CTRF Final Report for Project **CTRF 2015-2a** (October 2015 - December 2019)
RESEARCHER: Dr. Tom Hsiang, University of Guelph
GRADUATE STUDENTS: Kate Stone and Matt Rudland

PROJECT TITLE: Testing lower risk fungicides for activity against turfgrass diseases

PURPOSE: The purpose of this work was to investigate the use of lower risk fungicides against turfgrass diseases. The specific practical objective is to quantify the extent by which common diseases such as dollar spot, Fusarium patch and snow moulds can be reduced in lab and field tests, using different application regimes of chemicals such as acetic acid (vinegar), borax, citric acid, garlic powder, hydrogen peroxide, iron sulfate, lime sulphur, phosphites, soaps, sodium chloride, and sulphur. These are all products classified by the Ontario Ministry of the Environment (OME) as Class 11, and available for cosmetic use against turfgrass pests in Ontario, and not on the "banned" list for cosmetic use that is found in OME Class 9. This issue should be of concern to turfgrass managers across Canada since most provinces in Canada have some sort of ban or are considering a ban on chemicals for cosmetic use on turf. The subsequent scientific objective was be to assess which treatments are efficacious, and for those that have efficacy to determine the mode of action, since such compounds may possibly affect diseases by directly inhibiting the pathogens, or indirectly through effects on the plant (e.g. activated resistance) or effects on microbial components which affect either the plant or the pathogen or both. The benefits of this type of research would be replacement of "higher risk" synthetic fungicide applications, by ones already deemed to be "lower risk", via a scientific assessment of how such substances are able to decrease disease. The deliverables from this project were the development of a disease control management regime (application rate, application timing) for important turfgrass diseases using lower risk fungicides that are available for use in Canada.

LAYMAN SUMMARY

There are strong societal pressures against the use of synthetic pesticides in our modern urban society, and this has lead governments to pass legislation which makes it more difficult to use such chemicals without administrative hurdles. In Ontario, there is a class of compounds available for cosmetic use again turfgrass pests, and not on the "banned" list. Similar listings are found in other Canadian provinces. The purpose of this work was to test the efficacy of the selected disease control substances considered to be less risky to the environment and human health for their ability to control the common turfgrass diseases, dollar spot and Fusarium patch, in lab and field tests.

In the first year (2016), we tested at least four different concentrations of over a dozen different substances in the lab. In most cases, creeping bentgrass that was inoculated (disease placed on the grass) had the highest level of yellowing except for some rates of garlic powder and borax. The yellowing and color change levels for citric acid, sodium chloride and sulphur treatments were noticeably less. These trials were repeated again with similar results. From these lab tests, we selected the lowest rate that provided highest disease control for each of the compounds, and tested these in the field. These results demonstrated that weekly applications of the products gave results ranging from 1.5% to 10.5% area diseased compared to 17.5% for the inoculated control. In order of efficacy, these were as follows: Iron Sulfate, Standard fungicide (Banner), Citric acid, Hydrogen peroxide, Sulfur, Phosphite, Soaps, Sodium chloride, Garlic powder, Borax, and Acetic acid. These results demonstrated that most "home remedies" may have some suppressive effect, but not at levels to satisfactorily control the disease since only Iron Sulfate provided dollar spot disease suppression equivalent to the standard fungicide. During 2016-2017, 10 repeats of the same lab experiment on dollar spot disease suppression were done, and these continued to show significant differences between treatments.

Based on these results, field trials against *Microdochium nivale* were set up in September 2017, and were continued through winter. The disease levels were low in the fall, but the two treatments that showed significant reduction of disease were the standard fungicide control Banner (56% reduction), and Ferric Sulfate (87%). Through winter 2018, we continued to test different rates of these compounds against other turfgrass diseases in the lab. In spring 2018, we conducted another field trial using *M. nivale*, but disease levels were too low to properly evaluate the treatment.

In summer 2018, we conducted numerous trials of many different products at different rates against dollar spot disease on creeping bentgrass. These tests demonstrated that iron sulfate (both ferrous sulfate and ferric sulfate) can show high suppressive activity against dollar spot disease, with minor darkening. In addition, we continued to test Boric Acid and Garlic powder since these products showed significant disease suppressive activity in lab tests and previous field tests. Although they did provide some decrease in disease, they likely do not meet aesthetic standards as a field fungicide.

In 2019, we repeated field tests on efficacy of iron sulfate at different rates, and conducted more tests on phytotoxicity to examine how high levels of iron sulfate affect leaf blades. We found as in previous years, that rates of 500 g / 100 m^2 or higher applied on a weekly basis lead to unacceptable levels of blackening and even permanent scarring which was amplified with increasingly higher rates and with higher daytime temperatures. Even the 250 g / 100 m^2 rate at 3.5 day intervals could give some scarring. Microscopic examination of the darkened shrivelled leaf blades showed accumulation of orange balls (precipitates) on or inside leaf blades. Future research is needed to investigate what these orange clusters are and how they originate.

In summary, from the many lab and field tests over several years, we conclude that among over a dozen non-conventional treatments applied at different rates and intervals, only ferrous sulfate (and possibly ferric sulfate) applied at 250 g / 100 m^2 (52.5 g a.i. / 100 m^2) applied in 10 L water / 100 m^2 on a weekly basis can provide suppression of dollar spot disease equivalent to a standard fungicide control during low to moderate disease pressure periods. As well Microdochium patch can also be suppressed at this rate, but the disease suppression was not as great as that of the standard fungicide control. (NOTE: ferrous sulfate is available from several sources in Canada, but we used a 21% active from Crown Technology Inc., Vaughan, Ontario)

SUMMARY OF MAJOR RESULTS (by Report Date)

