OTRF Research Grant Final Report:

The effects of fertilization method on annual bluegrass (*Poa annua*) and silver thread moss (*Bryum argenteum*) competition with creeping bentgrass (*Agrostis stolonifera*) on golf course putting greens

Highlights:

The research has resulted in a better understanding of annual bluegrass competition on golf course putting greens. This understanding has been presented at numerous industry and academic presentations regarding managing golf greens to result in the desired species.

Industry Presentations and Publications Include:

Canadian Golf Superintendents Association Annual Meeting, 2008

Ontario Turfgrass Symposium, 2008

Ontario Turfgrass Symposium, 2010

Canadian Golf Superintendents Association Annual Meeting, 2011

Ottawa Turf Association, 2011

Ontario Golf Superintendents Association, 2012

Golf Course Superintendents Association of America, *TO be Presented*: 2012

Improving root growth: Chemical approaches. Green is Beautiful, Official Publication of the Ontario Golf Superintendents Association. Fall 2010.

Academic Presentations Include:

Lyons, Eric; O'Connor, Kelly; Hudner, Darra; Jordan, Katerina. 2010. Effect of irrigation frequency and fertilization application method on silvery thread moss encroachment in creeping bentgrass. *2010 International Annual Meetings: [Abstracts][ASA-CSSA-SSSA]. p. 61847*

O'Connor, Kelly; Hébert, François; Jordan, Katerina; Lyons, Eric. 2010. Leaf morphology of creeping bentgrass and annual bluegrass in relation to uptake of foliar nitrogen *2010 International Annual Meetings: [Abstracts][ASA-CSSA-SSSA]. p. 6188*

Deliverables:

Proposed	**Result**
The research proposed will increase the effectiveness of fertilization methods on golf course greens reducing waste and potential fertilizer pollution	The research showed that the use of ammonium based fertilizers was more effective in stimulating growth than urea based fertilizers. It also showed the spoon feeding and delivering nutrient to the soil through irrigation following a liquid application could increase root growth and select for the deeper rooted species.
Minimizing undesirable species by identifying proper fertilization methods	The research showed that creeping bentgrass growth was reduced with foliar application and annual bluegrass maintained growth regardless of fertilizer application type. The research also showed an explanation for why annual bluegrass was more effective at taking up foliar fertilization.
Recommendations for foliar fertilization techniques based on species and presence of moss	The research resulted in the conclusion that foliar fertilization selects for shallow rooted species such as annual bluegrass and moss. This information was conveyed through industry articles and numerous educational events throughout Ontario and Canada
Recommendations for fertilizer methods regarding maximizing root growth	The research showed that foliar fertilization could result in reduced root growth. This information was conveyed through industry articles and numerous educational events throughout Ontario and Canada
Pesticide free deterrence of moss invasion on golf course putting greens	This research showed that foliar fertilization at low rates may aid moss invasion and also showed confirmation on previous research looking at irrigation frequency and moss encroachment. This information was conveyed through industry articles and numerous educational events throughout Ontario and Canada
Training of a highly qualified graduate student in the field of turfgrass science	The work done on this project lead to Ms. Kelly O'Connor being awarded a MSc. in plant agriculture . Ms. O'Connor now works as field research coordinator for a agricultural company based in Southwestern Ontario. Huasong Xu also was able to run some experiment related to this experimentation that helped expand his knowledge of turfgrass systems, He completed his MSc. in Plant Agriculture in 2011
Increased understanding of the role of nutrient availability and plant competition	The research has resulted in numerous talks regarding plant competition and how fertilizer application can impact species composition of a grass sward
Increased understanding of the role of symbiotic fungi in plant competition between turfgrass species (dependent on NSERC funding)	NSERC funding was not attained but collaborations with university and industry researchers has started in 2011 that may lead to increased knowledge as the field plots used in this study are being maintained to explore the long term effects of foliar fertilization.

