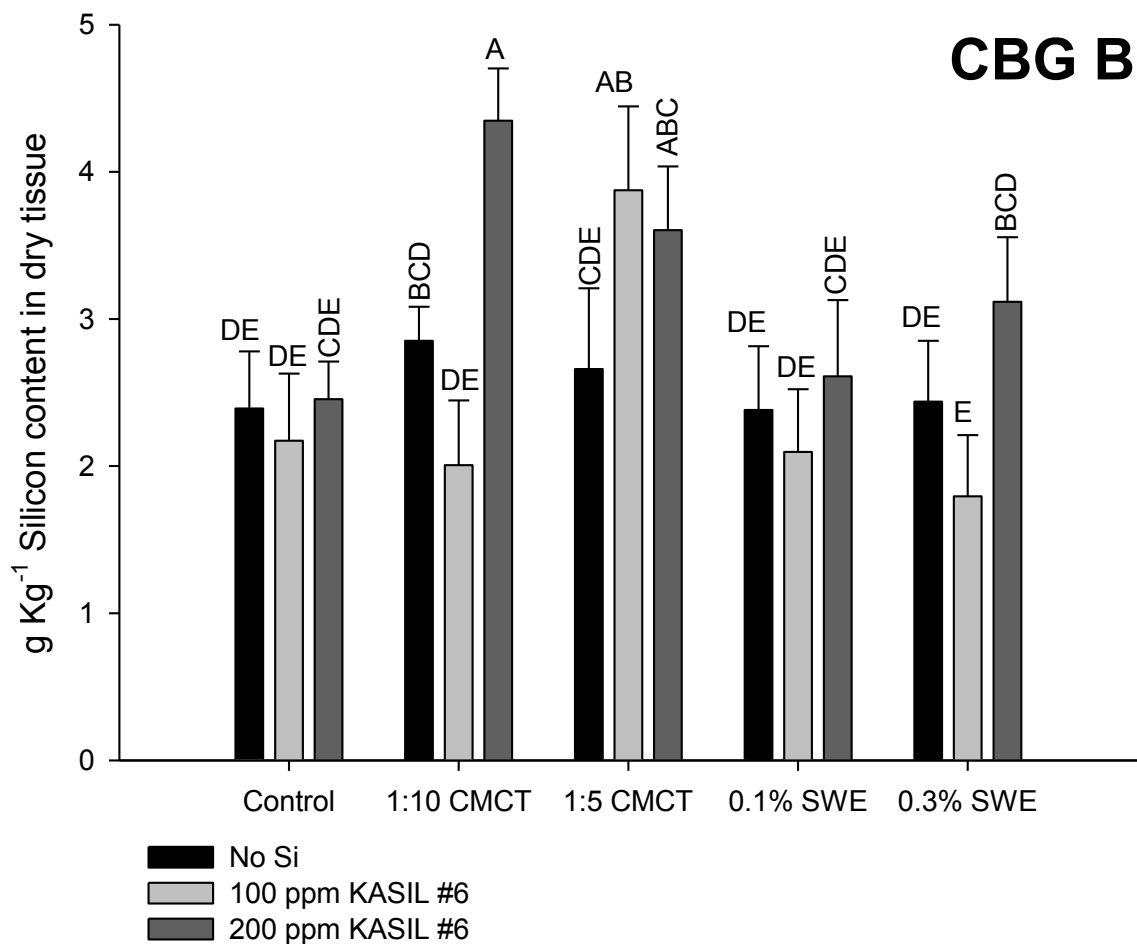

Fig 3.3 Effect of treatments on Tissue Si content in creeping bentgrass 'C' tissues (samples collected 15 days after treatments with dollar spot inoculation (10 DAI). Bars indicate mean ± standard error - P 0.0010. Bars followed by the same letter are not significantly different at 5% level.

Table 3.4 Creeping bentgrass 'C' Tissue Si content – 15 days after treatment with 10 days of dollar spot inoculation PROC Mixed - ANOVA TABLE

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatments	14	90	2.94	0.0010
Repetition	1	90	15.30	0.0002

Tissue Si content was significantly different across treatments in creeping bentgrass 'C' representing samples collected 15 days after treatments with 10 days of dollar spot inoculation (Fig 3.3). According to the letter groupings generated from LS means pair wise comparisons, highest Tissue Si content was in treatment #12 (200 PPM KASIL with 0.1% SWE) but was not statistically greater than other treatments but the trend appears to be similar to !5 days after treatment without dollar spot inoculation CBG B (Fig 3.2). however, there was no clear increasing trend in the Tissue Si content in creeping bentgrass 'C' with the 3 concentrations (0, 100 & 200 ppm) of KASIL applications.

3.3.4 Tissue Silicon Content in Perennial Ryegrass – PRG A– 5 days after treatment application

Fig 3.4 Effect of treatments on Tissue Si content in perennial ryegrass 'A' tissues (samples collected 5 days after treatments). Bars indicate mean ± standard error - P < 0.0001. Bars followed by the same letter are not significantly different at 5% level. These samples did not have any dollar spot inoculation.

Table 3.5 Perennial ryegrass 'A' Tissue Si content – 5 days after treatment application PROC
   Mixed – ANOVA TABLE

Type 3 Tests of Fixed Effects				
Effect	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Treatments	14	103	5.04	<.0001
Repetition	1	103	45.19	<.0001

Tissue Si content of perennial ryegrass was significantly different across treatments in

perennial ryegrass 'A' (Fig 3.1). From to the letter groupings generated from LS means pair wise

comparisons, higher Tissue Si content was in treatment #6 (200 PPM KASIL with 1:10 CMCT)

but not significantly different from all the other treatments. There was no clear increasing trend

in the Tissue Si content in perennial ryegrass 'A' with the 3 concentrations (0, 100 & 200 ppm)

of KASIL applications as it was like in bentgrass 5 days after treatments (Fig 3.1). From the

results, perennial ryegrass tissues 'A', 5 days after treatments had significantly increased silicon

content when KASIL was combined with CMCT. Although the mechanism was not

investigated, the treatment formulation with KASIL and CMCT worked synergistically to

increase Si accumulation in ryegrass shoot tissues when compared with control at 5 days after

treatments.

3.3.5 Tissue Silicon Content in Perennial Ryegrass – PRG B - 15 days after treatment application with no inoculation
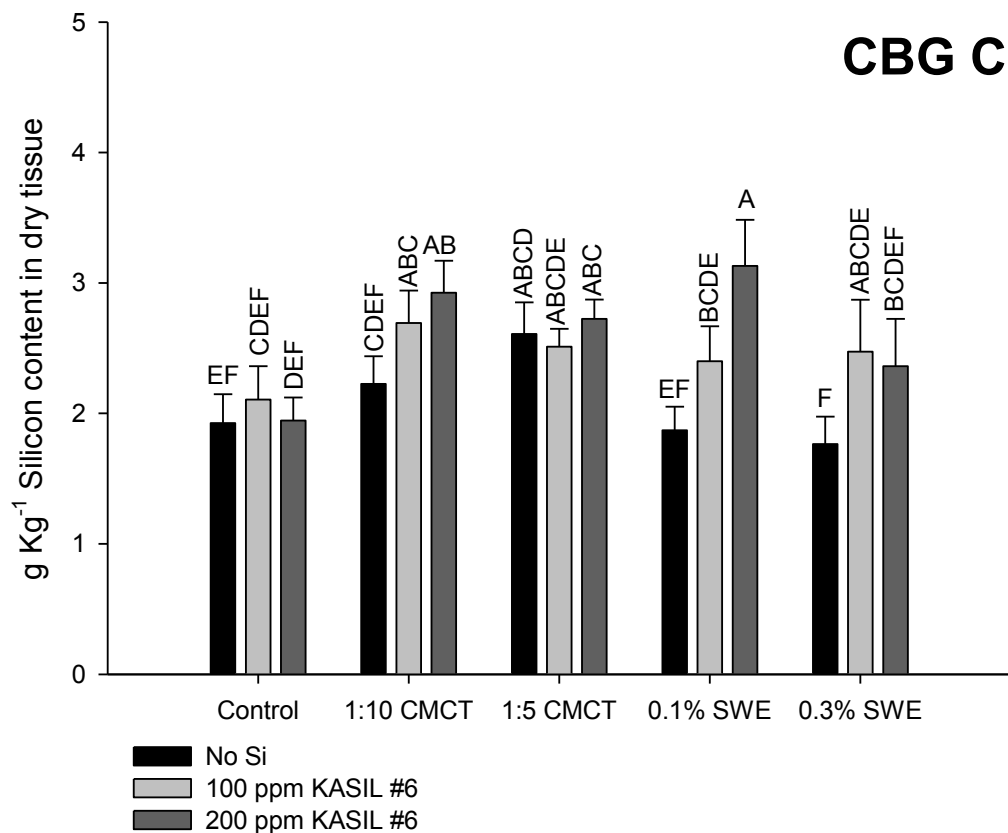

Fig 3.5 Effect of treatments on Tissue Si content in perennial ryegrass 'B' tissues (samples collected 15 days after treatments with no dollar spot inoculation). Bars indicate mean ± standard error - $P < 0.0156$. Bars followed by the same letter are not significantly different at 5% level.
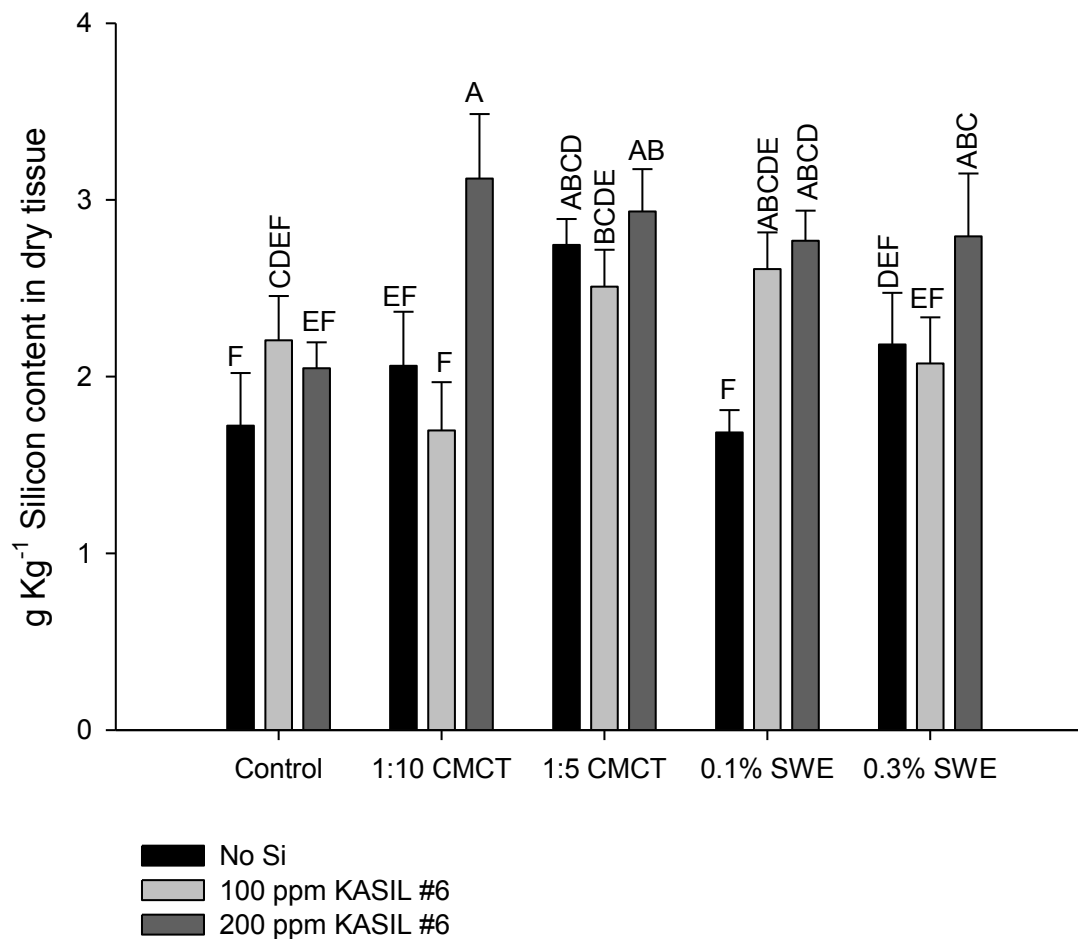
Table 3.6 Perennial ryegrass 'B' Tissue Si content – 15 days after treatment with no dollar spot inoculation PROC Mixed - ANOVA TABLE

