Discovery and Characterization of *Waitea circinata* var. *circinata* Affecting Annual Bluegrass from the Western United States

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ABSTRACT

de la Cerda, K. A., Douhan, G. W., and Wong, F. P. 2007. Discovery and characterization of *Waitea circinata* var. *circinata* affecting annual bluegrass from the western United States. Plant Dis. 91:791-797.

Waitea circinata var. *circinata* was identified as the causal agent of a new disease of annual bluegrass (*Poa annua*) in the United States. This pathogen is also known to cause brown ring patch on creeping bentgrass (*Agrostis stolonifera*) in Japan, but it had not been reported on any turf species outside of Japan. Symptoms on annual bluegrass caused by this fungus included regular to irregular yellow rings several centimeters to 1 m in diameter, typically at maximum daytime temperatures of 15 to 35°C. A total of 26 isolates were collected from diseased annual bluegrass. Twenty-two of these isolates were multinucleate, grew optimally at 25 to 30°C, and in culture formed irregular sclerotia approximately 2 to 5 mm in size that were white to orange and remained orange or turned brown to dark brown over a 28-day period. The remaining four isolates were characterized as being *W. circinata* var. *zeae* (*Rhizoctonia zeae*), which is a known pathogen of annual bluegrass in the United States. Sequence analysis of the internal transcribed spacer (ITS) regions of rDNA on a subset of isolates confirmed the identifications of *W. circinata* var. *circinata* ($n = 8$) and *W. circinata* var. *zeae* ($n = 1$) based on deposited sequences in GenBank. The identity of the remaining 14 isolates suspected to be *W. circinata* var. *circinata* was confirmed by *HapII* digestion of the amplified rDNA ITS region. Pathogenicity of four *W. circinata* var. *circinata* isolates was confirmed on both annual bluegrass and creeping bentgrass. This study is the first morphological and molecular confirmation of the presence of *W. circinata* var. *circinata* as a pathogen of turfgrass in the United States.

Waitea circinata var. *circinata* has been described as the causal agent of brown ring patch, a disease of creeping bentgrass (*Agrostis stolonifera*) in Japan (22,24). Two other varieties of *W. circinata* (27), *W. circinata* var. *oryzae* (anamorph = *Rhizoctonia oryzae* Ryker & Gooch) (18) and *W. circinata* var. *zeae* (anamorph = *Rhizoctonia zeae* Voorhees) (26), are also known to cause disease on turfgrasses and other graminaceous hosts (3).

In 2003, a new disease of annual bluegrass (*Poa annua*) began appearing in eastern Washington State at air temperatures above 28°C. Annual bluegrass is a turf species that is commonly found in many parts of the United States on golf courses in roughs, fairways, tees, and putting greens. Although often considered a weed, it often invades turf stands planted with other cool season species and is eventually cultivated as the dominant turf spe-

cies. The frequency of annual bluegrass use as golf turf is especially high in California and other western states as conditions are permissive for its perennial growth, and it often is the primary species found in older putting greens (25).

Symptoms of this disease on annual bluegrass were very similar to those of another turf disease, yellow patch, caused by *Ceratobasidium cerealis* (anamorph = *Rhizoctonia cerealis*) (2). Symptoms of the new disease typically start as thin yellow rings, several centimeters to 1 m in diameter (Fig. 1). Rings would either be circular or scalloped in appearance, and could stay yellow or become brown and necrotic over time. Unlike yellow patch, which often occurs in cool weather (10 to 18°C), this "new" disease of annual bluegrass was occurring under much higher temperature conditions and tended to become worse with increasing temperature. Symptoms were observed over a wide range of temperatures, anywhere from 15 to 35°C maximum daytime temperature.

Diseased plants appeared to be infected by a *Rhizoctonia*-like fungus that was found growing readily on leaves, stems, in crowns, and in the upper root zone and thatch. Initial characterization of the fungus indicated that it was not a species pre-

viously identified in California such as *R. solani* (brown patch) or *R. cerealis* (yellow patch) (F. P. Wong, *personal observation*). Since yellow patch was thought to be the dominant *Rhizoctonia*-incited disease on annual bluegrass in the West, it appeared as if this new disease was caused by a potentially undescribed or new *Rhizoctonia* species. Although initial observations suggested that the disease was caused by a *Rhizoctonia* anamorph of a variety of *Waitea circinata*, no information was available on the range of this species as a pathogen of turfgrass in the western United States. Therefore, the objective of this study was to collect, characterize, and identify the causal agent of this new disease in the western United States using morphological and physiological characteristics as well as molecular methodologies based on sequence analysis of the nuclear ribosomal DNA internal transcribed spacer (ITS) regions (10,24).