Summary of Greenhouse Studies

Golf course greens in North America are usually planted to creeping bentgrass (CB) but over time annual bluegrass (AB), a less desirable grass species, quickly invades. A growth experiment was conducted to measure the impact of foliar and soil applied urea and ammonium sulfate on the growth of CB and AB. Treatment with ammonium sulfate resulted in greater shoot growth and root growth for both species. For CB specifically, foliar fertilization reduced shoot growth when compared to soil applied fertilization, and root growth was significantly reduced with foliar application of ammonium sulfate. Annual bluegrass was shown to have less root mass overall although soil applied ammonium sulfate increased the root mass of both species in the top 6 cm by 250% over the foliar applied ammonium sulfate and application of urea with either method. In addition applying foliar urea reduced AB rooting below 6cm compared to soil applied urea. The grasses in the experiment as well as multiple samples of both CB and AB collected from golf courses were observed microscopically. Microscopy revealed no differences in stomatal number between the two species, however, treating the cuticle with silver nitrate showed that AB may have more locations for uptake of foliar applied nutrients through waxy extensions on its leaf margins. This research shows while CB has reduced growth under foliar fertilization AB is able to maintain constant growth possibly due to different leaf morphology and may explain some of the invasiveness of annual bluegrass on highly managed golf greens.

Research Methods for Greenhouse Studies

Foliar Fertilization Experiment.

Plant Material.

Although the experiment was run two times the dates described are for the second run of the experiment and all results reported are from the second run of the experiment. Data from the two experiments could not be pooled due to differences in the time to harvest and plant maturity at the time of harvest between the first and second experiment. L-93 creeping bentgrass (CB) was planted into trays of silica sand in March 2007. Cores of annual bluegrass (AB) were harvested from a golf green at the Guelph Turfgrass Institute, Guelph, Ontario, Canada. The cores contained approximately 80 % annual bluegrass, the remainder being creeping bentgrass. After proper identification, tillers of annual bluegrass were transplanted into a tray with silica sand. For both species, a single tiller was transplanted to each of 32 polyvinyl chloride (PVC) tubes (16 per species) with a diameter of 8 cm. The tubes contained 10 cm of gravel topped with 30 cm of silica sand meeting USGA recommendations (USGA 2007), to mimic a putting green root zone. All tubes were covered with aluminum foil, with the tiller protruding from the foil, to ensure that foliar-applied fertilizer remained on the plant and a 15 cm plastic tube was inserted to the soil level to allow application of water and fertilizer. Plants were grown in a greenhouse with supplemental light to provide a 16 hour day for the length of the experiment.

Fertilizer Treatments.

Two 18-3-18 fertilizer solutions were prepared for the experiment. The first one was urea-based and the second was ammonium sulfate (nitrogen) and both contained mono-potassium phosphate (phosphorus) and potash (potassium). Fertilizer was applied at a rate of 3.78 L per 100 m^2 at a concentration of 45.56 g of nitrogen per liter. All application methods received the same amount of nitrogen per application throughout the experiment. Aqueous magnesium sulfate (0.6 %) was added to all pots at week 4 due to sulfur deficiency symptoms that appeared in the first run of the experiment. This application eliminated sulfur deficiency as a cause for any growth differences.

For each foliar fertilization, 50 ml of deionized water was injected into the plastic tube before 0.2 ml of fertilizer was sprayed over plants. The sprayer was calibrated before the foliar fertilization by weighing a circle of paper towel (8cm in diameter) before and immediately after spraying water. For soil-applied fertilizer, 50 ml of deionized water and 0.2 ml of fertilizer were mixed and injected into the plastic tube.

Sampling and Measurements.

The variable fertilizer application methods were imposed for 8 weeks before the plants were harvested. Plants were separated into root and shoot biomass and tillers were counted and washed under tap water to remove residual fertilizer and soil particles. Roots were washed using a 1 mm sieve. Biomass was dried at 70˚C until constant mass and dry biomass were recorded. Total Kjeldahl N (TKN) was analyzed colorimetrically by spectrophotometry (Astoria A2 auto-analyzer, Astoria Pacific International, Oregon, USA) preceded by H_2SO_4-Se-K_2SO_4 digestion (Sen Tran and Simard 1993).

Experimental Design and Statistical Analyses.

The experimental design was a three-way factorial (species, application method, fertilizer solution) completely randomized with four replications. Analyses of variances (ANOVA) were performed using the MIXED procedure in SAS (version 9.1) (SAS Inc. Carey, NC, USA). Fisher's protected LSD tests were done (Steel et al. 1997) when interactions were significant at $\alpha = 0.05$.