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatments	14	104	2.13	0.0156
Repetition	1	104	1.88	0.1736

Tissue Si content was significantly different across treatments in perennial ryegrass 'B' representing 15 days after treatment without dollar spot inoculation (Fig 3.2). According to the letter groupings generated from LS means pair wise comparisons, highest Tissue Si content was in treatment #6 and #9 (200 PPM KASIL with 1:10 and 1:5 CMCT) but not statistically different from all the other treatments. There was no clear increasing trend in the Tissue Si content in perennial ryegrass 'B' with the 3 concentrations (0, 100 & 200 ppm) of KASIL applications. From the results, perennial ryegrass tissues 'B', 15 days after treatments had increased silicon content when KASIL was combined with CMCT but not significant different from all the other treatments.

3.3.6 Tissue Silicon Content in Perennial Ryegrass – PRG - C- 15 days after treatment application under 10 days of dollar spot inoculation
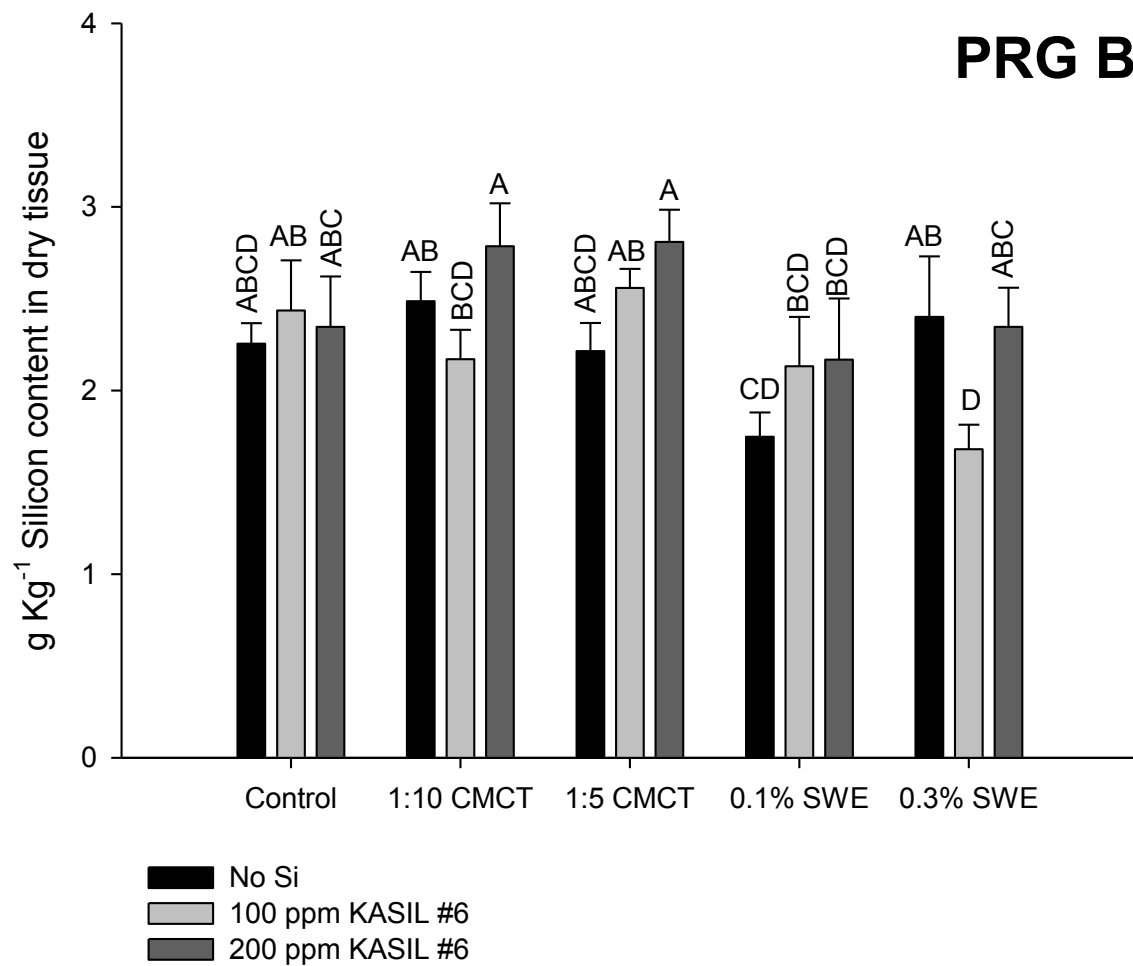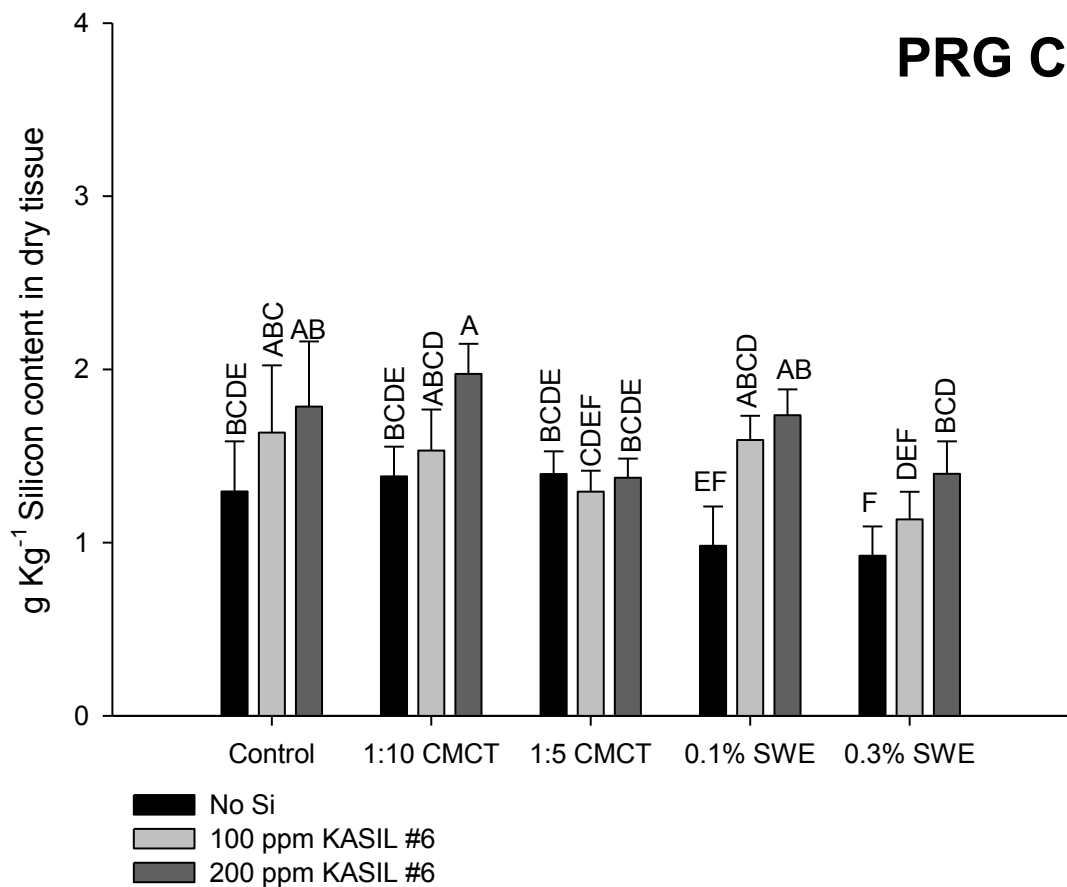
Fig 3.6 Effect of treatments on Tissue Si content in perennial ryegrass 'C' tissues (samples collected 15 days after treatments with dollar spot inoculation (10 DAI). Bars indicate mean ± standard error - P 0.0001. Bars followed by the same letter are not significantly different at 5% level.

Table 3.7 Perennial ryegrass 'C' Tissue Si content – 15 days after treatment with 10 days of dollar spot inoculation PROC Mixed - ANOVA TABLE

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatments	14	95	3.53	0.0001
Repetition	1	95	75.81	<.0001

Tissue Si content was significantly different across treatments in perennial ryegrass 'C' representing 15 days after treatments with 10 days of dollar spot infection (Fig 3.3). According to the letter groupings generated from LS means pair wise comparisons, highest Tissue Si content was in treatment #6 (200 PPM KASIL with 1:10 CMCT) but not statistically different from all the other treatments. There was no clear increasing trend in the Tissue Si content in creeping bentgrass 'C' with the 3 concentrations (0, 100 & 200 ppm) of KASIL applications. There was no testing to compare time points 'B' and 'C', but under infection no difference in tissue Si content was found across the treatments.