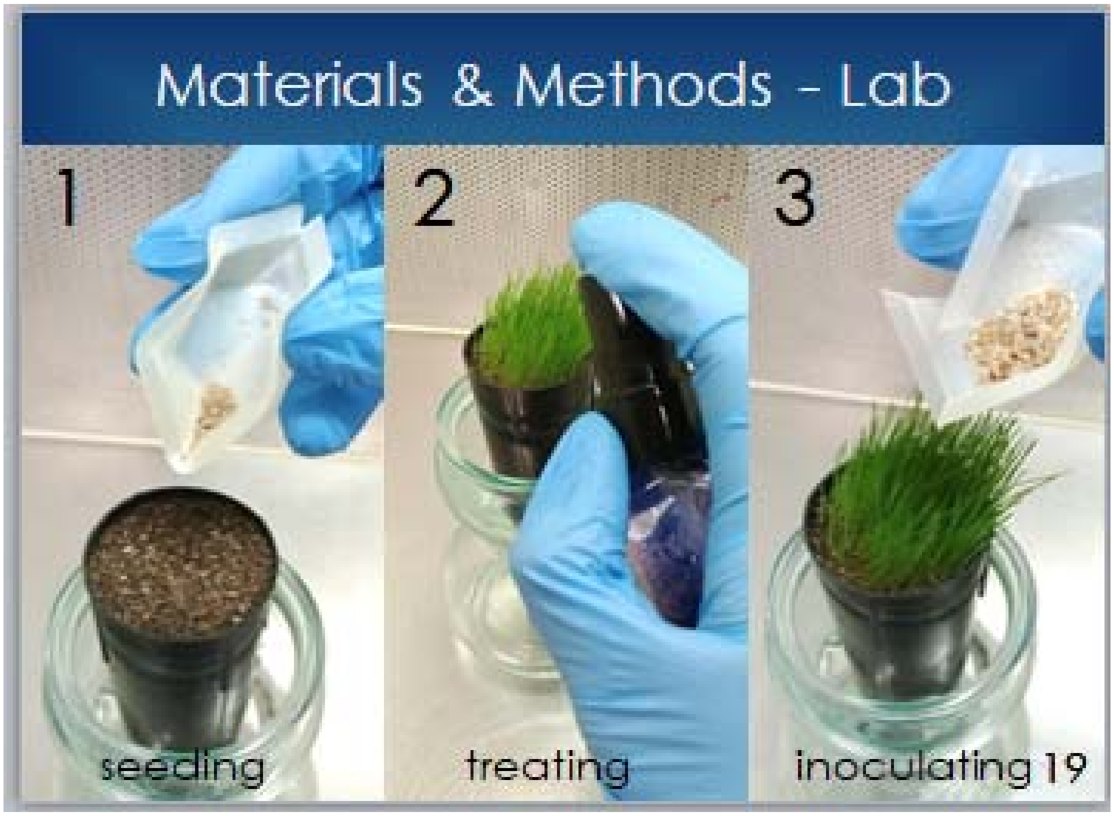
April 2016 Report - During this first 5 months of this project, we compared garlic powder, hydrogen peroxide, iron sulfate, acetic acid, borax, citric acid, dishwashing soap, sodium chloride, sulphur and phosphite on *Agrostis stolonifera* cv. Penncross in pots in the growth chamber for assessing dollar spot disease. We tested at least four different concentrations of each substance. In most cases, inoculated Penncross without treatment had the highest level of yellowing except for some rates of garlic powder and borax.

Sept 2016 Report - In field trials weekly applications of 12 products gave results ranging from 1.5% to 10.5% area diseased compared to 17.5% for the inoculated control. In order of efficacy, these were as follows: Iron sulfate, Standard fungicide (Banner), Citric acid, Hydrogen peroxide, Sulfur, Phosphite, Soaps, Sodium chloride, Garlic powder, Borax, and Acetic acid. These results demonstrated that most "home remedies" may have some suppressive effect, but most not at levels to satisfactorily control the disease in commercial use.

Sept 2016 Figure 1: Dollar spot field trial with 12 different treatments in early August 2016 at the Guelph Turfgrass Institute. The greener plots are Iron Sulfate. Dollar spot is clearer visible in some treatments.

March 2017 Report - We continued field trials in Fall 2016 testing activated resistance against Microdochium patch, as well as against Pink Snow Mold and Grey Snow Mold over the winter (2016-2017). The results from the fall trials were inconclusive since there was insufficient Fusarium Patch disease pressure (fall 2016 was much too warm). Similarly, winter 2016-2017 started off well with abundant snow in December 2016, but this melted by January and we saw record high temperatures. The inconsistent snowfall and snowcover has not allowed for grey snow mold development, but Pink Snow Mold and Fusarium Patch are visible on the field plots

October 2017 Report - **A**lthough there were significant differences between cultivars in disease development, the treatments (inoculated control, phosphite alone and Civitas-Harmonizer) did not show significant differences between treatments. During the last year, 10 repeats of the same lab experiment on dollar spot disease suppression have been done, and these have yielded significant differences between treatments. The most significant results are that in comparison to the inoculated control, these statistically significant reductions in disease were seen: Banner (78%), Sunlight Soap Liquid (55%), Acetic Acid (34%), Table salt (33%), Hydrogen Peroxide (32%), Phosphite (28%), Garlic powder (24%), and Iron sulfate (21%). Products not showing any significant suppression of disease caused by *Sclerotinia homoeocarpa* include Citric Acid, Borax and Sulfur.

