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 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 September of 2015, we have collected over 30 samples from golf courses in Ontario and Alberta that showed symptoms of take-all patch and began culturing hyphae from the root tissue. As of the date of this report, we have been able to determine that the causal agent is likely not *Gaeumannomyces graminis* var *avenae* based on our molecular work. Furthermore, using non-specific fungal primers and sequencing our results from PCR amplification, we have determined that we are likely dealing with a different pathogen – *Gaeumannomyces cylindrosporus*. This is a well-known pathogen of cereal crops and Bermudagrass but it has historically not been considered an important pathogen of creeping bentgrass. We are currently in the process of conducting a pathogenicity assay to confirm that the organism that we have isolated from the root tissue of creeping bentgrass plants is, indeed, the causal organism of many of the cases of take-all patch in Canada.

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). We are not observing this phenomenon, however, on golf courses in southwestern Ontario or in the provinces of Manitoba, Saskatchewan, or Alberta. Soil pH is typically variable in Ontario (Lauzon *et al.*, 2005) and above 7.0 in much of the other three provinces. It is likely that we are seeing reduced effect of antagonism in these areas due to the alkaline soils that exist in central to western Canada. 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: At this point, we are confident that the pathogen that has been isolated from at least 10 of the samples we have collected is not *Gga*, based on morphological growth on artificial media as well as on molecular identification. We had originally chosen polymerase chain reaction (PCR) primers that were specific to *Gga* but over the course of 6 months, had been unable to get any amplification of the fungal DNA. We then went to non-specific fungal primers and were able to obtain a band on gel electrophoresis following PCR amplification and we had this band sequenced. Using an online database, we have been able to identify the fungus isolated from 5 of the sample as *Gaeumannomyces cylindrosporus (Gc).* We are currently in the process of performing Koch's postulates to confirm that the isolated fungus is, indeed, pathogenic on creeping bentgrass turf. We are in the 2nd week of the pathogenicity trial and have noted symptoms of yellowing and thinning in the plots inoculated with 3 of the isolates we have. These symptoms are not present in the non-inoculated plots, suggesting they are a result of the pathogen.

Next steps: Once we are able to confirm pathogenicity, we will continue to collect isolates from Ontario through Alberta and determine whether all of the take-all patch symptoms in Canada are a result of *Gg* or if there is *Gga* present in the soils as well.

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: Now that we are relatively confident of the causal organism, Ernest has begun the greenhouse trial in the hopes of completing it by February. Pots have been prepared and seeded and inoculum is being prepared from the 3 isolates that we believe to be responsible for the disease in Ontario and Alberta. Treatments should begin in early December and the trial will continue through the winter.

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

Success to date: Based on the results of the greenhouse trial, we will be initiating the field trial in the spring.

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 and Alberta. 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* was amplified using PCR (conditions to be optimized for BioRad iProof Taq Polymerase) but with no success. 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. Based on the lack of amplification, we began to use less specific fungal primers, specifically NSA3 and NLC 2. Following amplification with these primers, the expected band was purified and sent in to Laboratory Services for sequencing. Using an online database of known genetic sequences, we match our unknown to known fungal organisms.

Once the organism is identified, the next step is assess the organism's pathogenicity,

or its ability to cause disease. This is done through a series of steps known as Koch's postulates:

1. Note symptoms of affected plant, describe and record
2. Isolate pathogen from affected plants and culture
3. Inoculate healthy host and describe symptoms; Symptoms should be the same as observed in Step 1.
4. Isolate the pathogen from the newly inoculated host; morphology must be the same as observed in Step 2.

Following confirmation of identity and pathogenicity, all greenhouse and field trials will be conducted.

Goals for Completion [Interim Report Only]
By the end of this term, it is our goal to confirm the identification of 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. Finally, we will have completed at least one repetition of the greenhouse trial and will be planning for the field trial to commence in the spring.

Graduate Student
Ernest has completed his coursework and met the conditions of his provisional acceptance. He has worked on optimizing the molecular methods to identify the pathogen responsible for the symptoms we have been seeing. Ernest has been collecting samples from Ontario and other parts of Canada and working hard to isolate and identify the pathogens present in the roots. Thanks to Ernest's hard work over the past year, we are very close to identifying the causal organism and this will help with developing an effective management scheme for take-all patch.