Microscopy.

Plant Material.

Samples of creeping bentgrass and annual bluegrass from the original experiment were used. Additional samples were collected from golf greens at three different sites in Guelph, Ontario. Two of the three sites are golf courses (Victoria Park East, Cutten Club) while samples of the third site were taken from two Guelph Turfgrass Institute (GTI) research greens. At each golf course, we collected four samples of each species on four different greens for a total of 16 samples per golf course. The same procedure was done at the GTI, but from two different greens for a total of eight samples. We harvested the samples with a knife and placed them in plastic bags with a wet paper towel to maintain soil moisture. Each tiller was transferred into pots filled with sand and placed in a greenhouse with a temperature cycle of 24˚C/16˚C day/night and a 16-hour photoperiod. The samples were placed under automatic misters (15 sec per hour) to ensure survival and growth and were fertilized by watering with a 20-20-20 solution weekly. Plants were clipped once a week to a height of 6 cm.

Stomatal Count.

Tissue samples were cut with scissors from fully expanded leaf blades on growing plants. A 2 cm section of a leaf blade was then selected between the sheath and the leaf tip. All procedures were conducted at room temperature during the day under normal room lighting. To obtain average stomatal count, clear nail polish was painted onto the leaf surface by fixing the ends of the leaf blade with tape on a microscope slide. Once the nail polish dried, it was peeled from the leaf blade and placed on a slide with a drop of water and a cover slip, which was sealed with nail polish. Four counts were taken from each peel under a compound microscope (Olympus BX51) at 200 X, and photographed with an Olympus DP71 camera (Olympus Corporation, Tokyo, Japan). The stomatal count was an average count per 1 mm^2. The same procedure was used for both species and for the abaxial and adaxial surfaces.

Silver Nitrate Uptake.

Silver nitrate uptake was performed on leaves with pigment removed to determine the presence of ectodesmata on the leaf surface of both species (Schlegel *et al.* 2005). A 2 cm section of a leaf blade was selected between the sheath and the leaf tip. The leaves were cleared by submerging them in household bleach for four hours. After leaves were cleared, they were rinsed in deionized water for 10 minutes. Cleared leaves were washed with hand soap to reduce surface tension of the cuticle and placed in a petri dish on top of filter paper. A micropipette was used to place 40 µl of a 0.02 M solution of silver nitrate. After silver nitrate was placed on the samples, the petri dish was closed for one hour. The leaves were then rinsed with deionized water to remove excess silver nitrate on the leaves, mounted on a microscope slide with a drop a glycerol and sealed with nail polish. Slides were placed under a compound microscope (Olympus BX51) and leaves were photographed with an Olympus DP71 camera (Olympus Corporation, Tokyo, Japan).

Summary of Results:

Foliar Fertilization Experiment.

There was a significant interaction between the species and the fertilization method for shoot dry mass (Table 1). Differences between application method were observed only for CB where shoot dry weight was 80 % higher with the soil application method compared to the foliar application (Figure 1a). Shoot dry weight was 2 times higher when species were fertilized with ammonium sulfate (Table 1, Figure 1b). Nitrogen analysis revealed a significant increase in shoot nitrogen for plants grown with foliar applied fertilizer compared to soil applied fertilizer (P=0.0249). Foliar applied plants had a nitrogen concentration of 2.17% compared to soil applied plants, which had a content of 1.78%.

Root mass distribution was significantly affected by species (P=0.0046). Creeping bentgrass had 50% of its total root mass in the upper 6 cm of the soil profile (0.171g (SE=0.03)) compared to AB, which had 66% of its total root mass (0.117g (SE=0.025)) in the upper 6cm of the soil profile. Plants grown with ammonium sulfate showed a 20% increase in root mass compared to those fertilized with urea (P=0.0008) (Figure 2). Creeping bentgrass had an increase in root weight in the upper portion of the soil profile from 0.14g (SE=0.01) with urea to 0.20g (SE=0.039) with ammonium sulfate. Creeping bentgrass showed an 11% increase in the proportion of root mass in the upper portion of the soil profile when fertilized with ammonium sulfate. In the upper 6cm, AB was not significantly affected by application method; however, CB had a 35% reduction in root growth under foliar fertility 0.13g (SE=0.01) compared to soil applied fertilizer 0.20g (SE=0.03) (Figure 2).

