Identification of a New *Waitea circinata* Variety Causing Basal Leaf Blight of Seashore Paspalum

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Abstract

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Seashore paspalum (*Paspalum vaginatum*) is a saline-tolerant, warm-season turfgrass species popular for golf course use in tropical and subtropical climates. A new variety of *Waitea circinata* (proposed as *W. circinata* var. *prodigus*) is described as the causal agent of basal leaf blight, a novel disease of seashore paspalum. Foliar necrosis and canopy thinning of seashore paspalum were observed on three different golf course fairways in Florida over an 18-month period. Five isolates with profuse, pink to yellow mycelia and small, salmon-colored or yellow to light-brown sclerotia were cultured from diseased turf foliage. Isolates grew rapidly over a temperature range of 25 to 35°C and were initially identified as an uncharacterized variety of *W. circinata*. Internal transcribed spacer sequences of rDNA from the isolates were

compared with sequences from previously described *W. circinata* varieties. The paspalum isolates formed a phylogenetic clade that was distinct from the other *W. circinata* varieties. Pathogenicity was confirmed on 'SeaDwarf' and 'SeaIsle Supreme' seashore paspalum, 'Penncross' creeping bentgrass (*Agrostis stolonifera*), 'Senesta' bermudagrass (*Cynodon dactylon*), and 'Dark Horse' roughstalk bluegrass (*Poa trivialis*). The geographical distribution and potential impact of basal leaf blight is unknown. However, the range of potential turfgrass hosts and environmental conditions conducive for disease development suggest that the pathogen may infect other species in addition to seashore paspalum.

Seashore paspalum (SP), *Paspalum vaginatum* Swartz, is a warm-season grass best adapted to tropical and subtropical climates (25). The species is halophytic (saltwater tolerant) and has been found in South Africa growing along seashores and in brackish waters, but the origin of the grass is uncertain (8,25). Numerous synonyms have been recognized since 1759, such as *P. distichum*, *P. foliosum*, *P. reptans*, and *P. reimarioides*, to name but a few (9). SP produces a very robust root system with deep, dense rhizomes and stolons (22). Suitable uses range from low-maintenance or remediation turfgrass for saline soils to higher-maintenance plantings such as lawns and golf course turf (9). The plant grows vigorously and is capable of producing a dense, dark-green canopy suitable for amenity turfgrass uses. SP was introduced into the turfgrass market in 1996 as a "next-generation" turf for golf courses that requires less water and fertilizer inputs to maintain a high-quality foliar canopy compared with other warm-season turfgrass species.

SP is susceptible to a range of fungal pathogens and nematodes that affect other warm-season turfgrass species. In greenhouse studies, SP was found to be as susceptible as bermudagrass, *Cynodon dactylon* (L.) Pers., to the parasitic nematodes *Belonolaimus longicaudatus* and *Hoplolaimus galeatus* (15). Samples of SP exhibiting dollar spot symptoms caused by *Sclerotinia homoeocarpa* F. T. Benn. are routinely received in the University of Florida Extension Plant Disease Clinic (P. Harmon, *personal communication*). Dollar spot has been reported as a disease of *P. vaginatum* in

China (19). Take-all root rot, caused by *Gaeumannomyces graminis* var. *graminis* (Sacc.) Arx & D.L. Oliver, an ectotrophic, root-infecting fungus of warm-season turfgrass, also has been diagnosed on SP (11). Large patch, caused by *Rhizoctonia solani* AG 2-2 'LP', occurs frequently on SP and may require preventative fungicide applications to prevent turfgrass loss (2,14,20).

In 2007, an unusual disease of SP was observed on three golf courses in Florida. The disease was observed three times during the coldest period of the year for South Florida, in January, but also twice during the hotter periods of the year, in July and September. Symptoms were observed on SP during cold and hot weather, following low and high fertility, and during wet and dry periods. Symptoms included irregular-shaped, blighted patches of turfgrass foliage ranging from 15 to 150 cm long and 5 to 90 cm wide. A *Rhizoctonia*-like fungus, tentatively identified as *Waitea circinata*, was isolated from necrotic leaves and stolons from samples taken from these locations. *W. circinata* is recognized as the teleomorph of a number of *Rhizoctonia*-like fungi (1,7,18,32,34,36,38). Previous work has suggested the use of variety designations for *W. circinata* to further differentiate the species based on biological and molecular genetic differences. Varieties *agrostis*, *circinata*, *oryzae*, and *zeae* (7,18,30,34) cause distinct diseases on graminaceous hosts but have not been reported on SP. The objective of this study was to assess the biology, pathogenicity, and genetics of isolates of *W. circinata* sampled from SP in Florida.

