Otherwise known as: Bacterial Wilt, Bacterial Decline, Bacterial Etiolation, Etiolated Turf Syndrome

A better understanding (I hope)

By Adam Bagwell, CGCS

Table of Contents:

Background Information

There are very few bacterial diseases of turfgrass, and even fewer incidences in the history of turf pathology in which we have hard data on the causal organisms. In 1980 D.L Roberts and J.M Vargas identified a bacteria that caused decline on C15 creeping bentgrass on greens in the 1970's (other varieties such as Seaside and Toronto were also affected), *Xanthamonas species.* Initial symptoms were described as "rapid wilting of leaves that initially appear blue-green, shriveled and twisted, but soon change to brown as the entire plant becomes a rotting mass of mush"(D.L Roberts, J.M Vargas, Botany &Plant Pathology, MSU).

In 2009, a mysterious decline on greens height bentgrass reared its ugly head once more. In many samples, *Acidovorax avenae sub avenae* was identified. Symptoms seemed to affect only certain varieties of creeping bentgrass, and in a mixed sward of bentgrasses, not all had symptoms. Elongated leaves with light-green coloration and a capacity for scalping were noticed.

Over time, some plants seemed to senesce and have a water-soaked appearance, thinning the stand of turf. To this day,

Koch's Postulates[1] have been partially satisfied (Vargas), although infection in the field has not been reproduced (Macdonald). The sticking point for most scientists seem to be that infection can be produced by inoculating plants from a culture produced from other plants in the greenhouse under high heat and humidity regimes, but the symptoms don't necessarily match those in the field.

Along the way, decline of Poa annua with similar symptoms has also occurred, identified as *Xanthomonas transclucens pv. poae*. Again, light-green to yellow elongated leaves that grew faster than the rest of the canopy were noticed.

The simple fact that three different organisms have, and can cause similar symptoms has caused confusion in the turf pathology community. In addition, the symptoms seem to occur under high temperature, high moisture conditions, leading some pathologists to questions whether bacteria present is not just a parasitic response to weakened, unhealthy turf.

[1] **Koch's postulates are the following:**

1. The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.
2. The microorganism must be isolated from a diseased organism and grown in pure culture.
3. The cultured microorganism should cause disease when introduced into a healthy organism.
4. The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.

Understanding Bacterial Wilt of Annual Bluegrass

Bacterial wilt of annual bluegrass is caused by *Xanthamonas translucens.* Mitikowski of URI has been quoted as saying "It seems to be heat driven. If you have a period of extended heat it will pop up." Most researchers agree that bacterial wilt is driven by environmental stressors. Mitikowski says "if you get it, your greens look like garbage and play like garbage. If you get a break in the weather, give it three weeks and the turf will bounce back." When compared to important turf diseases of annual bluegrass like ancthranocse, bacterial wilt does not seem as serious, as most of the time the turf recovers.

The first symptoms of bacterial wilt in annual bluegrass appears to be the formation of etiolated shoots of grass. Some researchers have described this symptom as mad tiller disease or etiolated turf syndrome (ETS), further noting that the elongated shoots are a disease itself, but merely a symptom. Elongated shoots are followed by thinning and puffy turf prone to scalping, that turns into dead turf. Roots seem to die back(Reitman, Getting a Handle on Bacterial Wilt, TurfNet.com, 9/2/2011). Dissection of a

leaf causes bacterial streaming under an electron microscope.

Bacteria in the Xanthomonas family, produce Xantham gum in the plant. This exopolysaccharide is a virulence factor that contributes to the bacteria's ability to cause disease. This product used in commercial food products such as bubble gum and ice cream causes the xylem transport system in the plant to plug. The plant collapses shortly thereafter (Chavez). Xanthomonas bacteria can also be characterized as having a yellow pigment in their membranes which protects them form UV degradation.

There are few cures once the bacteria have invaded the plant. Roberts and Vargas noted that Xanthomonas compestris (causal organism of C15 decline or the bentgrass version of bacterial wilt) sprayed with Mycoshield or Oxytetracycline an antibiotic (also used in the treatment of humans, mostly used in orchards for fire blight of apples), at 2.5lbs of formulated ingredient (in other words out of the bag) in 50 gallons of water per thousand square feet was effective for 3-4 weeks. Unfortunately for turf growers, no antibiotics are currently labeled for use on turf. Other treatment options are Actiguard otherwise

known as acibenzolar in a preventative use only. Reasearch conducted by Chavez at URI and Syngenta have shown a 77% reduction in the occurrence of bacterial wilt of annual bluegrass if sprayed on the plant before the onset of symptoms. The turf product marketed with acibenzolar is Daconil Action. Anecdotally, Fosetyl-Al or Chipco Signature, is also thought to suppress the disease. Bill Brooks at Hanover Country Club in Pennslvania has suggested that regular use of copper based fungicides like Junction can act as a bacteriacide to prevent the occurrence of the disease. Compounds containing copper can cause phytotoxicity in high heat.

Understanding Bacterial Wilt of C15 Toronto, and Seaside Bentgrass

Xanthomonas compestris has been identified as the bacteria that caused “C15 decline” as it was known in the late 1970’s and early 1980’s (Roberts, Vargas, The new threat:Bacterial Wilt of Toronto Creeping Bentgrass). Serious investigation of the disease occurred as a result of an outbreak two weeks prior to the 1980 Western Open at Butler National Golf Club in Oak Brook, Illinos. Using an electron microscope, Vargas and Roberts found that there were “numerous bacteria limiting the water transport in the xylem of the plant” in the early stages of the disease that would explain the wilt symptoms. Vargas, and Roberts also found that the progression of the disease went from leaf tip to the crown, which eventually became brown and decomposed. As noted in the previous chapter, Vargas and Roberts found that control could be found with Mycoshield at a rate of 2.5 pounds of actual product in 50 gallons of water per thousand square feet. Vargas and Roberts also satisified Koch’s Postulates in the lab “under specific environmental conditions that inoculated plants produced similar symptoms in the field”. In “The new

threat:Bacterial Wilt of Toronto Creeping Bentgrass" by Vargas and Roberts, they mention that this genus of bacterium, the first one found to infect turfgrass in the United States bore resemblance to a European variety that cause bacterial wilts throughout Europe. They suggest that the introduction to America from Europe was "quite possible because it could be transported on seed, which is frequently exchanged between North America and Europe." The last point will be discussed at length in future chapters, because it accounts for some of the arguments between pathologists on Bacterial Wilt, Decline, or Etiolation. Are Bacteria a primary or secondary pathogen?