Significant root mass differences were found between species in the lower portion of the soil profile (P = 0.0083). Roots in the lower portion of the soil profile fertilized with ammonium sulfate had a 19% increase in root mass compared to those fertilized with urea (P=0.0024) (Figure 3). Urea fertilization also led to a 54% decrease in root production in CB and a reduction of 40% in AB (Figure 2). Creeping bentgrass exhibited a 52% reduction in root mass from 0.21g (SE=0.05) under soil applied fertilizer to 0.11g (SE=0.01) under foliar applied fertilizer.

Both AB and CB grown with soil applied ammonium sulfate had increased root weight compared to all other conditions (P=0.0432). Creeping bentgrass grown with soil-applied ammonium sulfate had a higher root weight at 0-6cm depth (P=0.0044) compared to all other conditions (data not shown). Below 6cm CB again had a higher root mass under soil-applied ammonium sulfate compared to all other conditions (Figure 3).

Microscopy.

Stomatal Counts.

No difference between species and populations was found for stomatal count for either the abaxial (P = 0.173) or the adaxial (P = 0.805) surfaces with a mean count of 122.39 (± 42.13) stomates per mm^2 on the adaxial side and 109.19 (± 46.66) stomates per mm^2 on the abaxial side.

Silver Nitrate Uptake.

Both AB and CB absorbed the silver nitrate near the stomates (Figure 4b, 4d). The silver nitrate resembled black spots and seemed to be concentrated between the guard cells, not outside them. Some cultivars of CB such as L-93 are known to have "barbed-like extensions" (Williams and Harrell 2005) from the waxy cuticular layer, which resemble trichomes (Figure 4c). On leaves of CB plants these structures were present on the margins of the leaves. Annual bluegrass also possessed these structures and they were present on the margins, midrib and veins of the leaf surface. We found higher absorption of silver nitrate by AB compared to CB around these barbs. Silver deposits for AB are located at the base and head of the barbs (Figure 4a). Silver deposits were occasionally observed on CB barbs but it did not seem to enter them as with AB. Creeping bentgrass and AB had these consistent morphological traits regardless of the population (or the ecotype) harvested.

Table 1. Summary of ANOVA results for shoot dry mass measured on creeping bentgrass and annual bluegrass submitted to various fertilization solutions and application methods. Bold indicates significance (*P* < 0.05). ndf = numerator degrees of freedom.

Source of variation (fixed)	ndf	Shoot dry mass		Root dry mass (above 6 cm)		Root dry mass (below 6 cm)	
		F	*P*	*F*	*P*	*F*	*P*
Species (S)	1	0.23	0.633	12.53	**0.002**	11.47	**0.002**
Fertilization solution (F)	1	27.81	**< 0.001**	8.26	**0.008**	0.70	**0.004**
Application method (A)	2	4.63	**0.042**	4.23	0.051	7.28	**0.013**
S × F	1	1.30	0.265	0.19	0.670	2.63	0.118
F × A	1	4.05	0.056	10.79	**0.003**	4.56	**0.043**
S × A	1	5.07	**0.034**	2.89	0.102	2.48	0.129
S × F × A	1	1.84	0.181	0.55	0.586	4.77	**0.018**

