Research *You Can Use*

Characterizing the Cause of Bentgrass Yellowing and Decline on Putting Greens

Bacteria may be responsible.

BY PAUL R. GIORDANO, ARIELLE M. CHAVES, NATHANIEL A. MITKOWSKI, JOSEPH M. VARGAS JR.

The USGA and concerned golf facilities provided funding to university scientists investigating the yellowing and thinning decline of creeping bentgrass greens during periods of stressful summer temperatures and humidity. The results provide clues that bacteria may be responsible for damaging bentgrass greens; however, work will continue to help provide the best possible information on how to properly diagnose and manage this problem.

Note: The information in this article has been adapted from original work published in *Plant Disease* titled "Identification, Characterization, and Distribution of *Acidovorax avenae* subsp. *avenae* Associated with Creeping Bentgrass Etiolation and Decline" (Giordano et al., 2012, 96:1736-1742).

During the past few years in many regions of the United States, extreme weather conditions and summer stress made managing creeping bentgrass exceptionally challenging. Many of the problems were due to disease complexes, stress factors, or other disorders. Among these problems, bacterial etiolation (e-'tee-o-lay-'shun — discoloration and elongation of grass blades) and decline of creeping bentgrass was a mysterious topic widely debated by university researchers and industry professionals since the early reports of outbreaks in the summer of 2010. The debate may have started sometime in 2010, but the problems related to this disease have plagued putting greens for several years. For nearly a decade, golf course