Understanding Bacterial Etiolation on Bentgrass caused by *Acidovorax avenae sub avenae*.

The first reported incidence of the disease occurred in 2009 from a Japanese scientist who published there report under a heading called “bacterial brown stripe of creeping bentgrass”. (Bacterial Decline of Creeping bentgrass, GCM 7/12). Interestingly enough, *Acidovorax avenae sub avenae* also cause a bacterial stripe disease in rice(Song Hwang Kim Schaad,2004). Damage to bentgrass has only been recorded on greens height, but Crane Creek CC in Boise, ID has experience dieback on bentgrass at collar height (.300in) as well. Symptoms generally resemble those caused by *Xanthomonas translucens* on annual bluegrass, with etiolated tillers light green in color, followed by wilting and in some cases death of the plant. Puffiness is common which can lead to further mechanical damage to the turfgrass. Symptoms seem to arise on almost any cultivar of bentgrass under prolonged heat, drought stress, and less than ideal environments such as shade, low air movement etc. Common cultural practices like topdressing with dragging, vertical mowing, aggressive grooming, anything that causes a wound in the plant increases the ability of

turfgrass to get an infection. Bacterial infections are passive, which means that the plant has to have an opening (either through stoma or a wound) for bacteria to colonize. Latin contests that the disease is actually quite rare. Of two hundred submitted samples in 2011, and 2012, only a small percentage actually had damage associated with Acidovorax. Further, Latin noticed that damage was noticed in samples that had high levels of bacteria, samples with lower levels did not necessarily display symptoms.

Figure 1: Confirmed Locations of Bacterial Wilt/Etiolation/Decline in 2010-2011. (Map courtesy of USGA)

According to Tredway(The Wilt that Wasn't GCI, 5/15/2012) copper containing fungicides like Junction and products

that contain Mancozeb may provide some benefit, but as he says in the article “none of these treatments are what we call effective.” Latin suggests that Acibenzolar (Daconil Action) and Chipco Signature may provide some plant health benefit through systemic acquired resistance, but would rather see superintendents limit stress on turfgrass by raising mowing heights, syringing, and rolling more. In an interview in late July conducted with Michael Agnew, PhD (Syngenta), Agnew implied that Daconil Action had provided up to a 77% suppression of symptoms. Giordano and Vargas agree by alleviating stress during summer months, increasing air movement, raising mowing height during stressful periods, avoiding aggressive cultivation practices like aerification and topdressing during peak outbreak times, and avoiding morning mowing to reduce the spread of the bacteria through dew and guttation water, these are the best management practices that lead to the best control(Giordano, Vargas A New Bacterial Disease Problem on Creeping Bentgrass Putting Greens, Golfdom’s Turfgrass Trends, Feb 2012). Steve Macdonald in an interview also suggested that a switch to light frequent applications of nitrate containing fertilizers would be beneficial. Anecdotally, when Macdonald has tested samples

(running soil analysis) of Acidovorax affected turf he's found in most samples, when compared to a non-symptomatic sample from the same course, a higher level of ammonium. For example, 20-30ppm in affected turf vs. 2-3ppm in unaffected turf. Macdonald feels that excessive fertilization with ammonium based fertilizers, and perhaps urea, contribute to, or are associated with the bacteria's success. Macdonald has also thought that as more superintendents employ more frequent rolling and skip mowings, that the 1/3 rule is being violated frequently. By skipping mowings the next cut actually "scalps" and more than 1/3 of the height of the plant is being removed. The increased wounding gives more vectors into the plant for the bacteria.

The Bacterial Argument, Primary or Secondary pathogen? Why no one knows quite yet.

The advent of Bacterial disease on turf has been a frequent topic of discussion. It most likely stems from a superintendent's unwillingness to want to explain why their grass is fading away without the common fungal symptoms. It is human nature to point to this mysterious new disease. And, since the pathologists around the country keep saying "there is no proof" that bacteria is actually causing plant degradation due to the fact that Koch's postulates haven't been satisfied 100%, then the discord is pronounced. Vargas has produced symptoms in plants with innoculations. However, the symptoms have not matched those seen in the field, and no field innoculations have produced any symptoms. Vargas states a lack of proper environmental conditions in Michigan as part of the problem (Giordano, Vargas A New Bacterial Disease Problem on Creeping Bentgrass Putting Greens, Golfdom's Turfgrass Trends, Feb 2012). The lack of ability to produce the exact environment on a green in a greenhouse is an issue as well. Another problem is funding! Until this bacteria cause millions of

dollars of damage to the industry, chemical companies won't throw much funding at solving the problem.

Vargas is currently working on providing a quick and accurate diagnostic tool for Acidovorax. Bacteria due to their size, sheer number of species, are notoriously hard to group. The Acidovorax genus has only recently been renamed from Pseudomonas avenae, due to new classifications based on DNA-rRNA homology (Song, Kim, Hwang and Shaad, 2004). In addition, after speaking with Dr. Vargas in July 2012, he hinted that he suspects that the bacteria is being introduced into plants from interseeding bentgrass into putting greens, a practice that has become more common in the past few years. Vargas suspects that Acidovorax, a disease found in wheat, transfers to grass seed from wheat chaff and stubble from previous crop rotations. Bacteria transfers from rice and cucurbit seeds to seedlings in agricultural settings. A 2004 study by Song,Kim,Hwang and Schaad entitled "Dectection of Acidovorax avenae ssp. Avenae in Rice Seeds Using BIO-PCR" describes a technique for enriching populations of Acidovorax in seed washings by incubating the seeds for 12 hours. Populations of saprophytic bacteria remained stable thus not interfering

with the PCR test. One of the issues identifying disease causing bacteria is separating what you'd like to study, with all the other bacteria that can affect the study. Populations of Acidovorax routinely reached detectable levels for PCR (polymerase chain reaction) testing. PCR is the basis for genetic fingerprinting, looking a segments of DNA for comparative purposes. The point of the brief description of a new fairly quick process for diagnosing bacteria on seed lots, is that Vargas would be able to substantiate his claim, and perhaps Acidovorax is imported on grass seed grown in Oregon. This idea cannot be easily dismissed based on evidence from agriculture.