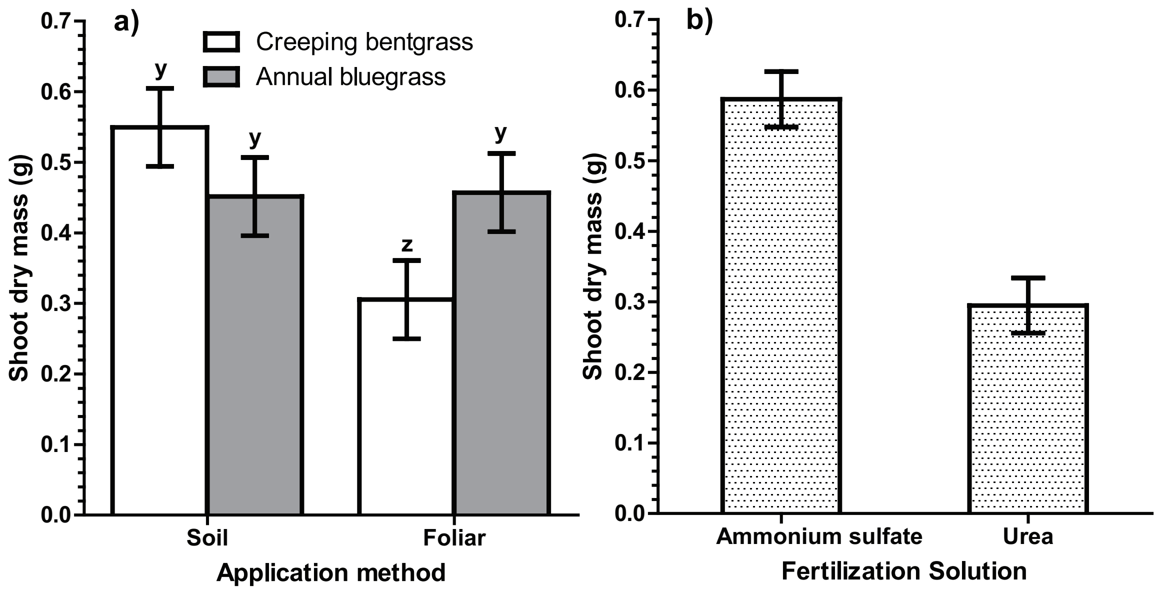

Figure 1. a) The effect of application method of nitrogen fertilizers on the shoot dry mass of creeping bentgrass and annual bluegrass with the fertilizer type pooled b) the effect of fertilizer type on shoot dry mass when the species are pooled. Error bars represent standard error of 4 replicates in a factorial experiment.

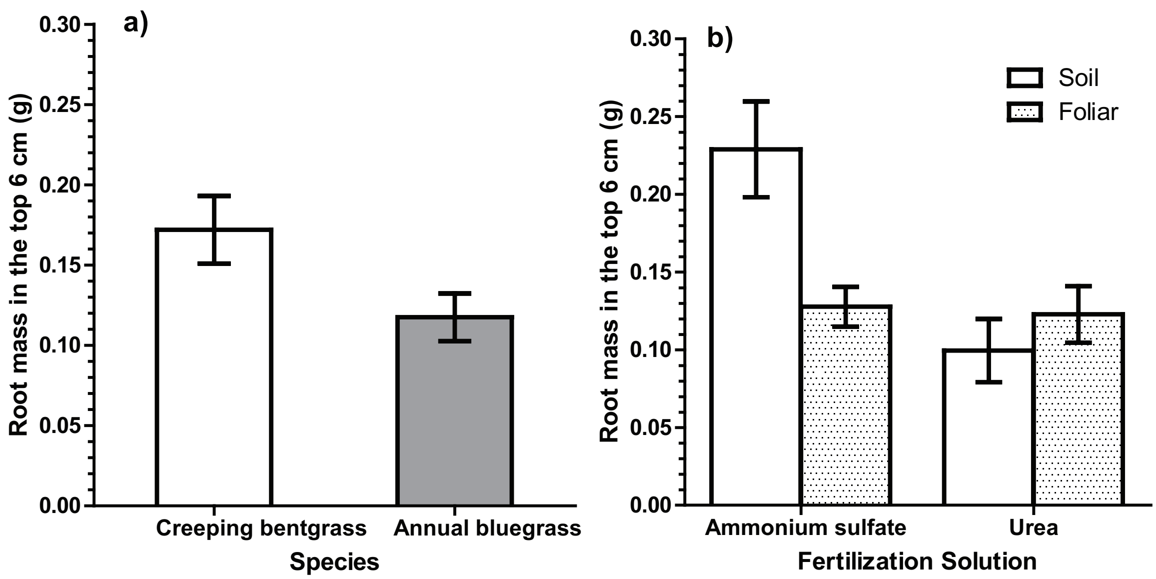

Figure 2. a) Root mass in the top 6 cm of creeping bentgrass and annual bluegrass and b) the root mass in the top 6 cm of soil and foliar applied ammonium sulfate and urea. Error bars represent standard error of 4 replicates in a factorial experiment.

