BY PAUL R. GIORDANO AND JOSEPH M. VARGAS JR.

Yes, Virginia, there is a new bacterial disease

While research – and questions – about bacterial wilt continues, some puzzle pieces are falling into place.

Typical symptoms of etiolation on an annual bluegrass putting green in late summer.

An article entitled "The Wilt that Wasn't" was published in GCI that attempted to provide insight into the tumultuous world of plant pathology and the academic quarrels that are often encountered in scientific research. That particular article touched on "bacterial wilt" of creeping bentgrass, initially hypothesized to be caused by the bacterium *Acidovorax avenae*; an issue that has been at the forefront of superintendent's minds for the last few years. At the time of publication, very little research pertaining to *Acidovorax* in creeping bentgrass had been published. Additionally, anecdotal accounts from various regions around the country made for a non-cohesive, convoluted summary that was confusing.

This article is intended to shed a bit of light, rather than heat, on the subject of bacterial etiolation and decline of creeping bentgrass by providing some historical context, and more importantly, empirical evidence of the current and ongoing research with this emerging disease.

In the 1980's, a new disease was identified that changed the way we think about turfgrass pathogens. The disease was bacterial wilt of 'Toronto' creeping bentgrass, caused by a *Xanthomonas* bacterium. This was important, as it was the first major bacterial disease of amenity turfgrass, and for several years a good deal of controversy surrounded this new disease and its cause. Since then, a few researchers have dedicated much time and effort characterizing that and another closely related *Xanthomonas* pathogen of annual bluegrass. Today, the vegetative cultivars of creeping bentgrass such as Toronto, and the diseases that affect them are largely a thing of the past; however, bacterial wilt on annual bluegrass greens, tees and fairways is still a common problem every year, all around the country. One distinct characteristic of the disease on annual bluegrass is the yellowing and overgrowth or etiolation (Ee-tee-oh-lay-shun) that is often observed during cooler weather conditions.

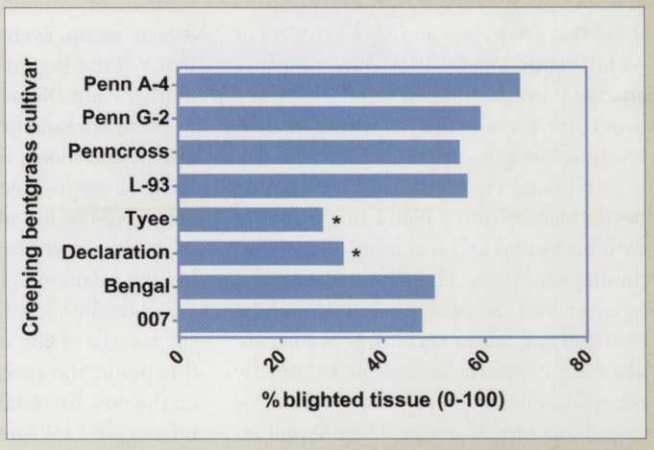
As they say, history repeats itself. Nearly 30 years after the 'Toronto' C-15 bacterial wilt scare at Butler National, a new controversy began to emerge with its focal point being a championship golf course in the greater Charlotte, N.C., area. A recurring problem of yellowing, etiolation, and eventual decline of irregularly-shaped areas on the creeping bentgrass putting greens was plaguing the club, and even with an unlimited budget to manage it, nothing seemed to be working. For several years, samples were sent to labs around the country in an attempt to get a disease diagnosis, still nothing seemed to work. In 2009, we received a sample exhibiting unique symptoms with a letter and an overview of management practices. After ruling out the "usual suspects" and having little luck making sense of the situation, our efforts turned to looking for a bacterial pathogen. This notion was based on the similar symptoms in the creeping bentgrass sample to those often seen on annual bluegrass affected with the bacterial wilt pathogen, *Xanthomonas translucens*.

A naturally infected creeping bentgrass putting green with symptoms of etiolation and yellowing in irregular areas.

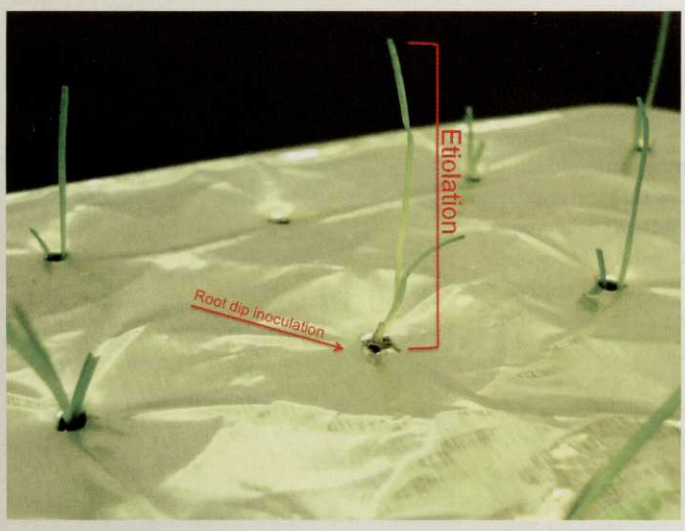
Enough storytelling, how about some science?