Further frustrating the satisfaction of Koch's postulates concerning whether Acidovorax or Xanthomonas is a primary disease causing organism, or whether it is merely a result of other factors causing a weakened immune system in the plant, is the virulence factor of bacteria. Unfortunately for all academics, *Acidovorax avenae ssp. avenae* has many different strains, all of which have different levels of pathogenicity. Hu,Young,Triggs and Wilde (Pathogenic relationships of the subspecies of *Acidovorax avenae,* 1997) took strains isolated from different crops like sweet corn,

maize, oats and sugarcane, and re-infected them. Strains of *Acidovorax avenae ssp. avenae* were more virulent on sweet corn than maize, and almost non-virulent on oats.

Table 1 Strains of *Acidovorax* spp. used in this study showing host plant, country, year of isolation, originator, and other collections where held

ICMP No.	Host plant	Country and date	Originator (and number)
Acidovorax avenae subsp. ***avenae*** (Manns 1909) Willems *et al.* 1992			
7083	*Eleusine coracana* (L.) Gaertn.	Japan 1975	K. Nishiyama 75D-1
7084	*Eleusine coracana* (L.) Gaertn.	Japan 1975	K. Nishiyama 75D-4
3960	*Oryza sativa* L.	Japan 1955	K. Ohata = ATCC 19882: NCPPB 1392
251	*Saccharum officinarum* L.	Mauritius 1955	G. Orian. = ATCC 12529: NCPPB 359 – rec. as *P. rubrilineans*
252	*Saccharum officinarum* L.	Mauritius 1955	G. Orian = ATCC 12530: NCPPB 360
254	*Saccharum officinarum* L.	Reunion 1960	A.C. Hayward B385 = ATCC 19307: NCPPB 920 – rec. as *P. rubrilineans*
255	*Saccharum officinarum* L.	Australia 1961	A.C. Hayward B476 = NCPPB 931 – rec. as *P. rubrilineans*
3139	*Saccharum officinarum* L.	India 1963	Y.P. Rao – rec. as *P. rubrilineans*
3106	*Zea mays* L.	India 1970	S.R.S. Dange M9 – rec. as *P. rubrilineans*
3107	*Zea mays* L.	India 1969	S.R.S. Dange M4 – rec. as *P. rubrilineans*
3166	*Zea mays* L.	India	S.R.S. Dange M5 – rec. as *P. rubrilineans*
3167	*Zea mays* L.	India	S.R.S. Dange M6 – rec. as *P. rubrilineans*
3178	*Zea mays* L.	Japan 1970	M. Goto Corn-1
3179	*Zea mays* L.	Japan 1970	M. Goto Corn-4
3180	*Zea mays* L.	Japan 1970	M. Goto Corn-5 – rec. as *P. rubrilineans*
3183	*Zea mays* L.	USA 1958	A.J. Ullstrup = ATCC 19860: CFBP 2425: LMG 2117: NCPPB 1011– **Type strain**
3184	*Zea mays* L.	Japan 1968	M. Goto Corn-A
3185	*Zea mays* L.	Japan 1968	M. Goto Corn-B
5811	*Zea mays* L.	Brazil 1976	J.R. Neto – rec. as *P. rubrilineans.*
1656	*Zea mays* L. var. *rugosa* Bonaf.	USA 1963	C. Wehlburg 63-1 – rec. as *P. rubrilineans*
3168	*Zea mexicana* (Schrad.) Reeves and Mangelsd.	India	S.R.S. Dange T15 – rec. as *P. rubrilineans*
3181	*Zea mexicana* (Schrad.) Reeves and Mangelsd.	Japan 1970	M. Goto Teosinte-1
3182	*Zea mexicana* (Schrad.) Reeves and Mangelsd.	Japan 1970	M. Goto Teosinte-2
3186	*Zea mexicana* (Schrad.) Reeves and Mangelsd.	Japan 1968	M. Goto Teosinte-A
3254	*Zea mexicana* (Schrad.) Reeves and Mangelsd.	Japan 1968	M. Goto Teosinte-B

Table 1: An illustration of the many different strains of acidvorax (courtesy of Hu,Young,Triggs and Wilde

(Pathogenic relationships of the subspecies of *Acidovorax avenae,* 1997)

This variation in the bacteria's virulence on different cultivars and species could explain why at Crane Creek CC in Boise, Idaho that only one cultivar of creeping bent rapidly died, while others seemed unaffected, even though they showed the typical etiolated symptoms. The virulence difference could also explain why when Acidovorax is isolated in the lab from, say, Charlotte, North Carolina, on G2 creeping bentgrass, and inoculated on A4 creeping bentgrass in Michigan, that the symptomatic expression is completely different. It would also seem to explain the inability to infect turf in a field setting. To get infection you need the correct climatic conditions, host, but you must also have the specific strain of bacteria. All *Acidovorax avenae ssp. avenae* is not created equal.

If virulent bacteria is transmitted on grass seed and causes bacterial wilt/etiolation/decline, what might be done to treat the seed to prevent the spread? Two experiments have been done trying to kill *Acidovorax avenae ssp. citrulli* on cucurbit seed. A Japanese study by Kubota, Hagiwara, and Shirakawa (Disinfection of Seeds of Cucurbit Crops Infested with *Acidovorax citrulli* with Dry Heat,

2012) looked at heat treatment of seeds and how much heat it would take to disinfect seeds without affecting germination percentages. The idea may cross into turf, but I doubt the results of their experiment will. Complete disinfection of watermelon, cucumber, and small-seeded squash was achieved at 85 degrees centigrade (185 degrees Fahrenheit) for 3-5 days. Consistency of the heat in the oven was found to be extremely important.

Another study done by Hopkins,Thompson,Hilgren, and Lovic (Wet Seed Treatment with Peroxyacetic Acid for the Control of Bacterial Fruit Blotch and Other Seedborne Diseases of Watermelon, 2003) looked at a wet seed treatment using Peroxyscetic acid at low concentrations. Treatments at 1600 micrograms/ml for thirty minutes were effective in preventing seed transmission of bacterial fruit blotch and gummy stem blight. Hydrochloric acid treatments at 10000 micrograms/ml, while effective, adversely affected germination. After treatment seed drying was needed at a low humidity in a 40 degree centigrade drying oven for 48 hours.

