

CTRF Annual Progress Report for Project **CTRF 2015-2a** (covering Oct 1, 2016 to Sept 30, 2017)
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PROJECT TITLE: Testing lower risk fungicides for activity against turfgrass diseases (Oct 1, 2015 to Dec 31, 2019 proposed end date) [new items since last annual report (Sept 2016) shown in yellow]

PURPOSE: The purpose of the proposed work is 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 sulphate, 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 on chemicals for cosmetic use on turf. The subsequent scientific objective would be to determine the mode of action with efficacious treatments, 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 is 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 is 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. During this first year of this project, we have been comparing garlic powder, hydrogen peroxide, iron sulphate, 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 (Table 1). The yellowing levels for citric acid, sodium chloride and sulphur treatments were noticeably less (Table 1). These trials were repeated again with similar results. From these lab tests, we selected the lowest rate that provided highest efficacy for each of the compounds, and tested these in the field (Figure 1). The results for 12 products at single rates against dollar spot on a creeping bentgrass putting green are presented in Table 2. 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 on 24 Aug 2016. 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. We continued field trials in Fall 2016 testing product efficacy 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

did not allow for consistent snow mold development in the field, and although there were significant differences between cultivars in disease development, the treatments (inoculated control, phosphite alone and Civitas-Harmonizer) did not give 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 sulphate (21%). Products not showing any significant suppression of disease caused by *Sclerotinia homoeocarpa* include Citric Acid, Borax and Sulfur. Based on these results, field trials against *Microdochium nivale* have been set up in September 2017, and will be continued through winter. We are continuing to test different rates of these compounds against other turfgrass diseases, and will conduct further field tests through 2018 and 2019.

Funding: (REVENUE) for three year study starting October 1 2015 - September 30 2018
 CTRF: \$35,000/yr to Univ. Guelph [TF52548], total \$105,000

Expenditures

Item	Jan16 - Sept 16	Oct 16 - Sep 17	TOTAL
Personnel	0	16,327	16,327
Travel & Field Work	0	44	44
Supplies + Lab Work	5,231	535	5,766
Growth room charges	200	0	200
TOTAL	5,820	16,906	22,338

Total Revenue from CTRF: \$52,500 (since Oct 1, 2015). First funds were received Jan 2016.

NOTE: Because of problems getting the Trust Fund established at the University of Guelph, there were no charges to this project in 2015, although I had labour and other experimental costs incurred. In 2016, I had to spent funds from another grant which was ending, and hence many expenses (including labour costs) were directed to the other grant. At this rate of expenditure, we will have a surplus by the end of this grant period (Oct 2018). I propose that we change the payment schedule and lengthen the duration of this grant (same total amount, but we will work on this project for a longer period, particularly adding another field season in 2019).

Current payment & report schedule	Amount	Proposed payment & report schedule	Amount
October 1, 2015 (no report)	17,500	October 1, 2015 (no report)	17,500
February 15, 2016 (progress report)	17,500	February 15, 2016 (progress report)	17,500
September 15, 2016 (annual report)	17,500	September 15, 2016 (annual report)	17,500
February 15, 2017 (progress report)	17,500	February 15, 2017 (progress report)	none
September 15, 2017 (annual report)	17,500	September 15, 2017 (annual report)	17,500
February 15, 2018 (progress report)	none	February 15, 2018 (progress report)	none
September 30, 2018 (final report)	17,500	September 30, 2018 (annual report)	17,500
		February 15, 2019 (progress report)	none
		Dec 31, 2019 (final report)	17,500
TOTAL	\$105,000		\$105,000

As well, we propose to place the end date of this project for Dec 31, 2019 with a final report to be submitted at that time (and no payment).

I took on a new student in January 2017 (Katherine Stone) who is working full time on this project. There is the potential for this turn into a Ph.D. project, and hence another reason for the request to lengthen the project (but at no additional cost to CTRF).

