BY LARRY STOWELL, PH.D. AND COLLEAGUES

Shed light on rapid blight

Cooperative effort leads to a better understanding of impact and strategies

Rapid blight discovery is the story of a new plant disease whose unusual biology, inconspicuous morphology and apparently low economic impact almost consigned it to oblivion. It's also a story of what can be so rewarding about plant pathology – the excitement of discovery; the appeal of unraveling the complex interactions among plant, pathogen and environment; and the joy of collaboration. For it was only through a cooperative effort among a privately funded researcher, a publicly funded university, USDA researchers and the golf course industry that the identity of a new terrestrial pathogen was discovered, its economic impact better understood and strategies for its management developed.

EARLY OBSERVATIONS

During the winter of 1995, David Zahrte, the golf course superintendent of Santa Ana Country Club in Southern California, submitted a sample to the PACE diagnostic lab. Zahrte, who manages 19 *Poa annua* (annual bluegrass) putting greens, was worried that his greens, which seemed fine on a Friday afternoon, were suddenly covered with mysterious-looking patches of dead turf on Monday. (Fig. 1)

Fig. 1. Initial observation of a mysterious disease (later to be named rapid blight) on an annual bluegrass putting green in 1997 illustrating large coalescing areas. Photo: Larry Stowell

The affected turf's foliage was yellow and brown and had a water-soaked appearance, yet the roots seemed unaffected. A microscopic observation of the sample revealed none of the usual winter time Poa diseases such as Fusarium patch. There were no obvious signs of fungal pathogen invasion such as mycelia or spores. No insect or nematode pests or their damage were observed, whereas nutritional and cultural problems such as anaerobic soils or nutrient imbalances were also ruled out. The only detected abnormality was the presence of many thin-walled, spindle-shaped cells, measuring 6 by 16 μm and packed inside the foliage mesophyll cells.

Were it not for the sheer number of the spindle-shaped cells, they easily could have been mistaken for cellular organelles belonging to the plant. In fact, one mycologist suggested the spindle cells of the suspected pathogen might be just that, plant chloroplasts. However, when turfgrass samples were maintained in the lab in a moist chamber, the disease spread from diseased to healthy plants. There was a constant association between the presence of the spindle cells and diseased plant tissue. Unfortunately, this organism didn't resemble any other type of documented turfgrass pathogen.

A SERIES OF DEAD ENDS

Early attempts to identify the organism causing the new disease were frustrating. Identification through standard taxonomic keys and attempted isolation on standard culture media were fruitless. Initially, the organism was tentatively identified based on the morphology of its cells as a chytridiomycete. But attempts by Jim Adaskaveg of the University of California, Riverside, to isolate a chytridiomycete on specialized media from infected samples were unsuccessful.

Mycologists suggested the organism might be a protozoan, a single-celled animal, instead. Protozoologists countered suggesting the organism most likely was a chytridiomycete. Both were mistaken. It was discovered this organism was a unique pathogen to turfgrasses and was the first observation of this organism type attacking any kind of land plants.

PUTTING OUT THE FIRE

From 1995 through 1998, an increasing number of infected annual bluegrass samples began to arrive at PACE's diagnostic laboratory from locations throughout California and Colorado. Additional samples of rough bluegrass (*Poa trivialis*), used for overseeded Bermudagrass putting greens, arrived from Nevada. At first, the problem appeared to be spreading; but it's equally likely word began to spread about a new and mysterious disease, making superintendents anxious to see if their turf was being damaged by the disease. Control methods for the disease were needed even though the causal organism hadn't yet been identified.

The late Houston Couch of Virginia Tech was contacted for input on potential control strategies. Based on PACE's descriptions of the thin-walled, spindle-shaped cells, Couch suggested mancozeb might be an effective control agent because of its mode of action as a general membrane disruptor. His guess was correct, and shortly afterwards, mancozeb became the first recognized treatment for prevention and to limit the spread of the disease. This material was used under FIFRA Section (2ee), which permits the use of a registered pesticide on a pest that doesn't appear on the label, as long as the product is used on a labeled crop and all use and handling conditions on the label are followed.