Materials and Methods

Turfgrass sampling and isolate maintenance. Eight golf courses in South Florida with either 'SeaIsle 2000', 'SeaDwarf', or 'SeaIsle Supreme' SP fairways were visited nine times approximately every 2 months from August 2007 to January 2009. One sample, 10.75 cm in diameter and approximately 15 cm in depth, was removed from an area exhibiting foliar blight or thinning on each of the eight golf course fairways or roughs at each of the nine

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visits (Fig. 1A and B). Each sample was placed in a polyethylene bag, and the bagged samples were placed on ice. The soil and dead organic matter were washed from each sample. Samples were sliced in half approximately 0.5 cm below the soil surface, separating the underground stolons, rhizomes, and roots from the above-ground leaves and stolons.

Sixteen aboveground and sixteen belowground necrotic tissue pieces (each approximately 1.5 to 2 cm in length) were removed from each plug, surface sterilized in 70% ethanol for 30 s, and rinsed twice for 30 s with sterilized, deionized water. Samples were blotted dry on sterile tissue and transferred to petri dishes containing either water agar (Bacto agar [Difco Laboratories, Detroit] at 15 g/liter of deionized water), one-fifth-strength potato dextrose agar (1/5-PDA; 7.8 g of PDA (Difco Laboratories), 10 g of Bacto agar, 150 µg of rifampicin [Fisher Scientific Co., Fair Lawn, NJ], and 0.5 g of ampicillin [Sigma Chemical Co., Steinheim, Germany] per liter of deionized water), or PDA + thiophanate-methyl (Topsin-M 70WP; Cerexagri Inc., King of Prussia, PA; 39 g of PDA, 0.1 g of thiophanate-methyl, 150 µg of rifampicin, and 0.5 g of ampicillin per liter of deionized water).

Samples were incubated at 25°C and were observed for up to 5 days. Nonsporulating, *Rhizoctonia*-like colonies with hyphae that had characteristic right- and acute-angled branching were transferred to PDA + rifampicin + ampicillin (31). Additional hyphal tip transfers were made to obtain pure cultures. Isolates were maintained in the dark at 25°C. For extended storage, isolates were transferred to sterilized oat seed that had been autoclaved for three consecutive days for 30 min each time. The inoculated oat seed was incubated for 2 to 3 weeks, dried in autoclaved paper bags, and stored at 15°C.

Colony and fungal characteristics. Size, color, and morphology of sclerotia were noted for five uncharacterized *W. circinata* (UWC) isolates from diseased SP on four golf courses after the fungi were grown at 25°C for 28 days on PDA. To measure hyphal width and the number of nuclei per cell, isolates were transferred to sterilized microscope slides coated with 2% water agar and incubated at 25°C in the dark for 2 days. Mycelia on the agar-coated slides were stained with 6 ml of 0.5% (wt/vol) safranin-O (Fisher Scientific Co.) in distilled water, 3.0% (wt/vol) KOH, and 5 ml of glycerol. Diameters of nine hyphal strands per isolate were measured, and nuclei per cell were counted (3).

Internal transcribed spacer region DNA sequencing. Ribosomal DNA (rDNA), including the internal transcribed spacer (ITS)1, the 5.8S ribosomal subunit, and ITS2, were sequenced from the five UWC isolates and compared with the sequences of other plant-pathogenic fungi, including *Thanatephorus*, *Waitea*, *Ceratobasidium*, and *Sclerotinia* spp. Approximately 50 mg of aerial mycelium from each isolate was removed from agar plates and macerated in 20 µl of sterile, distilled, deionized water for 1 min with a 0-3200 SPM Mini-Bead beater operated at medium speed (Biospec Products, Bartlesville, OK). A Qiagen Quick Clean-Up DNA extraction kit (Qiagen Inc., Valencia, CA) was used to extract DNA from the macerated samples according to the

manufacturer's instructions. Approximately 100 ng of template DNA was used for each 50-µl polymerase chain reaction (PCR) reaction with 20 pM of each oligonucleotide primer (ITS1 and ITS4) and the RED Extract-N-Amp PCR reaction mix (Sigma-Aldrich, St. Louis; 39). An Eppendorf AG 22331 thermocycler (Hamburg, Germany) was set for an initial 3-min cycle at 94°C; followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min; with one final cycle of 72°C for 10 min and a 4°C indefinite holding cycle. PCR product (5 µl) was electrophoresed in a 1.5% agarose gel with 0.005% ethidium bromide. The presence of the expected 650-bp amplicon was visualized with UV light after ethidium bromide staining.

