Title	Management of Take-all Patch in High pH Soils
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Executive Summary

Take-all patch (TAP) is a fungal disease of creeping bentgrass (*Agrostis stolinifera* L.) most commonly caused by the fungus *Gaeumannomyces graminis* var. *avenae (Gga)* Saccardo. Between 2012 and 2014, 44% of diseased turf samples analyzed by the Guelph Turfgrass Institute (GTI) Diagnostic Laboratory at the University of Guelph were diagnosed with TAP, 47% of which were from Ontario. Developing management strategies to reduce TAP severity would be beneficial to the turf industry. In some agricultural and turfgrass systems, a natural decline in disease severity has been observed over time and is believed to be due to soil suppression. However, take-all decline has yet to be observed in Ontario. The absence of decline phenomena may suggest a variety of situations, including: 1) Ontario turf TAP symptoms are not caused by *Gga* and 2) soil conditions (specifically high pH) are not conducive to the development of suppressive soils. This study is being conducted to both confirm the causal agent of TAP in Ontario and to investigate alternative methods of disease management through cultural practices. It can be postulated that cultural practices that aim to improve plant health in tandem with creating suppressive soils can effectively reduce the development and severity of TAP on *A. stolinifera*.

Background

Creeping bentgrass (*Agrostis stolinifera* L.) is considered to be an optimal turfgrass for golf course greens in cool-season environments (Lawson *et al*., 2012). Healthy stands of creeping bentgrass spread vigorously, tolerate low cutting heights, and recover quickly from damage (Lawson *et al.*, 2012). The uniformity of shoot and leaf density makes creeping bentgrass an ideal rolling surface for golf and lawn bowling (Croce *et al.,* 1995). The presence of creeping bentgrass prevents the growth of weeds, including weaker grass species that tend to require higher maintenance and nutrient inputs (Smiley *et al.,* 2005).

Creeping bentgrass is susceptible to the fungal pathogen *Gaeumannomyces graminis* var *avenae* (*Gga*), a known causal agent of take-all patch (TAP). *Gga* is a destructive pathogen capable of killing healthy stands of high quality turf (Clarke and Gould, 1993; Smiley *et al*., 2005). Although take-all patch is a serious disease of creeping bentgrass, the occurrence of this disease often wanes over time. Under acidic soil conditions, a phenomenon known as take-all decline has been observed (Cook, 1981; Bockus and Tisserat, 2000), whereby the presence of the pathogen will decline over five to seven years following its appearance (Smith, 1957; Clarke and Gould, 1993). Soil pH is typically variable in Ontario (Lauzon *et al.*, 2005). Severe

Gga infections reportedly occur in soils with pH above 7.0 in the first 2.5 cm of the soil profile (Smith, 1957; Clarke and Gould, 1993), thus it is possible that this disease may be mediated through the application of acidifying soil amendments.

The purpose of this investigation is twofold: to confirm the causal agent of take-all patch in Ontario using both classic and molecular techniques, and to determine the efficacy of various cultural and chemical practices, including soil and water acidification, on the control of *Gga*.

Objectives

Objective 1: Confirm the causal agent of TAP in Ontario using classical and molecular identification techniques.

Success to date: As mentioned in the previous report, we had successfully identified the causal agent of TAP through molecular techniques using samples from Alberta, Saskatchewan, and Ontario. The next step was to confirm the results through classical techniques, specifically performing Koch's postulates. The study was initiated in November of 2016, but the pathogen is extremely slow-growing and less virulent (takes longer to infect the host than *G. graminis* var. *avenae*) so the study is not complete yet. Plants were infected with the pathogen on November 11, 2016 but signs of the pathogen did not become evident until the middle of January 2017. The plants were removed from the pots, slides of the roots were made and examined under the microscope. When a positive identification of runner hyphae was made the section was marked. The marked sections of roots were surface sterilized using a protocol involving silver nitrate. Roots were plated on selective media and incubated at 18°C. It was often necessary to subculture from these plates to reduce the incidence of contamination. Due to the slow-growing nature of the pathogen it will be several more weeks before we can confirm the identity of the isolated pathogen. We do have promising results from one of the four isolates tested but these need to be confirmed with molecular techniques.

Update for April 2017: We have successfully cultured a fungus from the roots of the plants in the Koch's postulates experiment. Based on morphological features, we believe it to be the same fungus (*G. cylindrosporus*) that we used to inoculate the plants. However, we are currently waiting for cultures from the ATCC so that we can confirm the identity of the isolated fungi using molecular techniques.

Objective 2: Conduct a greenhouse trial to investigate the combined effect of nitrogen formulation and acidified irrigation on disease development and severity.