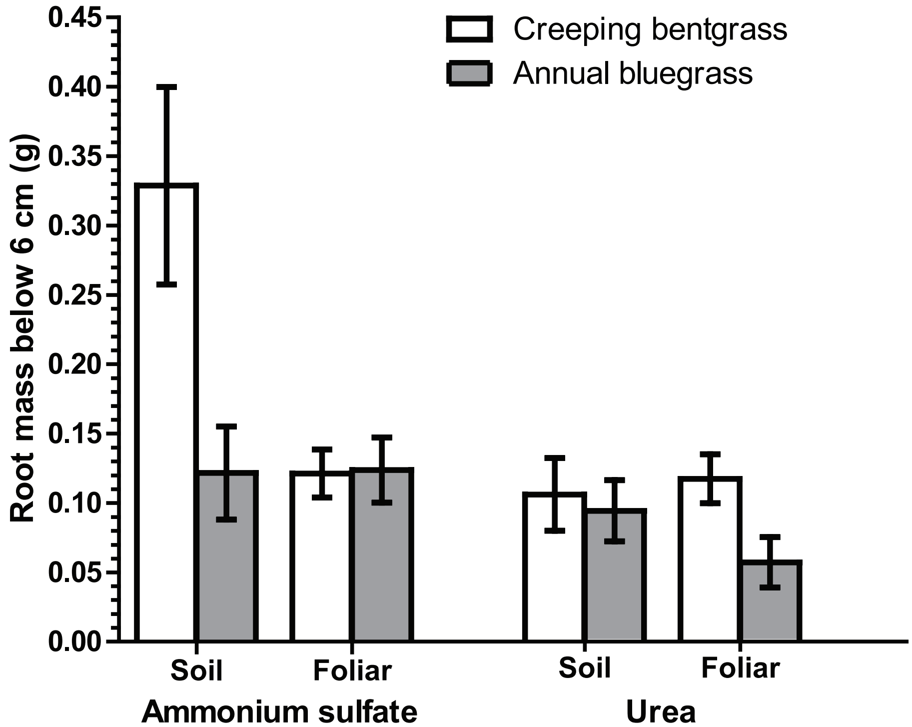

Figure 3. Root mass below 6 cm of creeping bentgrass and annual bluegrass when ammonium sulfate and urea are applied to the foliage or to the soil. Error bars represent standard error of 4 replicates in a factorial experiment.