DNA cloning. The PCR product for each isolate of UWC was cleaned with the QuickLyse Miniprep 250 kit (Qiagen Inc.) according to the manufacturer's directions. The PCR products for each isolate were inserted into *Escherichia coli* and cloned with a Topo TA Kit (Invitrogen, Carlsbad, CA). Five white bacterial clones per isolate were cultured in Luria-Bertani (LB) broth in a shaker incubator at 37°C for 14 to 16 h. The transformed bacterial clones were lysed and cleaned with a miniprep kit according to the manufacturer's directions (Qiagen QuickLyse Miniprep kit; Qiagen Inc.).

The purified plasmids were submitted for sequencing to the Interdisciplinary Center for Biotechnology Research at the University of Florida in Gainesville. All sequences were aligned using the Clustal W method in Mega Build 4.02 (Center for Evolutionary Functional Genomics, Tempe, AZ). After removing the primer sequences, consensus sequences were derived using three or more clone sequences for each isolate. A phylogenetic tree was constructed in Mega Build 4.02 using a neighbor-joining algorithm for the Kimura two-parameter model, and a genetic distance matrix was determined (5). Bootstrap values in Mega Build 4.02 were determined based on 1,000 random samples of the data set.

Temperature and growth studies. The five UWC isolates, an isolate of *W. circinata* var. *zeae*, and an isolate of *W. circinata* var. *circinata* were evaluated for colony growth rate at each of six temperatures. A mycelial plug (5 mm in diameter) was removed from the edge of a colony of each isolate on PDA, transferred to a 9-cm-diameter petri plate of PDA, and incubated at 15, 20, 25, 30, 35, or 40°C in the dark. Colony diameter was measured every 24 h for 4 days or until the colony filled the plate. There were three replicate plates for each isolate, and the test was performed twice. Growth rates (millimeters per day) were calculated for the first 24 h and rates were averaged across the six temperatures. Average growth rate data were analyzed with analysis of variance (ANOVA) in SAS (version 9.0; SAS Institute, Cary, NC). Fisher's least significant difference (LSD) test was used to separate means by isolate ($P = 0.05$).

Pathogenicity studies. Inoculations with the five UWC isolates were made onto two cultivars of SP (SeaDwarf and SeaIsle Supreme). A qualitative disease incidence assessment was made 3 days after inoculation, and the test was repeated two times. In subsequent inoculation experiments, isolate SK-PSA-TM4 was inoculated onto SeaDwarf and SeaIsle Supreme SP, 'Senesta' seeded

Fig. 1. Symptoms of basal leaf blight of seashore paspalum on A, 'Sealsle Supreme' and B, 'SeaDwarf' in south Florida.

bermudagrass (*C. dactylon*), and ‘Dark Horse’ roughstalk bluegrass (*Poa trivialis* L.). Disease severity was rated 3 days after inoculation, and the experiment was repeated. In a final inoculation experiment, the five UWC isolates were inoculated onto ‘Penncross’ creeping bentgrass (*Agrostis stolonifera* L.). Disease severity was assessed 3 days after inoculation. The experiment was repeated. In all inoculation experiments, three replications per isolate and host combination were used. Data from multiple repetitions of experiments were combined where statistically appropriate and unless otherwise specified.

Cool-season grasses and bermudagrass were seeded into 7.5-cm-diameter pots filled with calcine clay (Turface MVP; Profile Products LLC, Buffalo Grove, IL). The grass was grown for at least 3 weeks with up to one foliar trimming prior to but not the day of inoculation. Granular fertilizer was applied once at 0.3 g/pot (6-12-8 starter fertilizer; Harrell’s LLC, Lakeland, FL).

SP was sprigged into either 7.5- or 10.0-cm-diameter pots by placing five to eight stolon pieces of SP on the surface of each pot filled with calcine clay, and the pots were irrigated. The SP was fertilized twice with 6-12-8 fertilizer at 0.3 g per 7.5-cm-diameter pot and 0.5 g per 10-cm-diameter pot 1 month after sprigging. The SP was trimmed weekly for approximately 3 months, until the turfgrass canopy covered each pot. Inoculations were made approximately 4 months after sprigging.