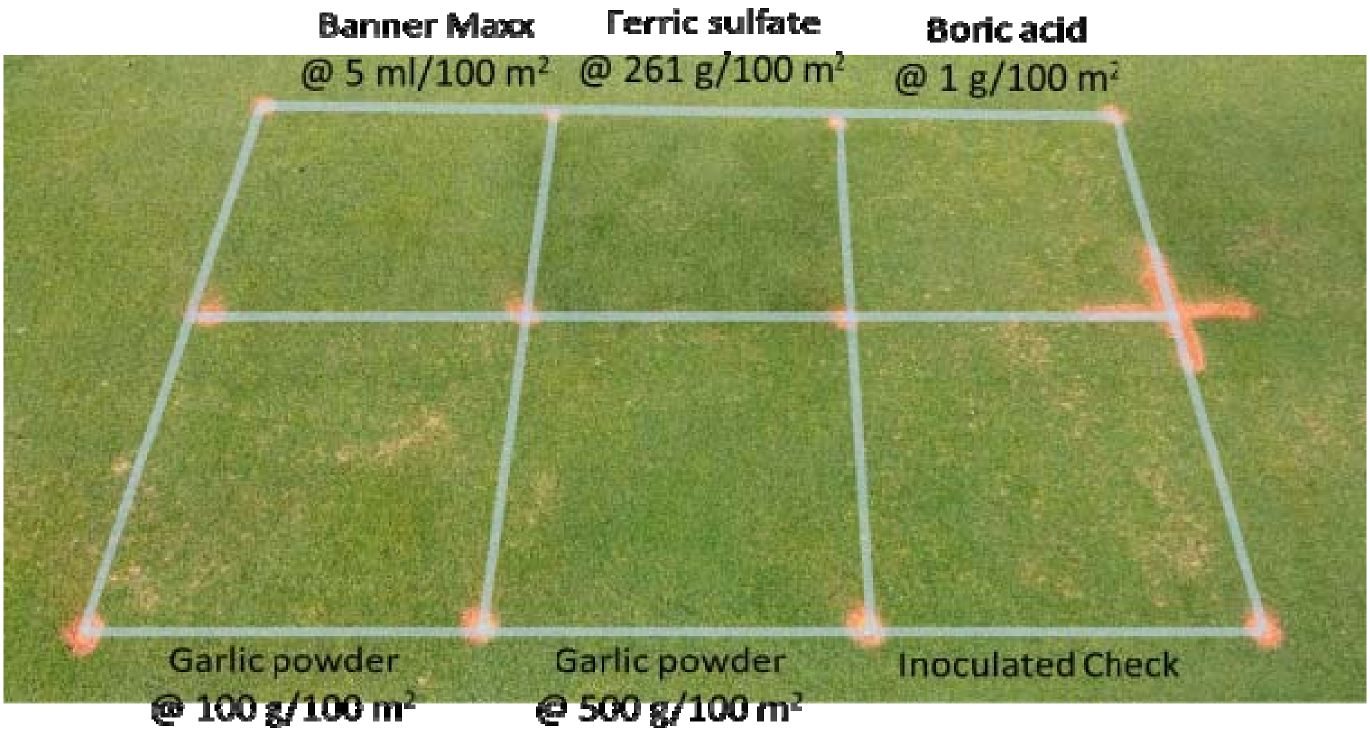


Oct 2017 Figure 1: Lab testing using grass grown in Cone-tainers and inoculating with a pathogen

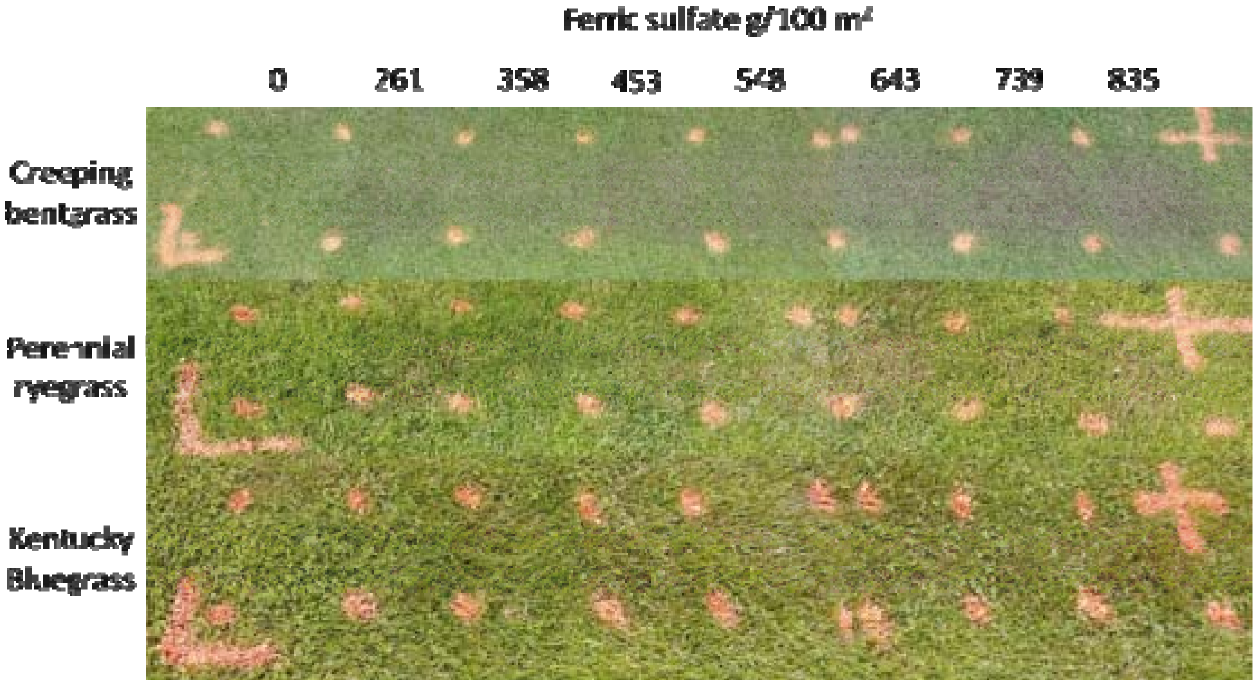
Oct 2017 Figure 2: Lab testing using grass grown in Cone-tainers at 2 wk after inoculation

June 2018 Report - Field trials against *Microdochium nivale* were set up in September 2017, and were continued through winter. The disease levels were low in the fall, but the two treatments that showed significant reduction of disease were the standard fungicide control Banner (56% reduction), and Iron Sulfate (87%). The full data are presented in Table 6. We are continuing to test different rates of these compounds against other turfgrass diseases, and will conduct further field tests through the rest of 2018 and through 2019. Because ferrous sulfate has shown the highest efficacy, this compound will be a major focus of our remaining work.

