Detection of the rapid blight pathogen *Labyrinthula terrestris* on non-symptomatic *Poa trivialis*

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Abstract

Rapid blight is a new disease of cool season turf grasses caused by Labyrinthula terrestris. It is problematic in Arizona and ten other states in cool season turfgrasses at sites with elevated salinity of soil and/or irrigation water. L. terrestris colonizes Tifgreen bermudagrasses in the field, but causes no apparent disease. Laboratory trials have shown that as concentrations of sodium chloride in irrigation water increase, disease severity increases, and when calcium and potassium salts are used to increase salinity, disease is greatly reduced or not observed. In preliminary field assays of cool-season turfgrasses irrigated with effluent, L. terrestris was observed in laboratory cultures from non-symptomatic turfgrass. To further substantiate if L. terrestris and/or other Labyrinthula species were present in non-symptomatic turfgrass in the field and to determine if disease could be induced by increased salinity, a trial was conducted at the Karsten Turfgrass Research Facility of The University of Arizona. In August 2006, field plots were established in bermudagrass "Tifway 419" and overseeded with Poa trivialis "Laser" in October. Plots were treated with potassium chloride, potassium sulfate or sodium chloride salts to increase soil salinity. Other plots treated with fungicides that are ineffective in controlling rapid blight as well as a sulfur treatment also were included in the assays. Poa trivialis was sampled in December 2006 and April 200. In laboratory assays using a semi-selective medium, Labyrinthula was detected in all treatments. Incidence was significantly higher in the untreated control and fungicide treated plots than in the salt treated plots. Results show that increasing soil salinity did not induce disease or result in an increase in detection of Labyrinthula at this site. Results of this study on Poa trivialis and previous studies on Tifgreen bermudagrass suggest that Labyrinthula may be widespread in non-symptomatic turfgrasses.

Introduction

Rapid blight is a new disease of cool season turf grass on golf courses in Arizona. It is caused by *Labyrinthula terrestris*, an organism in a group referred to as the marine slime molds. However, it is not a true slime mold at all, but more closely related to organisms such as diatoms (Bigelow, et al., 2005; Olsen, 2007). Early symptoms of disease include patches of turf that appear water soaked, slightly sunken and darker than healthy turf. Diseased turfgrass may yellow and die after only a few days. Rapid blight has been reported in eleven states (Stowell et al., 2005) and the United Kingdom (Entwistle, 2005). In most cases in Arizona, disease has occurred on golf courses using irrigation water with moderate to high salinity (EC > 2.0 dS/m). It has been observed in overseeded commercial lawns and sport turf as well. Rapid blight is problematic on turf varieties used for overseeding bermudagrass such as rough bluegrass (*Poa trivialis*), annual bluegrass (*Poa annua*), perennial rye (*Lolium* *perenne*), and colonial bent (*Agrostis tenuis*). Tifgreen bermudagrasses (*Cynodon* spp.) apparently are unaffected but are known hosts (Olsen and Kohout, 2006).

Field observations and laboratory data indicate that as soil and water salinity increase, disease increases. Rapid blight may disappear altogether after substantial rainfall of about one inch or more. Laboratory trials indicate that NaCl is required for disease development while increased potassium, calcium and magnesium salts are not (Olsen, et al., 2005). In the field, *Labyrinthula* was isolated from a perennial rye overseed in research plots at The University of Arizona Karsten Research Facility in which a study of salinity tolerance was being conducted, and extra salts were applied to the plots to increase salinity (Olsen, unpublished data). These observations led to the proposal that rapid blight could be induced in turfgrasses when soil salinity is increased indicating that the pathogen is present but not pathogenic, and that *L. terrestris* and/or other *Labyrinthula* species may be cosmopolitan in turfgrasses.

A trial was initiated at the Karsten Turfgrass Research Facility of The University of Arizona to further substantiate the presence of *Labyrinthula* in non-symptomatic turfgrass and to determine if disease could be induced by increased salinity. Assays also were conducted in plots treated two broad spectrum fungicides that have no effect on rapid blight (Stowell et al., 2005) and in plots treated with sulfur since it had shown promise for disease control in previous field trials (Olsen, et al., 2006).

Materials and Methods

Field plots.

Plots were established at the Karsten Turfgrass Research Facility at the Campus Agricultural Center in August 2006 in an established field of Tifway 419 bermudagrass. The field was irrigated with effluent water with a salinity of about 0.8-1.0 dS/m. Plots were 3 ft x 12 ft with eight replications of each treatment in a randomized complete block design. Half of each plot (6 ft of the plot length) was over-seeded with *Poa trivialis* on October 20, 2006.