GAINING STEAM

Initially, there appeared to be little interest in this new turfgrass disease in the academic world or the agrichemical industry. However, as the disease was identified in additional hosts and from additional locations, interest slowly grew.

Rapid blight symptoms on ryegrass in Arizona. Photo: Larry Stowell

In 1999, four years after the disease was first described from California *Poa annua* greens, golf course superintendent Mick Twito of Estrella Mountain Ranch in Phoenix submitted samples from a third host, perennial ryegrass (*Lolium perenne*). And in December of 2000, the first sample from the eastern United States was diagnosed when Tommy Witt, then superintendent of Cassique Golf Course in Johns Island, S.C. and president of the Golf Course Superintendent's Association of America, submitted a sample of rough bluegrass containing the same spindle-shaped cells in diseased foliage.

The occurrence of the disease in South Carolina was important because it brought Bruce Martin, Ph.D., of Clemson University into the project. Martin's lab took rapid action in 2001 by initiating a series of chemical management, host range, cultural, biological and molecular studies. Steven Alderman, a USDA forage pathologist at Oregon State University also became involved, investigating the potential for infection and transmission within the perennial ryegrass and rough bluegrass production seed fields in the Pacific Northwest. Fortunately, he ruled out seed-borne transmission as the cause of the initial disease outbreaks.

To further support the productive collaboration and information exchange that was emerging among superintendents and turf researchers, a working group was formed in 2001. The group consisted of superintendents from 60 golf courses throughout the country that were affected by this disease

Martin and Stowell were uncomfortable with the fact that golf course superintendents and others were using the name 'chytrid' to describe this unknown disease. It was suspected to be caused by an unknown organism resembling those in the Chytridiomycota, but it hadn't been confirmed. So, Martin and Stowell coined the name 'rapid blight,' which adequately described the consequences of the disease when it occurred in epidemic proportions.

The working group's first action was to provide funds to support Martin's initial research about disease control. Although the funding was insufficient to support a full-fledged project, the U.S. Golf Association soon provided a substantial grant to Martin's group to support more research. At the same time, Kurt Desiderio, a PACE working group member from Saticoy Country Club, was frustrated by the incomplete control of the disease with mancozeb. Based on

a quick screening trial that he conducted on the golf course, he found trifloxystrobin (Compass) was effective at stopping the disease. This was a surprise because repeated tests with the closely related azoxystrobin (Heritage) had yielded no positive results. But Martin immediately followed up by placing Compass and pyraclostrobin (which was soon to be labeled Insignia) in his next round of screening tests, and Desiderio's observation was confirmed. Trifloxystrobin was the first material shown to be more effective than mancozeb for controlling rapid blight. In 2003, Insignia was labeled for turf disease control and included rapid blight on the label.

IDENTIFYING THE CAUSE

The collaboration among scientists and superintendents received another boost when Mary Olsen, Ph.D., a plant pathologist at the University of Arizona, became involved because of an increasing number of Arizona golf courses that were suffering with the disease on greens, fairways and roughs. Working closely with Donna Bigelow, Dave Kopec and Robert Gilbertson, Olsen initiated lab and field research about rapid blight in 2002. The PACE working group was able to provide some funds to support the research. Olsen's work soon led to the long-awaited identification of the organism and the naming of a new species.

