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)*. 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* Saccardo (*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: Ernest has worked towards identifying the causal agent of TAP in Ontario using samples obtained from various participants. He has continued applying the skills he has obtained from learning molecular techniques from classes and under the advisement of his committee members. Ernest's preliminary results from molecular techniques suggest that the presence of another pathogenic organism, *Gaeumannomyces cylindrosporus,* within creeping bentgrass root tissues may result in patch-like symptoms. Ernest is conducting a Koch's postulates trial using samples from the field containing runner hyphae to inoculate plants grown from seed to confirm the potentially pathogenic organism.

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

Success to date: Ernest has initiated a greenhouse trial at the beginning of the year (2016). Pots containing creeping bentgrass plants have been inoculated with isolates of *G. cylindrosporus* from three sampling sites. This current trial is ongoing and will continue until plants have shown symptomology and have been harvested for analysis.

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

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 in Ontario. 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 20^{o} C for 14 days.

DNA extraction has been optimized and conducted using a standard DNA extraction protocol for plant DNA. Polymerase chain reaction was used to amplify DNA sequences specific to fungal organisms. Molecular identification of each sample was conducted using common primers among fungal organisms. These primers contain the Internal Transcribed Spacer (ITS) region of DNA, and this region is conserved amongst 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 was conducted by constructing columns of USGA greens to inoculate with suspected samples of *G. cylindrosporus*. Each individual column containing turf was defined as one experimental unit. Specification of turf columns was as follows: 45cm tall columns containing a 30cm root zone mix (composed of 80% sand and 20% peat), with a 10cm drainage layer containing mixed gravel. Creeping bentgrass variety Penn A4 was grown in the columns and was applied at a rate of 1.25-1.5 pounds per 1000 square feet (~900g/100 m^2). A fertilizer mix 19-25-5 (N-P-K) at 0.4kg/100m^2 was applied up to the second week after seeding.

Four fertilizer treatments were applied to two groups of plants, one group receiving acidified irrigation and the second group receiving non-amended irrigation. Columns irrigated with acidified water were watered once a day in the morning until a pH of 6.5 was reached. Fertilizer treatments were as follows: negative control with no fertilizer, urea (46-0-0), ammonium sulfate (21-0-0), and ammonium nitrate (33-0-0). Columns containing creeping bentgrass received urea, ammonium sulfate and ammonium nitrate at rates of 0.39kg/100m^2, 0.76kg/100m^2, and 0.48kg/100m^2, respectively. Fertilizer treatments were applied bi-weekly at a target rate of 0.16 kg N/100m^2.

Inoculum was prepared from suspected *G. cylindrosporus* samples obtained from the GTI under the protocol developed by Dewan and Sivasithamparam, (1989). Seeds of Kentucky bluegrass were used as an inoculum vector and sterilized in autoclave in 250ml flask at 120^{0}C for 50 min. Each flask was inoculated with fungal cultures using of 5 discs (5mm in diameter) obtained from the margins of colonies of fungi cultured on PDA plates. Inoculated seeds were incubated for 14 days at 20+/-2^0 C,

and then applied to healthy turf contained in soil columns.

Over the course of the experiment, observations of plant health and diseases severity were taken once a week. In addition, soil pH was tested using a pH probe at both the beginning and completion of the trial. This experiment conducted over a period of 6-8 weeks. At the conclusion of this experiment, destructive sampling of roots was used to confirm and quantify pathogen presence.

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. The research design will be a randomized complete block with a total of 48 plots. There will be twelve 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, addition 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. Our current timeline for the project goals have been reassessed with the intention of completing this project by August 2017. By the end of the 2016 spring semester, it is our goal to publish a disease note for *G. cylindrosporus*. In addition, the winter 2016 greenhouse trial will continue until August 2016, and we intend to commence the field trial portion during fall 2016. Finally, we intend to expand our search for study participants by contacting the Ontario Golf Superintendents' Association, along with former submitters to the GTI Diagnostic Laboratory.

Graduate Student

To accomplish the aforementioned 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 has decided to take a leave of absence for the summer 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 brought up to speed on the project and is currently trying to finish up the molecular work.

An amended timeline is as follows:

August 2016: Complete molecular work and barring any unforeseen complications, definitively identify the causal agent of TAP in Ontario and Canada

July 2016: Initiate growth chamber study (greenhouse study will be moved to growth chamber to ensure conducive conditions for disease development). Study will be completed by mid-fall 2016 and will focus on soil pH adjustment and its effect on TAP.

September 2016: Initiate field trial to asses a variety of cultural practices for management of TAP.

A more detailed timeline will be included with September's interim report.