Sept 2018 Report - In summer 2018, we conducted numerous trials of many different products at different rates against dollar spot disease on creeping bentgrass. These tests demonstrated that ferrous sulfate can show high suppressive activity against dollar spot disease, and is well below the threshold for phytotoxicity. In addition, we continued to test Boric Acid and Garlic powder since these products showed significant disease suppressive activity in lab tests and previous field tests. Although they do provide some decrease in disease, they likely do not meet aesthetic standards. Turfgrass species differ in their tolerance to phytotoxicity induced by Iron Sulfate.



Sept 2018 Figure 4: Field trial 1 (above) at DPI28 comparing the inhibition of dollar spot by the treatments.



Sept 2018 Figure 8: Ferric sulfate damage trial (above) looking at turfgrass damage at DPT21 on three grass species. Creeping bentgrass shows greatest sensitivity to higher rates of Iron Sulfate.

Feb 2019 Results

Based on previous lab trials from January 2018 until June 2018, ferrous sulfate, para-aminobenzoic acid, Polyoxin-D, humic acid and a registered biocontrol agent (*Chlonostachys rosea*) were selected for outdoor efficacy tests as these compounds showed potential to significantly reduce dollar spot disease on creeping bentgrass in lab tests. Starting in August 2018 and continuing until October 2018, multiple field trials were conducted on creeping bentgrass at the Guelph Turfgrass Institute to determine whether or not these compounds were able to significantly reduce dollar spot disease in a field setting. The first trial was completed over 9 weeks, starting in August 2018, on creeping bentgrass cv. Penncross in order to find out which of these compounds would effectively reduce disease. The results of this first trial (Figure 1) indicated that two ferrous sulfate compounds at different rates, para-aminobenzoic acid, and Polyoxin-D all significantly reduced dollar spot disease. However, humic acid, at two different rates, as well as *C. rosea* did not significantly reduce dollar spot disease.

We also conducted a ferrous sulfate tolerance test from August 2018 to October 2018 on 10 creeping bentgrass cultivars including: MacKenzie, penncross, Focus, 007, Alpha, Tyee, L93, V8, T1 and A4. The results of this trial (Figure 5&6) indicated that the 1x rate resulted in very little blackening of the leaves overall, whereas the 5x rate gave some darkening that was reduced to low levels by 1 week post application. However, the 10x and 15x rates resulted in severe darkening both 1 day and 1 week post treatment application. Due to this observation, we would not suggest a treatment rate of ferrous sulfate heptahydrate over 5x, as it will reduce the overall appearance of the turfgrass significantly.

We also examined the effects of various compounds on Microdochium patch. Creeping bentgrass plots at GTI were treated twice with each compound in October 2018. They were then inoculated with *M. nivale* in late October and late November, 2018. Pictures of the plots from November 2018 are shown in Figure 7, demonstrating differences in the ability to suppress Microdochium patch or even enhancing it (Boric acid).

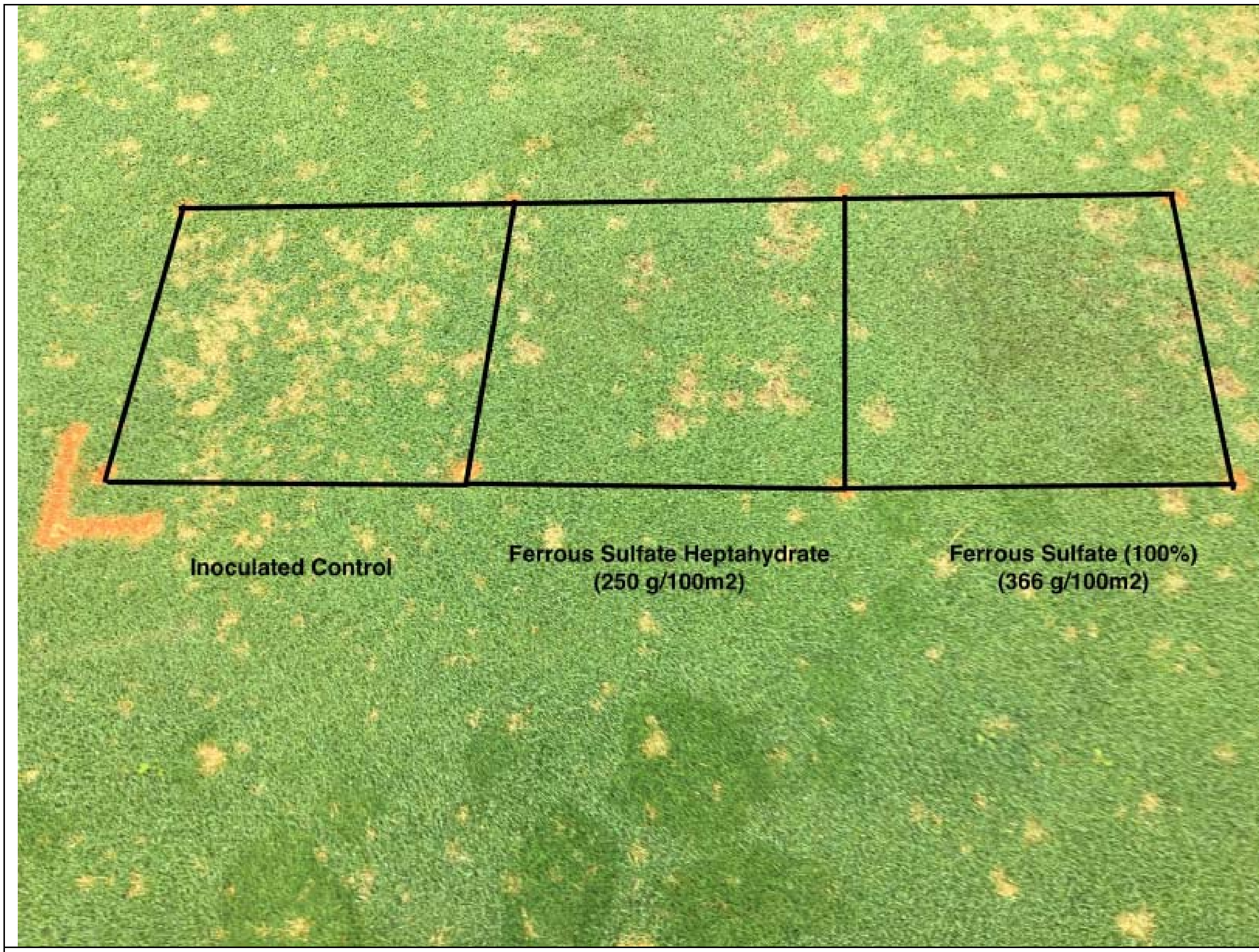
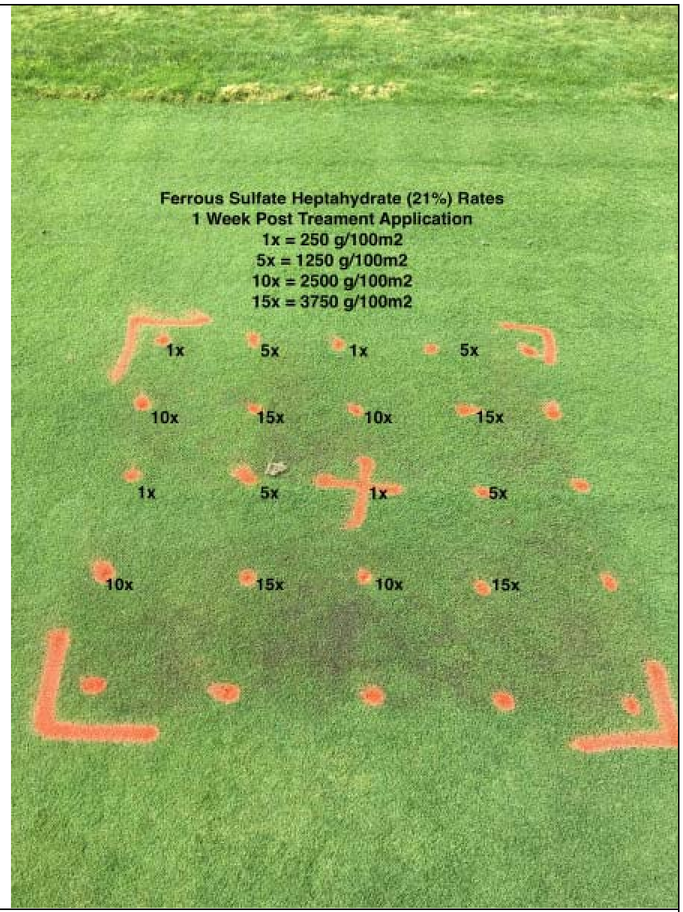


