

Occurrence and Identification of an Emerging Bacterial Pathogen of Creeping Bentgrass

Joseph Vargas Jr., Paul Giordano, Ron Detweiler, and Nancy Dykema, Michigan State University



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Objectives:

1. Evaluate problems associated with *Acidovorax 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.

Bacterial etiolation and decline is a newly described disease of creeping bentgrass characterized by symptoms of yellowing, abnormal shoot elongation, and eventual necrosis or thinning of the stand in irregular-shaped areas on putting greens. Since the identification of

Acidovorax avenae subsp. *avenae* (Aaa) in symptomatic creeping bentgrass from Quail Hollow G.C. in 2009, research has aimed to characterize the infection process and potential control measures for this disease. The pathogen has been isolated from dozens of golf courses around the country, and our research has shown susceptibility of numerous cultivars of creeping bentgrass, particularly at temperatures exceeding 86 F.

Bacterial diseases of turfgrass are rare, thus, characterizing the etiology and epidemiology of this pathogen is imperative to developing measures to treat the disease. We have confirmed the general pathogenicity of Aaa on creeping bentgrass through inoculations with a dipped scissor technique by clipping plants with bacterial soaked scissor blades. This technique has elicited symptoms of discoloration, necrosis and decline but likely

does not mimic natural plant invasion, and subsequently fails to induce etiolation.

Recent efforts have explored the elusive symptom of etiolation, by employing an alternative, root-dip inoculation strategy that has been successful in eliciting a quantifiable symptom

Pre-treatment of healthy cups of creeping bentgrass with various products prior to inoculation with *Acidovorax avenae* subsp. *avenae*; only the oxytetracycline and streptomycin treatments resulted in significantly less necrotic turfgrass than the untreated

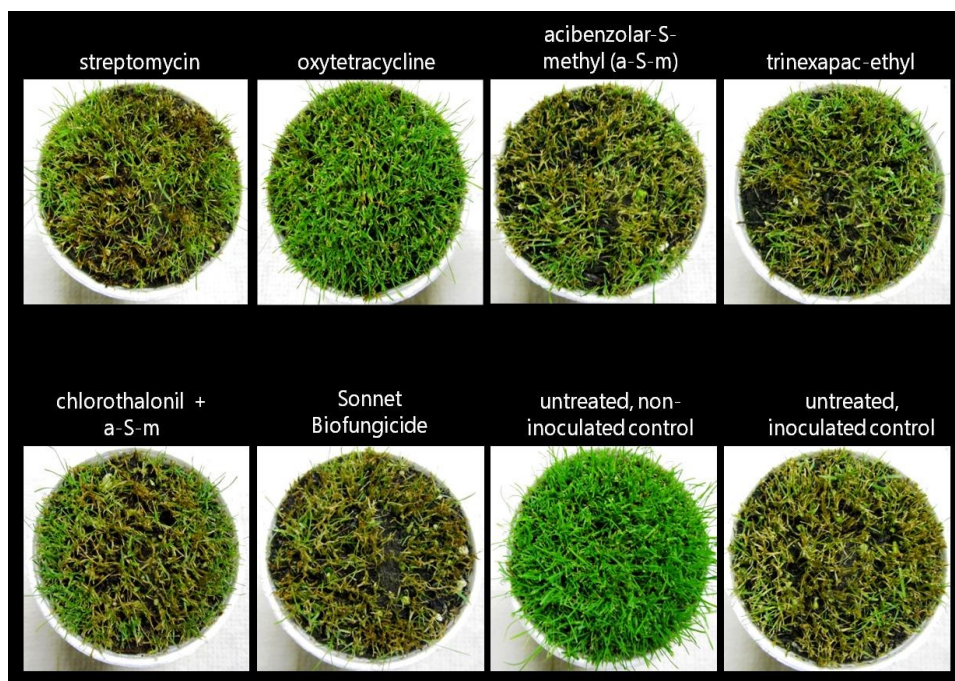


Figure 2. Close-up view of induced etiolated creeping bentgrass plants from the inoculated research site at Michigan State University. Microscopic thumbnail view of the etiolated plant shows heavy bacterial colonization and streaming from the cut end of the leaf blade.