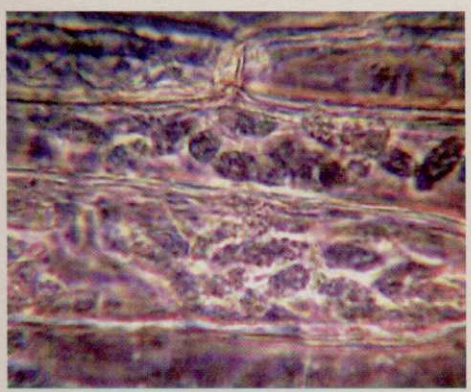
In 2002, Robert Gilbertson, professor emeritus of mycology at the University of Arizona, recognized the spindle-shaped cells in a rapid blight affected rough bluegrass sample from a golf course in central Arizona, similar to those that cause a disease of eelgrass in marine estuaries. The organism causing the wasting disease of eelgrass, *Labyrinthula zosterae*, is a marine slime mold. On the basis of shared morphological characteristics associated with the size and shape of its spindle-shaped vegetative cells and growth characteristics, Olsen and her associates proposed the rapid blight pathogen was a member of genus *Labyrinthula*.

Olsen subsequently was able to grow the cells isolated from diseased turfgrass tissue on an artificial medium developed specifically for *Labyrinthula*. Cells harvested from cultures were used to inoculate healthy *Poa trivialis* and perennial ryegrass. Symptoms identical to those observed in the field developed on all inoculated plants. *Labyrinthula* was then re-isolated from the inoculated diseased turfgrass. This technique of isolation, pure culture and reinfection is known as Koch's postulates and is considered proof the organism is the true pathogen and cause of disease. Olsen's group named the rapid blight-causing organism *Labyrinthula terrestris*. It's the only *Labyrinthula* known to attack a land plant. All others occur in marine environments.

LABYRINTHULA DESCRIPTION

Labyrinthula has been classified in different ways since it was first described in 1867. It's now placed in the kingdom Chromista (also called the Stramenopiles) with organisms such as diatoms and the Oomycetes (species of *Pythium* and *Phytophthora* are in this group), but it isn't closely related to these organisms.

Labyrinthula terrestris vegetative cells are fusiform, averaging about 6 by 16 μm and usually divide longitudinally. Labyrinthula terrestris forms digitate colonies in an extracellular net-



Spindle-shaped cells of the rapid blight pathogen in leaf tissue. Photo: Larry Stowell

work produced by specialized organelles called bothrosomes and can move along these networks at a notable speed. Cells contain various sized vacuoles, numerous lipid droplets and have a central nucleus with a large nucleolus. As cells multiply, colonial networks are formed and expand to as wide as 5/32 of an inch (4 millimeters) in 24 hours on agar culture media. After about a week, the cells migrate into rounded clumped aggregates ranging from 0.1 to 0.5 mm in diameter. Sori or reproductive cells have never been seen.

Given the quick emergence and increasing incidence of rapid blight disease on golf course turf, a full characterization of the pathogen through DNA studies was critical. Paul Peterson, a postdoctoral fellow in Martin's group, collected isolates of *L. terrestris* from rapid blight-affected turfgrasses from the East Coast and West Coast as part of a USGA assisted, nationwide survey ("Rapid Blight - Disease, Water and Soil Survey") that Martin's group initiated in 2003. In close cooperation with the Fungal Genomics Laboratory at N.C. State University under the direction of Ralph Dean, the rapid blight *Labyrinthula* sp. were found to differ from other described and some nondescribed species of Labyrinthula such as *L. zosterae*, which causes wasting disease of eelgrass. This work confirmed and supported the original morphological characterization and identification of the rapid blight pathogen by Olsen. Additional gene sequencing to examine genetic diversity among rapid blight pathogens is under way.

CONDITIONS FAVORING RAPID BLIGHT

In most cases, rapid blight has been associated with saline irrigation water and an accumulation of salt in the soil. To further evaluate this relationship, Martin's group sent out a nationwide request to golf courses with suspected rapid blight to submit samples of affected turf, irrigation water and soil for analysis. Evaluation of the samples was carried out as part of the above-mentioned survey, and a database about soil, water and weather parameters associated with disease outbreaks was compiled. Interestingly, in the Carolinas, rapid blight outbreaks occurred concurrent with drought and applications of high salinity irrigation water (greater than 2.5 dS/m or greater than 1,600 ppm). However, on some Western golf courses, the disease might also occur at lower salinity levels (0.5 to 1.5 dS/m or 320 to 960 ppm) based on the survey.