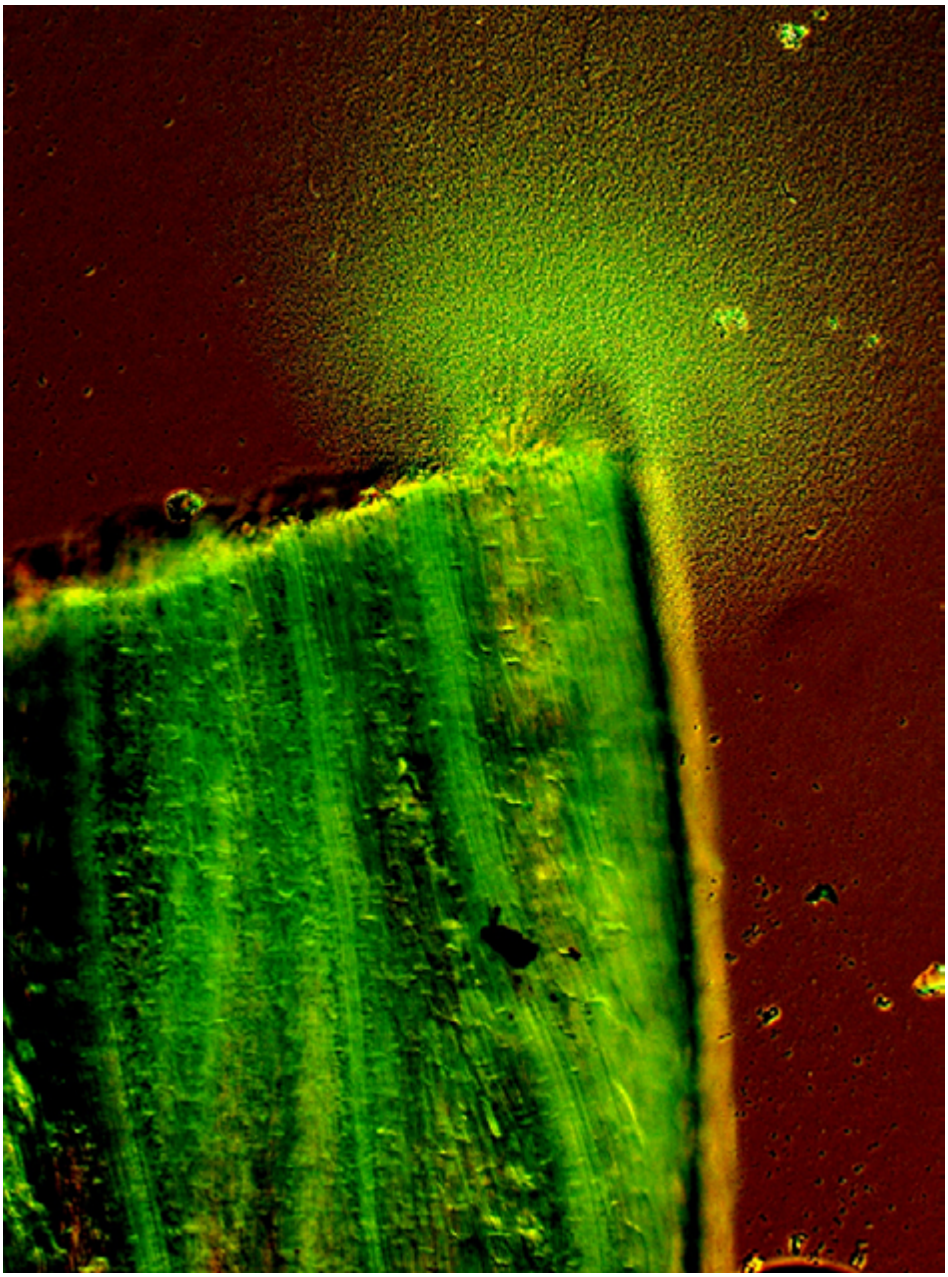


Figure 1. Heavy streaming of Acidovorax avenae *subsp.* avenae *cells out of the cut end of naturally infected creeping bentgrass stems.*

Green Section Record Vol. 51 (1)
January 11, 2013
TGIF Record Number 213968

Figure 2. Typical early symptoms of etiolation observed on a golf course putting green affected by Acidovorax avenae *subsp.* avenae*. Note the chlorotic irregular area with excessive elongation of affected plants. Etiolation is characterized by elongated yellow blades of grass with stems that extend 0.25 to 1.5 inches above the turfgrass canopy.*

superintendents have noticed unique symptoms of elongation, yellowing, and eventual decline on their creeping bentgrass putting greens; however, turfgrass pathologists kept coming up empty and attributed much of the problem to heat stress or other stress-related problems.

Things changed a bit when frustrated researchers at Michigan State University took a different approach by searching for atypical disease culprits after having no luck with the usual suspects. This led to observations of extremely high levels of bacteria pouring out of symptomatic stems of creeping bentgrass plants from a golf course in the transition zone (Figure 1). Not since the 1980s and the days of Toronto C-15 decline had these amounts of bacteria been observed inhabiting creeping bentgrass. This warranted further investigation, which subsequently identified a perpetrator: the bacterium *Acidovorax avenae* subsp. *avenae* (Giordano et al., 2010).

Bentgrass putting greens with symptoms of bacterial etiolation and decline typically show small (6 to 12 inches), irregularly shaped areas of leaf discoloration progressing from green to a yellow appearance. Often associated with this discoloration is the rapid growth or etiolation characterized by elongated yellow blades of grass with stems that extend 0.25 to 1.5 inches above the turfgrass canopy (Figure 2). Initially just a cosmetic problem, high summer temperatures lead to thinning and death of turf that results in irregularly shaped areas of dead grass on the putting green. Damage is most severe on intensively managed putting greens, and symptoms tend to first appear on the peripheral or outer edges of the putting greens that are typically more stressed due to frequent and repetitive mowing and rolling.

Although initial isolation determined that *Acidovorax* could produce these symptoms, there was limited published information on the infection and characterization of the disease. Recently, researchers from Michigan State University and the University of Rhode Island collaborated on research published in the December 2012 issue of *Plant Disease*. The project, funded largely by the USGA and several concerned golf clubs around the country, focused on bacterial isolation from creeping bentgrass samples, the dissemination of *Acidovorax* on golf courses around the United States, and the different hosts that *Acidovorax* may be capable of infecting.