Inoculum was produced on oat seed. Seed (50 g) was soaked in 50 ml of distilled water for 24 h in a 250-ml glass flask with a foam plug. The imbibed seed was autoclaved for 30 min once a day for three sequential days. Sterilized seed was inoculated with five agar plugs transferred from the leading edge of a 1-day-old PDA culture of each isolate. Flasks were maintained in a dark incubator at 25°C for 2 to 3 weeks. Approximately every 2 days, the flasks were vigorously shaken to mix the mycelia with the seed. The colonized oat seed was transferred to sterilized paper bags and dried for 2 weeks in a fume hood. Inoculum was stored at 5°C in sterilized 100-ml glass vials sealed with Parafilm.

Turfgrass was inoculated by placing five colonized oat seeds equidistant in the foliar canopy of a pot that had been irrigated with 100 ml of water. Each pot was placed in a 30-by-50-cm polyethylene bag containing a moist paper towel to maintain high humidity. The bags were sealed and placed in an incubator for 3 days at 30°C with 12 h of light per day. Pots inoculated with sterile oat seed served as a control treatment for all inoculation experiments.

Disease incidence was assessed 3 days after inoculation of the SP cultivars. Disease severity was assessed on pots of turfgrass utilizing the Horsfall-Barratt rating scale for inoculation experiments with SK-PSA-TM4 and the five UWC isolates on bentgrass (16). Data were subjected to logit transformation and converted to a 0-to-100% severity scale after ANOVA, and means separation was performed according to Fisher’s LSD utilizing SAS version 9.0. At the conclusion of these studies, eight pieces of host tissue from at least one replication of each inoculated turfgrass species were excised, surface-sterilized with 70% ethanol, rinsed, and transferred to 1/5-PDA and water agar. Isolated fungi were trans-

ferred into pure culture and identified by morphological characteristics as described above.

Results

Colony morphology and characterization. A summary of the morphological characteristics of UWC isolates SK-PSA-TM4, SK-PMA-WA1, SK-OA-W3-I, SK-OA-TM1, and SK-HBA-W1 is presented in Table 1. All UWC isolates had right- and acute-angle hyphal branching and produced extremely profuse, light-yellow to pink mycelial growth on PDA (Fig. 2A). The aerial mycelium of each isolate clumped, clustered, and attached to the underside of the petri dish lid. No basidiospores or other sexual (teleomorphic) structures were either found associated with the symptomatic turfgrass samples or in vitro at any time up to 120 days after isolation. Irregularly shaped, salmon-colored to brown sclerotia (Fig. 2B), 3 to >7 mm in diameter, were observed embedded in the PDA

Fig. 2. Characteristics of previously uncharacterized *Waitea circinata* (UWC) isolates. A, Colony on potato dextrose agar following incubation for 28 days in the dark at 25°C; B, differences in sclerotial characteristics, left to right, of *W. circinata* var. *circinata* (SK-SMD), UWC (SK-PSA-TM4), *W. circinata* var. *oryzae* (SK-30A-W1-I), and *W. circinata* var. *zeae* (SK-HBA-W1).

Table 1. Comparison of hyphal and sclerotial characteristics of isolates from symptomatic seashore paspalum in south Florida

Isolate	Colony morphology ^x	Hyphae ^w		Sclerotia	
		Diameter (μm)	Nuclei	Characteristics ^y	Size (mm) ^z
SK-PSA-TM4	Yellow-pink, aerial clumping, monilioid cells	5 ± 0.5	7 ± 1.1	Many, clumped, irregular, salmon, in agar	5 ± 1.7
SK-PMA-WA1	Yellow-pink, aerial tufted growth	5 ± 0.4	7 ± 1.4	Many, irregular, yellow-salmon, in agar	3 ± 1.0
SK-OA-W3-I	Yellow-pink, aerial clumping, monilioid cells	5 ± 0.6	7 ± 1.7	Sparse to none, yellow, spherical, in agar	5 ± 1.0
SK-OA-TM1	Yellow, aerial clustered clumping, monilioid cells	5 ± 0.3	6 ± 2.5	Sparse, clumped, irregular, yellow-orange, in agar	7.4 ± 2.6
SK-HBA-W1	Yellow-pink aerial clumping	5 ± 0.4	7 ± 1.7	Erratic, abundant, salmon-cream, catenulate, scattered in agar	3.4 ± 1.6

^w Mean and standard deviation ($n = 9$) of hyphae diameter and number of nuclei per cell after 2 days on 2% water agar.

