Title	Management of Take-all Patch in High pH Soils
Principle Research and Affiliation	Katerina S. Jordan
Technician	Taylor Wallace
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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: We have successfully identified the causal agent of TAP through molecular techniques. Samples from Alberta, Saskatchewan, and Ontario have been positively identified as *Gaeumannomyces cylindrosporus*. The confirmation of these results through classical techniques is currently underway.

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 will be conducted in the winter semester of 2017 by Josh Callaghan, an undergraduate student. Josh is doing an undergraduate research project with Dr. Jordan and has decided study TAP. He will be conducting the greenhouse trial under the supervision of Dr. Jordan and Taylor Wallace.

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

The results from the greenhouse experiment will help to determine the treatments used in the field trial. The field trial will be conducted in the spring of 2017.

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 bentrgrass with samples of *G. cylindrosporus*. The project will begin in January of 2017. To date the inoculum has been made and the treatments are being selected.

Inoculum has been prepared for several of the isolates of *G. cylindrosporus*. The inoculum was prepared by soaking a mixture of several grass seeds (Kentucky bluegrass and various fescues) overnight. The water was then drained from the seeds and approximately 300 mL of wet seed was placed into 1000 mL beakers and covered with aluminum foil. These beakers were then autoclaved 3 times, at 121 °C for 45 minutes, over the course of three days. The pathogen which was isolated from field samples was cultured on potato dextrose agar, amended with the aforementioned antibiotics, for several weeks (at least 6). In a sterilized flow hood, one isolate at a time, five plates of each isolate were sliced into cubes (0.5 cm by 0.5 cm) and placed into the beakers with the autoclaved seed. The seed and agar chunks were mixed using a sterilized metal implement and the aluminum foil was replaced. The fungus was given three weeks to infest the seed and reproduce. The infested seed was then laid out on sterile petri plates and allowed to dry completely the course of several days in the sterile flow hood. Finally the inoculum was placed into paper bags and put into the freezer for future use.

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

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 decided to take a leave of absence for the summer semester and is not expected to 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:
Fall 2016: Finish Koch's postulates to confirm that *Gaeumannomyces cylindrosporus*

is a pathogenic organism capable of causing the symptoms seen in the field.

January 2017: Initiate growth chamber study (greenhouse study will be moved to growth chamber to ensure conducive conditions for disease development). Study will be completed by April 2017 and will focus on soil pH adjustment and its effect on TAP.

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

The completion of this project is expected to be on time, August 2017.