OTRF Project No. ____________

OTRF Funded Research Project

☐ Interim Report ■ Final Report

Title	Eco-inoculants: A paradigm shift technology to suppress dollar spot disease in turfgrass using a mixture of beneficial microbes
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Executive Summary & Background rationale

There is interest in replacing synthetic pesticides with bio-based alternatives. Today, 35% of all fungicides applied to turfgrass is to control a disease called dollar spot which may lead to environmental effects on natural ecosystems. The total cost to control dollar spot disease globally may be as high as $400 million/year. The goal of this project was to create and test eco-friendly technologies to suppress dollar spot disease in turfgrass, in collaboration with scientists at the University of Guelph. The project is attempting to develop a foliar spray based on beneficial microbes that naturally live inside different grasses (including corn and finger millet). During this 6-month project, the project conducted extensive field trials during the 2013 summer season to test two microbes (called A12 and C11) as foliar sprays that were previously shown to suppress dollar spot disease under replicated greenhouse experiments. A total of 180 field subplots were tested. The field results showed significant variability, with turfgrass sprayed with one microbe (C11) in one field block showing excellent health, but not in others. The results suggest that the foliar sprays need to be optimized in terms of the dosages of the beneficial microbes or other improvements to the formulation, for further testing. Another problem may be that the beneficial microbes are being outcompeted by natural soil microbes, or they cannot tolerate variability in outdoor temperatures. To make further progress in this area, a DNA fingerprinting technology was optimized to be able to measure the persistence of the beneficial microbes in turfgrass over time after spraying. The results showed that microbes A12 and C11 colonize turf shoots but not roots. Now that the A12 and C11 detection technologies have been optimized, in future studies, the effect of ecological competition or temperature on the persistence of A12 and C11 can be systematically studied. A second project was also undertaken during this 6-month period: the short-term goal was to optimize and use DNA fingerprinting techniques to measure the growth of 250 different potentially-beneficial microbes in turfgrass after parallel spraying; the longer-term objective was to identify defensive microbes that could be pyramided together to create a complex foliar spray (called an "eco-innoculant") to outcompete harmful microbes that might attempt to invade turfgrass. The project successfully infected 660 different turf plants, isolated DNA from 1320 root and shoot samples, and optimized the DNA fingerprinting technology as already noted. The samples were sent to a private company for analysis. The quantity of the inoculated microbes in turfgrass was very low, but it was shown that by double-amplifying each microbial signal (two rounds of quantitative PCR), microbes could be quantified turfgrass shoots and roots, which is a positive step forward for this technology. The project is awaiting the final report from the private company after which we will proceed to pyramiding

the microbes and testing them as a complex foliar spray to combat a wide spectrum of diseases in turfgrass. Adoption of either of these technologies will depend on results from further field trials but the project has moved forward rapidly in 6 months. The lessons learned from this project apply to the use of bio-based fungicides in related grain-producing grasses such as corn and wheat.

Objectives & Results	

Objective 1. Field trial of single inoculants
This objective aimed to conduct field trials at the Guelph Turfgrass Institute to test the effectiveness of each beneficial microbe (endophyte) to control Dollar spot disease on creeping bentgrass. The project proposed to conduct trials with 5 spatial replicates, using a randomized block design, and then repeating each trial twice during the summer season. The specific questions proposed were:
1.1 To test whether the beneficial microbes must be sprayed before or after fungal infection;
1.2 To test whether the beneficial microbes must be re-sprayed;
1.3 To test different ideal spray formulations;
1.4 To test spray efficacies following application of fungicides, fertilizers and rainfall to mimic real-world conditions on golf courses.
Objective 1 UPDATE:
Beneficial microbes C11 and A12, or appropriate controls (e.g. dollar spot fungus only) were sprayed on 180 subplots (30 treatment blocks) at the Guelph Turfgrass Institute (GTI) on creeping bentgrass and mixtures of turfgrass cultivars. The project conducted experiments using >5 spatial replicates in 2m x 3m subplots at two different fields.
1.1 As noted in the Objectives, the beneficial microbes were sprayed either 1-week before infection with dollar spot disease or 7-10 days after infection with dollar spot disease.
1.2 For this initial field test, it was decided to spray all plots every 2 weeks rather than a single spray in order to maximize colonization with the beneficial microbes.
1.3 We were only able to test single formulations because we decided to increase the plot sizes from 1m x 1m to 2m x 3m, because we noticed cross-contamination between adjacent subplots caused by the lawn mower: this caused us to focus the experiment as the amount of spray needed was increased 6-fold compared to the original plan.
1.4 The field trials mimicked real world conditions on golf courses except that the mowing frequency was reduced in order to minimize cross-contamination between the treatments which were adjacent to one another.
Objective 2. Eco-innoculant development and testing
The long-term objective here was to test the concept that spraying turfgrass with a complex mix of harmless microbes could suppress dollar spot disease in creeping bentgrass. The specific objectives for 6 months were:
2.1 To discover which of his 250 beneficial microbes (endophytes) most effectively colonize creeping bentgrass harmlessly using DNA quantification approaches (qPCR of bacterial 16S rDNA), 3 replicate pools (5-7 plants) per endophyte, two tissues (root and shoot separately), for a total of 1500 DNA tests. We proposed to subsequently pyramid together the best colonizers in different combinations and use qPCR to determine combinations of harmless microbes that are stable in creeping bentgrass.
2.2. We also proposed to conduct an initial growth chamber/greenhouse trial to test the effectiveness of each complex mixture of endophytes to suppress Dollar Spot disease.
Objective 2 UPDATE:
2.1 As proposed, a total of 250 beneficial endophytes were attempted but only 220 different microbes grew sufficiently for testing; these 220 microbes were inoculated into creeping bentgrass using 3 replicate pools (5-7 plants/pool per microbe), generating a total of 660 plants. Plant DNA (containing the DNA of each microbe) was isolated from dissected roots and shoots individually, which generated 1320 DNA samples. The DNA samples were sent to a private-sector DNA testing company (MOGene). In collaboration with the University of Guelph scientists, MOGene discovered that concentrations of the