METHODS - LAB TESTS



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



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

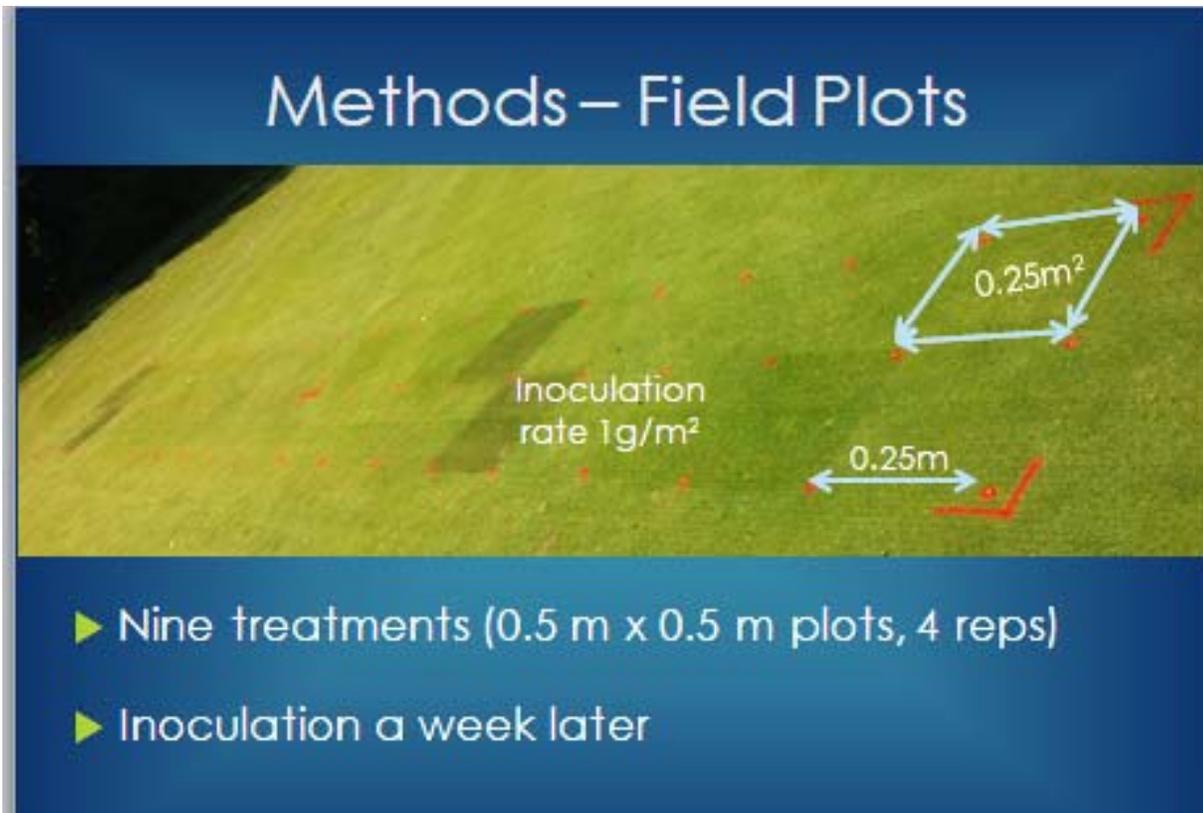


Figure 3: Example of field test with different treatments in a Randomized Complete block design.

RESULTS TO DATE (previously presented; see yellow section below for newer results)

Table 1: Effect of dollar spot infection on yellowing of *Agrostis stolonifera* cv. Penncross following treatment at 7 and 14 days after seeding with various lower risk fungicides. The plants were inoculated with *Sclerotinia homoeocarpa* at 21 days after treatment, and rated over the next 3 weeks for yellowing. For each chemical, the means for the different rates followed by a letter in common indicates that they are not significantly different at $p=0.05$.