If Vargas is right in assuming that Acidovorax avenae ssp. avenae is a primary pathogens, there is some evidence from

the agricultural sector to substantiate the claim. If the bacteria is transferred on seeds, then there are also some possibilities for seed treatment to limit the transference to germinating seedlings.

Other turfgrass pathologists seem to take a much different approach. Since Koch's postulates have not been satisfied for "bacterial wilt/etiolation/decline, they believe that the presence of bacteria is a result of a weakened plant. Macdonald points to the fact that he's found etiolated turf on every species of cool-season grass. Etiolation is common on tall fescue where clippings have been dumped. Etiolation is common around the edges of urine spots, and also on the edges of fairy rings. Interestingly enough, this makes sense, as the dead grass from fairy rings is caused by the build up of ammonia gas is the soil. Clippings can contain fairly high levels of Nitrogen as well, and in a concentrated area, cause rapid growth of turf, as does the "green area" associated with a fairy ring.

(Photo Courtesy of Steve Macdonald, Turfgrass Disease Solutions, LLC)

Urine, as we all know, does contain urea, and can cause a rapid growth of turf as evidenced by anyone with a dog in a back yard.

(Photo Courtesy of Steve Macdonald, Turfgrass Disease Solutions, LLC)

Macdonald has also seen some correspondence to high levels of ammonium in soil samples under affected turf with ETS/Bacterial Etiolation/Decline.

Soil Data and ETS Observations

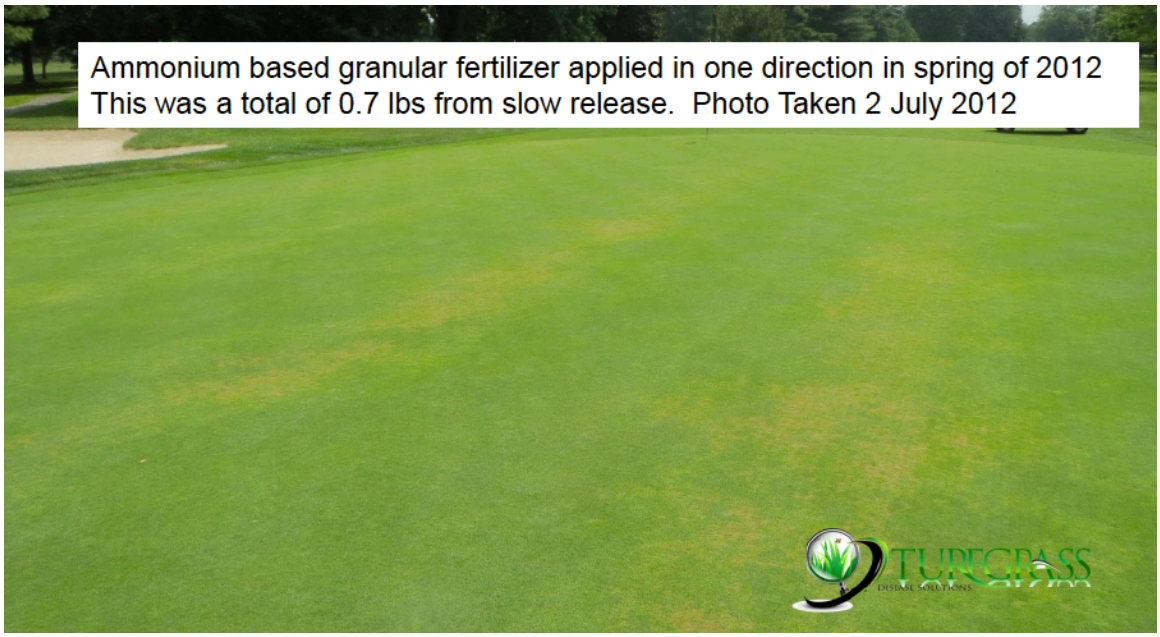
		Averages				
State of Origin	Symptom Expression	NH4 levels (ppm)	NO3 levels (ppm)	Total Available N (ppm)	Suggested NH4 ppm	Suggested NO3 (ppm)
Pennsylvania	YES	25.65	12.0	37.65	5	5
Pennsylvania	NO	8.0	9.6	17.65	5	5
New Jersey	YES	9.3	9.5	9.35	5	5
New Jersey	NO	0.7	1.6	2.4	5	5
Maryland 1	YES	18.4	10.1	28.5	5	5
Maryland 1	NO	8.5	5.8	14.35	5	5
Maryland 2	YES	11.5	1.1	12.6	5	5
Maryland 2	NO	<0.5	1.1	<1.6	5	5
Averages	YES	16.2	8.1	24.4		
Averages	NO	8.3	4.5	12.8		

Bacterial streaming was also evident in New Jersey and Maryland sample Samples were analyzed by Brookside Labs, Inc. Samples were taken on the same greens inches to a few feet away.

Table 2: Soil Sample N levels in affected and unaffected turf (Courtesy of Steve Macdonald, Turfgrass Disease Solutions, LLC)

Let's combine the theory of high nitrogen levels associated with the disease with the idea that Acidovorax or Xanthomonas may be a primary pathogen. Discounting the fact that Nitrobacter tend to die at temperatures above 49 centigrade (120 Fahrenheit) and the optimal range for

conversion of NO2 to NO3 is around 28 centigrade (82 Fahrenheit), an interruption in the nitrogen cycle due to high heat could be the reason for differentiation in the soils of table 2 (Holt,Hendricks, Bergey's manual of Determinative Bacteriology, 1993). The symbiotic relationship of Nitrobacter and bentgrass cultivar could be a contributing factor, some cultivars may not be as hospitable to the Nitrobacter as others. A research article published by Nalcaci, Boke, and Ovez (Potential of the bacterial strain Acidovorax avenae subsp. avenae LMG 17238 and macro algae Gracilaria verrucosa for denitrification, 2011) demonstrated that when provided with a carbon source like ethanol or glucose, *Acidovorax avenae ssp. avenae* strain LMG 17238 in a fixed bed batch system, can remove nitrogen from water. Does Acidovorax take advantage of high-N situations in the plant?