3.3.7 Disease Ratings

Table 3.8 Performance of creeping bentgrass and perennial ryegrass with 5 days of treatments and followed by inoculation after sampling and incubating for 10 days under high humidity

| Treatments | Turf quality under disease[1] average rate | |
	CBG[2]	PRG[3]
1. Control	4.3	6.3
2. 100 ppm KASIL	4.8	6.2
3. 200 ppm KASIL	6.3	7.0
4. 1:10 CMCT	5.8	7.6
5. 1:10 CMCT+ 100 ppm KASIL	5.1	6.1
6. 1:10 CMCT+ 200 ppm KASIL	4.5	6.7
7. 1:5 CMCT	5.1	7.0
8. 1:5 CMCT+ 100 ppm KASIL	6.0	6.9
9. 1:5 CMCT+ 200 ppm KASIL	5.7	6.9
10. 0.1% SWE	5.1	7.1
11. 0.1% SWE +100 ppm KASIL	4.4	6.2
12. 0.1% SWE + 200 ppm KASIL	5.5	6.6
13. 0.3% SWE	5.5	5.3
14. 0.3% SWE +100 ppm KASIL	5.1	6.9
15. 0.3% SWE +200 ppm KASIL	4.2	6.8

[1]9 = least disease and best turf quality, CBG[2] = P- value 0.8107, PRG[3] = P - value 0.6242
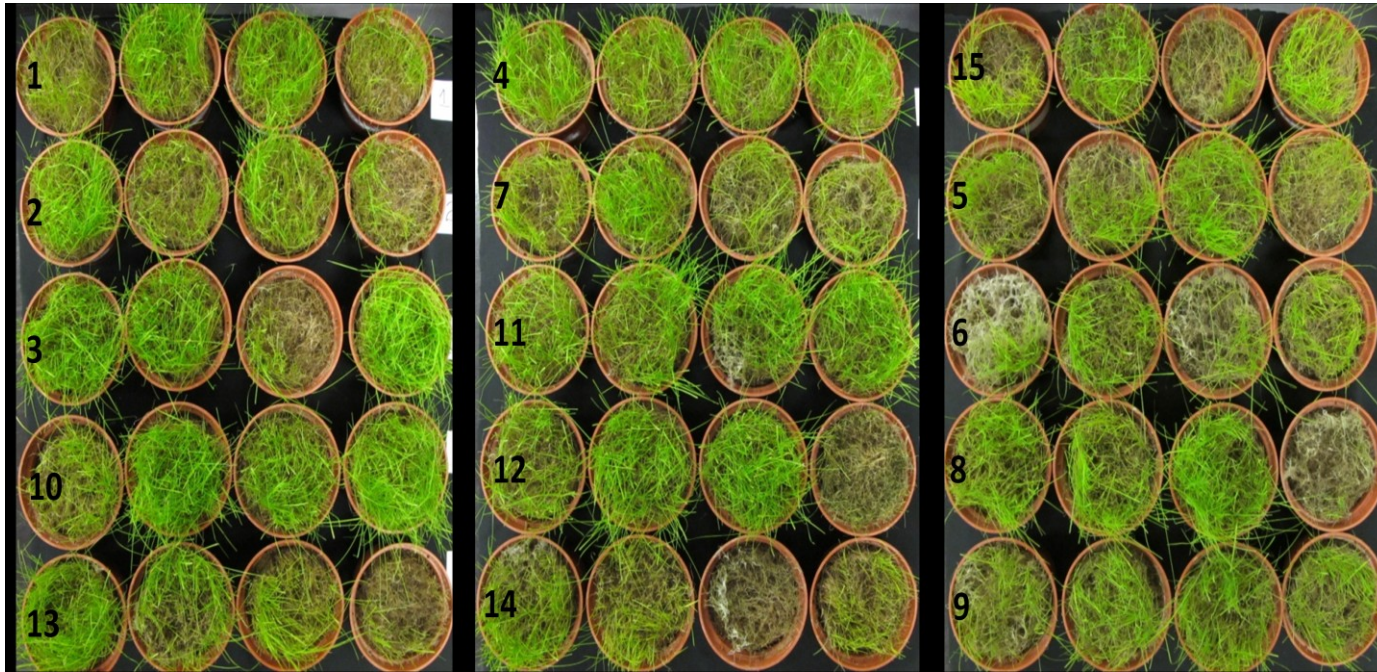
Fig 3.7 Performance of creeping bentgrass 10 days after dollar spot inoculation, the numbers in the pictures indicate their corresponding treatments as listed in Table 3.2. Horizontally arranged pots are four replicates within treatments.

Table 3.9 Disease ratings- Creeping bentgrass – 10 days after dollar spot inoculation- ANOVA TABLE

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatments	14	104	0.66	0.8107
Repetition	1	104	12.71	0.0006
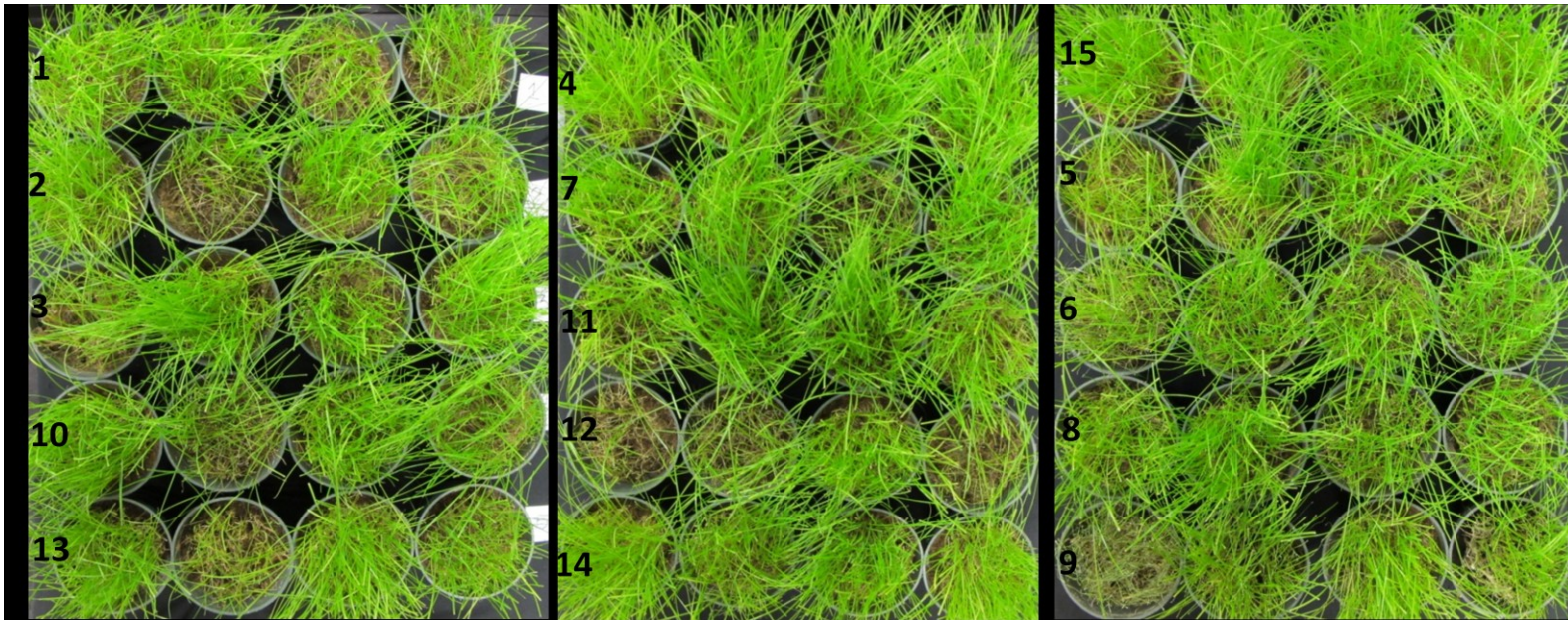
Fig 3.8 Performance of perennial ryegrass 10 days after dollar spot inoculation, the numbers in the pictures indicate their corresponding treatments as listed in table 3.2. Horizontally arranged pots are four replicates within treatments.

Table 3.10 Disease ratings- Perennial ryegrass – 10 days after dollar spot infection- ANOVA TABLE

Type 3 Tests of Fixed Effects				
Effect	Num DF	Den DF	F Value	Pr > F
Treatments	14	104	0.84	0.6242
Repetition	1	104	29.23	<.0001

There was no significant difference in the disease ratings for creeping bentgrass and perennial ryegrass (Table 3.2) among the treatments. Creeping bentgrass and perennial ryegrass pots with 10 days of dollar spot inoculation were photographed, and displayed in fig 3.7 and 3.8. Creeping bentgrass had more disease symptoms and turf quality was below the acceptable quality (rate 6.0). This may due to the high disease pressure, susceptible nature of the grass and the continuous high humid conditions maintained day and night for 10 days. The average disease severity rate across the treatment for creeping bentgrass was 5.2. Perennial ryegrass also displayed disease symptoms under infection but as it is moderately resistant, disease severity ratings were acceptable across the treatments. The average disease severity rate across the treatment for perennial ryegrass was 6.6 (rate '6' was acceptable).

3.4 Discussion

The treatment formulations used for this greenhouse study has shown alterations in pH of highly alkaline products. Boulter *et al*. (2000) stated that compost extracts are rich source of nutrients, microbial population and metabolites. Therefore, reduction in pH when alkaline: acidic combination (i.e. KASIL and CMCT) may be due to the synergy of organic acids, biologically active compounds and metabolites present in the CMCT with the potassium and silicate ions. Organic components and phytohormones are rich in seaweed extracts (Zhang and Erwin, 2008). The slight reduction in pH when SWE and KASIL are combined is due to the interaction of potassium and silicate ions with ions present in SWE. Lower pH is beneficial to plant growth and in addition this type of fortification can serve as means to reduce application of highly alkaline products. When combined these treatments are novel fortified products that have potential in plant health enrichment. Synergy of these product combinations may also translate to enhance plant protection against pathogens. These products can also be applied to other agricultural fields like hydroponics to replace toxic supplements.

From creeping bentgrass tissue Si content results, combining KASIL with CMCT will significantly increase Si content in the leaf tissue. This effect was minimally consistent but not significantly different than other treatments at 15 days after treatments with and without infection. The Si content of CMCT used was not measured in this experiment but it might have contributed to the tissue Si content or enabled increased Si absorption. Tissue Si content of perennial ryegrass had increased when KASIL was combined with CMCT. Similar but minimal impact of treatments can be seen 15 days after treatments with and without infection. In order to better understand the contribution of tissue silicon content, Si content of CMCT should be

measured – i.e. to explain the high levels of Si in treatments without KASIL, but with CMCT. To extend the impact of increased tissue Si content, treatments should be applied more frequently.

Cooke and Leishman (2011) stated that the role of plant Si in the dry matter is important for agricultural and plants living in natural ecosystems. Si is metabolically cheaper alternative than carbon in short lived leaves. Si is potentially important to function as a structural, stress alleviation and defense enhancing roles by substituting for carbon that could be allocated for growth and reproduction. Si has been studied with various plant pathogenic fungi for the reduction of fungal diseases (Fautex *et al*. 2005). The earliest proposed defense phenomenon was the "physical barrier" deposition of amorphous Si in the leaf apoplast to prevent entry of pathogenic fungi. This indicates the importance of tissue Si content in the leaf tissue. Inorganic salts have been increasingly used in plant protection in recent years, they are considered environmentally friendly when compared with fungicides. Silicon dioxide can be found in food consumed by humans. In recent years several other pathways have also been explored to test the ability of Si to help plants under various stresses. Studies conducted to estimate the tissue Si content in leaf tissue to correlate with disease resistance in plants are reported in the literature. Silicon content in the leaf tissue enhances the components of resistance in St. Augustinegrass when infected with grey leaf spot *M. grisea* (Brecht *et al*. 2007). Increasing tissue Si content in straw significantly reduced the severity of sheath blight (*Rhizoctinia solani*) in rice cultivars (Rodrigues *et al*. 2001). Fertilizing with calcium silicate reduced the severity of blight caused by *M.grisea* (Seebold *et al*. 2001). Calcium silicate application increased tissue Si content in the leaves and was effective in reducing downy mildew incidence in soya bean (Nolla *et al*. 2006). Root applications of potassium silicate KASIL resulted in consistent deposition of Si in the leaves of wheat plants; it also consistently suppressed powdery mildew in wheat plants (Guevel

et al. 2007). Si deposition in the leaf epidermis confers resistance against appresorial penetration by rice blast fungus *Magnaporthe oryzae* (Hayasaka *et al*. 2008). Tissue Si content increased with increased Si amendment in soil and decreased the disease incidence of grey leaf spot in perennial ryegrass turf (Nanayakkara *et al*. 2008). Potassium silicate application reduced the disease severity and tissue Si content increased with application in leaves of rose when infected with powdery mildew caused by *Podosphaera pannosa* (Shetty *et al*. 2011).