The research began by soliciting samples from golf courses in 2010 and 2011 to investigate the prevalence and dissemination of *Acidovorax* on creeping bentgrass. Over these two years, a collection of bacterial isolates was grown and frozen for further analysis. In total, 21 isolates from 13 states associated with these outbreaks on golf courses were confirmed as *Acidovorax* by pathogenicity assays and

DNA sequencing analysis (Figure 3). Since a turfgrass system is a dynamic mixture of fungi, bacteria, and other microorganisms, not all of the bacteria isolated were found to be *Acidovorax*. Because of this, pathogenicity testing of several unique bacterial isolates from creeping bentgrass samples exhibiting heavy streaming was carried out. Among the bacterial isolates tested, *Acidovorax* was the only one capable of heavily colonizing inside the plant and causing significant disease symptoms and decline (Figure 4).

Next, the host range of the pathogen was tested on 15 different creeping bentgrass cultivars, several other turfgrasses, and a few non-turfgrass species. Inoculations revealed isolates of *Acidovorax* from creeping bentgrass to be pathogenic on all *Agrostis* species and cultivars tested, with slight but significant differences in disease severity on cultivars such as Declaration and Tyee. Of the cultivars tested, these two appear to be the most resistant to *Acidovorax*. Other turfgrass species tested were only mildly susceptible to *Acidovorax* infection. This indicates that there is some genetic variation with regard to susceptibility of creeping bentgrass cultivars; however, it appears that absolute resistance to the bacterium may not exist. This is an important facet of initial research when we consider the Toronto C-15 problem that plagued creeping bentgrass in the 1980s. Back then, it was determined that the bacterium *Xanthomonas campestris* pv. *graminis* was the causal agent of the decline; however, this particular bacterium preferentially attacked only select cultivars of vegetatively established creeping bentgrass. *Acidovorax* will infect many bentgrass cultivars, but is generally less aggressive than *Xanthomonas*.

Bacterial diseases can be quite distressing for researchers, superintendents, and golfers. Generally, our knowledge and experience with these pestilent microbes is lacking on turfgrass due to the preoccupation of research and resources directed toward fungal diseases. This foundational research on an emerging problem helps to shed some light on the subject of *Acidovorax* infection on creeping bentgrass. However, many questions still remain unanswered, and excellent teams of researchers are currently working to solve the pertinent issues that remain unresolved.

Although this study has demonstrated the ability of *Acidovorax* to infect and cause disease on creeping bentgrass in controlled environments, the phenomenon of early-stage etiolation is still uncharacterized. It is important to note that while etiolation is one observed symptom associated with *Acidovorax* infection, the abnormal elongation of turfgrass can be seen on stands of different turf species and often is not associated with a bacterial disease. Etiolation of managed turfgrass seems to be a symptom manifested by many different factors, most