The light greens lines show where the fertilizer was overlapped, but also where the ETS/bacterial etiolation/decline occurred.(Photo Courtesy of Steve Macdonald, Turfgrass Disease Solutions)

Does this explain the chlorotic nature of the etiolated plant as the bacteria remove available nitrogen from the plant? Does a plentiful supply of food cause rapid bacterial growth which does indeed plug the xylem and cause the plant to wilt?

Lebanon Country Club has been fighting etiolation on perennial ryegrass fairways and coined the name “mad tiller disease.” Etiolation has also been found on Seashore paspalum, a C4 warm season turf. Since etiolation is common on grass in non-shaded conditions on a variety of cool and warm season grasses, a quick assumption would be that it is

indeed related to the environment, and further that etiolation of turf is merely an annoying symptom a because wide-scale turf loss rarely happens where these symptoms occur. Macdonald (interview, August 10,2012) states that in his experience, Etiolated Turf Syndrome, or ETS (his preferred name), arises due to a disease square, rather than what is generally accepted, the disease triangle, where the correct variety of grass that is under stress (mowing, cultivation etc.), coupled with severe weather (high heat typically), and a rapidly growing plant (perhaps due to over fertilization) yields the disease.

Host Stress

Weather Rapidly growing tissue

Figure 2: The disease square for Etiolated Turf Syndrome or Bacterial Etiolation/Decline

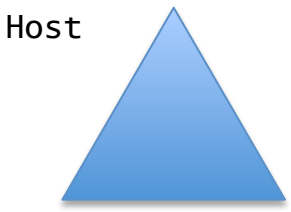


Figure 3: The disease triangle, in order to get a disease all three conditions must be met.

Macdonald has also seen ETS in areas of higher organic matter in more than on case, around irrigation heads and in the intermediate, two areas that do not get aerified on a regular basis. Perhaps high organic matter levels contribute to the correct environment for bacteria to thrive. More bacterial identification needs to be completed on a wide variety of turfgrass species, other than just on greens to support the “environmental stress causes secondary infection from bacteria” hypothesis.

Crane Creek Country Club Case Study

Crane Creek CC, Boise, Idaho, had the unfortunate circumstance of battling (for the first time) what we called Bacterial Etiolation. While turf decline and loss started around July 1,2012, Crane Creek set the stage in October of 2011 by applying 20oz/A of paclobutrazol to their *Poa annua*, Creeping bentgrass greens. Crane Creek has found that high rates of paclobutrazol applied in fall, has a multiplied suppressing effect on Poa the next spring, while leaving the bentgrass unscathed. Using the early season suppression of Poa (which tends to green up earlier and out-compete the bentgrass) to our advantage, Crane Creek then applied in 2 applications 2 weeks apart starting April 1, 2012 .75lbsN/M of 18-9-18 30% slow release Contec DG. Another application of .5lbsN/M 18-9-18 Contec DG followed May 5, 2012 prior to aerification. Aerification occurred on May 10, 2012 using 10in. solid tines on 3x5 spacing 1 direction, followed by a Planetaire slicing treatment in two directions. Heavy topdressing was applied and brushed in. Our preventative spray program for was applied 3 times the first week of April, May, and June. The mix consisted of Banner Maxx II at 2oz/M and Heritage at

.4lbs/M. Our weekly “soup mix” of liquid fertilizers was initiated on April 1. Our “soup mix” changed this year to include a locally built product, which is an enzyme extraction from healthy soil to stimulate soil bacteria reducing the need for as much N,P,K. Kelp and Humic/Fulvic acids are also part of the mix. Biostimulants containing gibberillins and cytokinins were included as well at labeled rates. A micronutrient package is included weekly as well. The application applies about .05lbsN/M/week. NpHuric acid started to be injected (28-0-0-27%). A proportioning meter applies around .15lbsN/weekly throughout the season on all surfaces. Around June 25th, the green committee chair wondered if we could reduce grain on the greens, which we rolling around 11.5-12 every day in another response to the charge “greener, firmer, faster”. There was not much grain. However, we lightly verticut. Around July 4th, in preparation for our men’s member guest, with one 3ftx3ft thinning spot showing on #14 green, we verticut lightly (3/16in. deep on 1in.) spacing again. Unfortunately, the greenskeeper on the verticutter did not heed the warning about avoiding any thinning areas. Since we had initially thought the thinning spot was due to drought stress, the spot was solid-tined on 2in.x2in.

spacing on July 5th to increase water infiltration. Shortly after these two treatments, turf loss spread with lightly colored etiolated turf creeping outward from the 3x3 spot to incorporate 200 sq. ft. In addition, spots on #18 green, #15 green and #16 green were noticed (light-green etiolation on one cultivar of bentgrass, symptoms were in patches, Poa in the patches was not affected, other cultivars of bentgrass were not affected).

Photo Courtesy of Jon Atkins CCCC, (#18 green bacterial etiolation on one cultivar of bentgrass)

We initially self-diagnosed bacterial etiolation, and sent samples to Rutgers (Dr. Buckley) 7/17/2012. The samples

(#14 collar, #14green, #18) were confirmed to have a ”fire hose” of bacteria streaming from the xylem. Buckley diagnosed symptoms consistent with Bacterial Wilt. After confirmation, immediate communication started with the membership. “No known cause and no know cure” was the common theme. We sent out an informative letter with what to expect in course changes to our Tuesday club enews, followed by the same letter posted on our Turf Science Facebook Page, which then automatically links to our club cell phone App. We described that reducing stress was our best defense against the spread:

- Raised height from bench height of .130in-.145in which slowed greens from around 10.7-11.5 to 9-10.
- Skipped mows on Mondays and Thursdays and rolled instead
- Rolled almost every day to keep green speed up
- Immediately applied granular 18-9-18 Contec DG 2 .33lbN/M applications 1 week apart.
- Started on a weekly application of Daconil Action/Chipco Signature application to help suppress the disease
- Syringed affected turf 3x daily

- Cut down trees behind #14 to remove most afternoon shade
- Sodded collapsed areas (about 300 sq. ft on #15, 1200sq. ft on #14

Photo Courtesy of Jon Atkins CCCC, (#14 green, showing the spread. The initial area was the sod patch)

- Called Agnew, Macdonald and Vargas and discussed at length
- In August switched to foliar N applications every 3 days to KNO3 and CaNO3 at .1lbN/M
- Switched mowing patterns so that the most affected greens were mowed last.