^x Maintained on potato dextrose agar (PDA) at 25°C in the dark for 28 days.

^y In agar means sclerotia were produced at and under the surface of the agar medium.

^z Mean ($n = 9$) and standard deviation of diameter of sclerotia after 28 days on potato dextrose agar.

after 2 to 4 weeks of incubation. Isolate SK-3-OA-W3-I produced sclerotia that were light yellow, whereas SK-HBA-W1 produced light-brown sclerotia. Hyphal diameter averaged 5 µm with six to seven nuclei per cell for each UWC isolate.

Maximum growth rates for the UWC isolates occurred at 30°C (Fig. 3). When comparing rates of growth on PDA encompassing temperatures from 15 to 40°C, SK-PSA-TM4, SK-OA-TM1, and SK-OA-W3-I had statistically similar growth rates that were significantly greater than the growth rates of isolates SK-PMA-W1, SK-HBA-W1, and SK-SMd (Table 2). The growth rate of isolate SK-BA-W1 was significantly greater than that of all the other isolates tested. The growth rate of isolate SK-SMd was significantly less than that of all other isolates tested (Table 2).

rDNA-ITS sequence analysis. Isolates used for the analysis and GenBank accession numbers are given in Table 3. Genetic pairwise distances for all ITS sequences according to the Kimura two-parameter model had a range of 0.000 to 1.697 (*data not shown*). When pairing sequences, the smaller the number, the greater the similarity between the sequences, and the larger the number, the greater the dissimilarity between the sequences. The minimum and maximum genetic distance among the UWC isolates was 0.000 and 0.0021 (100 and 99.79% similarity, respectively), with no more than 1 bp difference in the 589 bp sequenced. A 9- to 14-bp

difference was detected between UWC isolates and *W. circinata* var. *agrostis* isolates (30).

The UWC isolates formed a distinct phylogenetic clade with 99% support (Fig. 4). The clade formed by the UWC isolates was closest to the clade formed by *W. circinata* var. *agrostis*, followed by the *W. circinata* var. *zeae* clade and the *W. circinata* var. *circinata* single-isolate clade. The *W. circinata* var. *oryzae* clade was most distant from the UWC clade (Fig. 4).

Turfgrass pathogenicity studies. Both cultivars of SP developed symptoms following inoculation with each of the UWC isolates. Symptoms included water soaking, chlorosis, and then necrosis of the lower leaf blades and sheaths 3 days after inoculation. Abundant aerial mycelium was observed in the lower leaf canopy for each isolate (Fig. 5). Symptoms developed in

Table 2. Mycelial growth rates of *Waitea circinata* isolates after 24 h on potato dextrose agar

Isolate	Growth rate (mm/day) ^z
SK-BA-W1	22 a
SK-PSA-TM4	20 b
SK-OA-TM1	20 b
SK-OA-W3-I	20 b
SK-PMA-W1	19 c
SK-HBA-W1	17 d
SK-SMd	11 e

^z Mean ($n = 36$) growth rate. Isolate SK-BA-W1 is *W. circinata* var. *zeae* and SK-SMd is *W. circinata* var. *circinata*. Means followed by the same letter are statistically similar according to Fisher's least significant difference ($\alpha = 0.05$).

Fig. 3. Comparison of mycelial growth rates (millimeters per day) of *Waitea circinata* varieties on potato dextrose agar at 15, 20, 25, 30, 35, and 40°C. SK-BA-W1 is variety *zeae*, SK-PSA-TM4 and SK-HBA-W1 are variety *prodigus*, and SK-SMd is variety *circinata*.