Since the Si content in leaves is an important component of plant disease protection, it is also important to create products that will increase Si accumulation. Si is the second most abundant element in the earth crust, but the inability to uptake by plants may be due to the unavailable form of Si in the rhizosphere or silicon sources resistant to weathering. Although the mechanism of accumulation was not studied here, the treatments used in my research indicates that KASIL and CMCT synergistically work together to increase Si accumulation in plants. However the treatments did not have a significant effect on the disease severity of dollar spot on both creeping bentgrass and perennial ryegrass. The deviations in replicates maybe are due to high disease pressure and high humid conditions or presence of insects like fungus gnats interfering with uniform disease establishment.

In conclusion, pH of the treatments is altered when treatment combinations are formulated in the method used in this experiment. A soil pH around neutral or below is beneficial to plants and microorganisms in the rhizosphere. It is also a means to prevent alkalinity of the soil by applying highly alkaline sources. Combining KASIL with CMCT (1:10 or 1:5) or SWE (0.1%) increases tissue Si content in creeping bentgrass. The increase is minimally consistent even 15 days after treatments and with infection under green house conditions. In perennial ryegrass combining KASIL with 1:5 CMCT or SWE (0.1 and 0.3%)

positively affects the tissue Si concentration. The increased effects were not consistent at 15 days after treatments with and without infection. Perennial ryegrass is a fast-growing grass therefore with time the treatment effects are not clearly seen. The effectiveness may be increased by frequent application for the effects to extend through the growing season. However this study did not effectively control the dollar spot disease severity, as such further research is required in order to recommend the use of these product combinations in the field conditions. For other benefits such as an eco-friendly means to increase physiological health by increasing tissue Si content these fortified treatments may be incorporated in turfgrass management programs.

CHAPTER 4

Potassium Silicate with Compost Tea or Seaweed Extracts in Creeping Bentgrass on Induced Defense Responses for Dollar Spot Management

4.1 Introduction

Products with multiple disease control mechanisms are increasingly important in regards to dollar spot disease (*Sclerotinia homoeocarpa* F. T. Bennet) management, especially given that the disease developed resistance to fungicides in a relatively short period of time. Plants have evolved complex defense mechanism to resist attack by fungal pathogens that are mostly activated once the pathogen enters the plant (Lee *et al*. 2003). Organic chemicals and non fungicidal compounds can be applied on plants to stimulate similar defense responses. Such products can also be referred as 'biostimulants' meaning "products that contain one or more of a broad range of ingredients including nutrients, organic acids, hormones, vitamins, microbial inoculants, plant extracts etc" (Lee *et al*. 2003). As environmental stewards, use of biostimulants should be recommended when possible to reduce reliance on fungicides. Alternative tools used to manage dollar spot disease include composts and biocontrol agents (Powell *et al*. 2000). Disease management by biocontrol agents in *in vitro* and field studies is often attributed by production of diffusible metabolites that activate defense response in plants. For example, volatile organic compounds (VOC's) secreted by some plant growth promoting rhizobacteria (PGPR) are found to induce resistance in creeping bentgrass (Cortes-Barco *et al*. 2010).

Silicon is a bioactive element that induces both mechanical and physiological properties while alleviating biotic and abiotic stresses in plants (Fautex *et al.* 2005). The role of silicon in regulating plant defense mechanisms is complex. Apart from mechanical barrier mechanism, it can modulate and influence the timing and extent of plant defense responses at the biochemical

level. Fautex *et al.* (2005) states that silicon can bind to proteins involved in signal transduction or interfere with cationic cofactors of enzymes that influence pathogenesis related events. Silicon may induce natural defense mechanisms by accumulation phytoalexins in various monocots and dicots plants. Silicon treated rice plants had higher levels of momilactone phytoalexins in leaf extracts reduced disease blast disease severity (Rodrigues *et al.* 2004). This indicates that the role of silicon is not strictly as a physical barrier. In the *Arabidopsis thaliana* – powdery mildew pathosystem, silicon treatment upregulated many of the defense related genes but did not modify gene expression with no infection (Fautex *et al.* 2006). Therefore the role of silicon is induced defence response and its beneficial properties can be used to manage pathogen related stress. Silicon treatment in the cucumber-downy mildew investigation led to reduction of disease index by 60% compared to the control by activation of major defense-related enzymes (Yu *et al.* 2010).

Composts water extracts or compost tea can play an important role in the plant disease resistance. Compost tea has been used for decades to reduce severity of several foliar diseases; it contains biochemical agents as well as unidentified chemical factors that are beneficial to plants (Zhang *et al.* 1998). Rhizobacteria present in compost can potentially induce systemic resistance and activate pathogenesis-related (PR) proteins. Compost tea made form agro-waste suppressed *Choanephora cucurbitarum*, a casual agent of wet rot in okra by inducing resistance-related enzymes (Siddiqui *et al.* 2009). Water extracts of compost or compost tea from commercial composts significantly reduced the disease incidence and severity in pepper against *Phytophthora capsici* (Sang *et al.* 2010). Compost water extracts enhanced the expression of pathogenesis-related genes that corresponds to the induced systemic resistance (ISR). Seaweed extracts from marine seaweeds are used as nutrient supplements and biostimulants to increase plant growth and yield. Although the exact mechanisms of disease control by seaweed extracts

are still being explored, seaweed extracts are reported to affect the physiology of plants by influencing rhizosphere microbial community (Khan *et al*. 2009). Algal extract treated alfalfa increased resistance to *Colletotrichum* by the upregulation of plant defense genes that are involved in phytoalexins, pathogenesis-related (PR) proteins, and cell wall protein pathways (Cluzet *et al*. 2004).

Since these products when individually used are potential in inducing defence response, their combined used may be an exciting strategy to enhance disease resistance against dollar spot disease. Therefore, the main objective of this research was to evaluate the differences in biochemical properties such as chlorophyll content, total phenolics, defense related enzymes when potassium silicate (soluble Si source) was applied alone or as combined products with cow manure compost tea CMCT or SWE and study its effect on of dollar spot management. The experiments in this chapter are 1) Evaluate the effect of treatments on chlorophyll content, phenolics and defense-related enzyme activity at three different time points. 2) Comparison of the effect of treatments on the lesion area on creeping bentgrass tissue. 3) Mycelia inhibition study on treatment- amended potato dextrose agar (PDA) plates.

4.2 Materials and Methods

4.2.1 Experiment 1 – Biochemical Analyses

This experiment was conducted in growth chamber room located in Department of Environmental Sciences, Faculty of Agriculture - Dalhousie University. Transparent MK5 Caisson boxes (Caisson Labs, North Logan, UT, USA) were filled with approximately 100 ml (v/v) wetted potting mixture, consisting USGA (United States Golf Association) Certified sand (Shaw Resources, Nova Scotia, Canada) and peat (Professional PRO- MIX 'BX' MYCORISE ® PRO – Premier Promix, Quebec, Canada) in 4:1 ratio. The mixture was supplemented with turf

starter fertilizer 16-32-6 (N-P-K) (Nu-gro Golf, Brantford, ON. Canada). The boxes were covered with transparent lids and autoclaved twice (3 days interval) for 15 min at 121°C and 15 psi. Lids were opened in a laminar flow chamber to prevent contamination and seeds of creeping bentgrass (*Agrostis stolonifera* L.) were manually sprinkled at the rate of 0.2 g per pot. After seeding, the boxes were randomized and placed under conditions of 12h light and dark cycle with light intensity of 100 μmol m^{-2} s^{-1} for two weeks. The humidity was maintained above 95% at all times and opened and closed immediately during treatment applications only.

4.2.2 Experimental Design and Treatments

The experiment was a completely randomized design with six treatments and each treatment had three replicates therefore 18 experimental units. Treatments were applied at the end of two weeks. Treatments were formulated by combining two concentrations of silicon source KASIL® 6, 26.5% SiO$_2$ National Silicates, Toronto Canada, (0, 100 ppm) and three additives (No additive, 1:10 compost tea (described in 3.2.4), 0.1%SWE {SWE Acadian® (powdered alkaline extracts of *Ascophyllum nodosum*; Acadian Seaplants Limited, Dartmouth, Nova Scotia, Canada)}). The whole experiment had 3 time points for destructive sampling therefore had 54 experimental units. This experiment was repeated twice.

Table 4.0 List of Treatments used in the experiment

Sl. #	Treatment
1	Control/ dis. water
2	0.1%SWE
3	1:10 compost tea
4	100 PPM KASIL
5	0.1% SWE+ 100 PPM KASIL
6	1:10 compost tea+ 100 PPM KASIL

Treatments were filtered, 20 mL/pot was applied as soil drench using sterile syringes. Treatments were applied once and samples were collected at three different time points after treatments were applied. First sampling was collected three days after treatments with no inoculation (represented as time point A), rest of the samples were inoculated with dollar spot powder (described in 3.2.5) when 'A' was sampled. After three days of inoculation, the second sets of samples were collected (represented as time point B). The sampling time point (represented as C) was inoculated with dollar spot powder for six days. All samples were collected by removing the shoot tissue. Fresh tissue samples collected were weighed and used for chlorophyll estimation, phenolic content and enzyme analysis. This experiment was repeated twice.

4.2.3 Estimation of Chlorophyll

Chlorophyll was extracted using the method described in Fu *et al.* (2009) with some modifications. Chlorophyll was extracted by soaking about 0.05 g of fresh tissue and immersed in 5 mL dimethyl sulfoxide for 72 hours in the dark. Absorbance was measured at 663 nm

(OD_{663}) and 645 nm (OD_{645}) against blank dimethyl sulfoxide using a BioTek Power XS2 microplate reader (VT, USA) with Gen5™ software. Chlorophyll a and b was quantified using the equations

Chlorophyll a = ($12.25 \times OD_{663} - 2.79 \times OD_{645}$)/ fresh weight

Chlorophyll b = ($21.50 \times OD_{645} - 5.10 \times OD_{663}$)/ fresh weight

4.2.4 Estimation of Total Phenolics

The total soluble phenolics were determined by an assay described by Sarkar *et al*. 2010 with some modifications. Fresh creeping bentgrass tissues 0.05 g was immersed in 2.5 mL of 95% ethanol and was incubated in the freezer for 48 h. The samples were homogenized and centrifuged at 12,000 X g for 10 mins after freezing. Then 0.25 mL of sample supernatant and 0.25 ml of distilled water was mixed and transferred into a test tube and 0.5 mL of 95% ethanol and 2.5 mL of distilled water was added. In each sample, 0.25 mL of 50% (v/v) Folin-Ciocalteu reagent was added and mixed. After 5 min, 0.5 mL of 5% Na_2CO_3 was added to the reaction mixture and allowed to stand for 60 min. A blank was prepared with 0.25 mL distilled water instead of sample. After 1 h absorbance was read at 725 nm against blank using a BioTek Power XS2 microplate reader (VT, USA) with Gen5™ software. The absorbance values were converted to total phenolics and were expressed in grams equivalents of gallic acid per gram fresh weight of the sample. Standard curves were established using various concentrations of gallic acid in 95% ethanol.