- Aerified with knifing tines on August 8th (temp 105 Fahrenheit) coupled with a dusting of topdressing that was watered in.
- Increased greens moisture (from 19%-22%VMC to 25%-38%VMC average daily) and went to a light frequent watering schedule to reduce stress on turf. Anecdotally, it seemed the disease was worse where repeated wilt and perennial dry areas on greens occur.

The staff at Crane Creek kept up this program until August 15th, when shorter day lengths and cooler temperatures reduced symptoms and severity of the disease. By the second week of September, no visible damage remained.

The weather played a strong role. Boise recorded it's 3rd hottest July on record. 11 days this summer exceed 100 F, and over 30 days were over 90 F. A sample of weather data follows. The first 2 columns represent average daily soil temperatures, the next 2 columns represent data from our weather station.

STempUp	STempLow	Prev Day Air Temp Hi	Prev Day Air Temp Lo	GDD	Accum. GDD	Prev Day ET
68.37	64.40	92.7	48.1	20.4	531.05	0.2091
99.00	99.00	87.3	63.1	25.2	556.25	0.2066
99.00	99.00	80.2	51.8	16	572.25	0.2043
99.00	99.00	83.1	48.2	15.65	587.90	0.2048
99.00	99.00	83.5	48.0	15.75	603.65	0.2071
58.37	60.23	68.0	50.1	9.05	612.70	0.1553
99.00	99.00	80.5	40.9	10.7	623.40	0.1891
99.00	99.00	89.9	49.4	19.65	643.05	0.2069
99.00	99.00	84.8	50.7	17.75	660.80	0.1806
99.00	99.00	92.1	50.6	21.35	682.15	0.2084
99.00	99.00	84.2	58.7	21.45	703.60	0.1737
99.00	99.00	92.0	53.0	22.5	726.10	0.1995
99.00	99.00	79.8	60.9	20.35	746.45	0.2073
70.13	66.89	86.0	45.1	15.55	762.00	0.1980
65.86	68.55	89.1	54.2	21.65	783.65	0.1814
66.02	69.19	93.9	58.9	26.4	810.05	0.1876
65.61	68.83	96.3	58.4	27.35	837.40	0.2191
65.24	68.61	95.6	58.5	27.05	864.45	0.2070
66.88	69.96	103.2	60.8	32	896.45	0.1836
66.45	69.85	95.2	61.8	28.5	924.95	0.2056
67.56	70.55	95.2	58.7	26.95	951.90	0.1761
68.59	71.40	104.0	62.3	33.15	985.05	0.2136
66.17	68.52	86.6	62.9	24.75	1009.80	0.1244
65.92	68.87	91.7	62.0	26.85	1036.65	0.1506
66.94	69.61	85.4	59.3	22.35	1059.00	0.1743
64.17	67.75	86.5	61.3	23.9	1082.90	0.1339
64.09	68.43	93.8	58.1	25.95	1108.85	0.2044
66.52	69.95	91.6	55.7	23.65	1132.50	0.1994
67.21	69.81	99.3	60.6	29.95	1162.45	0.1757
64.56	69.28	93.7	59.9	26.8	1189.25	0.2064
64.61	68.73	91.3	53.5	22.4	1211.65	0.1963
67.14	71.26	97.2	56.4	26.8	1238.45	0.2104
64.78	68.90	86.2	57.0	21.6	1260.05	0.1910
64.83	68.54	88.1	54.4	21.25	1281.30	0.1831
65.97	69.87	93.7	55.3	24.5	1305.80	0.1855
66.76	70.47	96.5	59.2	27.85	1333.65	0.2116
65.56	70.12	94.4	58.7	26.55	1360.20	0.1696
64.58	69.38	92.0	55.3	23.65	1383.85	0.2019
64.66	69.52	95.1	55.2	25.15	1409.00	0.1899
63.83	68.93	92.8	55.1	23.95	1432.95	0.1995
63.86	68.90	93.6	53.2	23.4	1456.35	0.2004
62.87	68.26	91.5	53.4	22.45	1478.80	0.2006
62.57	67.72	88.3	52.5	20.4	1499.20	0.1996
66.05	70.43	85.8	52.0	18.9	1518.10	0.1947
67.36	71.65	90.4	51.4	20.9	1539.00	0.1912
65.49	69.97	95.6	57.3	26.45	1565.45	0.1885
68.29	71.83	95.5	65.0	30.25	1595.70	0.1966
63.18	68.59	102.7	60.5	31.6	1627.30	0.1922
62.55	68.07	92.5	64.4	28.45	1655.75	0.1561