Concentrations	Visual Yellowing Percentage (by DPI=days after inoculation)				
	Dpi 3	Dpi 7	Dpi 10	Dpi 14	Dpi 21
5% Garlic powder	23 a	32 a	41 a	48 a	56 a
1% Garlic powder	15 b	24 b	33 b	50 a	54 a
0.5% Garlic powder	3 d	8 d	15 d	33 b	38 b
0.1% Garlic powder	0 d	2 e	6 e	15 c	24 c
Water	8 c	18 c	28 c	38 b	44 b
10 mM Hydrogen peroxide	1 b	5 c	9 d	15 d	20 c
1 mM Hydrogen peroxide	2 b	6 bc	11 cd	21 c	28 c
0.5 mM Hydrogen peroxide	4 b	10 b	15 bc	28 b	48 a
0.1 mM Hydrogen peroxide	3 b	9 bc	17 b	31 b	38 b
Water	8 a	18 a	28 a	38 a	44 ab
200 mM Iron sulphate	3 b	8 bc	18 b	27 b	35 ab
100 mM Iron sulphate	3 b	9 b	16 bc	30 ab	36 ab
50 mM Iron sulphate	2 b	8 bc	17 bc	34 ab	41 a
10 mM Iron sulphate	1 b	4 c	11 c	25 b	31 b
Water	8 a	18 a	28 a	38 a	44 a
1% Acetic acid	15 a	30 a	36 a	46 a	45 ab
0.1% Acetic acid	12 ab	24 ab	28 ab	30 ab	47 ab
0.05% Acetic acid	9 b	20 b	25 b	35 ab	50 ab
0.01% Acetic acid	8 b	16 b	19 b	21 b	41 b
Water	15 a	30 a	36 a	43 a	72 a
0.05% Borax	16 bc	35 bc	42 b	46 b	53 a
0.01% Borax	18 bc	36 bc	44 ab	49 ab	59 a
0.002% Borax	24 a	48 a	52 a	64 a	64 a
0.001% Borax	20 ab	39 b	44 ab	52 ab	72 a
Water	15 c	30 c	36 b	43 b	72 a
4% Citric acid	28 a	34 a	36 a	36 b	14 b
3% Citric acid	19 b	23 b	26 bc	31 bc	21 b
1% Citric acid	16 b	22 b	23 c	24 c	16 b
0.05% Citric acid	25 a	28 ab	30 b	32 b	19 b
Water	16 b	25 b	36 a	52 a	51 a
0.5% Soaps (Dawn dishwashing)	20 ab	23 a	28 b	36 b	35 ab
0.1% Soaps	12 c	15 b	16 c	19 c	22 b
0.01% Soaps	15 bc	20 ab	23 bc	30 b	32 b
0.001% Soaps	21 a	23 a	26 b	30 b	21 b
Water	16 abc	25 a	36 a	52 a	51 a
2% Sodium chloride	18 a	20 ab	23 b	27 b	21 b
1% Sodium chloride	16 a	19 ab	24 b	29 b	23 b
0.5% Sodium chloride	17 a	19 ab	21 b	23 b	19 b
0.1% Sodium chloride	16 a	18 b	22 b	28 b	16 b
Water	16 a	25 a	36 a	52 a	51 a

2% Sulphur	13 a	19 b	16 b	20 cd	19 c
1% Sulphur	15 a	25 ab	17 b	19 d	18 c
0.5% Sulphur	17 a	29 a	23 b	27 b	28 b
0.2% Sulphur	15 a	24 ab	21 b	26 bc	25 b
Water	15 a	25 ab	58 a	60 a	81 a
2*10 ⁻³ g/mL phosphite	8 b	14 b	13 b	22 c	23 b
5*10 ⁻⁴ g/mL phosphite	8 b	15 b	14 b	21 c	22 b
5*10 ⁻⁵ g/mL phosphite	10 b	18 b	18 b	20 c	20 b
1*10 ⁻⁵ g/mL phosphite	11 ab	18 b	12 b	30 b	24 b
Water	15 a	25 a	58 a	60 a	81 a

Table 2: Effect of dollar spot infection on yellowing of *Agrostis stolonifera* and *Poa annua* at greens height (GTI California Green) with weekly treatments from 04 Aug 2016 onwards. The plants were inoculated with *Sclerotinia homoeocarpa* a day after first treatment (05 Aug), and the 0.5 m x 0.5 m plots were rated weekly for percent area affected. An ANOVA followed by a protected LSD was based on four replicate plots per treatment.