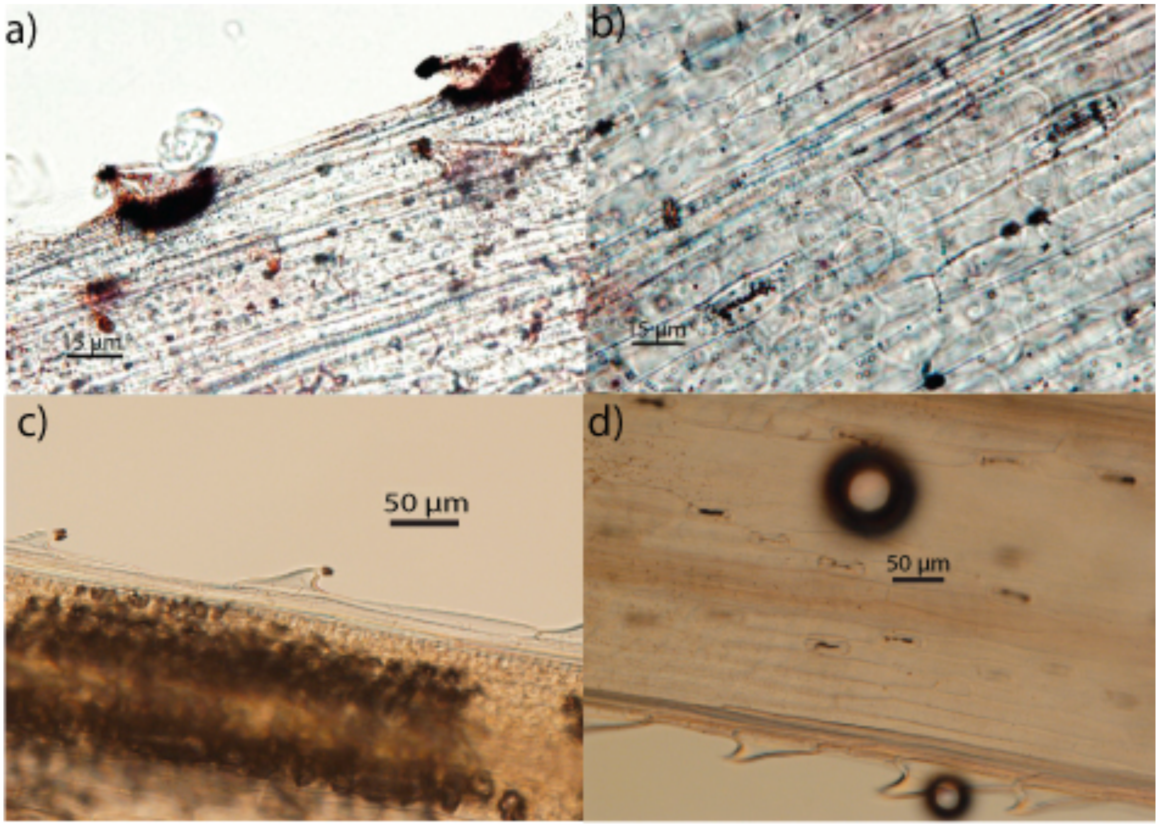

Figure 4: Microscopy pictures of cleared leaves of annual bluegrass (AB) (a and b) and creeping bentgrass (CB) (c and d) under light microscopy after treatment with silver nitrate ($AgNO_3$). Characteristic silver deposits are in black a) barbs b) stomata c) barbs d) stomata.

Summary of Field Trials and Moss Trials

The field plots have been under continuous foliar, soil applied liquid and granular fertilizer application since 2007. To this date there are minimal measurable differences in species composition between the species. This coming growing season root analysis will be taken for the final time and interactions with mycorrhizal fungi will be assessed as part of industry collaboration. The field research has resulted in the confirmation that foliar fertilization can reduce root growth although not for extended periods and only certain sections of the rootzone were affected (Figure 5, 6).

Greenhouse trials involving moss encroachment were difficult to interpret although the research did result in the success of a related project on turfgrass irrigation frequency funded through a different funding source. Evidence showed that when moss was first establishing it benefited from foliar fertilization but as the amount of moss increased differences between the application methods were minimized (Figure 7).

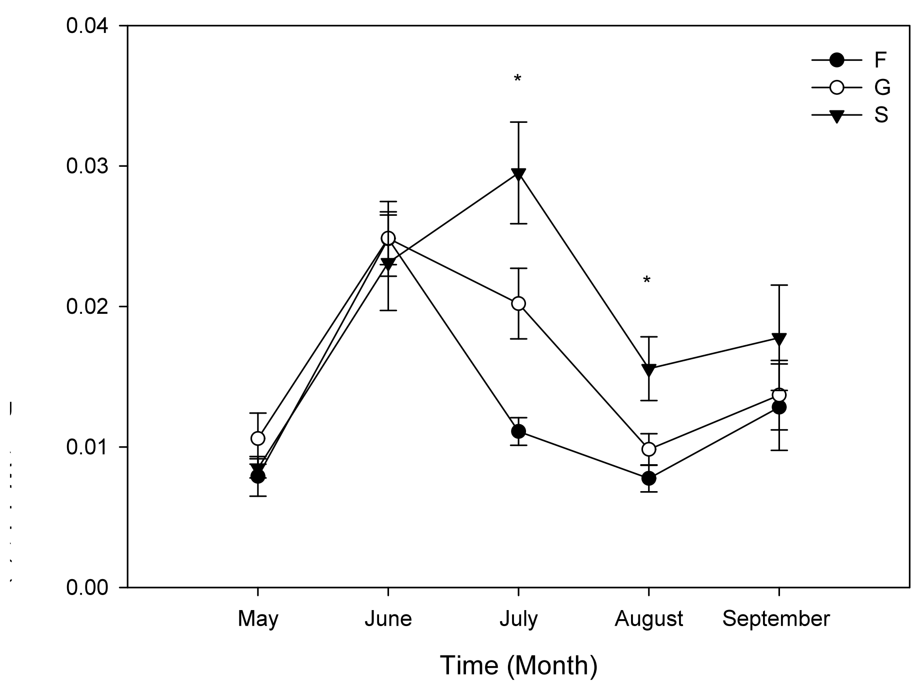

Figure 5. The root mass of annual bluegrass at 3-6 cm of the soil profile under Foliar (F), Granular (G) and soil applied (S) fertilizer in the field. Asterisks denote significant differences between fertilizer application methods at alpha=0.05 where N =12.