MATERIALS AND METHODS

Isolate collection and maintenance.

For this study, isolates were collected from samples from various locations affected by the disease on *P. annua* in 2005 (Table 1). Isolates were obtained by placing a single symptomatic leaf blade onto ¼-strength potato dextrose agar in 100-mm petri plates (¼-PDA; 4.95 g potato dextrose agar, 5.63 g granulated agar [Fisher Scientific, Pittsburgh, PA] per 500 ml of deionized water). Plates were maintained at room temperature, and 24 to 48 h later, a 1- to 2-mm hyphal tip from colonies typical of *Rhizoctonia* was excised and transferred to a new plate of ¼-PDA. For the duration of the study, isolates were maintained on ¼-PDA with transfers to new media every 21 days unless otherwise noted.

Morphological characterization. Isolates were transferred to full-strength PDA and maintained at 28°C in an incubator without supplemental lighting. The widths of several vegetative hyphae from 1-week-old cultures were measured with an optical micrometer. Sclerotial formation was monitored every few days for up to 28 days; color and morphology were noted during this period. Three cultures of each isolate were used for hyphae and sclerotial characterization.

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Accepted for publication 28 January 2007.

Cellular nuclear number was measured using 4',6'-diamidino-2-phenylindole (DAPI) staining of nuclei using the methodology described by Martin (14). Briefly, isolates were grown on glass slides coated with 2% water agar for 24 h, fixed with 3% formaldehyde, stained briefly with 1 µg/ml DAPI, and rinsed with sterile distilled (sd)H₂O to remove excess stain. Stained specimens were visualized using a Zeiss UV-epiillumination system (Zeiss USA, Thornwood, NY), and numbers of nuclei in young vegetative hyphae were recorded, counting three cells per slide. This was repeated three times for all of the isolates used in the study.

Effects of temperature on growth were determined for all isolates by placing a 5-mm-diameter plug from a culture onto a 100-mm petri plate of PDA. Plates were incubated at 5, 10, 15, 20, 25, 30, and 35°C (Precision 818, Precision, Winchester, VA). Colony diameters were measured after 2 days and the experiment was replicated three times.

ITS region sequencing. Ribosomal DNA ITS sequence analysis was performed for nine of the isolates collected in this study (AVCC 12.1, BSCC 17.1, DMLV 1.1, EDHG 1.1, EMCC 1.1, KRCC 1.1, MGCC 1.1, OCGC 1.1, and TRGC 1.1) and compared to sequences from the three known varieties of *W. circinata* (Table 2). The

Table 1. Isolates used for this study

Isolate	Location	Host	Collection date	Maximum daytime temperature range (°C) ^z	
				Lower	Upper
AVCC 12.1 AVCC 12.3	Apple Valley, CA	<i>P. annua</i>	5/5/2005	14	27
BSCC 17.1 BSCC 17.2	Stockton, CA	<i>P. annua</i>	5/20/2005	19	28
DMLV 1.1 DMLV 2.1	Las Vegas, NV	<i>P. annua</i>	4/25/2005	20	27
EDHG 1.1 EDHG 2.1	El Dorado Hills, CA	<i>P. annua</i>	5/23/2005	13	26
EMCC 1.1 EMCC 3.1 EMCC 4.1 EMCC 5.3	El Macero, CA	<i>P. annua</i>	5/17/2005	18	29
KRCC 1.1 KRCC 1.2	Kingsburg, CA	<i>P. annua</i>	5/1/2005	20	29
MGCC 2.1 MGCC 4.1	Spokane, WA	<i>P. annua</i>	6/1/2005	20	30
NVCC 9.1 NVCC 9.2	Napa, CA	<i>P. annua</i>	7/15/2005	26	37
OCGC 1.1 OCGC 2.1	Irvine, CA	<i>P. annua</i>	11/3/2005	19	33
TGCC 17.1 TGCC 17.2	Tracy, CA	<i>P. annua</i>	7/14/2005	29	37
TRGC 1.1 TRGC 2.1 TRGC 3.1 TRGC 4.1	Tustin, CA	<i>P. annua</i>	4/1/2005	17	26

^z Range of maximum daytime temperatures 7 days before symptoms of disease were observed based on historical data from the California Irrigation Management Information System.