63.23	68.35	94.2	56.6	25.4	1681.15	0.1799
62.74	67.90	89.5	61.4	25.45	1706.60	0.1143
62.36	67.59	88.0	53.8	20.9	1727.50	0.1873
63.31	68.24	91.3	55.9	23.6	1751.10	0.1630
62.17	67.45	92.1	56.7	24.4	1775.50	0.1628
60.98	66.62	91.5	56.2	23.85	1799.35	0.1676
64.67	68.57	92.7	55.7	24.2	1823.55	0.1688
63.10	67.79	89.2	56.4	22.8	1846.35	0.1691
64.95	68.45	89.7	55.0	22.35	1868.70	0.1688
61.34	66.65	92.3	53.8	23.05	1891.75	0.1629
59.20	64.91	85.8	62.2	24	1915.75	0.1135
57.25	64.08	90.2	57.2	23.7	1939.45	0.1512
54.61	61.54	89.5	61.4	25.45	1964.90	0.1354
59.43	62.91	86.4	54.2	20.3	1985.20	0.1591
62.81	66.28	87.1	52.5	19.8	2005.00	0.1673
60.39	65.94	77.4	45.4	11.4	2016.40	0.1637
57.83	64.07	86.7	44.7	15.7	2032.10	0.1394
56.76	62.72	95.2	55.5	25.35	2057.45	0.1448
58.01	63.05	89.7	59.5	24.6	2082.05	0.1541
60.24	64.64	91.1	55.4	23.25	2105.30	0.1685
53.87	61.02	79.2	48.7	13.95	2119.25	0.1372
54.36	60.87	84.3	46.4	15.35	2134.60	0.1423
55.33	61.33	85.3	52.4	18.85	2153.45	0.0957
53.87	61.02	81.3	55.4	18.35	2171.80	0.1507
54.36	60.87	81.8	43.7	12.75	2184.55	0.1514
55.33	61.33	85.0	44.6	14.8	2199.35	0.1432
55.45	61.07	81.6	46.8	14.2	2213.55	0.1517
57.25	62.42	87.8	47.6	17.7	2231.25	0.1460
54.67	59.94	81.4	51.2	16.3	2247.55	0.0754
55.50	60.84	84.1	48.1	19.3	2266.85	0.1423
60.84	63.62	90.5	49.4	19.75	2286.60	0.1376
57.14	63.02	90.1	61.1	18.9	2305.50	0.1547
53.22	60.13	76.7	47.7	9.2	2314.70	0.1334
48.46	56.92	70.7	39.3	7.55	2322.25	0.1233
49.80	56.77	75.8	36.7	9.5	2331.75	0.1296
51.32	57.33	82.3	42.2	15.25	2347.00	0.1471
54.18	58.97	88.3	44.5	15.7	2362.70	0.1408
56.14	61.35	86.9	48.4	15.3	2378.00	0.1379
53.21	59.10	82.2	47.7	13	2391.00	0.1392

Table: 3 Crane Creek Summer Weather Data

Anecdotal evidence suggests that extreme, prolonged heat creates a beneficial environment for bacterial etiolation.

Crane Creek exceeded the average growing degree days in July by 166GDD.

Growing Degree Days (GDD) for Boise, ID

Jul. 1 - Jul. 31

2012 = **967.5** GDD

2011 = **788.5** GDD

Average** = **801.0** GDD

Table 4: Actual GDD for the period July 1, 2012-July 31, 2012, July 2011, and Average GDD for Boise in July

Crane Creek will track the accumulation of GDD in the with respect to the initiation of Bacterial Etiolation on greens. To predict BE, if it is as affected by heat, would enable a model to be made of a period of years, an important tool for timing of preventative cultural and chemical measures.

Samples were submitted to Vargas at Michigan State, partly because after a phone call, he'd never seen a case as far west as Boise. Unfortunately, confirmation was never received. Soil samples were also submitted to Steve Macdonald at Turfgrass Disease Solutions, LLC for ammonium concentration analysis.

lb/A

BROOKSIDE LABORATORIES, INC.

58483-1

SOIL AUDIT AND INVENTORY REPORT

Name Crane Creek Country Club City Boise State ID

Independent Consultant Turfgrass Disease Solutions, LLC Date 08/09/2012

Sample Location	GREEN		15 ETS	15 NO			
Sample Identification			d 4 in	d 4 in			
Lab Number			0494-1	0495-1			
Total Exchange Capacity (ME/100 g)			5.47	5.53			
pH (H_2O 1:1)			6.3	6.3			
Organic Matter (humus) %			3.08	2.74			
Estimated Nitrogen Release		lb/A	54	50			
ANIONS	SOLUBLE SULFUR*	ppm	17	20			
ANIONS / PHOSPHORUS	MEHLICH III	lb/A P as P_2O_5	250	278			
		ppm or P	82	91			
	BRAY II	lb/A P as P_2O_5	360	278			
		ppm or P	118	91			
	OLSEN	lb/A P as P_2O_5	79	76			
		ppm or P	26	25			
EXCHANGEABLE CATIONS	CALCIUM*	lb/A	877	875			
		ppm	658	656			
	MAGNESIUM*	lb/A	96	96			
		ppm	72	72			
	POTASSIUM*	lb/A	233	257			
		ppm	175	193			
	SODIUM*	lb/A	84	89			
		ppm	63	67			
BASE SATURATION PERCENT							
Calcium		%	60.15	59.31			
Magnesium		%	10.97	10.85			
Potassium		%	8.20	8.95			
Sodium		%	5.01	5.27			
Other Bases		%	5.10	5.10			
Hydrogen		%	10.50	10.50			
EXTRACTABLE MINORS							
Boron* (ppm)			0.49	0.52			
Iron* (ppm)			129	170			
Manganese* (ppm)			46	48			
Copper* (ppm)			4.15	3.81			
Zinc* (ppm)			11.27	11.60			
Aluminum* (ppm)			122	130			
OTHER TESTS	Soluble Salts (mmhos/cm)		0.18	0.12			
	Chlorides (ppm)		18.53	5.01			
	NO_3-N (ppm)		18.7	2.3			
	NH_4-N (ppm)		28.9	8.2			

d - specific depth

* Mehlich III Extractable

Table 5: Soil Analysis of #15 green, 2 samples one with Bacterial Etiolation/ETS, one without.

Notice the NO3 ppm for affected turf (18.7ppm) and unaffected (2.3ppm). The same trend follows for NH4 affected (28.9ppm) vs. unaffected (8.2ppm).

Other samples were submitted to Leah Brilman, PhD, Seed Research of Oregon. Crane Creek asked Brilman to see what clone or cultivar was being affected. Crane Creek had been interseeding T1, and Tyee creeping bentgrass for the past 4 years, and prior to that Dominant Plus was used to repair some damaged greens. Her reply was that she thought that the affected variety was most likely seed used at establishment (1963) or at least have been there for many years based on clonal plant size. Brilman also noted that she's seen Bacterial Wilt/BE/ETS reported on almost every variety, some more frequently than others, but thought that it may be related to certain cultivars used in geographic areas more than others. Brilman noted that the affected turf had shorter roots.