Treatments	Rate	Percent Area affected				
		05-Aug	09-Aug	17-Aug	24-Aug	30-Aug
Standard fungicide (Banner)	26 g/100m ²	0.0	0.0	3.0	2.0	2.8
Iron sulfate	100 mM	0.0	0.0	1.8	1.5	8.0
Citric acid	3.0%	0.0	0.0	4.0	6.8	9.8
Hydrogen peroxide	10 mM	0.0	0.0	3.0	7.3	12.8
Phosphite	0.002%	0.0	0.0	3.3	7.8	13.8
Sulfur	1%	0.0	0.0	2.8	7.8	13.8
Soaps	0.50%	0.0	0.0	3.5	8.0	14.5
Garlic powder	1.0%	0.0	0.0	3.5	8.3	15.0
Borax	0.01%	0.0	0.0	4.3	10.0	16.3
Sodium chloride	0.10%	0.0	0.0	3.5	8.0	16.5
Acetic acid	0.1%	0.0	0.0	2.8	10.5	18.5
Inoculated Check	--	0.0	0.0	8.0	17.5	27.5
LSD (p=0.05)		0.0	0.0	1.9	3.8	4.9

The shaded cells are significantly less than the Inoculated Check

Conclusions from 2016 testing

These results to date are very promising, but they are generally not as efficacious as standard fungicides. We still need to test these products against other turfgrass pathogens using this lab system, and also during the next growth season, we will test different rates of select chemicals in the field.

RECENT RESULTS (Since Sept 2016)

Table 3: Effect of dollar spot infection on yellowing of *Agrostis stolonifera* cv. Penncross following treatment at 7 days after seeding with various lower risk fungicides. The plants were inoculated with *Sclerotinia homoeocarpa* at 14 days after treatment, and rated over the next 11 days for mycelial growth (mycel) and yellowing (yellow). For each treatment, means which were significantly less than the inoculated control ($p < 0.05$) are shown with green shading.

Treatment	Rate	Mycel4	Mycel6	Mycel8	Mycel11	Yellow4	Yellow6	Yellow8	Yellow11
Banner (standard)	26 g/100m ²	33.3	20.0	15.0	16.7	3.0	5.7	8.0	13.3
Iron sulfate	100 mM	23.3	20.0	16.7	15.0	3.0	8.0	11.7	16.7
Soap (Sunlight)	3.0%	16.7	14.0	10.0	10.0	4.3	15.0	16.7	16.7
Sodium chloride (salt) 10 mM		23.3	20.0	18.3	11.7	4.3	11.7	18.3	21.7
Hydrogen peroxide	0.002%	53.3	33.3	28.3	22.3	5.0	13.3	18.3	21.7
Sulfur	1%	18.3	15.0	12.3	9.7	4.3	20.0	21.7	21.7
Garlic powder	0.50%	30.0	21.7	16.7	14.0	4.3	20.0	23.3	25.0
Citric acid	1.0%	65.0	36.7	33.3	26.7	5.0	20.0	25.0	23.3
Phosphite	0.01%	60.0	51.7	45.0	40.0	4.7	21.7	25.0	28.3
Borax	0.10%	--	--	--	--	--	--	--	--
Acetic acid (vinegar)	0.1%	--	--	--	--	--	--	--	--
Inoculated Check	--	75.0	41.7	36.7	23.3	5.0	18.3	33.3	28.3
LSD ($p=0.05$)		34.0	29.8	28.0	23.7	12.5	13.7	16.0	17.9

Table 4: Spring 2017 *Microdochium* patch disease severity (AUDPC) on bentgrass cultivars inoculated with *Microdochium nivale* and treated with (W) water, (P) phosphite or (C+H) 5% Civitas+ 0.3% Harmonizer. [Data from S. Stricker MSc thesis]