Fig. 1. Symptoms on annual bluegrass (*Poa annua*) caused by *Waitea circinata* var. *circinata*. Symptoms typically start as thin yellow rings, several centimeters to 1 m in diameter, that can turn necrotic and brown or remain yellow. (Photo courtesy of Pat Gradoville, Palos Verdes Golf Club)

rDNA ITS region of these isolates was amplified using ITS 1F (5' CTTGGTCAT TTAGAGGAAGTAA 3') (7) and ITS 4 (5' TCCTCCGCTTATTGATATGC 3') (28).

Briefly, for each isolate, mycelia scraped from 7-day-old cultures grown on PDA in petri plates was ground with mortar and pestle in liquid nitrogen, and approximately 50 mg of ground tissue was used for DNA extraction using the PureGene DNA extraction kit (Gentra Systems, Minneapolis, MN). Approximately 100 ng of DNA was used for PCR in a 20- μ l reaction volume with a final concentration of 0.2 mM each of dATP, dCTP, dGTP, and dTTP, 0.375 μ M of each oligonucleotide primer, 20 mM Tris-HCl, pH 8.4, 50 mM KCl, 2.5 mM MgCl₂, and 0.5 units of *Taq* polymerase (Invitrogen, Carlsbad, CA). Amplification was performed in a PTC-100 thermocycler (MJ Research, Waltham, MA) using an initial cycle of 94°C for 3 min, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min. Five microliters of each PCR reaction was resolved by electrophoresis in 1.5% agarose-TBE gels to check for successful amplification of the expected approximately 650-bp product.

The PCR products were cleaned using ExoSap-IT (USB, Cleveland, OH) following the manufacturer's instructions and sequenced in both directions using Big Dye Terminator v3.1 chemistry (Applied Biosystems, Foster City, CA). Sequencing was performed at the Core Instrumentation Facility (CIF) of the University of California's (UC) Institute of Integrative Genome Biology at UC Riverside. The sequences were edited using Sequencher (version 4.6, Gene Codes Corporation, Ann Arbor, MI), aligned using Clustal X (version 1.81) (23), and visually edited in MacClade version 4 (13). Ambiguous regions in the alignment were removed prior to the analysis. Maximum Parsimony (MP) analysis using the heuristic search procedure with 1,000 random-addition-sequence replicates and tree-bisection-reconnection branch swapping was conducted using PAUP* version 4.0 beta 10 (21). Confidence in tree topology was examined using bootstrap with 1,000 replicates under the heuristic option.

Restriction enzyme digestion analysis of amplified ITS-sequences. Previous work has demonstrated that the ITS-rDNA region could be digested with *HapII* (synonym *HpaII*) to differentiate among *W. circinata* var. *circinata*, *W. circinata* var. *oryzae*, and *W. circinata* var. *zeae* (24). Therefore, 1 μ g of our amplified ITS-rDNA product was digested with 10 units of *HapII* (Takara Bio Systems, Otsu, Shiga, Japan) and resolved on 2% agarose-TBE gels by electrophoresis as described by Toda et al. (24).

Pathogenicity of *W. circinata* var. *circinata* isolates to *Poa annua* and *A. stolonifera*. The ability of *W. circinata* var. *circinata* to cause disease on *P. annua* had not

been previously demonstrated, but it was known to be pathogenic to *A. stolonifera*. To confirm that the isolates collected from the western United States were identical to those previously reported as pathogens of *A. stolonifera* in Japan, four isolates of *W. circinata* var. *circinata* (DMLV 2.1, EDHG 1.1, MGCC 2.1, and KRCC 1.1) were used to inoculate both plant species. A single isolate of *W. circinata* var. *zeae* (OCGC 1.1) was also included for comparison.

Isolates were grown on cornmeal-sand (20) using 4 g of cornmeal and 96 g of silica sand. The mixture was hydrated with 20 ml of sdH₂O, placed into 500-ml polystyrene specimen jars (Nalgene, Rochester, NY), and autoclaved for 1 h on each of two consecutive days. Agar plugs from 7-day-old cultures were used to inoculate the cornmeal-sand, which was maintained at 28°C for 14 days with daily agitation before use.