Crane Creek's plan for next year will be drastically different from this year's. According to all the theories about ETS/BE/Decline, we had the best weather for the disease. Higher fertilizer levels due to trying to grow bentgrass at the expense of Poa this spring, and high

levels of growth regulators which can add to plant stress, certainly contributed as well. Verticutting and mowing at an air temperature of 90 F, while disease was present wounding the plants and giving a greater opportunity for further spread, was also a poor choice. Sometimes a little grain is okay. Crane Creek will not try to suppress Poa by spraying fall 2012, thereby removing PGR stress in the spring. A reduction in the necessary nitrogen to push the bentgrass will result. Crane Creek plans on removing many microbiological stimulants from the weekly "soup mix" (Rossi, Unintended Consequences 8/2012) relying on nitrates, and micronutrients for the bulk of the nutritional needs on greens. Installing more soil moisture sensors will give us a broader understanding on problem greens than we currently with respect to irrigation needs. Greens will most likely be kept at higher moisture level, to avoid repeated drydown cycles. Irrigation intervals may be shortened from once every 5 days to once every 2-3 days. Crane Creek will educate the membership and reset green speed expectations back to water our 2008 green speed study indicated, rather than try to get 50 year old push up Poa/bent mix greens with old bent grass cultivars to 11.5 on a daily basis. Smoothness will be a focus. Mowing

everyday will be a priority to avoid scalping in aggressive growth conditions a per Macdonald's thoughts about substituting more rolling into cultural programs in place of mowing. No aggressive grooming or topdressing practices will take place when temperatures approach ninety degrees. A preventative program with Daconil Action and Chipco Signature will start in Mid-June and end at August 1st bi-monthly, 2 products that may suppress the disease. Our efforts at Crane Creek CC will be predicated on managing healthy turf.

Summary

Settling the argument of whether Bacterial Wilt/Decline/ETS is a primary or secondary pathogen will be a long, involved, task. Bacteria needs to be genetically assayed, an expensive process. Recent work has shortened the length of time for identification using BIO-PCR assays (Song,Kim,Hwang and Schaad, Dectection of *Acidovorax avenae ssp. Avenae* in Rice Seeds Using BIO-PCR,2004). I fully believe that both camps are right. In agricultural commodities including relatives to grass, bacterial infections cause widespread and significant damage. I doubt that Koch's postulates will ever be satisfied unless a green can be replicated in a lab. Too many uncontrollable factors determine symptom expression. If the genus and species and sub species of a bacteria is identified, the strain determines virulence, as does the host, and environmental factors.

The argument that a bacterial infection is a secondary disease due to stressors that create additional vectors into the plant, also makes sense. Extended periods of high air and soil temperatures seem to spur on the disease, while recovery usually occurs when the temperatures cool.

Interdependent variables and associations like higher than normal N levels under affected turf might be explained by Acidovorax avenae ssp. avenae's ability to feed off Nitrogen, as long as there is a carbon source nearby (Nalcaci, Boke, and Ovez, Potential of the bacterial strain Acidovorax avenae subsp. avenae LMG 17238 and macro algae Gracilaria verrucosa for denitrification, 2011). Whatever differences pathologists may have on whether *Xanthomonas transclucens* or *Acidovorax avenae* actually cause decline in turf, they all agree on the cure—grow healthy grass, employ correct cultural practices, and avoid stressing turf as much as possible.

I'd like to point out that the current "names" confuse the issue. Barring the discovery of any bacteria as a primary disease, and I feel confident that etiolated perennial ryegrass is associated with a different bacteria than *Acidovorax avenae* on creeping bent, or *Xanthomonas translucens* on poa annua, that the turfgrass industry accepts Etiolated Turf Syndrome (ETS). The name describes a common symptom found in all turfs, and all associated bacteria, without implying bacterial cause. And for now,

with our very small fund of knowledge about bacterial infections on turf, I submit that is all we can do.

References:

1. Brown, Kyle. 5/15/2012. The Wilt that Wasn't. Golf Course Industry.
2. Latin PhD, Richard. Bacterial Decline of Creeping Bentgrass. Golf Course Management. July 2012
3. Giordano, Paul. Vargas, PhD, Joe. A New Bacterial Disease Problem on Creeping Bentgrass Putting Greens. Golfdom's Turfgrass Trends. February 2012.
4. Reitman, John. Getting a Handle on Bacterial Wilt. Turfnet.com. 9/2/2011
5. Rossi, PhD, Frank. Unintended Consequences. Frankly Speaking. Turfnet.com. 8/3/2012
6. Song,Kim,Hwang and Schaad, Dectection of *Acidovorax avenae ssp. Avenae* in Rice Seeds Using BIO-PCR, J. Phytopathology 152, 667–676 2004 Blackwell Verlag, Berlin. ISSN (0931-17852004).
7. Nalcaci, Boke, and Ovez, Potential of the bacterial strain Acidovorax avenae subsp. avenae LMG 17238 and macro algae Gracilaria verrucosa for denitrification, 2011. journal homepage: www. elsevier.com/locate/desal
8. Holt,Hendricks, Bergey's manual of Determinative Bacteriology, 1993
9. Hopkins,Thompson,Hilgren, and Lovic. Wet Seed Treatment with Peroxyacetic Acid for the Control of Bacterial Fruit Blotch and Other Seedborne Diseases of Watermelon, 2003 Publication no. D-2003-1007-03R © The American Phytopathological Society
10. Kubota, Hagiwara, and Shirakawa.Disinfection of Seeds of Cucurbit Crops Infested with *Acidovorax citrulli* with Dry Heat, J Phytopathol 160:364–368 (2012)

11. Hu,Young,Triggs and Wilde. Pathogenic relationships of the subspecies of *Acidovorax avenae, Australasian Plant Pathology* (1997) 26: 227-238
12. Chavez, Arielle M.. Induced Resistance Against Bacterial Wilt of Annual Bluegrass (Poa annua L.) caused by Xanthomonas translucens pv poae. URI 2012.UMI # 1516631
13. Roberts, D.L., Vargas, J.M. Occurrence of Bacterial Wilt on Poa Annua and Other Turfgrasses MSU update pg 56-57
14. Roberts, D.L., Vargas, J.M. The New Threat: Bacterial Wilt of Toronto Creeping Bentgrass. MSU update pg 35
15. Macdonald, Steve, M.S, ETS Update July 2012. Interview Aug 10,2012
16. Brilman, Leah, PhD Seed Research of Oregon Email correspondence August 27,2012
17. Settle, Derek, PhD, Chicago Golf Association Email Correspondence, July 25, 2012
18. Agnew, Michael, PhD Syngenta interview, July 18, 2012
19. Buckley, Richard, PhD, Plant pathologist Rutgers U., interview, July 18, 2012