Figure 1: Ferrous sulfate and inoculated control (dollarspot) on creeping bentgrass plots at the Guelph Turfgrass Institute. Plots were first treated 18/08/02, with repeated weekly treatments until 18/09/05. They were inoculated with the dollar spot fungus on 18/08/09. This photo was taken on 18/09/18. Data are shown in Table 1.

	
Figure 5: Phytotoxicity trial with various ferrous sulfate treatments on creeping bentgrass plots at the Guelph Turfgrass Institute. Plots were first treated 18/08/15, with repeated treatments weekly until 18/09/06. This photo taken on 18/08/23, one day post treatment application. Data are shown in Tables 9 - 11.	Figure 6: Phytotoxicity trial with various ferrous sulfate treatments on creeping bentgrass plots at the Guelph Turfgrass Institute. Plots were first treated 18/08/15, with repeated treatments weekly until 18/09/06. This photo taken on 18/08/30, one week post treatment application. Data are shown in Tables 9 - 11.

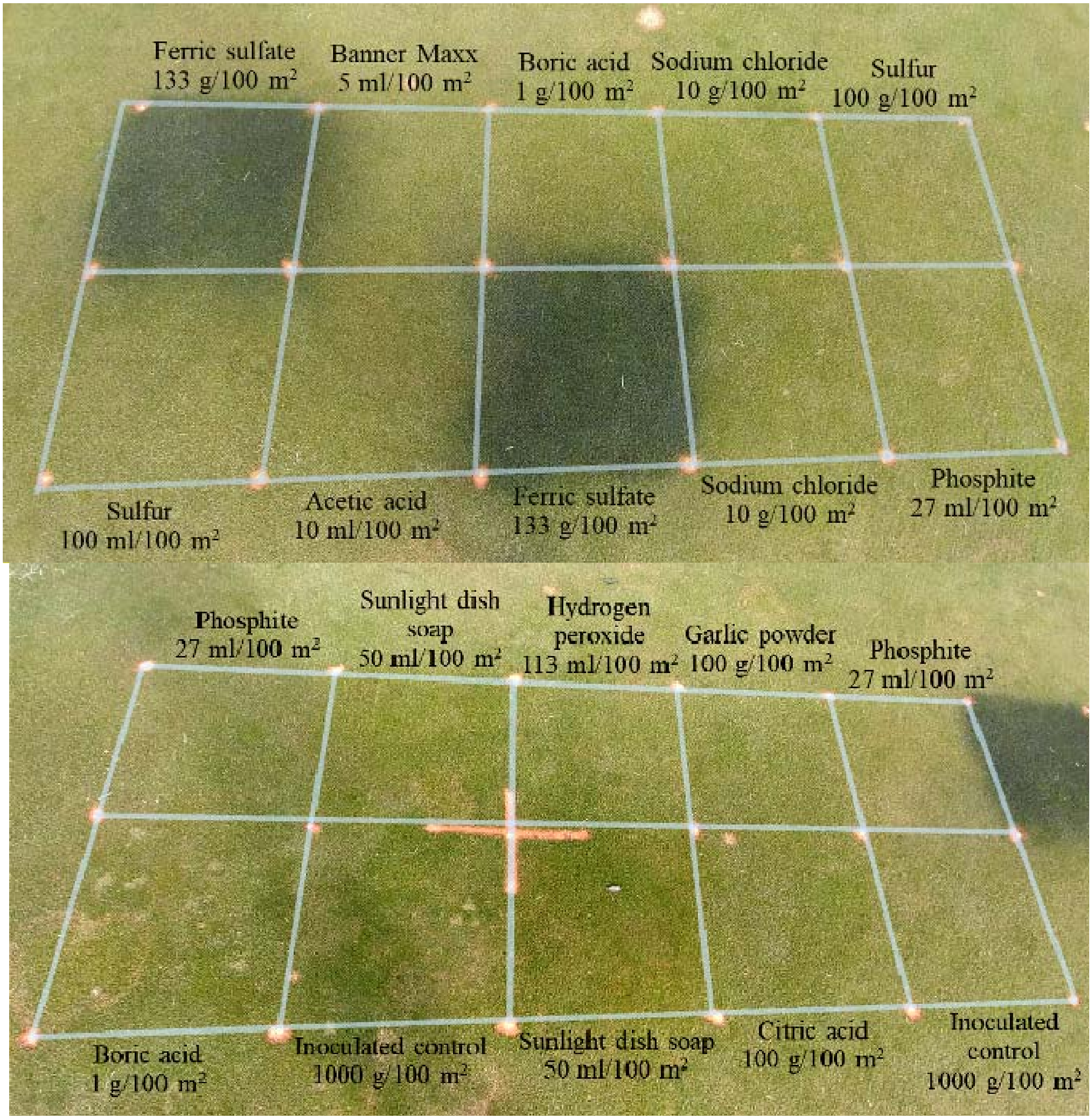
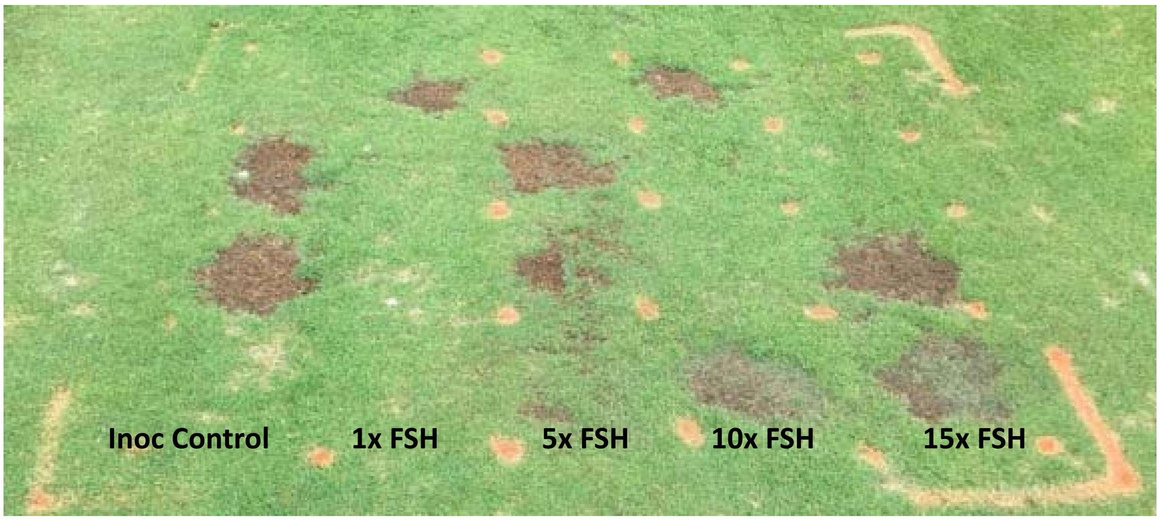


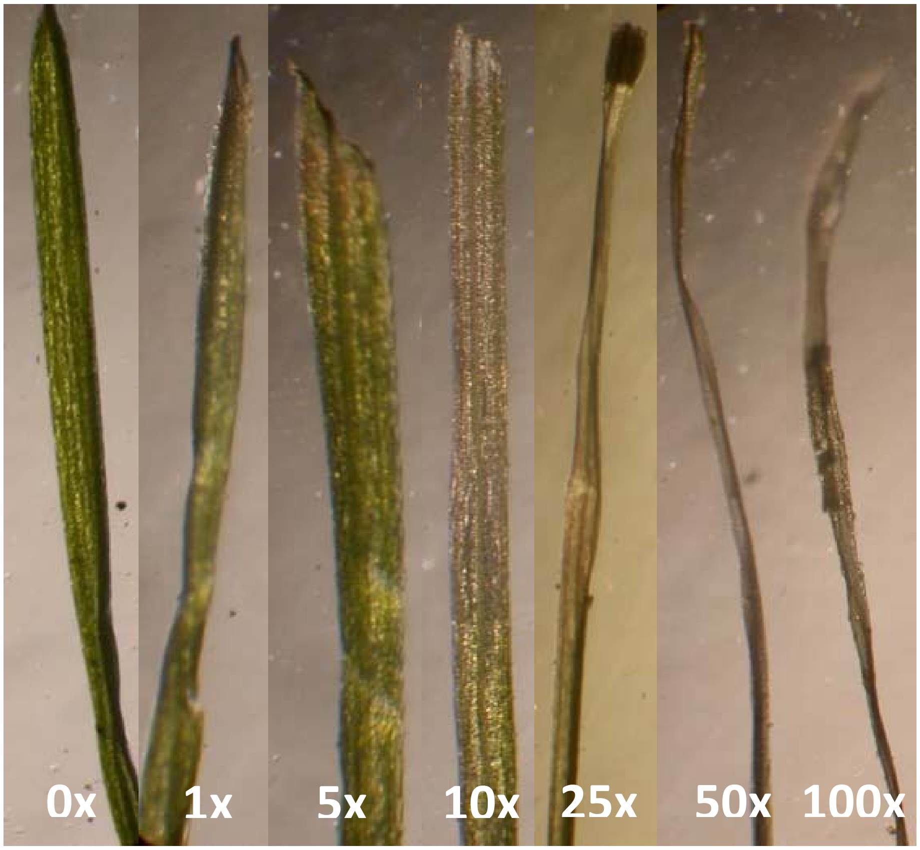
Figure 7: Creeping bentgrass plots at GTI treated twice with each compound in October 2018. They were then inoculated with *M. nivale* in late October and late November, 2018. Pictures were taken in November 2018.