To our surprise, when the affected stems of bentgrass were cut into, heavy amounts of bacteria were observed microscopically streaming out of the cut end of the plant. The quantities of bacteria observed in the affected bentgrass plants were alarming and warranted further investigation without a doubt. From that point, research efforts were underway to isolate and characterize this bacterial culprit.

Since there are roughly thousands of different bacterial species that live in turfgrass systems, and all of them are microscopic, the job of discerning which one is truly the problem can be challenging. Measures must be taken to isolate the most prevalent bacterium from the samples. To achieve this we employed a technique known



Disease ratings on tested cultivars of creeping bentgrass subjected to *Acidovorax* inoculations. Disease ratings were taken 14 days after inoculation and incubation at 86° F.



Etiolation symptom induced after root-dip inoculation strategy. Note the yellowing and extended growth of the plant.

Etiolation observed on inoculated field plots at Michigan State University. Confirmation of bacterial colonization in plants (top left).

as a serial dilution where samples were diluted up to 1:1,000,000 with sterile water before plating onto agar media. This process selects for organisms in high enough abundance to survive the dilution, and disfavors those that are in low numbers living on or within plants. Once a few viable candidates emerged on plates, they were grown and tested for their ability to infect healthy creeping bentgrass.

The initial tests consisted of dipping sterile scissors into a liquid suspension of each bacterium and trimming plants with the dipped scissors. This allows the bacteria to enter into the open wound created by the fresh cut, and is necessary because unlike fungi, bacteria are unable to directly penetrate plants and require a wound or natural opening to get in. These initial inoculations showed only one of the bacterial culprits to be capable of causing significant disease symptoms; excitingly named MSU1. Once this observation was confirmed with repeated inoculations, the bacterium was re-isolated out of the inoculated turfgrass, and DNA was sequenced to determine its identity. The bacterium was found to be in the *Acidovorax* genus, and had DNA most closely resembling that of Acidovorax avenae subsp. avenae. It was at this point that a "First Report" was published in the journal Plant Disease.

Word spreads fast in the turfgrass industry, and soon a firestorm of discussions and postings related to the new "bacterial wilt" could be found on the web. Rampant speculation and misdiagnoses followed during the infamous summer of 2010, which undoubtedly contributed to the controversial nature of the emerging problem. At this point, the research being conducted on the new bacterial disease was still in its infancy, and any information regarding the disease was largely anecdotal. Researchers around the country were beginning to look further into the matter, and the USGA took proactive measures to address the issue by funding projects at several universities around the country. MSU's research was undertaken with some specific objectives, many of which require a great deal of time and resources to accomplish. This is yet another factor that perpetuated the speculative nature of the disease; the lack of immediate data or published information. While research is ongoing, pieces of the puzzle are falling into place while progress is lacking on others.

WHAT WE KNOW. The bacterium *Acidovorax avenae subsp. avenae* is pathogenic on creeping bentgrass; this much we know. An early objective, however, was to determine if the disease was specific to particular bentgrass cultivars. We tested several common cultivars of creeping bentgrass for their susceptibility to *Acidovorax* infection, and found that all were susceptible to some extent. There were, however, some significant differences in the level of susceptibility of Declaration and Tyee when compared to the other cultivars. Research conducted at the University of Rhode Island by Dr. Nathaniel Mitkowski has confirmed similar results with several other cultivars of creeping bentgrass. These results confirm the non-specific nature of *Acidovorax* and its ability to infect creeping bentgrass; however, recommendations regarding particular cultivars to turf managers battling the disease are unwarranted until extensive field studies can confirm truly resistant or tolerant cultivars.

We also know that the disease is more widespread than Charlotte, NC, but still relatively sparse. To date, researchers at Universities such as MSU, URI, Clemson, NC State, Purdue and several others have successfully isolated the *Acidovorax* pathogen out of more than 50 infected bentgrass samples from golf courses across the country. A large majority of the samples at MSU have come from the transition zone and Mid-Atlantic regions of the U.S. However, several have come from golf courses in the upper and lower Midwest.