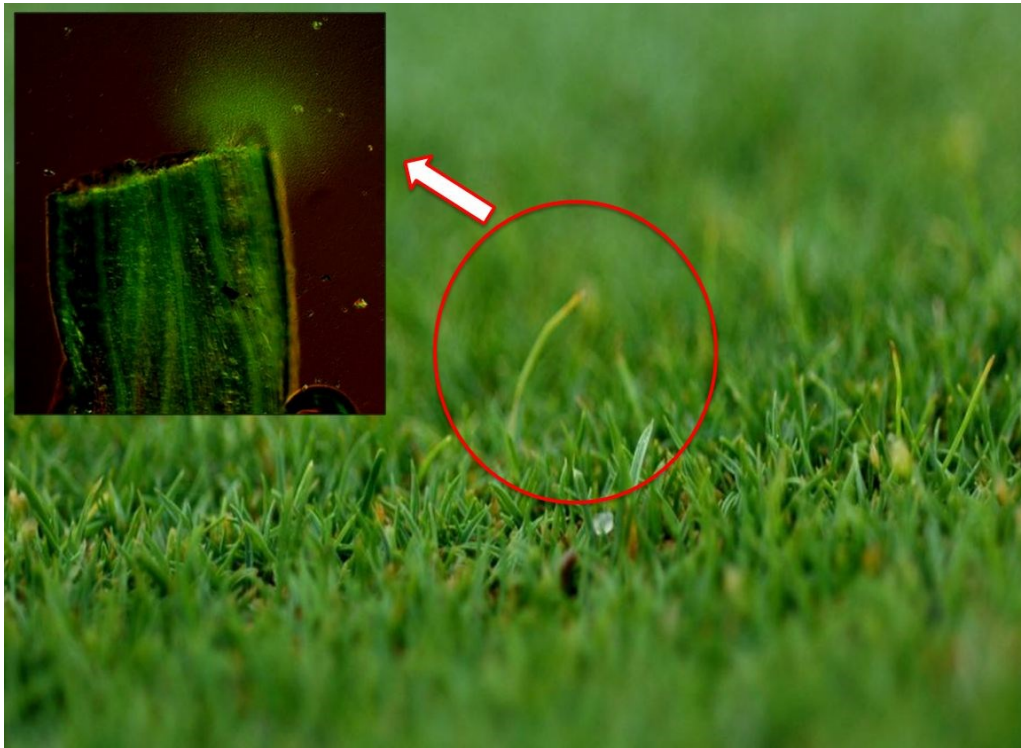


Figure 3. One of the four research areas set up on different putting greens at Moraine C.C. in Dayton, OH.



of overgrowth and chlorosis in creeping bentgrass plants. The inoculation technique is easily repeatable, and can be accomplished using various isolates of *Aaa* from different plant hosts. Only *Aaa* from turfgrass was able to cause an etiolation symptom, while *Aaa* isolated from maize failed to elicit significant disease symptoms when compared to a sterile water control. This work is currently in revision for publication in the journal *Plant Disease*.

Field studies have been undertaken for several years aimed at finding chemical solutions to disease symptoms caused by *Aaa* on creeping bentgrass putting greens. These studies have included a variety of products and have been conducted at various locations around the country. In 2013, we conducted trials at the MSU Hancock turfgrass Research Center (HTRC) as well as on a putting green at Inverness C.C. in Toledo, OH. Results from Inverness showed no significant differences among treatments when symptoms were present; however, a similar trend to 2012 was present, with trinexapac-ethyl (Primo) and ammonium sulfate treatments having the most pronounced etiolation among plots. The study at the MSU-HTRC in 2013 was successful in eliciting symptoms of etiolation through inoculation with *Aaa*. Significant increases in etiolation were observed in plots treated with Primo and ammonium sulfate compared to the untreated control. A significant reduction in etiolation was observed in plots treated with chlorothalonil+acibenzolar-S-methyl (Daconil Action). A recurring trend of symptom perturbation continues to be evident with the growth regulator trinexapac-ethyl and acidifying nitrogen fertilizer ammonium sulfate. At this time, recommendations for golf courses with bacterial

etiolation and decline are to avoid excess stress on putting greens and suspend the use of these products until conditions improve.

The USGA-funded project has leveraged additional funds for genetic investigations of *Aaa* on turfgrass from Project GREEN. A phylogenetic analysis of 30 turfgrass isolates and several other *Acidovorax* subspecies was conducted using a multi locus sequence analysis of a set of seven genes. This has led to further examination into discriminatory genetic relationships through the use of whole genome sequencing. Putative diagnostic primer sets have been developed through the use of a bioinformatics pipeline and genomic comparisons. These primers are currently being validated for future use as a rapid molecular diagnostic assay for confirmation of *Aaa* infection on creeping bentgrass.

Summary Points

- Symptoms of etiolation have been reliably reproduced through the development of an alternative inoculation strategy with *Aaa*.
- Field studies have confirmed etiolation to be significantly increased by applications of trinexapac-ethyl.
- Turfgrass isolates of *Aaa* are genetically distinct from other *Aaa* isolates from different grass hosts.
- Validation of discriminatory sets of PCR primers is currently underway for the development of a rapid molecular diagnostic assay.