Occurrence and Identification of an Emerging Bacterial Pathogen of Creeping Bentgrass

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Objectives:

Evaluate problems associated with *Acidovoarx avenae* subsp. *avenae* bacterial infection of creeping bentgrass on golf courses across the United States. This will be accomplished through field, greenhouse, and laboratory studies elucidating detection, infection, and control of the disease.

Start Date: 2011
Project Duration: 3 years
Total Funding: $59,608

Creeping bentgrass putting greens battling summer stress have been found to be heavily colonized by a bacterium identified in 2009 as *Acidovorax avenae* subsp. *avenae* (Aaa). Initially isolated from an *Agrostis stolonifera* cv. 'Penn-G2'/'Penn-A4' golf course putting green in North Carolina, the bacterium has now been isolated out of numerous samples from golf courses located in and around the transition zone. Symptoms include yellowing and etiolation of bentgrass plants in small (5-7 cm) to medium (7-15 cm) patches. Affected areas grow faster than surrounding areas and begin to thin after sustained periods of heat and humidity.

Research conducted has confirmed pathogenicity of the isolated bacterium on creeping bentgrass by the completion of Koch's postulates. Electron microscopy indicates a colonization of the bacterium in the vascular bundle of the naturally affected creeping bentgrass. Recently, several *Agrostis stolonifera* cultivars ('Penncross', '007', 'Bengal', 'Penn-A4', 'Penn-G2', 'Tyee', and 'Declaration') were screened for susceptibility under growth chamber conditions (33 C, 210 mE, 12-hr photoperiod, 75% RH). Results indicate the bacterium is virulent on all cultivars tested.

A collection of isolates from around the country is ongoing. Samples suspected of being affected are thoroughly diagnosed via microscopy, and upon detection of significant bacterial populations, isolations are conducted and strains stored for later use and identification.

Thus far, 16s rDNA molecular identification has verified Aaa in 7 of the 14 suspected samples from 2010. These include isolates from IN, MA, MD, NC, and TX. In 2011, a collection of 10 more suspected samples from infected creeping bentgrass putting greens have been isolated and stored for molecular analysis.

Additional to the standard 16s rDNA primers, a subset of primers have been obtained that are currently being evaluated for specificity to Aaa. Once the specificity of primers has been confirmed on a subspecies level, probes will be designed for quantitative PCR assays. This technique should allow for better diagnosis and population evaluations with regard to symptom development and disease progression.

Field work in 2011 at The Michigan State University Hancock Turfgrass Research Center was carried out from June-September. Weekly inoculations of Aaa bacterial suspensions on a 297-m^2 creeping bentgrass putting green were administered with a backpack sprayer after 3 days of bacterial growth in a nutrient rich broth. Mowing took place immediately after inoculations to encourage infection.

Inoculated (left) vs. non-inoculated (right) cups of 1 month-old creeping bentgrass. Inoculated with A. avenae *subsp.* avenae *and incubated at 33 C for 10 days.*

Treatments included products such as antibiotics and anecdotal treatments suspected to have affects on disease symptoms (Table 1). Minimal disease infection occurred on field plots as a result of the numerous inoculations. Sustained temperatures did not reach optimal levels for disease occurrence. However, valuable information could be obtained regarding quality and phytotoxicity of some of the non-turfgrass labeled products.

Greenhouse-grown plants will be subjected to treatments and inoculations before growth chamber incubation in order to test product efficacy in a controlled environment. Additionally, collaborative work has been set up with The Moraine Country Club in Dayton Ohio in order to conduct on-site product testing at a golf course with confirmed Aaa infection.

Treatment	Rate	Interval
Urea	0.10 # N/M	14 days
PK Fight (phosphite)	3 oz /M	14 days
Insignia (pyraclastrobin)	0.54 oz/M	14 days
Streptomycin sulfate	200 ppm	14 Days
Mycoshield (oxytetracycline)	200 ppm	14 days
Actiguard (Acibenzolar-S-methyl)	0.75 oz/acre	14 days
Reserve (Triticonazole+chlorothalonil)	3.6 oz/M	14 days
Primo (Trinexapac-ethyl)	0.12 oz/M	14 days
Reserve +Primo	3.6 oz/M + 0.12 oz/M	14 days
Sonnet Biofungicide (*Bacillus subtillus*)	0.75 oz/M	10 days
Control		

Table 1. *Treatment list for field study at MSU during 2010 for Aaa bacterial etiolation suppression.*

Summary Points

- Temperature and infection studies have determined pathogenicity of *Acidovoarx avenae* subsp. *avenae* on many creeping bentgrass cultivars.
- Continued progress has been made with obtaining samples and isolates from creeping bentgrass affected with *Acidovoarx avenae* subsp. *avenae*.
- Ongoing work to identify Aaa specific diagnostic PCR primers and probes.
- Continued field work with collaborators.