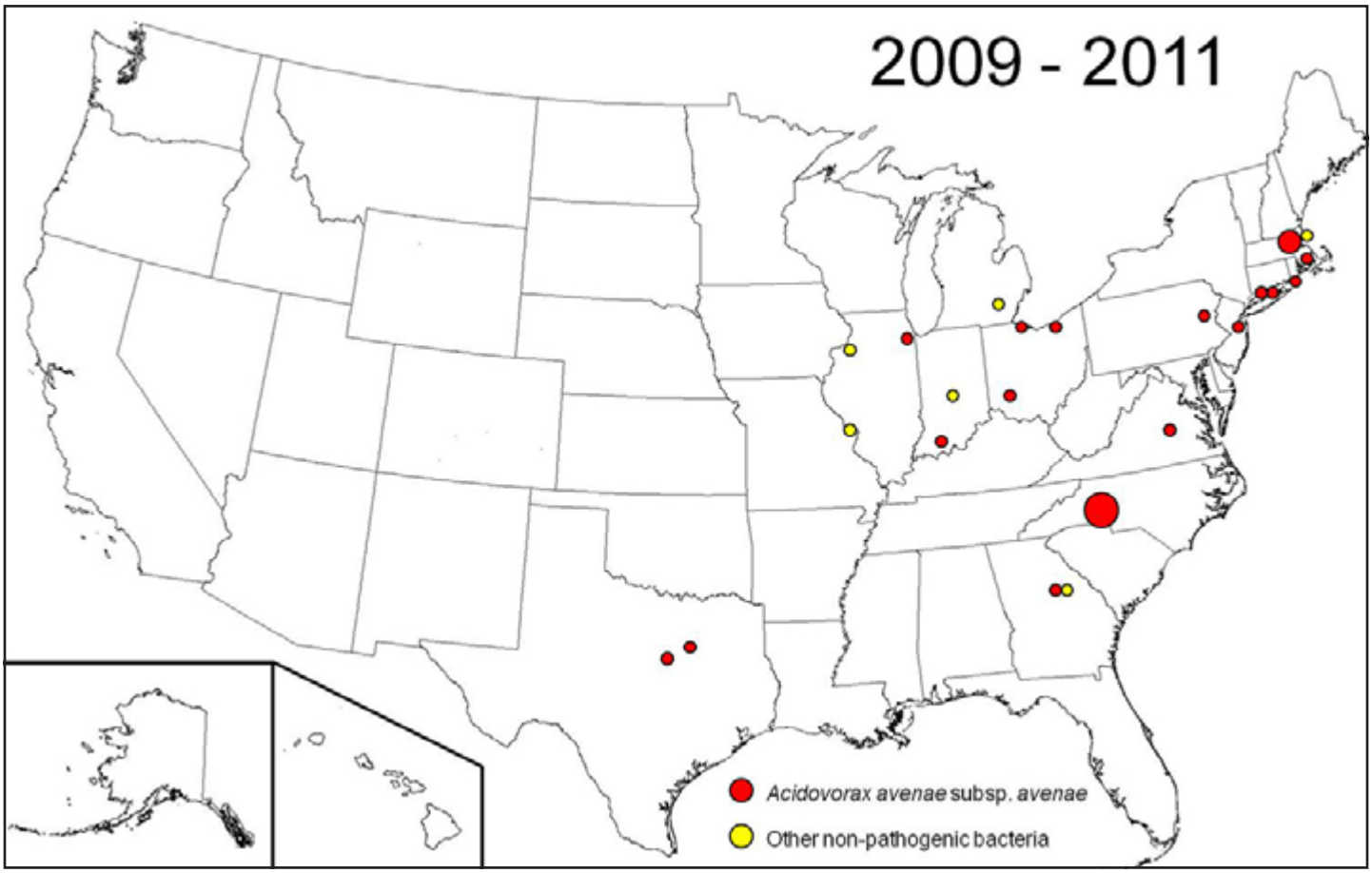
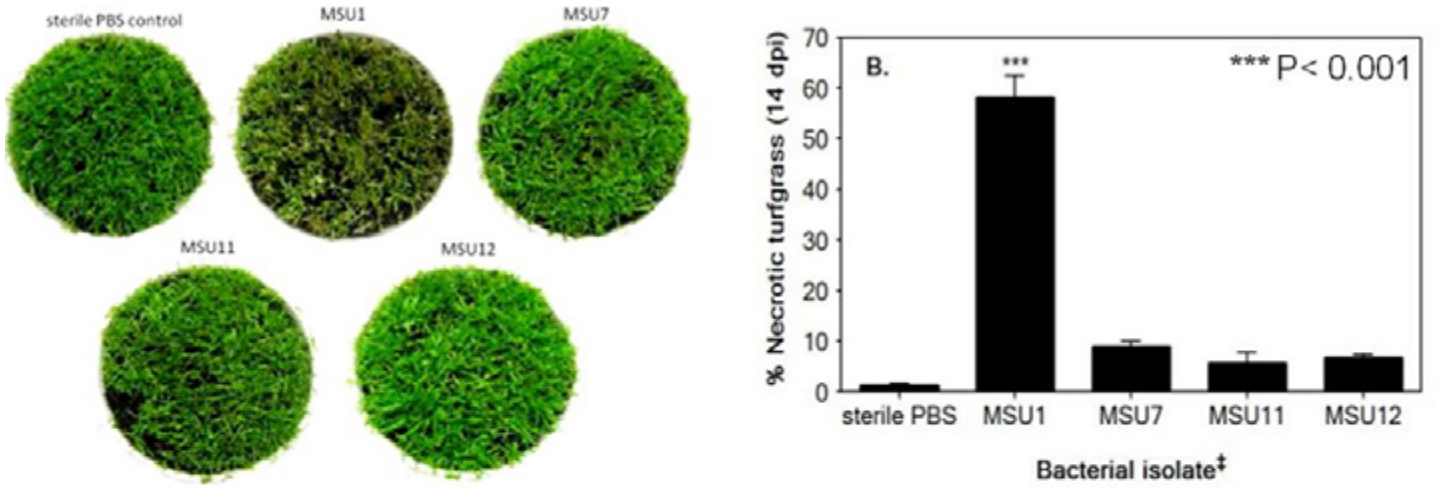