Plots were treated with sodium chloride (NaCl) and potassium chloride (KCl) or potassium sulfate (K_2SO4) to increase salinity. Salt was applied weekly from September 1, 2006 through April 8, 2007. Treatments on bermudagrass before overseeding were KCl and NaCl at two rates, the equivalent of 2.0 and 4.0 dS/m irrigation water. After overseeding, the two rates of salts were applied to the *Poa trivialis,* but K_2SO4 was applied to the *Poa trivialis* instead of KCl. DisperSul Pastille 90% sulfur was applied once to bermudagrass before overseeding at 10 and 20 lb/1000 ft^2, and NaCl at the 4 dS/m rate was applied to these plots thereafter. Salts and DisperSul were applied using a Gandy spreader. The amount of salt to apply to each plot was an estimate of the amount of salt applied in irrigation water with an EC of either 2 or 4 dS/m. Calculation was based on evapotranspiration data for the field from previous experiments (Gilbert, unpublished data) and the subsequent amount of water applied for irrigation on a weekly basis. Salt applications were continued weekly with rates based on evapotranspiration data and subsequent irrigation required for cool season overseed turfgrass (Gilbert, unpublished data; Brown, 2003).

Two fungicides that are not effective against rapid blight, azoxystrobin (Heritage) at 0.4 oz product /1000 ft^2) and flutolanil (Prostar) at 2.2 oz product /1000 $ft^{2,}$ were applied once on November 21 to both the cool season overseed *Poa trivialis* and the non-overseed bermudagrass. Fungicides were applied with a CO_2 pressurized sprayer (R&D Sprayers, Inc.) at 30 lb psi using a two nozzle boom equipped with 1804 Teejet flat fan nozzles. No salt applications were made with these treatments.

Soil salinity was determined from soil samples one to four inches below the thatch layer. In the October sampling, all replications of the salt treatments except the Disper-Sul treatments were sampled and tested individually. In the December sampling, all replications of the salt treatments and Disper-Sul treatments were sampled and bulked; in the April sampling, all replications of all treatments were sampled and tested individually. Salinity was determined by the method of Harivandi et al. (1992) by stirring deionized water into soil until soil glistens but with no run off. The electrical conductivity (EC) was measured with a portable meter (Milwaukee SM802 pH/EC/TDS meter) that was routinely calibrated using standard solutions (YSI, Inc., Yellow Springs, Ohio 45387). Saturated soil extract EC measurements were calculated using the formula (EC reading x 2.7) + 0.8 (Harivandi et al., 1992; Stowell, 2007).

Samples of the *Poa trivialis* overseed were taken on December 15, 2006 and again April 23, 2006. One sample was taken from each replication (8) of each treatment using a profile sampler (MPS2-S Heavy Duty Mascaro 7 inch Profile Sampler). The sampler was washed between each sampling, and samples were placed in individual sealed plastic bags and stored at 4˚C until processed.

Laboratory trials.

Presence of *Labyrinthula* was determined by microscopic observations of the distinctive morphology of *Labyrinthula* from sub samples of on semi-selective medium SIA+ (Olsen, et al., 2004). Samples were processed over a 2 week period and were assayed by block. Thirty plants of *Poa trivialis* were separated from the bermudagrass in water in plastic trays, rinsed with tap water on a screen, and floated again in a plastic tray to remove soil and debris. Plants were dried in a folded paper towel and placed on SIA+ medium. Each plant was observed daily for 2-3 days for growth of typical *Labyrinthula* cells. Attempts were made to isolate all observed Labyrinthula, but many observations could not be cultured since contaminating fungi often obscured efforts for successful pure cultures.

Results and Discussion

Soil salinity was increased in plots treated with NaCl and KSO_4 salts (Table 1). As early as 7 weeks after salt treatments began (Table 1, October sampling date), both NaCl and KCl applications resulted in significant increases over the non-treated control (ANOVA and pair-wise comparison of means). At the lower rate, calculated to represent irrigation of 2.0 dS/m water, the salinity averaged 3.5 dS/m in both KCl and NaCl treatments while at the higher rate, 4.0 dS/m, the salinity averaged 4.3 dS/m. The untreated control averaged 2.4 dS/m. By December, after the potassium treatment had been changed to KSO4, the salinity had increased slightly. By April the non-treated control and fungicide treated plots, none of which received salt treatments, were not significantly different from each other but were significantly less than salt treated plots which ranged from 5.7 to 7.9 dS/m. These soil salinities are equivalent to those in turf areas affected by rapid blight.

