

Isolation of the rapid blight pathogen *Labyrinthula terrestris* from Bermudagrasses in Arizona

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Abstract

*Rapid blight is a new disease of cool season turfgrasses that affects several important turfgrasses used for overseeding Bermuda in Arizona such as *Poa trivialis* and *Lolium perenne* (perennial rye). It is caused by *Labyrinthula terrestris*, an unusual organism that causes collapse of susceptible hosts and usually occurs in turfgrasses irrigated with moderate to high salinity irrigation water ($EC > 2.0$). Rapid blight has not been observed in the field in warm season grasses such as *Cynodon* sp. (Bermudagrass) although laboratory trials have shown that common Bermudagrass and other grasses may be hosts. Because the survival mechanism of *Labyrinthula terrestris* in the absence of cool season turfgrass hosts was unknown, this study was initiated to determine if *L. terrestris* “over-summers” in different Bermudagrass systems. Results of assays of several different Bermudagrass systems show that Bermudagrasses are good hosts for *L. terrestris* and explains why rapid blight is a perpetual threat year after year in the same locations.*

Introduction

Rapid blight is a disease of cool season turfgrasses such as *Poa annua*, *Poa trivialis*, *Lolium perenne* (perennial rye), *Agrostis tenuis* (colonial bentgrass) and *Agrostis palustris* (creeping bentgrass) (Bigelow, et al., 2005; Olsen, et al., 2003; Stowell, et al., 2005). In Arizona, commonly used varieties of *Poa trivialis* and perennial rye that are used for over-seeding Bermudagrass are very susceptible (Kopec, et al., 2005; Peterson et al., 2005). Rapid blight may cause seedling disease immediately after emergence of cool-season turfgrasses or develop on mature turfgrasses well into the spring. It has not been observed in the field in warm season grasses such as *Cynodon* sp. (Bermudagrass) although laboratory trials have shown that common Bermudagrass and other grasses may be hosts if inoculated and irrigated with high salinity irrigation water (Bigelow and Olsen, 2004a and b).

Rapid blight is caused by *Labyrinthula terrestris*, an unusual organism among turfgrass pathogens since it is neither a fungus nor a bacterium. It causes a rapid collapse of very susceptible hosts (Olsen, et al., 2003 and 2004). It grows best at salinities between about 2-8 dS/m, and most instances of severe disease occur at sites irrigated with water within this salinity range (Kohout, et al., 2005; Olsen et al., 2004). *L. terrestris* has been observed in host tissue and in laboratory culture only as microscopic vegetative cells, and no reproductive structures or survival structures such as are common among many plant pathogens have been observed. Its mechanism of survival in the field in the absence of a susceptible host is unknown.

In Arizona, rapid blight has been problematic in some of the same locations for several years, and our laboratory studies indicate that many other grasses including warm season turfgrasses are hosts for *L. terrestris*. We speculated that *L. terrestris* was established in Bermudagrass in areas where rapid blight occurs in cool-season turfgrasses used for over-seeding. The purpose of this study was to determine if *L. terrestris* “over-summers” in different Bermudagrass systems. Several sites with different cultural characteristics were sampled in mid summer when Bermudagrass was most active and all cool-season over-seed grasses had died out.

Materials and Methods

Two Arizona golf courses were sampled in July 2004. They are designated as golf course 1 (GC1) and golf course 2 (GC2). The salinity of irrigation water at GC1 is fairly constant at about 4.0 dS/m, and the golf course has a history of severe rapid blight disease that began shortly after the course was constructed in 1998. At GC2 the salinity of irrigation water varies from about 0.7 dS/m in the summer to about 1.3 dS/m in the winter as the irrigation source switches from well water in the summer to reclaimed water in the winter. GC2 is an established golf course where rapid blight was positively identified in 2003, but where disease was moderate and found only in a few isolated locations.

At GC1, two approaches to sampling were used: (1) samples were taken from a previously established research plot used for chemical trials, described as site GC1-1, where disease has been observed every year for the past 6 years; and (2) from 4 different sites around the golf course at which disease incidence varied from none observed to severe, described as sites GC1-2 through GC1-5. At GC2, two similar sites on the golf course were sampled at which disease was either moderate or had not been observed and are described as GC2-1 and GC2-2 respectively.

Site descriptions:

GC1-1: Practice tee with a history of severe rapid blight where fungicide trial plots were established in fall of 2003-2004. The tee is planted with Tifway 419 Bermudagrass and was over-seeded in winter 2003-04 with *Poa trivialis* "Laser". Replicated plots of two treatments were sampled for this study: (1) non-treated control with a high disease rating in spring 2004 and (2) effective fungicide treatment in which no disease was observed (Olsen and Gilbert, 2004).

GC1-2: Practice green with a history of severe rapid blight. The green is planted with MS Supreme Bermudagrass and was over-seeded in winter 2003-04 with *Poa trivialis* "Laser".

GC1-3: Driving range with no history of rapid blight. The driving range is planted with Tifway 419 Bermudagrass and had not been over-seeded for several years.

GC1-4: Fairway with a history of moderate rapid blight. The fairway is planted with Tifway 419 Bermudagrass and was over-seeded with perennial rye "Brightstar SLT" in winter 2003-04.

GC1-5: Fairway with no history of rapid blight. The fairway is planted with Tifway 419 Bermudagrass and was over-seeded with perennial rye "Brightstar SLT" in winter 2003-04.