Subsequent greenhouse trials showed that little disease occurred in susceptible perennial ryegrass and Kentucky bluegrass cultivars that received applications of irrigation water less than or equal to 1.3 dS/m. Disease severity increased with increased salinity. Results from Olsen's group were quite similar. Plants irrigated with low salinity water (0.5 dS/m) show no symptoms of disease but become infected. As salinity of the irrigation water increases from 0.8 dS/m to 4.0 dS/m disease severity also increases. Managing irrigation water to reduce salt accumulation in soils is an effective way to reduce rapid blight.

GEOGRAPHIC DISTRIBUTION AND HOST RANGE

Rapid blight was initially identified on golf

IMPACT ON THE BUSINESS

The rapid take on rapid blight

Santayana said that those who ignore history are doomed to repeat it. That's why it's enlightening to understand the detective work done by PACE labs and others in the late 1990s to identify the then-unknown pathogen that was eventually identified as "rapid blight."

After nearly a century of scientific research into turfgrass diseases, it's hard to believe that "new" pathogens are still being identified, yet it happens all the time. Now, with the application of genetic mapping and DNA technologies, we could be entering a time when we truly begin to understand the root causes of many diseases that were only vaguely defined previously.

Bentgrass decline and zoysia patch are examples of conditions that eluded us for years and just now are beginning to be understood. Spotting the symptoms is easy. It's finding and managing the actual pathogen that's so difficult.

BUSINESS APPLICATION

As a business issue, battling rapid blight could be a major factor in your fungicide budget. Rapid blight requires some serious treatments that will cost you big bucks if it gets out of hand.

More importantly, this disease mars courses in a way that can't be easily fixed. If you want to avoid ugly-looking spots on your course, preventive applications are key.

FIELD ASSESSMENT

If you manage annual bluegrass, rough bluegrass or perennial rye and you have saline irrigation or soils, you are potentially at risk for rapid blight. The rotation of your disease control tank-mix program will be critical, particularly in mid-spring and early summer.

A standard fungicide mix may not be enough, so make sure to consult with your local technical reps to make the right choice.

FUTURE OPPORTUNITY

Could genetically enhanced, disease-resistant turfgrasses be the answer? Perhaps. With so many chemical companies investing significantly in biotechnology (as opposed to traditional fungicide development), there's no question that resistant species are coming. But "minor" diseases like rapid blight are unlikely to be high on the research priority list, so traditional controls will be needed for the foreseeable future.

"In most cases, rapid blight outbreaks have been associated with saline irrigation water and an accumulation of salt in the soil."

– Larry Stowell

Symptoms of a disease, later identified as rapid blight appearing on bentgrass in Southern California. Photo: Larry Stowell

courses in the United States on annual bluegrass, rough bluegrass and perennial ryegrass. Although the disease has been documented on creeping bentgrass by Martin and Stowell, it has occurred rarely. By 2005, C. A. Entwistle, in cooperation with Olsen, described the disease attacking colonial bentgrass and annual bluegrass golf course greens in the United Kingdom. The report from the U.K. was notable for its northern location. Until then, rapid blight had been isolated only from 11 U.S. states.

Peterson evaluated 49 different cool-season turfgrass species in hopes of identifying specific turfgrass species and cultivars with tolerance to rapid blight. All the grasses examined were susceptible to rapid blight but at markedly varying levels. Mean disease severity levels among the cultivars tested ranged from less than 1 percent to greater than 90 percent. Bentgrasses (colonial & velvet), bluegrasses (annual and rough), most ryegrasses, crested dogstail, hairgrass and wheatgrasses were susceptible. The grass species most tolerant to rapid blight were the slender creeping red fescues, creeping bentgrasses and some alkaligrasses. Similar results were obtained by Olsen's group in Arizona.