Table 3. Isolates of *Waitea circinata* and related fungi that were examined utilizing ribosomal DNA internal transcribed spacer region sequences

Isolate no.	Host ^x	Origin	Date collected	Identity ^y	GenBank accession no.
01 SK-SMd	Annual bluegrass	Reston, VA	25 April 2008	<i>Waitea circinata</i> var. <i>circinata</i>	FJ154894
05 SK-BA1	'Sealsle 2000' SP	Boca Raton, FL	4 September 2007	<i>W. circinata</i> var. <i>zeae</i>	HM597140
09 SK-4OA3-1/5	'Sealsle Supreme' SP	Sarasota, FL	4 September 2007	<i>W. circinata</i> var. <i>zeae</i>	HM597139
14 SK-3OA-TM	'Sealsle Supreme' SP	Sarasota, FL	30 October 2007	<i>W. circinata</i> var. <i>zeae</i>	HM597141
36 SK-OA-W3-I	'Sealsle Supreme' SP	Sarasota, FL	9 July 2008	UWC	HM597147
39 SK-HBA1	'SeaDwarf' SP	Naples, FL	9 July 2008	<i>W. circinata</i> var. <i>oryzae</i>	HM597138
42 SK-0821 BG	'Tifeagle' bermudagrass	Columbia, SC	24 August 2008	<i>W. circinata</i> var. <i>oryzae</i>	HM597137
44 SK-PSA-TM4	'SeaDwarf' SP	Ft. Myers, FL	4 January 2008	UWC	HM597146
45 SK-OA-TM1	'Sealsle Supreme' SP	Sarasota, FL	4 January 2008	UWC	HM597144
46 SK-HBA-W1	'SeaDwarf' SP	Naples, FL	4 January 2008	UWC	HM597143
59 SK-OA-W1-II	'SeaDwarf' SP	Sarasota, FL	4 November 2008	<i>W. circinata</i> var. <i>oryzae</i>	HM597135
60 SK-4BWB-W1	'Sealsle 2000' SP	Boca Raton, FL	6 January 2009	<i>W. circinata</i> var. <i>oryzae</i>	HM597134
61 SK-PB-TM1	'SeaDwarf' SP	Boca Raton, FL	6 January 2009	<i>Ceratobasidium</i> sp.	HM597133
66 SK-PMA-WA1	'SeaDwarf' SP	Ft. Myers, FL	26 August 2008	UWC	HM597145
69 SK-3OB1W	'Sealsle Supreme' SP	Sarasota, FL	30 October 2007	<i>Thanatephorus cucumeris</i> AG2-2IIIB	HM597131
70 SK-OA-W1-I	'Sealsle Supreme' SP	Sarasota, FL	4 November 2008	<i>W. circinata</i> var. <i>oryzae</i>	HM597136
74 SK-820 BG	'Tifeagle' bermudagrass	West Palm Bch, FL	10 July 2008	<i>W. circinata</i> var. <i>zeae</i>	HM597142
76 3BWA-TM2	'Sealsle 2000' SP	Boca Raton, FL	6 January 2009	<i>Thanatephorus cucumeris</i> AG2-2LP	HM597132
90 ATCC MYA-4521 ^z	Culture collection	Manassas, VA	April 2009	<i>Sclerotinia sclerotiorum</i>	FJ810516
91 NUK-3BG ^z	Creeping bentgrass	Aichi, Japan	June 1999	<i>W. circinata</i> var. <i>agrostis</i>	AB213567
92 DAI-BG ^z	Creeping bentgrass	Hyoga, Japan	July 1998	<i>W. circinata</i> var. <i>agrostis</i>	AB213569
93 OHT-BG ^z	Creeping bentgrass	Chiba, Japan	June 1997	<i>W. circinata</i> var. <i>agrostis</i>	AB213578
94 SHO-BG ^z	Creeping bentgrass	Kanagawai, Japan	July 1999	<i>W. circinata</i> var. <i>agrostis</i>	AB213575

^x Annual bluegrass (*Poa annua* L.), SP = seashore paspalum (*Paspalum vaginatum* Swartz), bermudagrass (*Cynodon dactylon* (L.) Pers.), creeping bentgrass (*Agrostis stolonifera* L.).

^y UWC = previously uncharacterized variety of *W. circinata*.

^z DNA sequence and other information were obtained from GenBank.

each of the three repetitions of the experiment, and isolates were recovered from symptomatic tissue for each isolate on each SP cultivar. No symptoms were observed in the noninoculated control treatments.

UWC isolate SK-PSA-TM4 caused an average of 57 to 97% disease severity on SeaDwarf SP, Senesta bermudagrass, SeaIsle Supreme SP, and Darkhorse roughstalk bluegrass after 3 days of incubation at 30°C (Table 4). Disease severity was greatest on roughstalk bluegrass. For all the turfgrass cultivars inoculated, the lower leaves were blighted and the sheaths turned yellow to brown, with a water-soaked appearance. Mycelium was copiously present in the lower and upper turfgrass canopy. The infected turfgrass was uniformly blighted and water-soaked in appearance, with little to no healthy tissue remaining 3 days after inoculation. There were no individual or distinct leaf or sheath lesions present. Isolates used in the pathogenicity studies were recovered from necrotic foliage of the various turfgrass species inoculated.

