Occurrence and Identification of an Emerging Bacterial Pathogen of Creeping Bentgrass

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Turfgrass and Environmental Research Online
Volume 12, Number 2 | March–April 2013

Objectives:

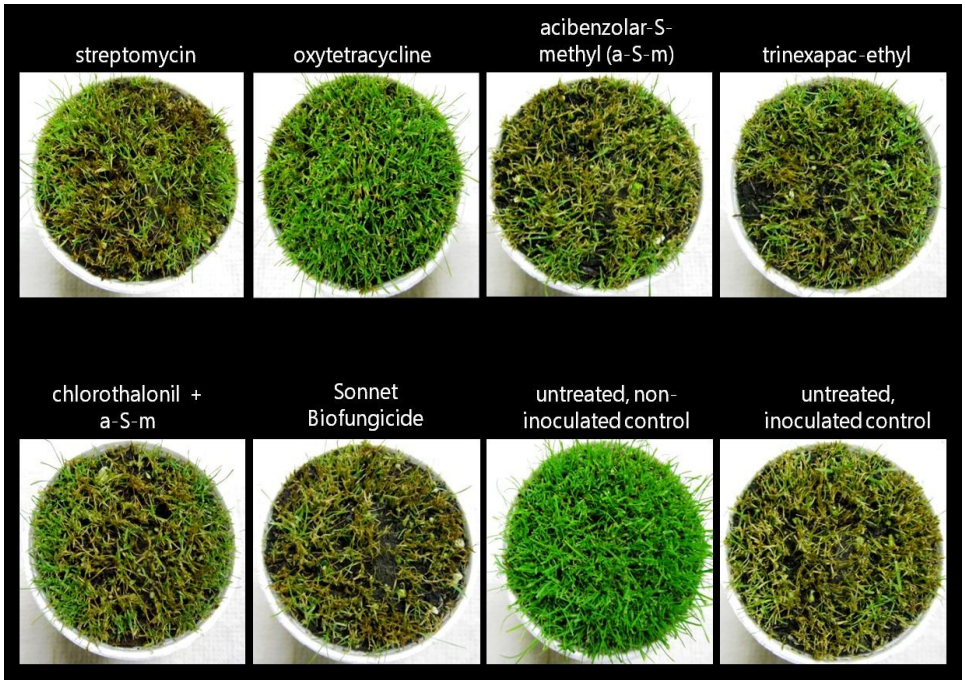
Evaluate problems associated with Acidovoarx avenae subsp. avenae bacterial infection of creeping bentgrass on golf courses across the United States. This will be accomplished through field, greenhouse, and laboratory studies elucidating detection, infection, and control of the disease.

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). The bacterium has been isolated out of dozens of samples from golf courses around the country. 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 and decline after sustained periods of heat, humidity and physiological stress.

Research thus far has confirmed pathogenicity of Aaa on creeping bentgrass by the completion of Koch's postulates in controlled environmental settings. Electron microscopy (EM) shows the bacterium colonizing in and between the vascular tissues of the creeping bentgrass. Solicitation of samples from golf courses from 2010–2012 was undertaken to investigate the prevalence and dissemination of *A. avenae* subsp. *avenae* on creeping bentgrass putting greens. An ongoing collection of at least 23 isolates from 13 states associated with these outbreaks on golf courses were confirmed as *A. avenae* subsp. *avenae* by pathogenicity assays and 16S rDNA sequencing.

Host range inoculations revealed isolates of *A. avenae* subsp. *avenae* to be pathogenic on all *A. stolonifera* cultivars tested (007, Bengal, Declaration, Tyee, L–93, Penncross, Penn G–2,

Figure 1. 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 inoculated



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USGA ID#: 2011–06–416
TGIF Number: 219659

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.