Labyrinthula was detected in at least one block (replication) of each treatment in the December sampling. The mean percentage of subsamples that were positive for *Labyrinthula* for all treatments ranged from 0 to 53%. An ANOVA and comparison of means indicated that there were no significant differences in the number of *Labyrinthula* observations among subsamples from salt treated plots. Except for the DisperSul 10 lb treatment, the numbers of *Labyrinthula* detections from the salt treated plots were significantly different from the untreated control and fungicide treated plots, none of which were treated with salts (Table 2). The plots were sampled again in April 2007. The mean percentage of subsamples that were positive ranged from 0 to 63% with high detections in the untreated control and the fungicide treatments (Table 2). Overall, the mean number of detections in the December assays was higher than in the April assays.

Although *Labyrinthula* was widespread, there were no disease symptoms in the *Poa trivialis*. *Labyrinthula* grew from lower senescing leaves of 72% of the plants assayed, indicating that the isolate at this location may be a good saprophyte and a weak parasite (Table 3). The isolates from the site vary slightly in morphology and growth habit from most of the isolates we have observed previously from diseased plants from other sites. The cells are smaller, and the colonies or networks that form are not as dense. Preliminary inoculation trials in the laboratory indicated that they were not as virulent but colonized *Poa trivialis* and perennial rye in laboratory assays.

Results show that it is possible to increase soil salinity by direct application of salts with minimal damage to the turfgrass. However, the salt applications did not induce disease and did not increase the incidence of *Labyrinthula* isolations from turfgrass samples. Compared to untreated plots, detection in salt treated plots was actually less in most assays. Results indicate that *Labyrinthula* may be widespread in turfgrasses and differences may exist in terrestrial isolates of *Labyrinthula* in turfgrass either as different ecotypes, pathotypes or even species. Further studies may elucidate differences in taxonomy and pathogenicity in *Labyrinthula* isolates from turfgrasses.

Acknowledgement

This work was supported by a grant from the Technology and Research Initiative Funds (TRIF) for The University of Arizona Water Sustainability Program (WSF).

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Table 1. Soil salinity (EC) of field plots after application of potassium and sodium salts. Values for the October and April assays are the average EC of all replications (N=8).

	Sampling date		
Treatment	October*	December**	April*

Non-treated control	2.4	c	2.4	3.8	c
NaCl 2 dS/m	3.5	b	3.5	5.7	b
NaCl 4 dS/m	4.3	a	5.1	7.7	a
KCl/KSO_4 2 dS/m	3.5	b	3.8	5.8	b
KCl/KSO_4 4 dS/m	4.3	a	4.6	7.9	a
Fungicide – Prostar 2.2 oz/1000 ft^2	_		_	3.7	c
Fungicide – Heritage 0.4 oz/1000 ft^2	_		_	3.4	c
Disper-Sul 10 lb/1000 ft^2 + NaCl 4 dS/m	_		4.9	7.5	a
Disper-Sul 20 lb/1000 ft^2 + NaCl 4 dS/m	_		5.7	7.9	a

*Within each sampling date, numbers followed by the same letter are not significantly different according to comparison of means using Least Significant Difference Test.
**No statistical analysis was done since December samples were bulked by treatment; values represent the EC of samples from all replications (N=8) mixed equally.

Table 2. Detection of *Labyrinthula* by microscopic examination of *Poa trivialis* tissue placed on semi-selective medium in the laboratory. Values are the mean number of positive observations out of 30 grass pieces sub-sampled from each replication of each treatment (N=8).

	Assay date			
Treatment	December*		April*	
Non-treated control	5.8	ab	1.8	ab
Fungicide – Prostar 2.2 oz/1000 ft^2	5.4	ab	2.0	ab
Fungicide – Heritage 0.4 oz/1000 ft^2	7.2	a	3.1	a
NaCl 2 dS/m	0.8	c	0.6	b
NaCl 4 dS/m	0.8	c	1.1	ab
KSO_4 2 dS/m	1.6	c	2.1	ab
KSO_4 4 dS/m	0.4	c	1.0	ab
DisperSul 10 lb/1000 ft^2 + NaCl 4 dS/m	2.6	bc	0.1	b
DisperSul 20 lb/1000 ft^2 + NaCl 4 dS/m	0.8	c	0.9	b

*Within each sampling date, numbers followed by the same letter are not significantly different according to comparison of means using Least Significant Difference Test.

Table 3. Percentages of *Labyrinthula* observed in microscopic examination in culture assays from different tissue of *Poa trivialis* in all replications of all treatments combined.

Tissue type	December assay	April assay
Lower senescing leaf	77	50
Roots	13	23
Stem	0	4
green leaf	6	21
entire piece	4	2