microbes in turfgrass were often low, so spent considerable time to optimize the DNA fingerprinting technology; however, they discovered that by double-amplifying the microbial signal (two rounds of quantitative PCR), the microbes could be quantified.
2.2 We are awaiting the final report from MOGene after which we will test the best colonizers in growth chamber/greenhouse experiments to determine which combinations of potentially-beneficial microbes are most stable in creeping bentgrass and can suppress dollar spot disease: this latter experiment can proceed rapidly (likely 2 months) since the qPCR and all other protocols have been optimized.

Suggestions for further study

Recommendations for further study:
1. The field trial results suggest that the foliar sprays of beneficial microbes need to be optimized in terms of the dosages of the microbes or other improvements to the formulation, for further testing. Another problem may be that the beneficial microbes are being outcompeted by natural soil microbes, or they cannot tolerate variability in outdoor temperatures. To make further progress in this area, DNA fingerprinting can likely measure the persistence of the beneficial microbes in turfgrass over time in the field. This combined approach (field testing with DNA fingerprinting of beneficial microbes) is certainly applicable to food crops, and may help to accelerate the inoculant industry which shows tremendous potential but few successes thus far (except for legumes such as soybeans).
2. After spraying the microbial sprays in the field, the concentrations of the microbes in turfgrass were often low. However, the project has discovered that by double-amplifying the microbial signal (two rounds of quantitative PCR), the microbes could be quantified (DNA fingerprinted) reliably. This lesson and the associated protocols that we will publish, can now help accelerate the entire turfgrass industry as inoculant sprays are already used routinely but with little tracking in the field as to their actual persistence over time and under different environmental or treatment conditions (e.g. fungicides, fertilizers, temperature, mowing).

ADDITIONAL NOTEWORTHY RESULTS

1. During the last 6 months, additional experiments were undertaken to discover additional microbes that could strongly suppress dollar spot disease: one new microbe was identified from an ancient corn that strongly suppressed dollar spot disease indoors. This will be tested in future greenhouse experiments and field trials, alone, or in combination with the previous two beneficial microbes.
2. In an unexpected and exciting result, it was discovered that one endophyte (C11) also suppresses dandelion seed germination, so perhaps it may be possible to develop a unique all-in-one biocontrol spray against weeds and fungal disease which would be very valuable to the turfgrass industry

Development of an eco-friendly alternative to fungicides to suppress dollar spot will help the turf sector remain competitive. Practical knowledge on how to control this fungal disease will reduce the usage of highly regulated control products, resulting in very positive environmental and economic impacts.
If the technologies prove to be robust, no doubt one or more commercial products will be developed which will be economically self-sustaining to provide benefits to the turfgrass industry

Project Communication

The intended benefit of the project will provide valuable information to all turfgrass (plant) managers (including the agricultural sector) since creeping bentgrass cultivars are commonly used across Canada, and Dollar Spot disease is a significant cold-season turf disease. In addition, the eco-innoculant technology pilot project in turf is a paradigm for other related grasses such as corn, wheat and barley, which impact at least seven provinces in Canada.

In addition to information posted here on the Ontario Turfgrass Research Foundation website, practical applications will be presented at the Ontario Turfgrass Symposium, and other national scientific meetings. Following completion of a related project involving these beneficial microbes, the research will be published in two peer-reviewed journal publications, one that describes the original two biocontrol agents, and a second publication that describes the testing of 250 microbes. The research will also be presented in 2014 at an international bio-control conference.