Management, host pathogenicity, and rapid identification of *Magnaporthe poae*, causal agent of summer patch on annual bluegrass and Kentucky bluegrass turf.

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Project summary:

With increasing demands placed on golf course putting green turf combined with increased temperatures over the last few years, the incidence of diseases such as summer patch have increased throughout Ontario. Summer patch is a root disease caused by the fungus *Magnaporthe poae* and it is most pathogenic on annual bluegrass (*Poa annua*), Kentucky bluegrass (*Poa pratensis*), and fine fescues (*Festuca* spp.). The pathogen grows best under conditions of warm air and soil temperatures and high soil moisture. Symptom development can occur at any time when the turfgrass is stressed, although the above conditions are usually when symptoms develop on turf as well.

The disease is managed primarily through preventative chemical applications in conjunction with cultural practices. However, the appropriate method of pesticide application (e.g. application volume, additional irrigation) and the effects of various cultural practices on pathogen survival and disease development are not well known for the disease on annual bluegrass putting greens. In addition, pathogenicity and natural resistance in annual bluegrass ecotypes are not known in Ontario. Finally, proper diagnosis of this disease relies on visual observation of symptoms and signs on the roots, both of which can sometimes be misleading.

The objectives of this study have been to develop best management practices for summer patch, determine host specificity and pathogenicity to gain a better understanding of disease development, and to develop a rapid and simple diagnostic tool for presence of *Magnaporthe poae* in the hopes of decreasing fungicide use by increasing the efficacy of preventive applications.

OTRF Final Report – January 2012

<u>Molecular work for identification of *Magnaporthe poae*</u>

Small sections of cup cutter sized-samples collected from various golf courses in Summer 2009/2010 that showed signs of colonization with fungi believed to be *Magnaporthe poae* were planted, grown and maintained in a growth chamber (set at 28^{o}C). Once plants began to show foliar symptoms, roots were examined microscopically. Roots containing dark runner hyphae (ectotrophic mycelia), believed to be colonized by *M. poae,* were isolated, sterilized and plated on antibiotic-amended potato dextrose agar (APDA). Plates were examined for visible fungal growth, reminiscent of *M. poae*. These cultures have been maintained on fresh media through periodic re-culturing.

To definitively identify *M. poae* in the samples collected, molecular/genetic work has begun. DNA was isolated from a sampling of the cultures and amplified through polymerase chain reaction (PCR) using a combination of universal fungal-specific primers (Internal transcribed spacers; ITS). To ensure PCR was successful, a small quantity of each PCR product was run on an agarose gel. As shown in Figure 1, three DNA bands (~ 520-530 base pairs (bp) in size) were evident and appropriate for *M. poae* according to previously published literature and personal communication with Dr. H. M. Fouly.

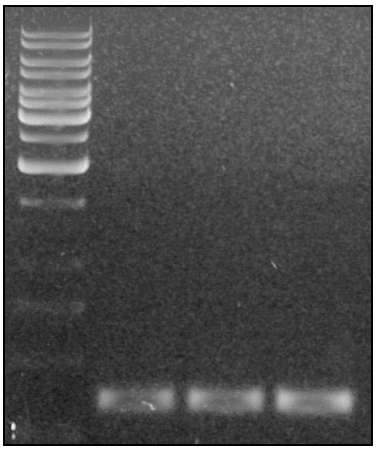

Figure 1. Three DNA bands (~ 520–530 bp) were produced through PCR. (Photo courtesy M. Bassoriello)

Once PCR products were confirmed, PCR purification was performed. Purified samples were sent to the University of Guelph Laboratory Services for sequencing. Recent sequencing results from Fall 2011 have generated up to 97% sequence match (DNA from collected cultures) to *M. poae*. To date, molecular/genetic work is continuing (PCR has been performed on approximately thirty samples; with approximately thirty more samples to be run) and samples are frequently being submitted to U of G Lab Services for sequencing results.

Field study 2010

A field study was conducted during the 2010 season at the Guelph Turfgrass Institute (GTI) on the *Poa annua* green established for this project. The randomized trial consisted of seven treatments including a variety of fungicide application regimes and untreated controls. Four replicates each of preventative application of azoxystrobin (watered-in and not watered-in) and curative application of azoxystrobin (watered-in and not watered-in) were performed. Various control treatments (no inoculum + no azoxystrobin controls, no inoculum + azoxystrobin, and inoculum + no azoxystrobin) were used.