4.2.5 Enzyme Analysis – Sampling and Storage

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

4.2.6 Extraction of Crude Enzyme

The enzyme analysis was conducted by following the protocol used in Kelloway 2012. 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.

4.2.7 Determination of Total Protein

Protein concentration was measured using the Coomassie Plus – The Better Bradford[TM] 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.

4.2.8 Estimation of Phenylalanine Ammonia Lyase Activity

Enzyme extraction was conducted following the protocol described in section 4.2.6. 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). PAL activity was defined as nMol of cinnamic acid h^{-1} mg^{-1} protein.

4.2.9 Estimation of Polyphenol Oxidase Activity

Enzyme extraction was conducted following the protocol described in section 4.2.6. The polyphenol oxidase (PPO) assay was based on methods described by Wang *et al.* (2011), 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. PPO activity was defined as ΔOD_{398} $min^{-1}mg^{-1}$ protein.

4.2.10 Estimation of Catalase Activity

 Enzyme extraction was conducted following the protocol described in section 4.2.6. 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. The change in absorbance ΔOD_{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:

$$\text{Units/mg protein} \ = \ \frac{(\Delta OD240/min) \ X \ 1000}{43.6 \ X \ mg \ protein \ / \ ml \ of \ reaction \ mixture}$$

4.2.11 Estimation of Peroxidase Activity

Enzyme extraction was conducted following the protocol described in section 4.2.6. 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. The change in absorbance ΔOD_{470} /min from the initial linear portion of the curve was calculated. POD activity was defined as $\Delta OD_{470} \ min^{-1} \ mg^{-1}$ protein.

4.2.12 Experiment 2 – Seedling Lesion Experiment

This experiment was conducted to determine the effect of treatments in inhibiting the dollar spot lesion formation on treated leaves. Plant material for experiment 2 was obtained by transplanting two week old seedlings (described in 4.2.1) into vermiculite from experiment 1 before treatment application. A tuft of grass seedlings with approximately 6-10 leaf blades were transplanted carefully using a forceps into 12×60 mm glass test tubes filled with three-quarter's moist vermiculite (Perlite Canada Inc., Montreal, Canada). Care was taken not to damage the roots while transferred; roots were placed close to the surface of the vermiculite. The transplanted grass was grown in tubes for twenty days with weekly fertilizer application 5 mL per tube of 1g L^{-1} fertilizer described in 3.2.1. On 20th day, when the grass blades attained desired blade width (between 0.3-0.5 mm) they were applied with 5 ml of treatments. The treatments used were same as described for experiment 1 in 4.2.2. The experiment was done once by detaching treated leaf blades from corresponding treatments.

4.2.13 Inoculation for Lesion Experiment

Whatman filter paper was placed in 9 cm Petri plates and wetted using 2 mL of sterile water. Creeping bentgrass tissues previously exposed to treatments for one week was sampled by detaching and was placed on the moist filter paper. The grass was inoculated with dollar spot mycelial plug (3mm diameter). The mycelia-covered plug was placed in contact with the cut-end of the grass blades; the plates were stored at 25°C for 5 days. The yellowing and discoloration of the leaves was photographed and scanned at the end of 5 days.

4.2.14 Imaging and Measurement of Lesion Area

After incubating for one week the inoculated and non inoculated leaves were scanned and lesion area was measured. The lesion area was calculated from the scanned .TIF files of the treatments by subtracting the green or healthy area from the area of the whole leaves using the ImageJ software (free software available online). Fresh inoculated and non inoculated leaves were photographed under UV light in the gel-doc to observe the accumulation of phenolic-like compounds at the zone of infection. Each treatment had 18 replicates.

4.2.15 Experiment 3 – Mycelial Inhibition Study

Potato dextrose broth (PDB) [HIMEDIA Lab, India, made for VWR) was mixed with Agar (Bioshop Canada Inc., Burlington ON) in the recommended dosage for full strength potato dextrose agar (PDA) media. Before autoclaving the media, the water added in PDA was amended with 10% of all the six treatments (described in 4.2.2). The media was autoclaved for 15 min at 121°C and 15 psi. The autoclaved amended and non-amended (served as control) treatments were poured (approximately 20 mL) into 9 cm Petri plates, cooled, sealed with parafilm and left in room temperature for 48 h to monitor any external contamination. The plates amended with treatments and control was infected using a 5-day old subculture of the dollar spot

fungus. A 0.5 mm diameter of actively growing dollar spot mycelia covered plug was placed in the middle of the plates. The plates were sealed using parafilm and incubated at 25 °C in dark until the mycelia reached the periphery of the plates. The mycelial spread was measured every 12 hours on the diameters marking perpendicular axes of the plate using vernier calipers. This experiment was repeated twice.

3.2.6 Data Analysis

As the method of sampling was destructive in nature, data from each sampling time points in experiment 1 for chlorophyll content, total phenolics, polyphenoloxidase, phenylalanine ammonia lyase, catalase, and peroxidase were analysed separately. All the data in this chapter was analysed as a completely randomised design because, the comparison was to estimate the individual effect vs. the collective or combined effect of the treatments rather than the interaction of the product combinations. The treatments used in this chapter were formulated before the treatment applications. The two repetitions of the experiments were arranged as random variable. Normality and constant variance was verified in MINITAB statistical software. Data from all the experiments in this chapter were analysed using PROC MIXED procedure of SAS statistical software (SAS Institute, Cary, NC), and significance was determined at $P \leq 0.05$. Least square means were used for the pair wise comparison of treatments and letter groupings of LS means was generated using a macro "pdmix800.sas" (Saxton, 1998).

4.4. Results

4.4.1 Estimation of Chlorophyll

Table 4.1 Effect of treatments on the chlorophyll content (means across replicates ± SE) of creeping bentgrass tissues at different sampling points

Treatments	No infection	3 Days after infection	6 Days after infection
Chlorophyll A (µg g^{-1} fresh weight)			
1. Control	261.00±13.7 A	189.70±27.40 B	199.10±14.70 A
2. 0.1% SWE	257.40±12.6 A	244.85±8.37 A	223.60±13.00 A
3. 1:10 CMCT	284.50±17.3 A	231.12±9.41 A	233.00±18.40 A
4. 100 ppm KASIL	281.10±13.9 A	260.84±6.58 A	232.50±14.40 A
5. 100 ppm KASIL+0.1% SWE	266.30±13 A	228.10±13.80 AB	230.36±8.03 A
6. 100 ppm KASIL+1:10 CMCT	260.10±18.1 A	258.75±7.85 A	224.90±20.10 A
P- value	0.699	0.014	0.628
Chlorophyll B (µg g^{-1} fresh weight)			
1. Control	54.06±4.34 A	44.23±6.40 B	48.64±3.81 A
2. 0.1% SWE	57.57±4.70 A	56.71±1.80 A	53.74±3.05 A
3. 1:10 CMCT	58.96±1.37 A	55.37±3.69 AB	51.82±3.70 A
4. 100 ppm KASIL	58.24±4.06 A	64.70±3.04 A	53.94±2.48 A
5. 100 ppm KASIL+0.1% SWE	61.81±8.56 A	57.46±5.70 A	52.99±3.04 A
6. 100 ppm KASIL+1:10 CMCT	51.65±4.04 A	62.88±3.04 A	49.09±4.30 A
P- value	0.749	0.031	0.807

- Values followed by the same letter are not significantly different at 5% level

The chlorophyll content was not affected by the treatments where no infection occurred (Table 4.1). Significant difference in treatments was seen three days after treatments in chlorophyll content when compared across the treatments. Three days after infection chlorophyll levels were lower in all the treatments except #4 (100 ppm KASIL). A similar, though not significant, trend was obtained six days after infection. From the results it is clear that the treatments have minimal impact on chlorophyll content of creeping bentgrass leaves.
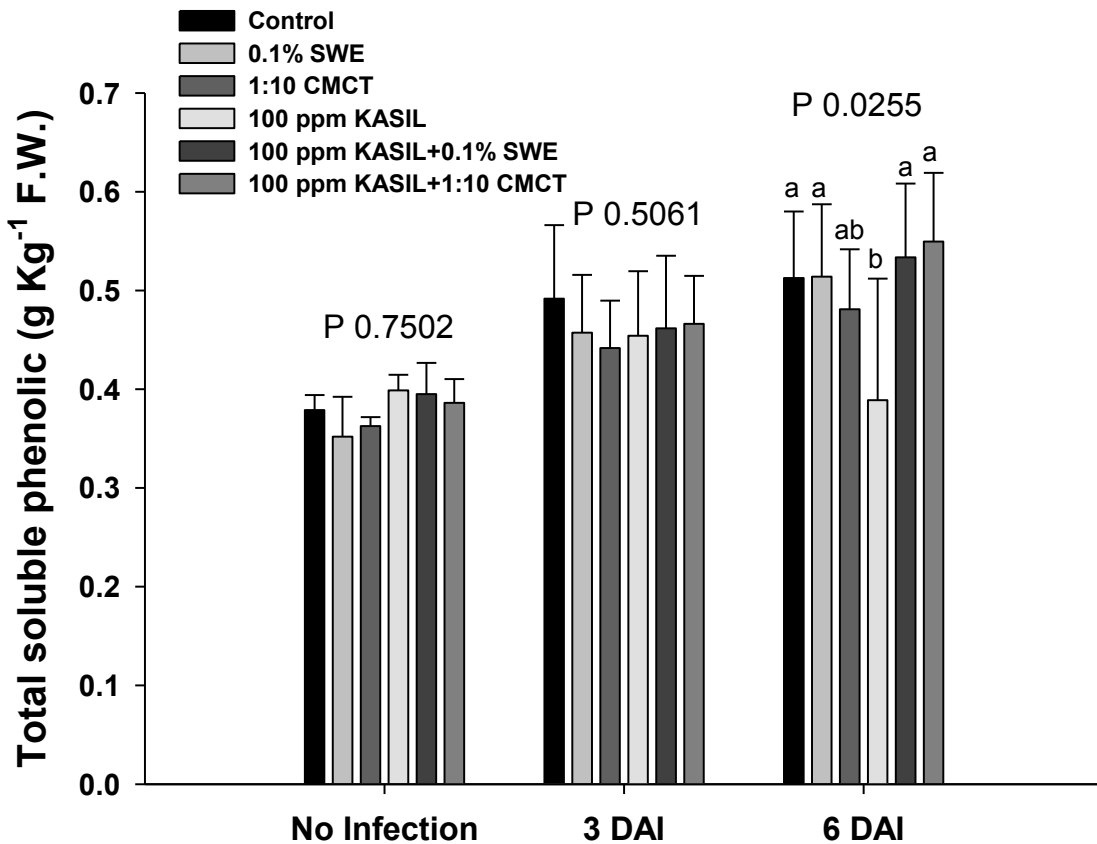
4.4. 2 Estimation of Total Phenolics

Fig 4.1 Total phenolic content of creeping bentgrass sampled at different time points with and without infection of dollar spot. RH ≥ 95% when leaves were sampled. Bars indicate mean ± standard error.

