CTRF Annual Progress Report for Project **CTRF 2015-2a** (covering Oct 1, 2017 to March 1, 2018)
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PROJECT TITLE: Testing lower risk fungicides for activity against turfgrass diseases (Oct 1, 2015 to Dec 31, 2019) [new items since last annual report (Sept 2017) 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.

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	Oct 17 - Mar 18	TOTAL
Personnel	0	16,327	6,009	22,336
Travel & Field Work	0	44	0	44
Supplies + Lab Work	5,231	535	155	5,921
Growth room charges	200	0	400	600
TOTAL	5,431	16,906	6,564	28,903

Total Revenue from CTRF: $70,000 (since Oct 1, 2015). First funds were received Jan 2016.

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

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 2016-2017, 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.

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/100m^2). 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.

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 Sulphate (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 sulphate has shown the highest efficacy, this compound will be a major focus of our remaining work.

CONTINUING AND 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 are conducting 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.

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

METHODS - FIELD TEST

Figure 3: Field testing at GTI using mature creeping bentgrass and inoculating with a pathogen

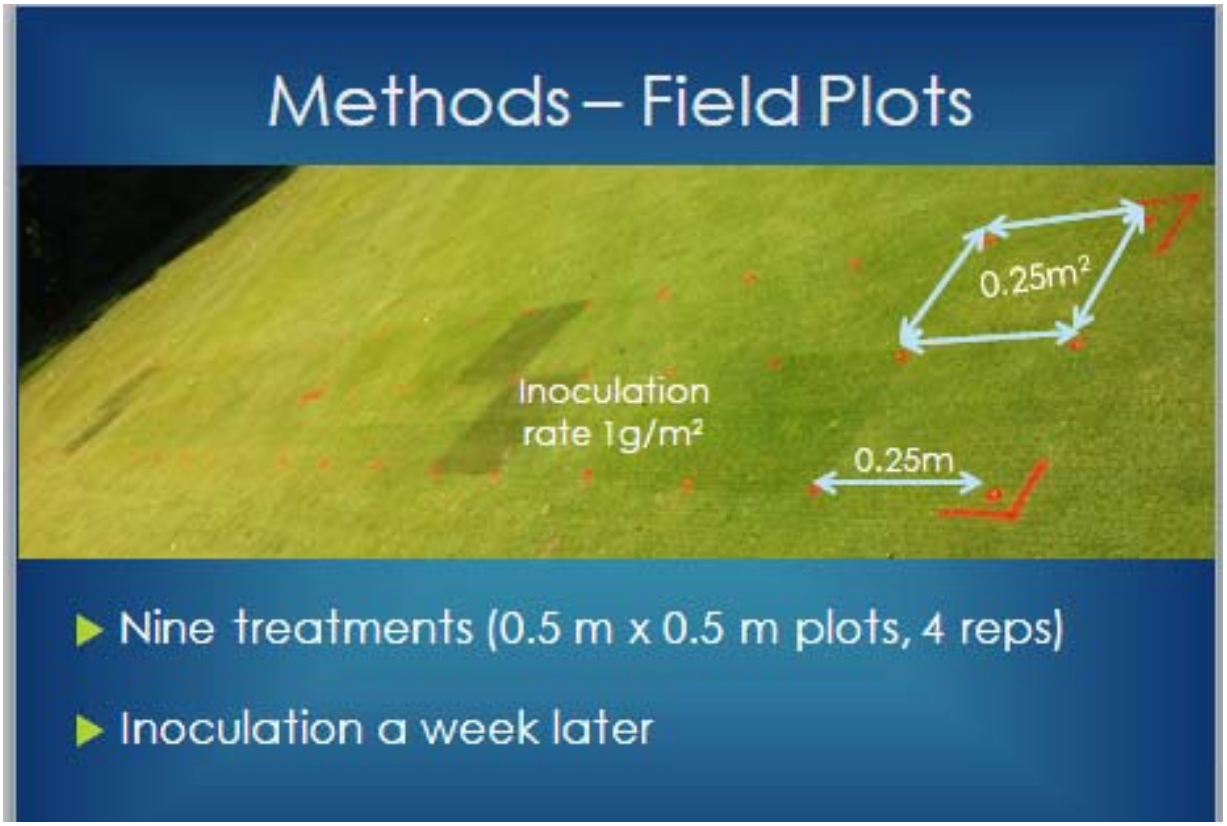

Figure 3: Example of field test with different treatments in a Randomized Complete block design.

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.

		Percent Area affected				
Treatments	Rate	05-Aug	09-Aug	17-Aug	24-Aug	30-Aug
Standard fungicide (Banner)	26 g/100m2	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

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/100m2	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]

	Disease severity[a] (AUDPC[b]) by treatment[c]							
	Non-inoculated				Inoculated			
Cultivar	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. Meta-analysis of 10 repeat lab 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 epresent percent suppression compared to the inoculated control in each of the 10 tests, in whch 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 7 days after inoculation (%)
Banner (standard)	26 g/100m2	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.

Table 6. Dollar spot field trial using methods and layout since in Figures 3 and 4, showing disease suppression compared to the inoculated control. Means with green shading show statistically significantly suppression of disease.

		Percent Disease Suppression		
Treatment	Rate (in 100 ml/ m2)	DPI 07	DPI 14	DPI 20
Inoculated Check	1 g / m^2	0	0	0
Ferric Sulfate	27.4 mM	8	21	87
Banner Maxx	5 ml/100 m2	64	78	56
Garlic Powder	1%	-10	24	17
Borax	0.01%	1	15	17
Hydrogen Peroxide	10 mM	26	32	-8
Acetic Acid	0.10%	27	34	-21
Citric Acid	1%	-10	17	-22
Phosphite	0.53 mg/ml	26	28	-24
Sodium Chloride	0.10%	17	33	-35
Sunlight Dish Soap	0.50%	33	55	-44
Sulfur	1%	-20	6	n/a
LSD (p<0.05)		36	21	42