The greenhouse trial is in progress currently. Josh moved the greenhouse trial into a growth chamber so that the conditions could be controlled more closely. He chose urea as a negative control and azoxystrobin as a positive control. Treatments included ammonium sulfate as an acidifying fertilizer; acid-treated irrigation; manganese sulfate; and a combined treatment of manganese sulfate, ammonium

sulfate and acidified irrigation. The experiments expected end date is late April 2017.

Update for April 2017: The growth chamber experiment has just been completed and the data analyzed. We noted a significant effect of the fungicide treatment and also noticed a reducing in the disease with the combined treatment that included ammonium sulfate, acidified irrigation and manganese sulfate. Although the cultural treatment did not significantly reduce disease at the 0.05 alpha level, we did see a significant reduction at the 0.10 level. This suggests that the cultural practices should be incorporated in the field trial that is beginning shortly. We may wish to repeat the growth chamber trial as well but need to let it run for longer since the newly identified pathogen takes longer to infect its host.

Objective 3: Conduct a field trial to investigate the disease suppression of several cultural practices.

The results from the growth chamber experiment have helped determine the treatments for the field trial. Due to the slow-growing nature of the pathogen the experiment will need to run for the duration of the summer and likely into the fall. This will ensure that the pathogen has adequate time to infiltrate the root tissues of the plants.

Update for April 2017: We are in the process of preparing inoculum for the field trial. At this time we plan to use a number of techniques for inoculation to ensure that there is adequate opportunity for the pathogen to infect the plants. We will perform core aeration on the research green and apply the inoculum into the holes as well as over the surface of the green. We plan to initiate the experiment in May and continue the experiment until the Fall.

Materials and Methods

Isolation, Culture, Molecular Identification of TAP causal organism

Samples of *A. stolinifera* either displaying symptoms or with a history of take-all patch were obtained from various golf course sites across Canada. Roots from samples were observed microscopically for evidence of runner hyphae on root tissues. Sections of the microscope slide where runner hyphae were located were designated with a marker for isolation. Approximately 1 cm of root tissue was obtained from each sample. Root tissues of *A. stolinifera* were surface sterilized with 1% silver nitrate. Sterilized root tissue was plated on potato dextrose agar amended with streptomycin, kanamycin, and gentamicin at 50ppm, 50 ppm, and 10ppm, respectively. Samples were incubated at 18^{o} C for 21 days. Samples were subcultured on to clean media to ensure that the culture was pure.

DNA was extracted from the homogenized fungal tissues using a kit purchased from Norgen. Polymerase chain reaction was used to amplify DNA sequences specific to

fungal organisms. Molecular identification, through sequencing, of each sample was conducted using primers designed to amplify the ITS region of fungal organisms. The primers used for the identification of fungal isolates are: NSA3: AAACTCTGTCGTGCTGGGGATA and NLC2: GAGCTGCATTCCCAAACAACTC (Martin *and Rygiewicz,* 2005), synthesized by University of Guelph Lab Services. The visualization of amplified DNA was done using 0.1% agarose gel electrophoresis (with an expected band size of ~1100bp). Successfully amplified DNA fragments were submitted to University of Guelph Lab Services for DNA sequencing. Each fungal organism will have some variation within its ITS region and this information can be used to conduct a search for the identity of the fungal organism using the National Centre for Biotechnology Information (NCBI) database.

Greenhouse Trial: Managing TAP with Acidifying Fertilizer and Acidified Irrigation

To test the preventative effects of acidified soils on TAP development and severity, a greenhouse trial will be conducted by inoculating pots of creeping bentgrass with samples of *G. cylindrosporus*. The project began in January of 2017. The pots were 10 cm x 10 cm and were prepared with 90% calcareous sand and 10% peat moss. A4 creeping bentgrass seed was applied at a rate of 0.25 g/pot. The plants were left under misters during the winter break and full germination was seen early January 2017. Plants were transferred to a growth chamber after six weeks of growth and the preventative treatments were started. Treatments were administered weekly as outlined in Table 1-A. Inoculum consisted of 4 different *G. cylindrosporus* isolates. Plants were inoculated with 4.5 g of *G. cylindrosporus* per pot. Using a pencil to penetrate the soil surface, three holes approximately 4 cm deep were created and filled with inoculum, followed by 100 mL of deionized water. Three weeks after inoculation, curative treatments were applied using same rate as preventative treatments. Eight weeks after inoculation the samples will be harvested and analyzed for runner hyphae, both incidence and severity will be documented. It is possible that there will not be sufficient disease infiltration after eight weeks, if that is the case the experiment will be extended for a few more weeks.

Table 1-A: Treatments were administered on Tuesdays in 60 mL. Plants are watered with 60 mL of tap water on Fridays. Acidified irrigation & combination treatment receive 60 mL of pH 4 water every watering.