The UWC isolates SK-PSA-TM4, SK-OA-TM1, SK-HBA-W1, SK-PMA-WA1, and SK-OA-W3-I caused 98 ± 1.4 , 92 ± 7.0 , 88 ± 7.3 , 85 ± 13 , and $78 \pm 15\%$ disease severity (mean $\pm$ standard deviation), respectively, on creeping bentgrass 3 days after inoculation. Prolific aerial mycelium of each isolate grew throughout the lower turfgrass canopy. Water-soaking and necrosis of the leaf blades and sheaths were visible by day 2. Disease symptoms with

all UWC isolates on all turfgrass species began as a lower (basal) leaf blight, quickly progressing to an overall crown and foliar blight. No leaf spots, lesions, or sclerotia were observed.

Discussion

The discovery of a “new” SP disease and associated *Rhizoctonia*-like fungus merits a discussion of the taxonomy of the group. Over the last 150 years, diverse groups of fungal species have been assigned to the form-genus *Rhizoctonia* s. l. (1,7,13,21,23,24, 26,27). However, taxonomic reclassifications and clarifications have resulted in the development of several new anamorphic genera, each associated with a distinct teleomorphic genus. For example, the anamorphs of the genera *Thanatephorus*, *Ceratobasidium*, and *Waitea* are currently recognized as *Rhizoctonia* (33), *Ceratohiza* (23), and *Chrysorhiza* (32), respectively. Taxonomy and nomenclature of the teleomorphs of *Rhizoctonia*-like fungi have been based on characteristics of basidiocarps, sterigmata, basidiospores, and other sexual structures (31,32). Teleomorphs of the *Rhizoctonia*-like pathogens of turfgrass rarely are observed; therefore, isolate identification tends to be based on the morphology of anamorphs, which lack asexual spores and fruiting bodies (4). Traditionally, characteristics such as mycelial color, the number of nuclei per hyphal cell, and the shape, size, and color of sclerotia have been used to identify these fungi (34–36). In addition, isolates

Fig. 4. Phylogenetic distance tree comparing nucleotide sequences from the ribosomal DNA of *Waitea circinata* isolates and related fungi. Confidence values on the branches were obtained for 1,000 bootstrap replicates with Mega version 4.02 software.

Fig. 5. Symptoms of basal leaf blight caused by *Waitea circinata* var. *prodigus* isolate SK-PSA-TM4 3 days after inoculation on **A**, 'Penncross' creeping bentgrass; **B**, 'Dark Horse' *Poa trivialis*; **C**, 'Senesta' bermudagrass; **D**, 'SeaDwarf' seashore paspalum; and **E**, 'Sealsle Supreme' seashore paspalum. **F**, Shoots of SeaDwarf seashore paspalum noninoculated (left) and inoculated (right) with isolate SK-PSA-TM4.

have been grouped based on the ability of hyphae to anastomose (6,13,17,27,33,37).

The hymenomycete *W. circinata* Warcup & P.H.B. Talbot (38) was described to include fungi similar to *Thanatephorus* spp. but having horn-like sterigmata shorter than *Thanatephorus* spp. and basidiospores that fail to undergo repetitive germination. Mycelia of *W. circinata* were considered to be saprobic (38) prior to the diagnosis of what is believed to be the anamorph, *Chrysorhiza zae* (Voorhees) T.F. Andersen & Stalpers (= *R. zae*; 12,26,32), as a pathogen of several members of the genus *Poaceae* (4,12). Isolates

of *C. zae* also have been referred to as *W. circinata* var. *zae* (7,34,36). The epithet *Chrysorhiza* was created to accommodate fungi with a *Waitea* teleomorph (32), while *Rhizoctonia* anamorphs are associated with *Thanatephorus* (33).