GC2-1: Green with a history of moderate rapid blight. It is planted with Champion ultra-dwarf Bermudagrass and was over-seeded with *Poa trivialis* "Laser" in winter 2003-04.

GC2-2: Practice green with no history of rapid blight. It is planted with Champion ultra-dwarf Bermudagrass and was over-seeded with *Poa trivialis* "Laser" in winter of 2003-04.

Sampling:

At GC1-1 five samples were taken from each of eight 10 m² replicated plots by taking cores at 20 cm intervals along a diagonal line across each plot. The other sites were sampled by taking 10 cores along a line at 60 cm spacing. All sites were sampled using a profile sampler (MPS2-S Heavy Duty Mascaro 7 inch Profile Sampler, Turf-Tec, www.turf-tec.com) and included the turfgrass thatch layer as well as roots and surrounding soil. Samples were placed in individual sealed plastic bags and stored at 4°C until processed.

Assays for *L. terrestris*:

For each sample, excess soil was shaken from the root layer, and roots and stolons were washed for several minutes under running tap water to remove all remaining soil. Roots and stolons were cut into 2 cm pieces and rinsed thoroughly again in tap water. Ten pieces of roots and stolons were placed on SIA+ medium (Olsen, et al., 2003) on each of three culture dishes and incubated at 23°C. After one to three days, dishes were examined microscopically for growth of *L. terrestris* from plant tissue. The total number of pieces from which *L. terrestris* was isolated at each sampling site was averaged for each plot and the percentage of the total pieces assayed was calculated.

Results and discussion

L. terrestris was isolated from Bermudagrass at both golf courses at all sites. Results are shown in Table 1 for all sites except GC 1-1. *L. terrestris* was isolated from sites GC1-2, GC1-4 and GC2-1 where rapid blight had been observed the previous winter in cool-season grasses used for over-seed. It also was isolated from GC1-5 and GC2-2 where rapid blight has not been observed on susceptible cool-season turfgrasses used for over-seeding and from GC1-3, the driving range that had not been over-seeded for several years. When results from this trial were subjected to ANOVA and comparison of means (Tukey test), the number of isolations from GC1-4 was significantly different from other sites. The cause of the increased number of isolations from GC1-4 is not known, but the observation may be useful in further elucidation of parameters that result in increased infection of Bermudagrass.

At site GC 1- 1 there was no significant difference in the number of roots and stolons from which *L. terrestris* was isolated in fungicide treated plots and plots that were not treated. Although there was excellent control of disease in the *Poa trivialis* turfgrass used to over-seed plots in winter 2003-2004 using a mixture of pyraclostrobin and mancozeb (Olsen and Gilbert, 2005), the number of roots/stolons from fungicide treated, non-symptomatic plots did not vary significantly from untreated plots. These results indicate that control of disease in susceptible turfgrass does not prevent *L. terrestris* from surviving in Bermudagrass throughout summer months. In field trials to date in which Bermudagrass has been treated with effective fungicides before over-seeding with susceptible cool-season grasses, no reduction in rapid blight has been observed.

Bermudagrasses at these golf courses appear to be symptomless hosts of *L. terrestris*, and they probably serve as large reservoirs of inoculum for susceptible cool-season turfgrasses. The presence of *L. terrestris* in Bermudagrass that had not been over-seeded in several years (site GC1-3) shows that the pathogen can survive for long periods on Bermudagrass alone. Field observations and microscopic examination indicate that *L. terrestris* can move fairly quickly, either on its own or by way of foot traffic or machinery (Olsen, et al., 2004). Given the speed with which *L. terrestris* can move or be moved and its apparent long term association with Bermudagrass, rapid blight is a perpetual threat to these turfgrass systems. Research currently is underway to further elucidate the nature of the association between Bermudagrasses and *L. terrestris* as well as to determine methods to reduce inoculum levels in Bermudagrasses prior to over-seeding with susceptible cool-season varieties.

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Table1. Percentage of Bermudagrass samples from sites on two golf courses from which *L. terrestris* was isolated.

Site	Variety of Bermudagrass/ winter over-seed	Previous record of rapid blight*	Average percentage of roots and stolons from which <i>L. terrestris</i> was isolated**
GC1-2	MS Supreme/ <i>Poa trivialis</i> Laser	yes	17 a
GC1-3	Tifway 419/ none	na***	10 a
GC1-4	Tifway 419/ <i>Lolium perenne</i> Brightstar SLT	yes	42 b
GC1-5	Tifway 419/ <i>Lolium perenne</i> Brightstar SLT	no	10 a
GC2-1	Champion Dwarf/ <i>Poa trivialis</i> Laser	yes	7 a
GC2-2	Champion Dwarf/ <i>Poa trivialis</i> Laser	no	5 a

* Rapid blight disease was observed and *L. terrestris* was isolated from cool season turfgrasses used to over-seed Bermudagrass the previous winter.

** Numbers followed by the same letter are not significantly different (N=10).

*** Not applicable since the site was not over-seeded the previous winter.

Table 2. Percentage of Tifway 419 Bermudagrass samples from fungicide treated and non-treated control plots at site GC1-1 from which *L. terrestris* was isolated in laboratory assays.

Treatment	Average percentage of roots and stolons from which <i>L. terrestris</i> was isolated*
Fungicide treated plots	15.9
Non-treated plots	8.5

*Not significant by ANOVA (N =8, p=0.16).