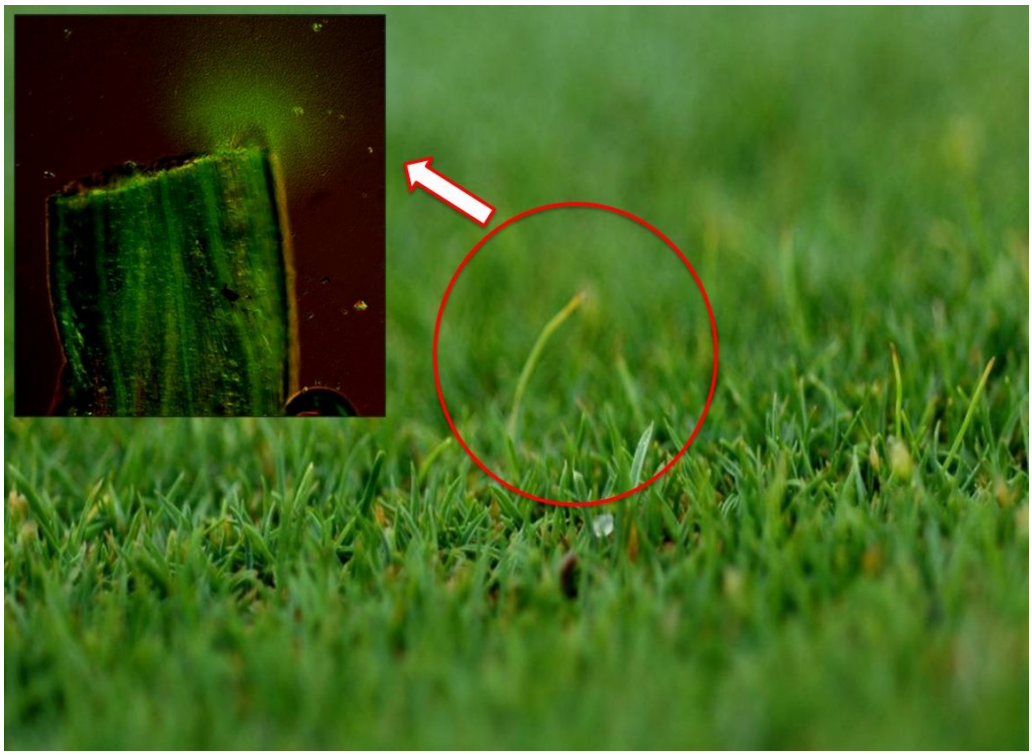

Figure 3. One of the four research areas set up on different putting greens at Moraine C.C. in Dayton, OH.

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Penn A–4, PennEagle, Crenshaw, Southshore, Macenzie, Cato, SR–1150, PennLinks, Providence) with slight but significant differences in disease severity on particular cultivars. Other turfgrass hosts tested were only mildly susceptible to *A. avenae* subsp. *avenae* infection.

Growth chamber inoculations have determined disease to be most severe between 30° and 35° C (86°–95° F) with temperature gradient inoculations. Pre–treatment of bentgrass pots with numerous different plant health products before inoculations have shown only the antibiotics oxytetracycline and streptomycin to be effective in suppressing disease in a controlled environment (Figure 1).

Field studies investigating a wide variety of control products (Table 1) were conducted on two separate sites in 2012: The Michigan State University Hancock Turfgrass Research Center, and on several putting greens with collaborators at Moraine Country Club in Dayton, OH. Very little differences in disease control were observed on affected areas at Moraine C.C., even during times of high disease incidence.

Weekly inoculations of Aaa bacterial suspensions on a creeping bentgrass putting green were administered at the MSU site between June–August. Eitolation due to Aaa infection was widespread across field plots during an extremely warm period of temperatures in early July and again in mid–July (Figure 2). Products tested for control did not seem to suppress disease symptoms. Treatments of ammonium sulfate and trinexapac–ethyl resulted in more pronounced etiolation and yellowing symptoms than others. This observation seems to be a commonality among studies currently being conducted by different researchers around the country.

Finally, the inaugural work related to the isolation, identification, and characterization of Aaa infection on creeping bentgrass has been accepted for publication in a coming issue of Plant Disease. Additionally, the research that has been made possible by this USGA initiative has helped leverage funding from Project GREEEN in a research grant aimed at whole genome sequencing and molecular characterization of the bentgrass Aaa against closely related Acidovorax species and subspecies. This high–throughput platform will accelerate the process of diagnostic assay development and undoubtedly clarify much of the ambiguity surrounding bacterial infection of creeping bentgrass on golf courses.

Summary Points

- Temperature and infection studies have determined pathogenicity of *Acidovoarx avenae* subsp. *avenae* (Aaa)on many creeping bentgrass cultivars.
- Only antibiotics have shown acceptable suppression of disease symptoms in controlled inoculation experiments. Further research must be done on the effects of trinexapac–ethyl and ammonium based fertilizers.
- Ongoing work to identify Aaa specific diagnostic PCR primers and probes through whole genome sequencing and bioinformatics.
- Continued field research with collaborators.

Table 1. List of various treatments evaluated for bacterial etiolation and decline control at the MSU and Moraine C.C. research sites.

Treatment	Rate	Interval
Mycoshield (oxytetracycline)	3lb/acre	14 days
KPhite (phosphite)	6 oz /1000 sq ft	14 days
Kasumin (kasugamycin hydrochloride)	1.5 oz/1000 sq ft	14 days
Agrimycin (Streptomycin sulfate)	2 lb/acre	14 Days
Daconil Action (Chlorothalonil+acibenzolar–S–methyl)	3.6 oz/1000 sq ft	14 days
Signature (Aluminum tris)	8 oz/1000 sq ft	14 days
Primo (trinexapac–ethyl)	0.12 oz/1000 sq ft	14 days
Trimmit (paclobutrazol)	0.7 oz/1000 sq ft	42 days
Junction (copper hydroxide/mancozeb)	4 oz/1000 sq ft	14 days
Zerotol (hydrogen dioxide)	60 oz/1000 sq ft	7 days
Ammonium sulfate	0.25 lb N/1000 sq ft	14 days
Ammonium sulfate +Primo (trinexapac–ethyl)	(0.25 lb N) + (0.12 oz)/1000 sq ft	14 days
Control		

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