Cultivar	Disease severity ^a (AUDPC ^b) by treatment ^c							
	Non-inoculated				Inoculated			
	Water	Phosphite	C+H	Treatment LSD ($p < 0.05$)	Water	Phosphite	C+H	Treatment LSD ($p < 0.05$)
PennA4+T1	534	475	383	911	510	798	442	1054
Alpha	376	407	256	257	298	306	267	383
Focus	0.0	0.0	6.8	14	6.8	0.0	0.0	8.1
L93	27	85	2.3	125	63	27	54	112
Mackenzie	161	69	97	360	44	35	65	109
OO7	35	59	33	50	97	24	46	105
PennA4	404	288	65	718	146	127	133	240
Penncross	2.7	3.5	109	218	44	61	87	187
T1	55	7.0	20	112	4.7	125	33	256
Tyee	59	70	107	112	128	74	100	161
V8	8.3	14	52	50	39	40	2.7	112
Cultivar LSD ($p < 0.05$)	436	319	170		380	356	231	

^a Disease severity was rated as percent yellowing every week from mid- January to early April for up to 3 replications. Means were subjected to ANOVA analysis, and Fisher's protected Least Significant Difference (LSD) test ($p < 0.05$).

^b AUDPC is area under the disease progress curve which represents cumulative disease severity over time and is calculate from multiple assessments of disease severity.

^c On 09 Mar and 29 Mar 2017, the plots were sprayed with 25 ml of either water, 50 mM phosphite, or 5% Civitas+ 0.3% Harmonizer. On 17 Mar and 05 Apr 2017, half of the plots were inoculated with 2.5 g of dried ground wheat seed inoculum.

^d Grey shaded boxes indicate a significant difference among treatments (row) or cultivars (column).

Table 5 summarizes the results of 10 repeat experiments, each lasting 6 weeks. The results show that most of the products chosen could result in significantly reduced yellowing, but none have been found to provide the level of control provided by Banner (26 ml/100m²). One of the disadvantages of these in vitro tests is that inoculum rate may be too high and it overwhelms the resistance provided by the fungicide alternative. But when inoculum rate is too low, then disease does not develop.

Table 5. Meta-analysis of 10 repeat experiments use conetainers (14 cm long with 70 g of topdressing sand) for growing creeping bentgrass 'Penncross' treated with products listed in Table 3, inoculated a week later, and evaluated 7 and 14 days after inoculation with *Sclerotinia homoeocarpa*. These values represent percent suppression compared to the inoculated control in each of the 10 tests, in which the inoculated control showed yellow ratings ranging from 10% to 33%. Means with green shading show statistically significantly suppression of yellowing.

Treatment	Rate	Disease Suppression at 7 days after inoculation (%)	Disease Suppression at 14 days after inoculation (%)
Banner (standard)	26 g/100m ²	63.6	77.9
Soap (Sunlight)	3.0%	33.0	55.1
Acetic acid (vinegar)	0.1%	27.1	34.3
Sodium chloride (salt)	10 mM	17.0	32.8
Hydrogen peroxide	0.002%	25.9	31.8
Phosphite	0.01%	26.4	27.7
Garlic powder	0.50%	-9.6*	24.1
Iron sulfate	100 mM	7.7	20.8
Citric acid	1.0%	-10.1	16.7
Borax	0.10%	0.05	15.3
Sulfur	1%	-16.0	6.0
LSD (p=0.05)		0.36	0.21

* Negative value show yellow levels above the inoculated control.

FUTURE WORK:

We will continue to replicate this work, and also test other pathogens of other diseases in the lab (brown ring patch, brown patch, anthracnose, Microdochium patch...). In spring 2018 and summer 2018, we will conduct field tests with select treatments against dollar spot disease, and maybe rust, red thread, and whichever other diseases might appear in the field. Although the 'home remedy' treatments do appear to be as efficacious as traditional synthetic pesticides, they might be able to reduce disease to levels that might become more acceptable in the future. We are also interested in how these compounds work, whether they have direct inhibitory activity against the pathogens or stimulate the plants to fight off disease or some other mechanisms.