A preliminary infiltration study was performed prior to the start of the trial, in which 20 L of water per $2m^2$ plot was applied for the watered-in treatments. Inoculum of *M. poae* was prepared using Kentucky bluegrass seed and various fresh cultures collected from courses throughout southwestern Ontario. The inoculum was grown for several weeks, dried and then weighed into separate envelopes for use in the field study. Plots were watered after the inoculum was applied (to the appropriate plots) and twice daily throughout the entire trial duration. Photographs, disease severity ratings, turf quality ratings and visual observation of the roots using core samples were taken during the trial to assess treatments. As well, cup cutter samples were taken from each plot at the end of the trial. Further research and data analysis using the disease severity ratings and turf quality ratings was completed. This trial will be re-established in summer 2012, along with newly developed trials examining various nitrogen-based fertilizers as well as a combination of cultural management practices. Unfortunately, trials that were started during the summer of 2011 were terminated for the season (unfavorable weather conditions (extremely hot and dry) and unintentional scalping of the trial green on numerous occasions) due to lack of apparent (or very few inconsistent) *M. poae* symptoms observed on the trial green.

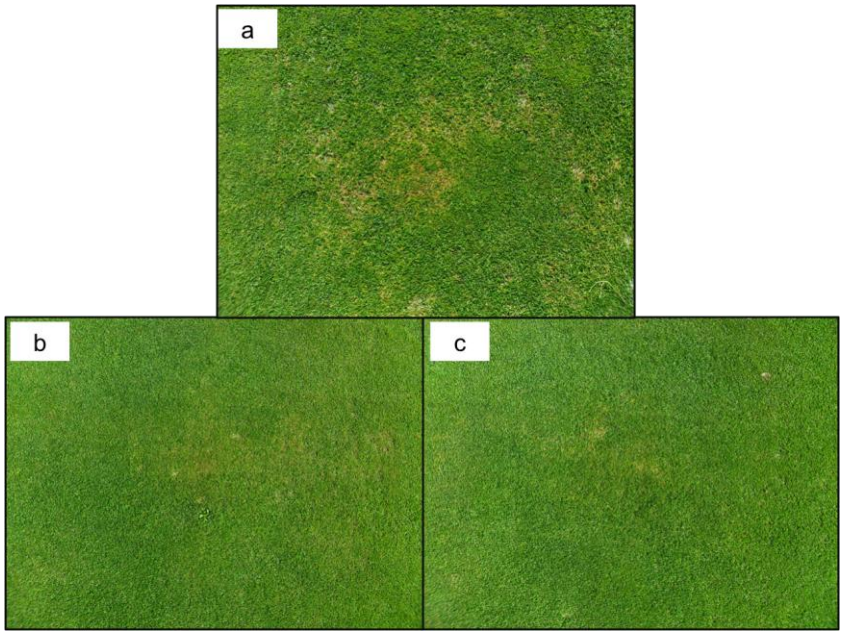

Figure 2. Results of 2010 field trial. a) no pesticide applied, b) azoxystrobin application, not watered in and c) azoxystrobin application, watered in. (Photos courtesy M. Bassoriello)

<u>Determination of pathogenicity and potential resistance:</u>

During summer 2010, a number of turf samples from golf courses were submitted through the turfgrass diagnostic laboratory. Samples with signs and symptoms suggestive of *M. poae* & summer patch, respectively, were collected. Roots containing

dark runner hyphae were isolated, sterilized and prepared on culture media. Cultures were grown and maintained (fresh & periodic re-culturing) as previously discussed.

Regular correspondence with volunteer golf course superintendants is also maintained for sample collection. In addition to collecting numerous isolates of *M. poae*, various ecotypes of *Poa annua* were collected and are being maintained for pathogenicity testing (ca. 1,500 individual *P. annua* tillers are being maintained (in conetainers) in a greenhouse). Pathogenicity tests will commence in Winter 2012 using *M. poae* cultures received from the American Type Culture Collection (ATCC) as well as with the isolates collected from Ontario golf courses. All cultures are grown and maintained on PDA media.

Executive Summary:

- *M. poae* has been identified according to Koch's postulates (from samples collected within Ontario)
- *M. poae* (isolated from collected samples) has 97% homology (similarity) to *M. poae* in National Center for Biotechnology Information (NCBI) database
- Preliminary field trial (using M. poae inoculum prepared from collected cultures) → visually, the watered in fungicide treatment appeared to result in less symptomatic foliage than the not watered in treatment
- Approximately 1,500 individual *P. annua* tillers maintained in greenhouse for pathogenicity testing

Future Research:

Once we have determined both morphologically and genetically (through DNA analysis) that *M. poae* is definitely present in the collected samples, development of a diagnostic assay will commence. The plans for the summer of 2012 are to inoculate *P. annua* in the field (at the GTI) and to establish fungicide, fertility and cultural management studies. Further pathogenicity testing will be repeated in 2012/2013. The collection of *M. poae* isolates and *P. annua* ecotypes will be continued.

References:

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