Figure 3. Map of the U.S. showing regions where Acidovorax avenae *subsp.* avenae *(red) was isolated out of affected creeping bentgrass. Larger dots indicate multiple isolates from the same or nearby golf courses.*



Isolate	I.D.
MSU1	*A. avenae* subsp. *avenae* (Aaa)
MSU7	*S. maltophilia*
MSU12	*R. pickettii*
MSU11	*H. seropedicae*
Control	Sterile PBS

Figure 4. Pathogenicity testing of bacterial isolates on seed-grown 10-week-old creeping bentgrass (adapted from Plant Disease *96: 1736-1742).*

of which are still poorly understood. We hypothesize that the natural infection of *Acidovorax* at low to moderate levels causes a physiological or hormonal response in the plant by which elongation of the newest shoot and leaf occurs. Observations of this unusual growth habit also can be observed in annual bluegrass infected with the bacterial pathogen *Xanthomonas translucens* pv. *poae*.

Extreme environmental conditions, particularly high temperatures, sustained humidity, and periods of heavy rainfall in many regions of the United States, have made an already intensively managed species such as creeping bentgrass increasingly difficult to manage in summer months. Infection of creeping bentgrass by *Acidovorax* and the rapid increase in tissues during high heat and humidity often result in necrosis and plant death. Much remains to be learned about latent infection of *Acidovorax* on creeping bentgrass. For example, rice plants can harbor "dormant" or undetectable infections of the bacterium that can be transmitted from plant to seed, and when high temperature and humidity occur, symptoms develop. It is likely these symptomless infections exist in turfgrass plants; however, research is still required to document if this is truly the case.

Finally, *Acidovorax* infection of creeping bentgrass has been considered a stress-related condition on golf course putting greens and is thought to be more severe at low heights of cut and aggressive cultural practices such as sand topdressing and grooming. The emerging problem associated with *Acidovorax* on turfgrass is likely stress induced. Since bacteria are passive invaders, they require a plant with wounds, openings, or abrasions as entry points. Daily mowing, aggressive cultural practices, and heavy traffic are effective means by which inoculum can spread to neighboring plants throughout a turfgrass stand. Disease symptoms often are not observed on turfgrass that is subjected to less rigorous maintenance regimes.

Unfortunately, control options for bacterial diseases in turfgrass are extremely limited. Research is underway to determine appropriate cultural and potential chemical control options for this emerging disease on golf course putting greens. With continued support and dedicated assistance from the USGA Green Section and interested golf facilities, teams of some of the best researchers and pathologists around the country will continue to seek out the answers.

PAUL R. GIORDANO, *Department of Plant, Soil, and Microbial Sciences, Michigan State University, East Lansing;* ARIELLE M. CHAVES *and* NATHANIEL A. MITKOWSKI, *Department of Plant Sciences and Entomology, University of Rhode Island, Kingston; and* JOSEPH M. VARGAS, JR., *Department of Plant, Soil, and Microbial Sciences, Michigan State University.*