Treatment	Weekly nitrogen application[1]	Weekly potassium application[1]	Additional treatment, quantity and source	Watering
Urea (Negative control)	3.553 mg urea per pot	0.77 mg mono-potassium phosphate fertilizer (MKP) per pot		tap water once per week
Heritage (Positive control)	3.55 mg urea per pot	0.77 mg MKP per pot	0.252 µL heritage per pot every 28 days in 20 mL of water	tap water once per week

Ammonium Sulfate (AS)	7.62 mg AS per pot	0.77 mg MKP per pot		tap water once per week
Manganese sulfate ($MnSO_4$)	3.55 mg urea per pot	0.77 mg MKP per pot	1.68 mg $MnSO_4$ in 20 mL of tap water every 12 weeks	tap water once per week
Acidified irrigation (AI) (pH 4)	3.55 mg urea per pot	0.77 mg MKP per pot	60 mL of acidified irrigation (pH 4)	All water applied was acidified, an additional 60 mL was applied once per week
Combination	7.62 mg AS per pot	0.77 mg MKP per pot	1.68 mg $MnSO_4$ in 20 mL of tap water every 12 weeks	All water applied was acidified, an additional 60 mL was applied once per week

[1] nutrient were applied in 60 mL of water, if multiple fertilizers were applied the fertilizers were mixed in the solution

Field Trial: Comparing Cultural and Chemical Methods of Disease Control

The purpose of field trials for this project is to evaluate the efficacy of cultural practices in reducing take-all patch severity. This research will be conducted at the Guelph Turfgrass Institute (GTI) in Guelph, ON. There will be several treatments applied to plots of *A. stolinifera* previously grown on the site. These treatments include: a control with no treatments, core aeration, irrigation acidification, application of acidifying fertilizers, additions of manganese amendments, soil-applied fungicides, and a combination of all aforementioned treatments. Over the course of the field study, data will be collected with respect to: turfgrass quality, soil pH measurements, disease severity ratings, and presence of pathogen in root tissue. Following the collection of data, a statistical analysis will be conducted comparing treatments.

Goals for Completion [Interim Report Only]

The goal of confirming the causal agent of TAP has been met with some challenges. These have included the difficulty of isolating pure cultures of fungal organisms from creeping bentgrass roots obtained from the field. This challenge was addressed by requesting fresh samples in the spring of 2016. These fresh samples were obtained from all over Canada rather than just Ontario which allowed for a broader scope of the inferences made. Our current timeline for the project goals have been reassessed with the intention of completing this project by August 2017. By June

2017, it is our goal to publish a disease note for *G. cylindrosporus*. In addition, the winter 2017 growth chamber trial will continue until April 2017, and we intend to commence the field trial portion during spring 2017.

Graduate Student

To accomplish the project goals, MSc. Candidate Ernest Urquico was selected in September of 2014 to undertake a research project. Ernest had experience as a lab technician taking on responsibilities such as data acquisition, experimental design, and disease diagnostics in turfgrass studies. Ernest was accepted to the OAC graduate program under provisionary status, which encouraged him to focus on his academics. Ernest was able to achieve competitive grades of 87% and 85% in his first and second semesters, respectively. Through his courses, Ernest has built a strong background on the subjects of plant disease, statistics, and molecular biology. Ernest has also obtained valuable experience in presenting his preliminary results to industry professionals at the Ontario Turf Symposium (OTS), and assisted with the preparation of the OTS Plant Disease Diagnostic Workshop held February 2016. In addition, Ernest served as a teaching assistant for the Diploma of Turf Management program, and learned a number of skills from this experience including exam administration, lab demonstration preparation, and assignment evaluation. Unfortunately, due to ongoing health issues, Ernest was not able to perform at his usual level during the winter semester of 2016 and as a result, he decided to take a leave of absence for the summer semester and did not return for the fall semester. As such, this project has been turned over to Taylor Wallace, who recently earned her MSc with Dr. Jordan in turfgrass pathology and nematology. Taylor has been actively working on this project as well as others within the lab.

An amended timeline is as follows:
May 2017: Finish analysis of growth chamber study and prepare to repeat over the summer

May 2017: Finish definitive diagnosis of causal agent of TAP and begin preparing disease note for publication in Plant Disease journal

May 2017: Begin field trial to test the treatments that successfully reduced TAP symptoms in the growth chamber study.

June 2017: Submit disease note in Plant Disease

The final report of this project will likely be delayed to December 2017 as we were unaware of how slowly the pathogen grows and develops in the plant until we were able to successfully completely our Koch's postulates. As such, although the field trial will be initiated as planned in the spring, we do no anticipate having recordable results until the fall, with a final report being prepared by the end of the year.