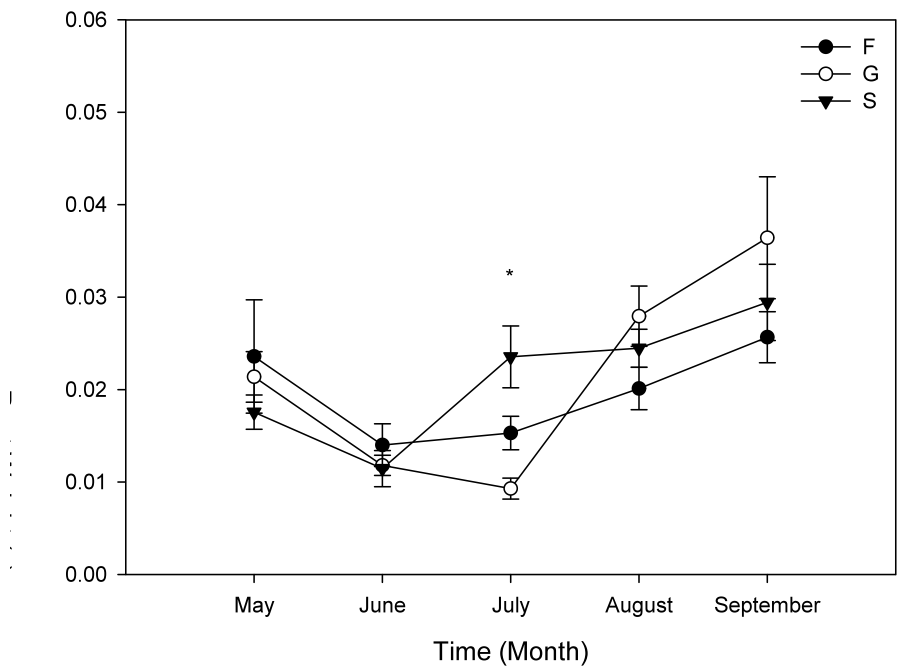

Figure 6. The root mass of creeping bentgrass at 3-6 cm of the soil profile under Foliar (F), Granular (G) and soil applied (S) fertilizer in the field. Asterisks denote significant differences between fertilizer application methods at alpha=0.05 where N =12.

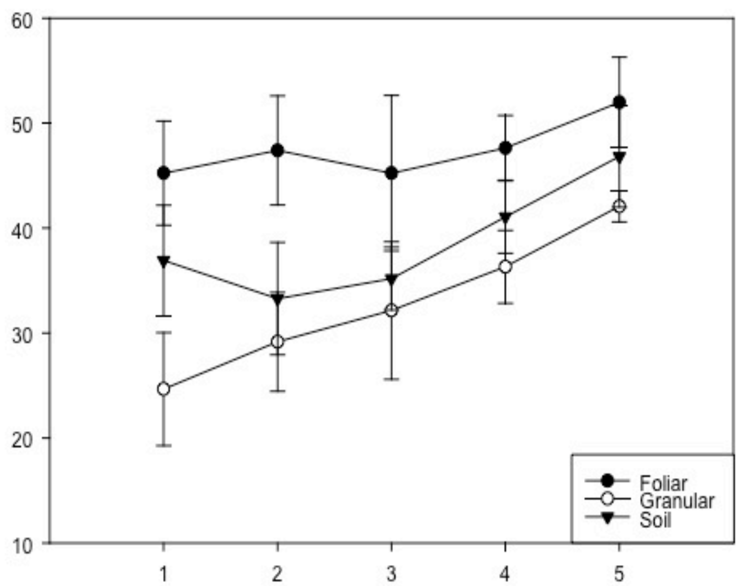

Figure 7. Percent cover of silvery thread moss (STM) under foliar, granular and soil applied liquid when grown with creeping bentgrass. Error bars represent standard error of 4 replicates.