According to Olsen, Bermudagrass shows no symptoms of rapid blight, but in a survey of two golf courses where rapid blight occurred in cool-season grasses used for overseeding, *L. terrestris* was isolated from Bermudagrass roots and stolons during the summer after the cool-season turfgrasses had died out.

The combined observations of these experiments showed several cool-season turfgrasses to be tolerant of rapid blight under conditions of moderately high salinity stress. Some of these grasses might be suitable for overseeding where rapid blight is a chronic problem. In South Carolina, some golf courses are using seed blends of rough bluegrass and alkaligrass with acceptable results. Potential exists for the use of certain creeping bentgrass cultivars or slender creeping red fescues for overseeding as well, although slow rates of germination and establishment might be complicating factors to consider.

OTHER FACTORS

A thorough knowledge of the biology and lifestyle of a disease organism is helpful when managing and controlling disease caused by that organism. For this purpose, Martin's and Olsen's groups conducted a series of controlled environment experiments to determine the growth characteristics of the pathogen.

In lab studies, Olsen found *L. terrestris* grew well at 15 C to 30 C but grew slowly at 4 C and not at all at 40 C. Peterson found similar results when he evaluated the relative growth of 14 different *L. terrestris* isolates collected throughout the United States under varying degrees of temperature and levels of salinity. Growth parameters were studied and measured on solid

media. The results of these experiments from Martin's lab indicated that L. terrestris grew best in a range between 22 C to 26 C.

L. terrestris isolates grew well over a relatively wide range of salinity levels from 3.5 to 10.5 dS/m. These results suggest that different *L. terrestris* isolates vary in their salt requirements and that East Coast isolates might require higher salinities for optimal growth than West Coast isolates.

Wounding isn't necessary for *L. terrestris* to enter the plant, and *L. terrestris* moves easily from infected plants to noninfected plants when only a few leaves are touching or when plants share common drainage water.

CHEMICAL AND CULTURAL CONTROL

Field trials to determine efficacy of selected chemicals for control of rapid blight have been conducted at several sites. The most effective chemicals for prevention of rapid blight identified so far are pyraclostrobin (Insignia), trifloxystrobin (Compass) and mancozeb (Fore, Protect). Compass or Insignia mixed or rotated with mancozeb gives good control if applied preventively, while curative applications of chemicals might contain the disease but don't eradicate it.

Cultural control requires a variety of strategies including leaching programs to reduce soil salts, but leaching alone isn't sufficient in many cases. For this reason, the selection of rapid blight-tolerant overseeding varieties holds promise. Blending fast-establishing susceptible grasses (rough bluegrasses or moderately susceptible grasses like the perennial ryegrasses) with tolerant grasses (alkaligrasses, creeping bentgrasses and slender creeping red fescues) might help to reduce the risk of devastating epidemics of rapid blight.

COMING AND GOING

If the preliminary results of molecular analysis by Martin's group continue to yield genetic sequence data that lacks variation, there might be a recent common ancestor of the rapid blight pathogens. That ancestor appears to be most closely related to a *Labyrinthula* species that attacks *Spartina alterniflora*, a true grass (family *Poaceae*) in marine environments. When and how the jump from marine environments to terrestrial plants was made might never be known, but the search for the answer is intriguing and will occupy plant pathologists for years.

There are many more questions to answer concerning rapid blight and *Labyrinthula* as a plant pathogen. From what we know, rapid blight affects a broad range of cool-season turfgrasses that show an increased severity of symptoms as soil salinities increase. Soil salinity problems are likely to increase as competition for high-quality water, increased use of recycled water on golf courses and drought conditions occur. As a result, turf managers will need to develop management strategies that cope with the potential for increased rapid blight attacks. In the meantime, a successful integrated approach has been identified that relies on a combination of cultural practices and chemical control. **GCI**

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Literature cited for this article can be found on our Web site, www.golfcourseindustry.com, posted with this article.