There was no significant difference in the total phenolic content of creeping bentgrass tissues between treatments and control before infection and 3 days after infection (Fig 4.1). Significance was obtained at time point six Days after infection (Fig 4.1). At six days after infection highest phenolic content occurred with 100 ppm KASIL + 1:10 CMCT, but this difference was not significant than that observed with the control and other treatments. The average total phenolic content was roughly between 0.35 to 0.6 g^{-1} kg fresh weight.
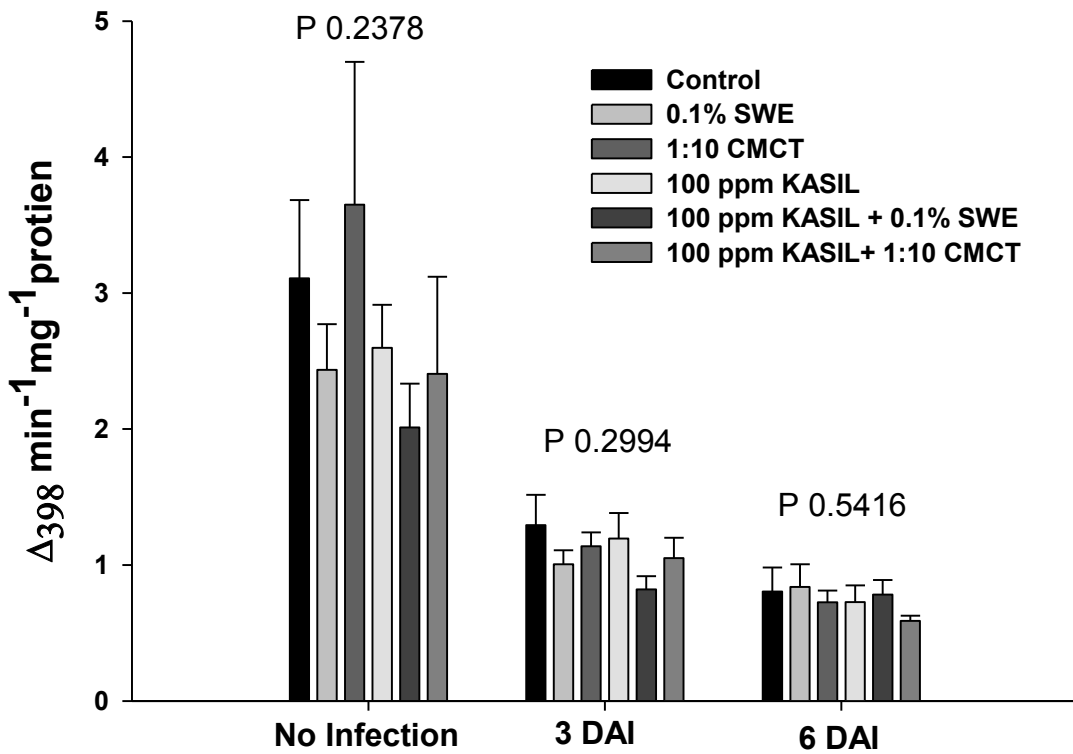
4.4.3 Polyphenol Oxidase Activity

Fig 4.2 Polyphenol oxidase activity of creeping bentgrass sampled at different time points with and without infection of dollar spot. RH $\geq$ 95% when leaves were sampled. Bars indicate mean $\pm$ standard error.

There was no significant difference in the polyphenol oxidase activity of creeping bentgrass between the treatments and control (Fig 4.2) within sampling periods. Little change in polyphenol oxidase activity was observed with respect to duration of treatments and with or without infection but each individual time point was not statistically different (Fig 4.2.) It is important to note that although the comparisons of different sampling points were not tested, there is a clear decline in the level of polyphenol oxidases with increasing time post-treatments.
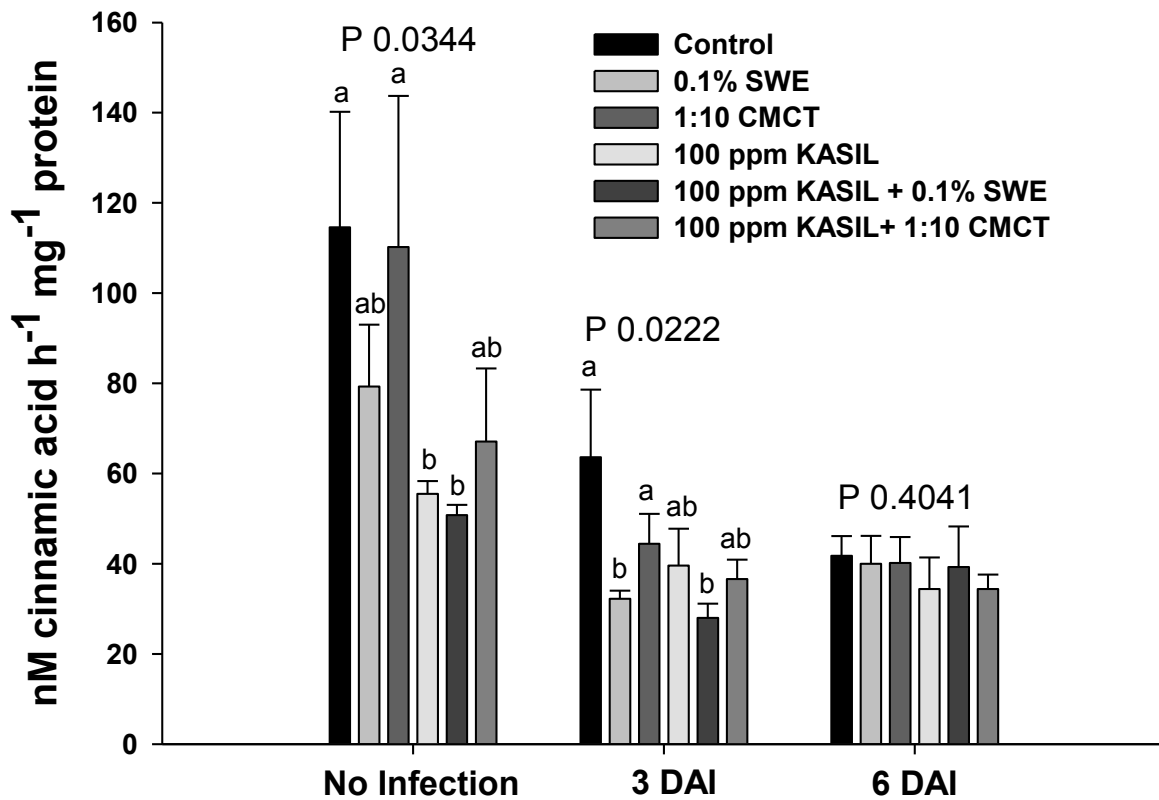
4.4.4 Phenylalanine Ammonia Lyase Activity

Fig 4.3 Phenylalanine ammonia lyase activity of creeping bentgrass sampled at different time points with and without infection of dollar spot. RH ≥ 95% when leaves were sampled. Bars indicate mean ± standard error.

There was significant difference in the phenylalanine ammonia lyase activity of creeping bentgrass before infection and 3 days after infection but no significant at 6 days after infection (Fig 4.3). Before infection the highest phenylalanine ammonia lyase activity was in control but not significantly different from the treatments # 2, 3 and 6 (Fig 4.3). Three days after infection, the highest phenylalanine ammonia lyase activity was control but not significantly different from the treatments # 3, 4 and 6 (Fig 4.3). It is important to note that although the comparisons of different sampling points were not tested, a decline in the trend appears in the level of phenylalanine ammonia lyase with increasing time post-treatments.
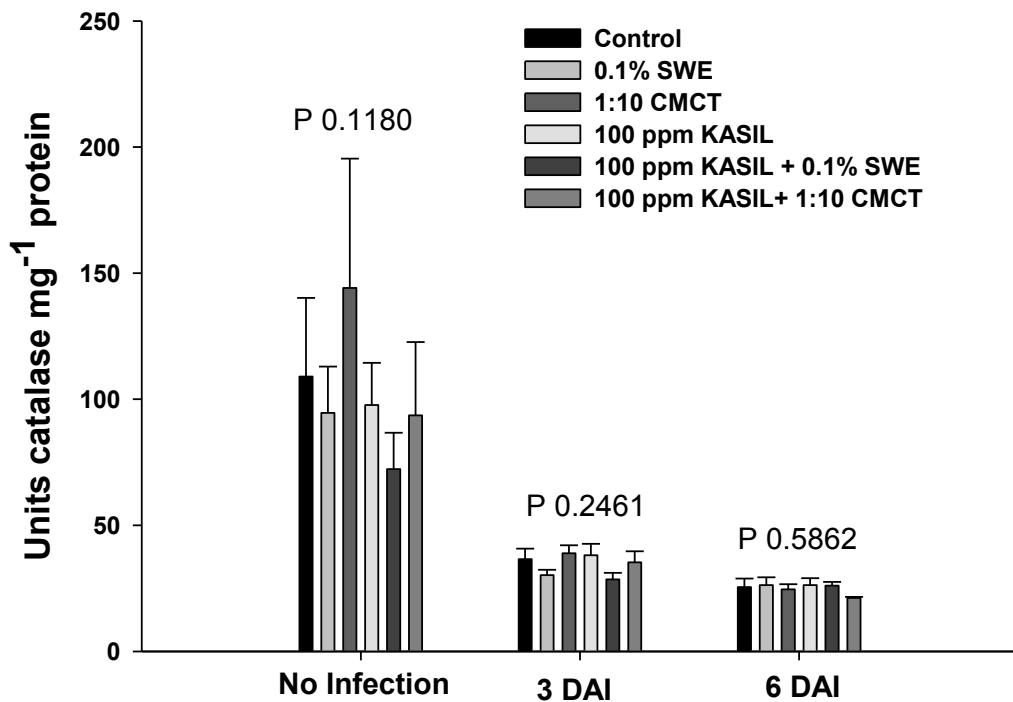
4.4.5 Catalyse Activity

Fig 4.4 Catalase activity of creeping bentgrass sampled at different time points with and without infection of dollar spot. RH $\geq$ 95% when leaves were sampled. Bars indicate mean $\pm$ standard error.

