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.) caused by the fungus *Gaeumannomyces graminis* var. *avenae (Gga)*. From 2012 to 2014, 44% of samples diagnosed by the Guelph Turfgrass Institute (GTI) Diagnostic Laboratory at the University of Guelph were suspected to have TAP, 47% of which were from Ontario. This disease is difficult to manage because once it is present in the soil and the root tissue, fungicide treatments are only partly effective. In addition, the disease is more severe and prolonged in high pH soils, which are are often what we find in Ontario and west to Alberta. Developing management strategies to reduce TAP development and severity is important in maintaining the desirable creeping bentgrass species on the greens. In agricultural and some turfgrass systems a natural decline in the disease is observed over time and this is believed to be due to soil suppression. However, take-all decline has yet to be observed on golf course greens in Ontario. The persistence of the disease may be due to a number of factors, but we are theorizing two likely reasons: 1) suppression of the disease may be hindered by the alkaline soils and/or 2) the causal agent in Canada is different than has been observed in the United States and therefore unaffected by soil antagonism. The objectives of this study are to confirm the causal agent of take-all patch in Canada and to assess cultural and more effective chemical management strategies for the disease. As of March of 2015, we have collected over 20 samples from golf courses that showed symptoms of take-all patch and began culturing hyphae from the root tissue. Much of the first 6 months of the project have been spent attempting to identify the isolated fungus from the root tissue. We have not yet been able to identify the organism, and have been working on optimizing molecular methods to do so. As of the date of this report, we have only been able to determine that the causal agent is likely not *Gaeumannomyces graminis* var *avenae* based on our molecular work. We are continuing to work on the molecular methods to identify the pathogen at which point we can then move forward with the management portion of the study.

Background

Creeping bentgrass (*Agrostis stolinifera* L.) is considered to be an optimal turfgrass species for golf course greens in cool-season environments (Lawson *et al*., 2012). Healthy stands of creeping bentgrass spread vigorously and can tolerate low cutting heights while quickly recovering 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*), the 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: In his first and second semesters, Ernest has invested time towards sampling the local areas in Ontario for participants in his project. With the obtained samples, isolation of potentially pathogenic fungi have been catalogued and maintained as cultures to be processed for identification using molecular biology techniques. In addition, the preliminary work for executing protocols for DNA analysis of the suspected fungal pathogen for TAP disease has been undertaken and will continue to progress. To date, we have not been able to identify the causal agent but are finding that the primers specific for *Gaeumannomyces graminis* var. *avenae* are not yielding results, suggesting we may be dealing with a different causal agent for the disease.

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 set the foundation for his greenhouse trial by preparing an experimental design and gathering the necessary materials and space to study the effects of fertility and irrigation management practices in creeping bentgrass turf.

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

Success to date: This will be initiated in the fall, providing we have confirmation of the causal agent and are able to produce enough inoculum.

Materials and Methods

Isolation, Culture, Molecular Identification of G. graminis var. avenae

Samples of *A. stolinifera* 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 conducted using the Qiagen DNEasy Plant and Fungus DNA extraction kit. Avenacin gene specific to *Gga* will be amplified using PCR (conditions to be optimized for BioRad iProof Taq Polymerase). The primers used for the identification of *G. graminis* isolates are: Gga: ACGGCGGTGGATGGCAAGAC and AV3rc: TGCTCATGGTGGTTCCTGCG (Rachdawong *et al.*, 2002), synthesized by University of Guelph Lab Services. The visualization of amplified DNA has been done using gel electrophoresis (with an expected band size of 617bp) and sequencing conducted by University of Guelph Lab Services.

Goals for Completion [Interim Report Only]

By the end of the summer semester, it is our goal to confirm and identify the causal agent of TAP disease from samples obtained across Canada. Positive identification of the causal agent of TAP will be accomplished by obtaining a positively identified control specimen for DNA sequence comparison. In addition, we intend to expand our search for participants in our study through contacting the Ontario Gold Superintendents' Association, along with former submitters to the GTI Diagnostic Laboratory. The proposed Greenhouse trial will commence once we have identified the causal organism as we need to confirm pathogenicity of the causal agent.

Graduate Student

To accomplish the aforementioned goals, MSc. Candidate Ernest Urquico was selected in September of 2014 to undertake this research project. Ernest has previously obtained experience as lab technician taking on responsibilities such as data acquisition, experimental design and diagnostic skills in turfgrass diseases. Ernest was accepted to the OAC Graduate program, but under provisionary status. As such, much of his focus during his first two semesters has been on coursework

due to the requirements surrounding his provisional status. During the first two semesters of his study, Ernest was able to achieve competitive grades of 87% and 85% in his first and second semesters, respectively. Through his courses, Ernest has also able to build a strong background on the subject of plant disease, statistics and molecular biology.