Dec 2019 Results - In Summer 2019, there were more repeats of field trials to confirm what was found in earlier years, plus attempts to assess the darkening of foliage at a microscopic level. Among 10 cultivars of creeping bentgrass, certain cultivars responded more effectively to the 1x FSH treatment than others. Cultivar 007 showed the highest average disease suppression and Cultivar Alpha had the lowest average disease suppression. In multiple tests with 1x FSH, 5x FSH, fungicide check (Banner), and other treatments, it became apparent that 5x FSH could achieve a disease suppression rate as high as Banner, but with an undesirable effect of darkening in the first week which dissipated in the second week. Rates 5x and above could also induce permanent phytotoxicity and scarring (Figure 1). A more detailed study using 1x, 2x, 3x, 5x rates at 3.5 day, 7 day and 14 day intervals showed that there was darkening and phytotoxicity at rates 2x and above that ranged from 5% to 30%, whereas the 1x weekly rate provide significant suppression compared to the inoculated control, but not as much as Banner applied every two weeks.

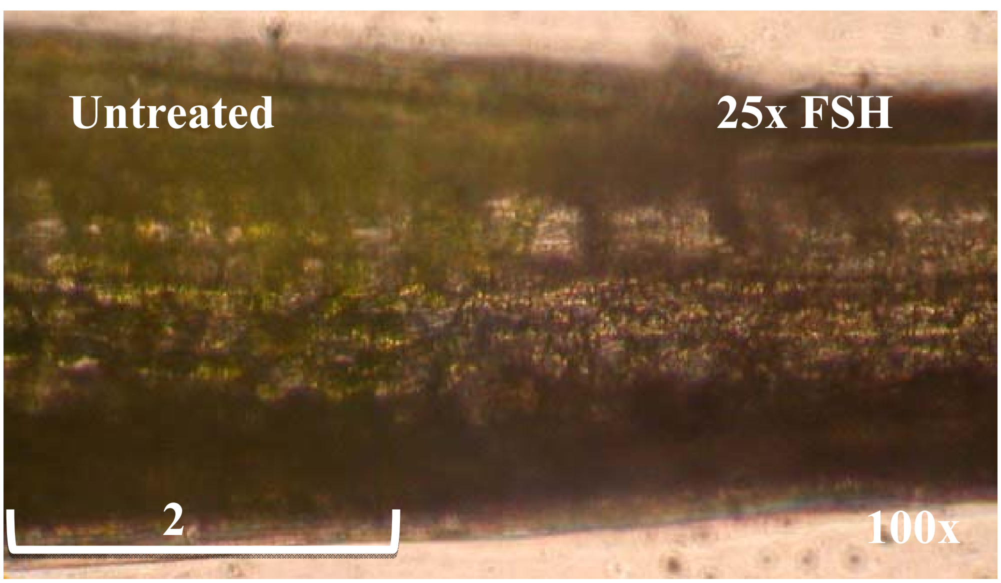


Dec 2019 Figure 1: Scarring from increasing rates of ferrous sulfate heptahydrate (FSH) on creeping bentgrass at fairway height (1x FSH = 250 g/100m^2). Photo taken 28 days post last treatment application.

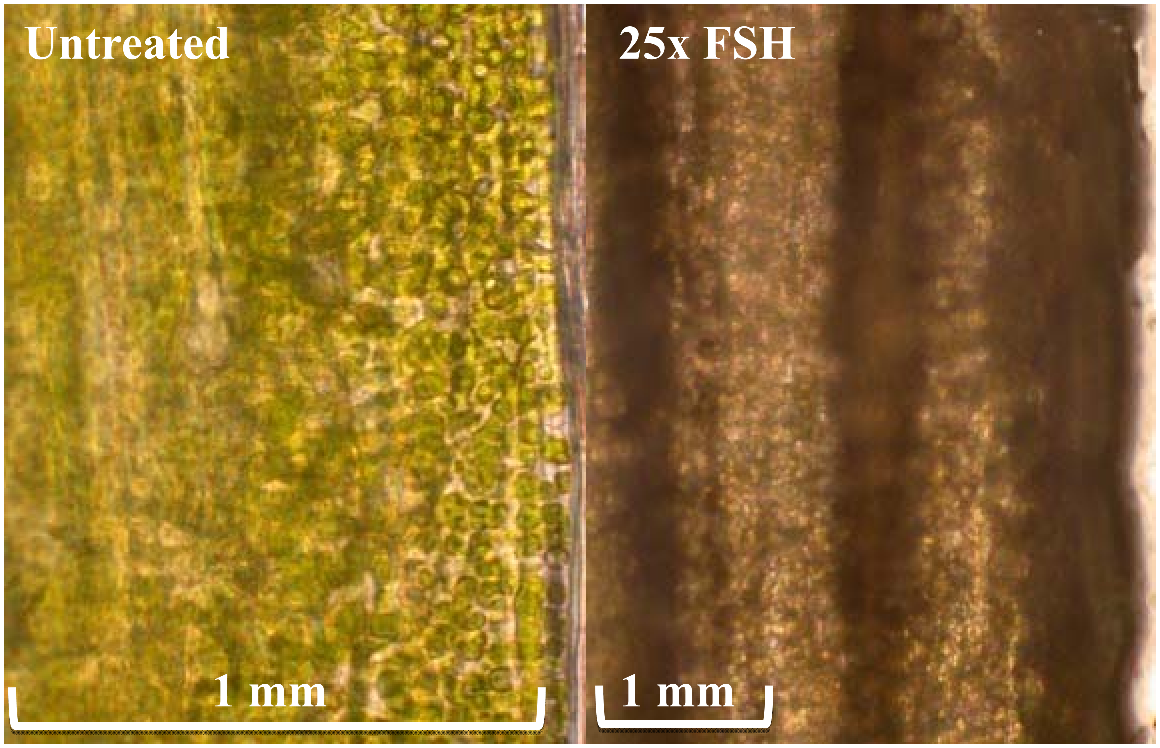
We also investigated phytotoxicity and darkening on individual leaf blades. Figures 2-5 show close-ups and magnified pictures of leaves after treatments. As seen in Figure 2, increasing rates of FSH caused increased blackening. The blackening started from the leaf tips and with high treatment rates, the blackening was more evident down the leaf blade. This implied that there was accumulation toward the tips where the the effect was most pronounced. There was a distinct border between blackened and non-blackened regions (Figure 3). Higher rates (2.50 kg, 6.25 kg, 12.5 kg and 25.0 kg/100m^2) caused a significant change in colour compared to the untreated control (Figure 4). In addition, higher FSH rates caused increased wilting in the leaf blades (Figure 2). The highest rate of 25.0 kg appeared to lead to orange precipitates which were visible on the outer surface of the leaf blade (Figure 5). In addition, as seen in Figure 5, small orange clusters (maybe of iron?) may have collected in the mesophyll.