There was no significant difference in the catalyse activity of creeping bentgrass tissues between the treatments and control (Fig 4.4). Although not significant it appears like there is a decline in the catalyse activity with respect to sampling time points post-treatments (Fig 4.4).
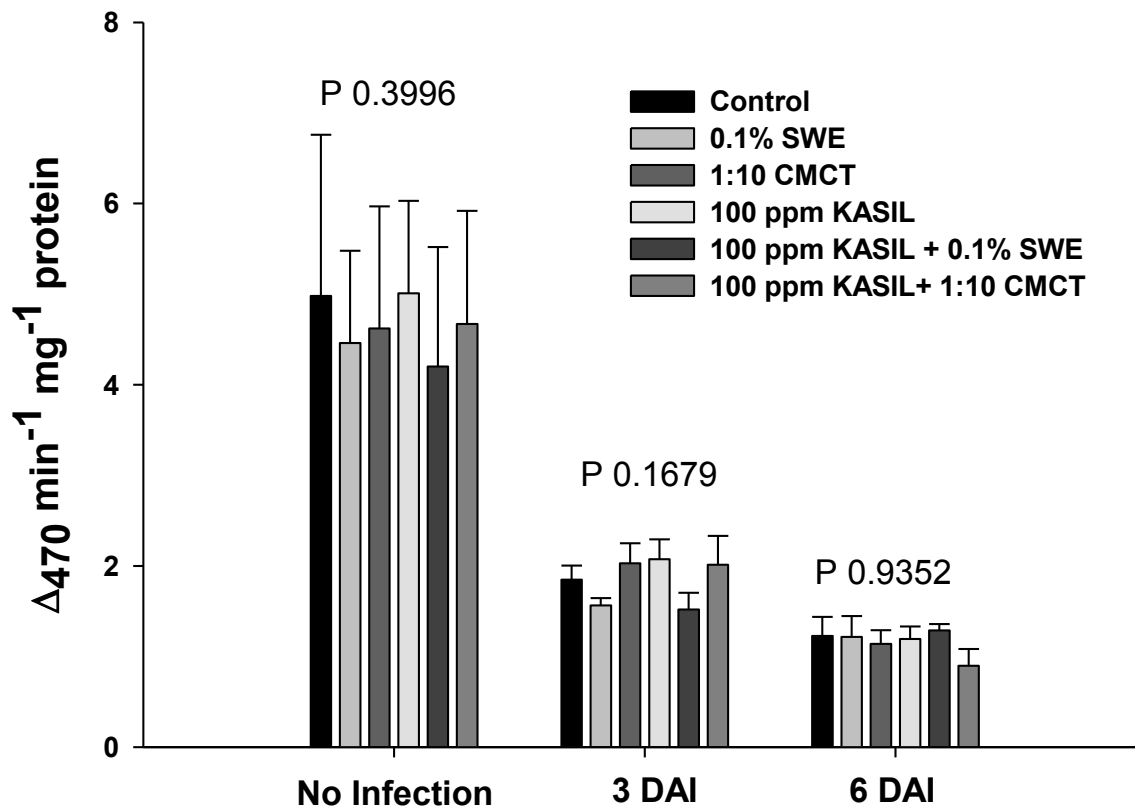
4.4.6 Peroxidase Activity

Fig 4.5 Peroxidase activity of creeping bentgrass sampled at different time points with and without infection of dollar spot. RH $\geq$ 95% when leaves were sampled. Bars indicate mean $\pm$ standard error.

There was no significant difference in the peroxidase activity of creeping bentgrass tissues between the treatments and control (Fig 4.5). Although not significant it appears like there is a decline in the peroxidase activity with respect to sampling time points post-treatments (Fig 4.5).
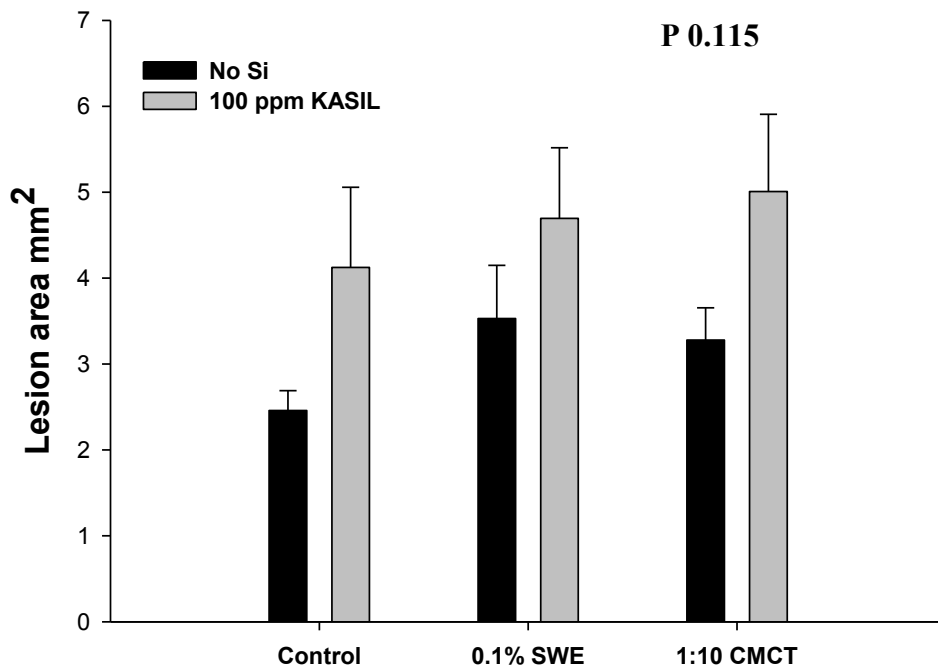
4.4.7 Experiment 2 - Lesion Area

Fig 4.6 Lesion area of one week treatment-exposed leaf tissues of creeping bentgrass measured at seven days after dollar spot inoculation. Bars indicate mean across replicates ± standard error.
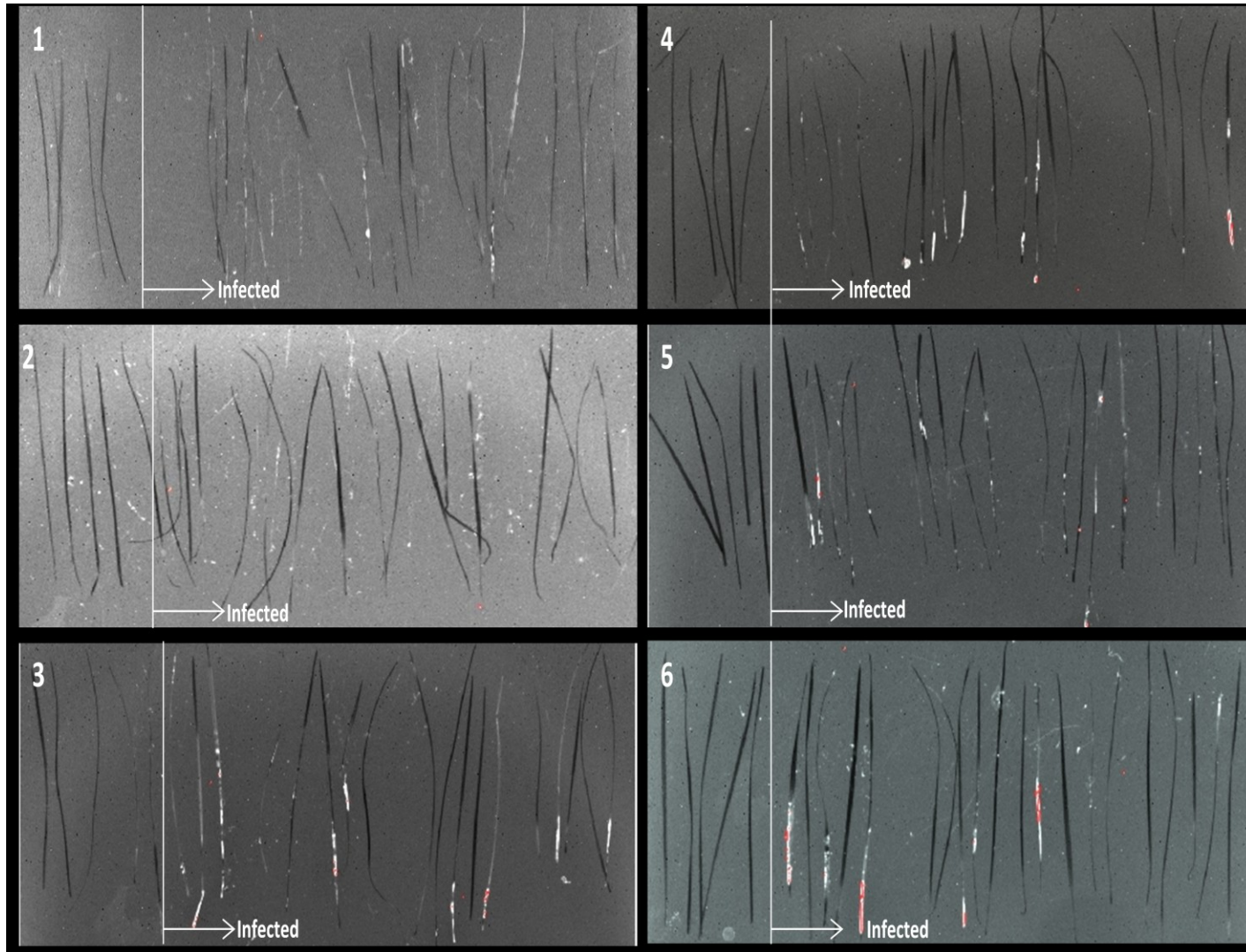
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Fig 4.7 Images of infected and non-infected creeping bentgrass under UV- light. The numbers on the left-top-corner represent corresponding treatments. The fluorescent parts of the leaves are phenolics-like accumulation at the zone of infection.

Infected area under UV-light shows bright accumulation of phenolics (Fig 4.7), but there was no significant difference in the lesion area on the creeping bentgrass leaves between the treatments and control Fig (4.6). The overall smaller width of the leaf balde may have influenced on the decrease in leaf area in the control with no Si. The area of the bleached tissue was not exactly represented by the data because the software was not able to capture the total bleached area.
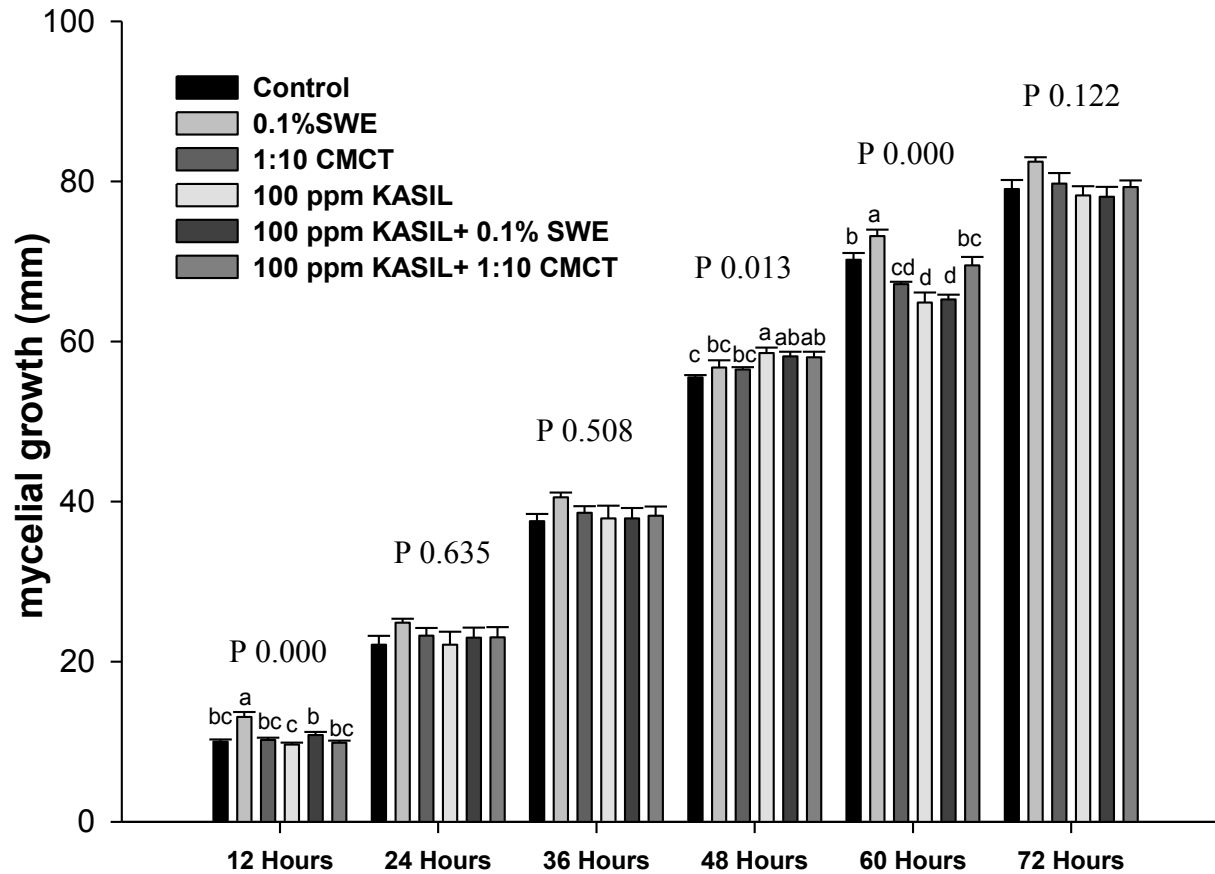
4.4.8 Experiment 3 – Mycelial Inhibition Study

Fig 4.8 Effect of treatments on the dollar spot mycelial growth on potato dextrose agar media mixed with 10% of the treatments. Mycelial growth was measured at different time points. Bars indicate mean ± standard error.