Observations made over the past several seasons have lead to the hypothesis that this new disease may be "stress-related." Since symptoms are most commonly seen on intensively managed putting greens and are often located in highly trafficked or mechanically stressed areas, the pathogen has been considered a bi-product of turfgrass stress. Understand that all pathogens

require some predisposition of the plant in order to infect and cause disease. Whether it is low nitrogen fertility combined with low mowing heights favoring crown rot anthracnose or lack of soil oxygen from rain or over watering combined with high temperatures favoring summer patch, particular conditions must occur in order for a disease to take hold. This "new" disease is no different. Research has shown Acidovorax to be most aggressive on creeping bentgrass at temperatures exceeding 30° C (86° F). Couple high temperatures with low mowing heights and other aggressive cultural practices like double cutting and rolling, and the disease typically worsens in the field.

Most pathologists are recommending alleviating any unnecessary stress, especially during summer months, to discourage the onset of bacterial etiolation and decline. This sounds like a great solution to the problem, but as long as golfers demand "fast greens" for every day play, and even "faster greens" for tournaments, no golf course superintendent is going to raise their greens mowing height to 3/16-inches or add extra nitrogen fertility; at least ones that want to keep their jobs won't.

WHAT WE DON'T KNOW. While we are able to incite turfgrass death by inoculating *Acidovorax* onto healthy plants in a controlled environment, the symptoms of etiolation have remained somewhat elusive. In July 2012, MSU field studies produced widespread etiolation symptoms after inoculating with *Acidovorax* during high-temperature periods. By cutting into these plants, it was clear to see the successful colonization of *Acidovorax* in the plant vascular tissue. This is an important step in confirming all symptomology associated with the disease. Research using alternative strategies to inoculate and encourage bacterial entry into plants have been successful in eliciting consistent plant etiolation. Much work remains regarding the nature of bentgrass etiolation as it pertains to bacterial infection and other potential environmental or chemical triggers.

Remedial treatments, thus far, have been sporadic and unpredictable. Certain antibiotics have shown some promise in controlled environments; however, these results have not necessarily translated well in field trials. Additionally, these products are not labeled for use on turfgrass and are strictly for experimental purposes only. Research regarding products for managing this disease is ongoing and largely inconclusive. There are, however, anecdotal and preliminary treatments that have been reported to provide some level of symptom control from several trials around the country.

MSU1 inoculated (left) and non-inoculated control (right) pots after incubation for 14 days at 86° F.

Another interesting facet of this disease is where or how the problem arises. The bacterium can be found all over the world and is likely an organism that naturally occurs in most root zone soils and turfgrass systems. Research has also shown the possibility of other bacterial pathogens being involved or working in tandem with *Acidovorax*, and it is likely that we are just scratching the surface in characterizing this complex biological phenomenon. This emerging problem has been referred to by some as the "Tournament Disease." Unfortunately, there is not a time in the foreseeable future when PGA, USGA, Invitational or even member/guest tournaments will cease to exist. With that said, bacterial etiolation and decline is a problem we are going to have to face collectively. **GCI**

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What's in a name?

Since the initial description of the disease, much confusion has revolved around the nomenclature of the new disease on creeping bentgrass. Researchers in the U.S. have coined the new disease on creeping bentgrass caused by *Acidovorax avenae* "bacterial etiolation" or "bacterial decline" based on symptoms commonly observed on the affected putting greens. Symptoms of chlorosis and etiolation are common, and the disease can often lead to a general decline of irregular areas of turf. Wilting is not often the dominant symptom associated with *Acidovorax* infection of creeping bentgrass.

Bacterial wilt is a disease of annual bluegrass caused by *Xanthomonas translucens*. The disease is known for its characteristic wilt symptoms, causing plants to turn blue-purple, twist and wilt from the tips down. Etiolation is a shared symptom, often observed in early stages of bacterial wilt on annual bluegrass.

The minor differences may seem trivial, but when discussing diseases, it is important to distinguish exactly which disease is of concern. Bacterial wilt and bacterial etiolation and decline are caused by two completely different bacterial organisms (*Xanthomonas* and *Acidovorax*, respectively), and occur on two completely different species of turfgrass (annual bluegrass and creeping bentgrass, respectively). Therefore, lumping all bacterial disease under the umbrella of "bacterial wilt" would be erroneous, akin to calling every patch disease affecting turfgrass "take-all patch." Different diseases are favored by different environments and require different management strategies. Therefore, identifying and distinguishing them with the appropriate name is the foundation of proper management.