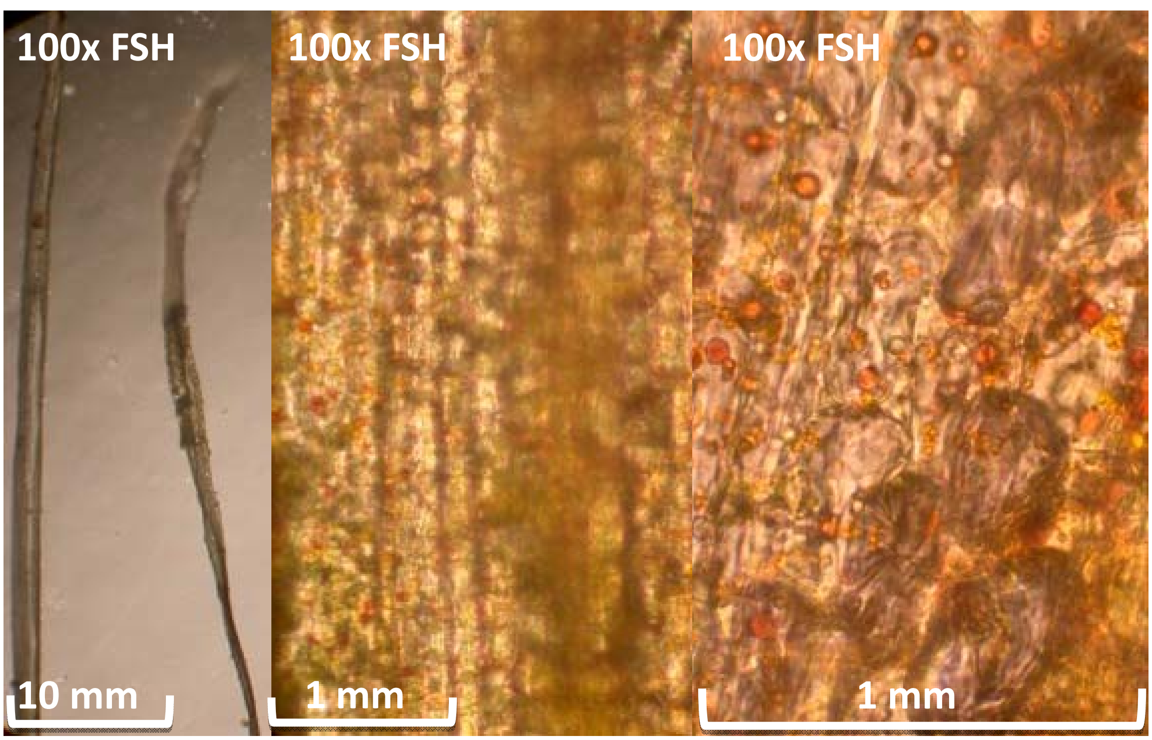
Dec 2019 Figure 2: Phytotoxicity on *A. stolonifera* cultivar Penncross leaf blades at 7 days post treatment application of increasing rates of ferrous sulfate heptahydrate (1x = 250 g/100m^2). Plants were seeded in conetainers and incubated for 10-14 days under 16-hr light / 8-hr dark at 20^{o}C before treatment, and again for 7 days after treatment.



Dec 2019 Figure 3: Phytotoxicity on *A. stolonifera* cultivar Penncross leaf blades at 7 days post treatment application ferrous sulfate heptahydrate (FSH) (25x = 6.25 kg/100m^2) under light microscopy. Plants were seeded in conetainers and incubated for 10-14 days under 16-hr light / 8-hr dark at 20^{o}C before treatment, and again for 7 days after treatment. Border between treated and untreated regions of the leaf blade is visible in the middle.



Dec 2019 Figure 4: Phytotoxicity on *A. stolonifera* cultivar Penncross leaf blades at 7 days post treatment application ferrous sulfate heptahydrate (FSH) (25x = 6.25 kg/100m^2) under light microscopy. Plants were seeded in conetainers and incubated for 10-14 days under 16-hr light / 8-hr dark at 20^{o}C before treatment, and again for 7 days after treatment. Untreated image only received water treatment.



Dec 2019 Figure 5: Phytotoxicity on *A. stolonifera* cultivar Penncross leaf blades at 7 days post treatment application ferrous sulfate heptahydrate (FSH) (100x = 25.0 kg/100m^2) under light microscopy. Plants were seeded in conetainers and incubated for 10-14 days under 16-hr light / 8-hr dark at 20^{o}C before treatment, and again for 7 days after treatment.