There was significant difference in the mycelial growth between the treatments and control at 12, 48 and 60 hours of incubation (Fig 4.8). Little change in mycelial growth was observed with 24, 36 and 72 hours but the differences were not significant (Fig 4.8). The lowest mean at each time points represents the effect of treatments to reduce the dollar spot growth on PDA. At 12 h, the lowest mean was treatment #4, 100 ppm KASIL, but statistically not different from treatment #1,3 and 6 (Fig 4.8). At 48 h, the lowest mean was treatment #1, control, but statistically not different from treatment # 2 and 3 (Fig 4.8). At 60 h, the lowest mean was treatment #4 and 5 (100 ppm KASIL and 100 ppm KASIL + 0.1% SWE), but statistically not different from treatment # 3 (Fig 4.8). Treatments had minimal impact when compared to the control but not significant.

4.5 Discussion

   Silicon mediated protection against plant pathogens are not only due to physical barrier mechanism. Si absorption into plant tissues provided protection to cucumber plants against *Pythium* root rot even without Si accumulation in the root tissue (Remus-Borel *et al*. 2005). Recent evidence suggests silicon can induce defense responses that are similar to systemic acquired resistance in function (Cai *et al* 2009). Si-treated plants can significantly alter the antioxidant enzyme activities against pathogenic fungi by producing antifungal compounds such as phenolics, phytoalexins and pathogenesis-related proteins. Therefore, experiment 1 was conducted in the growth chamber to study the effect of treatments on the chlorophyll content, phenolic content and enzyme activity in the creeping bentgrass tissues. Minimal but not significant effect of treatments was observed at 3 days after infection in the chlorophyll content. Dollar spot severity was not studied in the experiment but Si treatments in the literature has shown increased chlorophyll content and reduced dollar spot incidence in creeping bentgrass (Schmidt *et al*. 1999).

   Numerous reports on silicon supplements have reported potential activation of defense reaction in plants. Silicon treatments increased resistance in wheat to powdery mildew by accumulation of phenol-like deposits at the zone of infection (Remus-Borel *et al*. 2005). The reduced levels of blast disease severity in Si treated rice plants were due to the phenol-like compounds and phytoalexins (Rodrigues *et al*. 2004). The treatments used in this study have shown minimal impact on phenolic content 6 days after infection but not significant than control. The lesion experiments (experiment 2) demonstrated increased fluorescence or accumulation of phenolic- like compounds in the infected /lesion zones when compared with control (Fig. 4.7). These results are in accordance with fluorescence detected under blue light (488 nm) in the Si

treated plants at the zone of infection when wheat leaves were infected with powdery mildew (Remus- Boral *et al*. 2005).

Silicon plays a major role in the induction of defense mechanisms in response to pathogen attacks. Soluble Si treatment in cucumber plants resulted in rapid activation of peroxidises and polyphenoloxidase activity when infected with *Pythium* spp. (Cherif *et al* 1994). Silicon plays an important role in inhibiting downy mildew in cucumber by activation of major defense-related enzymes (Yu *et al*. 2010). Silicon treated plants significantly altered the activities of catalyses against rice blast disease (Sun *et al*. 2010). Fautex *et al*. (2006) reported that Si treatment does not affect the metabolism of unstressed plants, but provides a protective role in pathogen defense. In relation to this, results in this chapter have shown minimal impact on phenylalanine ammonia lyase activity before infection and 3 days after infection but the highest phenylalanine ammonia lyase activity was also found in control. Treatments did not affect other defense- related enzymes activity when analysed for polyphenoloxidase activity, catalase and peroxidise activity before or after infection. The reason may be due to the high humidity in the boxes used for conducting the experiments. Although not statistically different, it appears like there was a decline in the trend post-infection in the levels of enzyme activity of polyphenol oxidase, phenylalanine ammonia lyase, catalase and peroxidase activity. The reasons may be due to the other stresses affecting various physiological and biochemical processes in the plant when prolonged exposure to high humid conditions or high moisture or heat related stresses. These stresses sometimes interfere with the plants ability to combat the disease pressure by decreasing the capability of plants to synthesize the defense-related enzymes. Similar adverse effect on the enzyme activity was observed when zinc treated plants were exposed to salinity (Weisany *et al*. 2012).

From experiment 3, poison food technique, minimal effects were observed at 12, 48 and 60 hours of mycelial growth on treatment amended PDA. Shen *et al* (2010) reported that potassium silicate amended with PDA inhibits five soil-borne phytopathogenic fungi. In conclusion, the results from this study shows that potassium silicate fortified with CMCT or SWE may minimally influence the biochemical properties of creeping bentgrass tissues but not different from the control. Further research is required to understand the treatment effects on disease severity. Treatments also appear to play a minimal role in accumulation phenolic-like compounds in the zones of infection and 6 days after infection. Minimal impact was observed in phenylalanine activity before and 3 days after infection. No changes in the activity were observed in other enzymes studied. Therefore, potassium silicate combined with CMCT and SWE may have minimal impact on the induced defence-related properties in creeping bentgrass. Further investigation is required to understand the impact of treatments on disease severity.

CHAPTER 5

CONCLUSION

Apart from inherent resistance, plant diseases are also managed by biodiversity in natural ecosystems, the biodiversity that encompasses the availability of diverse genes, species and ecosystems. Therefore, interactions between plants, species or available nutrients in natural ecosystems may contribute to induce defense responses. With the spread of agriculture, some pathogens are economically significant to humans than others because of the acres of abundant monoculture or host-availability for the pathogens in close vicinity. From the literature, it is clear that dollar spot has gained resistance over potential fungicides that were used to control. Therefore it is important to amend management strategies when dealing with dollar spot disease in turfgrasses. Research on strategies that combine proven beneficial products may replace fungicides in the future. Formulation of treatments with diverse products may also artificially mimic a natural ecosystem in the monoculture. Although there may be a diverse group of beneficial microorganisms and nutrients in the rhizosphere, decades of application of synthetic chemicals may have adversely influenced their population.

Treatment formulation by combination used in this research is an example of using diverse compounds. The treatments used combined different products such as potassium silicate, cow manure compost tea and seaweed extract. Although not investigated, brewing principle that is used to make an aerated compost tea may contribute to the synergy. Recently literature consists of articles on composts that are fortified with biocontrol agents after or during the composting process to deliver enhanced plant protection. There are several biocontrol agents and beneficial products researched individually to manage dollar spot disease (described in 2.3.3) that may be fortified in the future to manage fungal disease such as dollar spot.

This study (chapter 3) indicates that the treatment formulations influenced on the pH of the highly alkaline KASIL to below neutral pH when combined with CMCT. Similarly slight reduction of pH when highly alkaline KASIL was combined with highly alkaline seaweed extract close to neutral. Reduced pH is important for cool season turfgrasses and the microorganisms in the rhizosphere of the Atlantic region. This synergy may also serve as a means to apply beneficial alkaline products such as KASIL or SWE. Results indicated significant changes in tissue Si content when KASIL combined with CMCT in creeping bentgrass. Minimal impact continued to occur 15 days after treatments and also with infection. Similar results were obtained for perennial ryegrass. In order to extend the beneficial properties of the treatments, frequent application should be studied in the future. However, the treatments did not affect the disease severity. Further research is required by changing disease conditions and increasing replicates to see the effect of treatments on disease severity. The synergy is not investigated in detain in this research, but silicon content in compost tea might have contributed to the silicon content in treatments with no KASIL or may have increased absorption ability of plants

The results from chapter 4 indicate minimal impact of treatments on chlorophyll at 3 days after infection. Phenolics were altered minimally 6 days after infection. More phenolic-like accumulation was observed in treatments compared to control in the lesion experiment. But the treatments did not affect the lesion area on individual grass blades. Similar observation of phenolic-like deposition was reported as the effects of soluble Si treatment in wheat-powdery mildew pathosystem by Remus-Borel *et al*. (2005). Phenylalanine ammonia lyase activity was minimally influenced by the treatments before the infection and 3 days after infection. Other enzymes studied were not affected by the treatments. The treatment amended PDA also showed minimal impact on the mycelial growth but not different from control. Further research is

required to study the effect of treatments on disease severity and biochemical parameters should be investigated in comparatively less humid conditions.

Treatment formulation by combination of synergistic products is an exciting strategy to influence plants to manage diseases. However, treatments did not affect the disease severity therefore further research is required for recommendations to use in field conditions. Research methodologies may be improved in the future by combining more potential products and may be evaluated under field conditions. Silicon is extensively used in agriculture; the combined formulations may be an exciting strategy to study their impacts in different crops. Silicon is increasingly used for plant protection in greenhouse production; it would be an interesting to study the effects of the synergy of the combined formulations of these products. Further research is required to understand the factors responsible for this synergy increase their impacts by combine more diverse and potential products. This management strategy may influence on the physiological properties such as tissue silicon content. With further research, product combinations of this kind may help reduce dependence on synthetic chemicals and protect the environment as well as people exposed.

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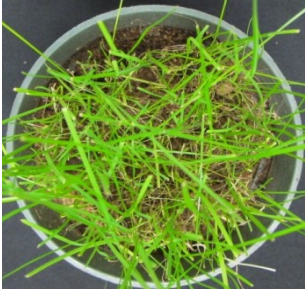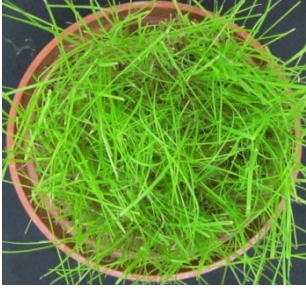
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Appendix A

1-9 rating scale (example)

Infected Creeping bentgrass	Disease Rating	Infected Perennial ryegrass	Disease rating
	1 – severe disease, very poor quality		**1 –** severe disease, very poor quality
	3 – low disease, poor quality		**3 –** low disease, poor quality
	6 – moderate disease, acceptable quality		**6 –** moderate disease, acceptable quality
	9 – least disease, high quality		**9 –** least disease, high quality