In addition to *C. zae*, two additional fungi, *R. oryzae* Ryker & Gooch (27) and a species with the suggested name *R. circinata* (18), are characterized as anamorphs of *W. circinata* (18,27,28). These *Rhizoctonia* spp. appear to fit the concept of *Chrysorhiza* but the original names were not published according to the tenets of the International Code of Botanical Nomenclature (1). Therefore,

Table 4. Pathogenicity of *Waitea circinata* isolate SK-PSA-TM4 on seashore paspalum, bermudagrass, and roughstalk bluegrass

Inoculum	Disease severity (%) ^x			
	'SeaIsle Supreme' SP	'SeaDwarf' SP	'Senesta' bermudagrass ^y	'Dark Horse' roughstalk bluegrass ^z
Noninoculated	10 b	5 b	5 b	3 b
SK-PSA-TM4	69 a	57 a	57 a	97 a

^x Ratings were taken 3 days after inoculation and incubation at 30°C. Means ($n = 6$) within columns followed by the same letter are statistically similar according to Fisher's least significant difference ($\alpha = 0.05$). SP = seashore paspalum (*Paspalum vaginatum* Swartz).

^y Bermudagrass (*Cynodon dactylon* (L.) Pers.).

^z Roughstalk bluegrass (*Poa trivialis* L.).

supplementary taxonomic work (i.e., designation of type, Latin diagnoses) is required before *R. oryzae* and *R. circinata* are recognized as *Chrysorhiza* spp. In the meantime, reference to *W. circinata* vars. *oryzae* and *circinata* is appropriate.

Sequences from phylogenetically conserved regions of DNA such as the rDNA ITS region have been used to distinguish varieties of *W. circinata* (7,12,18,29). Isolates may be identified based on the similarity of conserved sequences such as ITS1, the 5.8S ribosomal subunit, and ITS2 when teleomorphic structures are not present (7,29). The UWC isolates we found associated with SP in Florida appear to be morphologically and genetically distinct from previously described species affecting turfgrass. Morphological characteristics that distinguished the SP isolates from established varieties of *W. circinata* included abundant, yellow to pink aerial mycelium on PDA and salmon to yellow-brown aggregated sclerotia measuring 3.4 to 7.3 mm in diameter embedded in the agar medium after 2 to 4 weeks of incubation at 30°C on full-strength PDA. In addition, analyses of rDNA sequences revealed that the isolates from diseased SP formed a clade that was significantly distinct from that of isolates of *W. circinata* vars. *agrostis*, *circinata*, *oryzae*, or *zeae*. Variability in growth rates, host ranges, temperature optima, disease symptoms, and hyphal and sclerotia color reinforce the differentiation of the UWC clade.

Based on morphological and sequence data, we propose that these isolates from SP comprise a new variety designated *W. circinata* var. *prodigus*. DNA sequence data indicated that isolates of *W. circinata* var. *prodigus* were only 97 to 98% similar to *W. circinata* var. *agrostis* and formed a distinct clade with 99% support in an ITS phylogenetic tree. Isolates of *W. circinata* var. *agrostis* were recovered from diseased creeping bentgrass in Japan (30). They were not observed to produce abundant, yellow to pink mycelium, and the sclerotia were only 1 to 3 mm in diameter and dark brown in contrast to the sclerotial described above as characteristic of *W. circinata* var. *prodigus*.

Diagnostic differentiation among *Thanatephorus* and *Waitea* spp. anamorphs affecting SP could be assisted greatly by routinely plating samples on both standard and thiophanate methyl-amended (100 µg/ml) agar media. Growth of the LP strain of *R. solani* that causes large patch is inhibited on the amended medium. Isolates of *C. zeae* that cause leaf and sheath spot, and *W. circinata* var. *prodigus*, will grow on both media but the *prodigus* isolates appear more floccose within 48 h of plating. Misidentification of these pathogens could result in unnecessary applications of ineffective fungicides. For example, symptoms caused by *C. zeae* or *W. circinata* var. *prodigus* could be confused with brown patch or large patch caused by *R. solani*, resulting in ineffective management of the disease with applications of thiophanate-methyl (10). Accurate identification of the causal agent for fungicide selection is an important factor in turfgrass disease management.

The proposed common disease name of "basal leaf blight", caused by *W. circinata* var. *prodigus*, is reflective of the aggressive blighting caused by the pathogen on the oldest, basal leaves, progressing over time to form diffuse necrotic patches in turfgrass swards. The rapid colonization and blight of four turfgrass species at 30°C in controlled environmental conditions in this study and the rapid growth rate of *W. circinata* var. *prodigus* in vitro at 25 to 35°C suggest that this pathogen could be a potential threat to multiple species of turfgrass over a wide range of climatic conditions.

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