P. annua (Turf Merchants Inc., Tagent, OR) and *A. stolonifera* ('Penncross', Turf-Seed Inc., Hubbard, OR) were grown in 8-cm-diameter plastic pots filled with University of California soil mix. Pots were seeded at a rate of approximately 2 kg per 93 m² and maintained at ambient glass house conditions (approximately 26 to 30°C) without additional supplemental light and irrigated as needed on a daily basis. Plants were maintained at a height of 2.5 cm by regular clipping of plants three times a week. Five-week-old plants were used for the inoculation studies.

For each isolate, three pots of both *P. annua* and *A. stolonifera* were inoculated with 3 g of cornmeal-sand; three noninoculated pots of each plant species served as negative controls. Pots were incubated in a growth chamber with 12 h of fluorescent lighting per day (VWR 844, VWR West-

chester, PA) at 28°C with 80% relative humidity. Plants were misted with water four times per day to provide adequate leaf wetness for infection and disease development. Disease severity (percent disease) was evaluated 7 days after inoculation on a 0 to 10 scale with 0 = no disease and 10 = all plants dead; the experiment was performed three times. Severity data were converted to a 0 to 100% scale and normalized using an arcsine (y) transformation. Analysis of variance and multiple comparisons were performed using SAS v. 9.0 (SAS Institute, Cary, NC).

RESULTS

Colony morphology and characteristics. *Sclerotia formation.* Isolates OCGC 1.1 and 1.2, and NVCC 9.1 and 9.2, produced buff colored colonies with vegetative hyphae 6 to 9 μ m in width. On PDA plates, small, white, spherical sclerotia (0.5 to 1 mm in diameter) began forming underneath the agar surface in as little as 7 days and eventually all turned a dark red brown color by day 28. Morphologically, these isolates appeared to be identical to *W. circinata* var. *zeae* described as a pathogen of turfgrass (3,15).

The remaining isolates formed large, irregular sclerotia approximately 2 to 5 mm in diameter in as few as 3 days on PDA. These sclerotia were both submerged and on the agar surface, and were initially whitish beige to orange. Some groups of isolates (EDHG, DMLV) remained orange to orange-brown, while others turned brown to dark brown over the 28-day period. Colony color was buff turning brown over the 28-day incubation period. Morphologically, the isolates appeared to be similar to those of *W. circinata* var. *oryzae* (3) or *W. circinata* var. *circinata* (24).

Table 2. Isolates and rDNA internal transcribed spacer (ITS) region sequences used in this study for genetic characterization

Isolate	Morphological identity ^z	Host	Origin	GenBank accession no.	Reference
AVCC 12.1	Wcc	<i>Poa annua</i>	California, US	DQ900583	Current study
BSCC 17.1	Wcc	<i>Poa annua</i>	California, US	DQ900584	Current study
DMLV 1.1	Wcc	<i>Poa annua</i>	Nevada, US	DQ900587	Current study
EDHG 1.1	Wcc	<i>Poa annua</i>	California, US	DQ900592	Current study
EMCC 1.1	Wcc	<i>Poa annua</i>	California, US	DQ900585	Current study
KRCC 1.1	Wcc	<i>Poa annua</i>	California, US	DQ900589	Current study
MGCC 2.1	Wcc	<i>Poa annua</i>	Washington, US	DQ900588	Current study
TRGC 1.1	Wcc	<i>Poa annua</i>	California, US	DQ900586	Current study
Z1	Wcc	Soil	Alaska, US	AB213585	20
Z4	Wcc	Soil	Alaska, US	AB213586	20
Z41	Wcc	Soil	Alaska, US	AB213587	20
Ro36	Wco	<i>Oryza sativa</i>	Aichi, Japan	AB213588	20
RoTTS	Wco	<i>Oryza sativa</i>	Toyama, Japan	AB213589	20
C-301	Wco	Soil	Fukuoka, Japan	AB521590	20
Ro119	Wco	<i>Oryza sativa</i>	Aichi, Japan	AB521591	20
OCGC 1.1	Wcz	<i>Poa annua</i>	California, US	DQ900594	Current study
M008	Wcz	<i>Oryza sativa</i>	Aichi, Japan	AB213593	20
M009	Wcz	Soil	Wakayama, Japan	AB213594	20
C-504	Wcz	Soil	Ishikawa, Japan	AB213595	20

^z Identity for isolates collected in the current study based on colony morphology and sclerotia characteristics. Wcc = *Waitea circinata* var. *circinata*, Wco = *Waitea circinata* var. *oryzae*, Wcz = *Waitea circinata* var. *zeae*.

Optimal temperature range. All isolates of the unknown fungus grew optimally on PDA between 25 and 30°C (Table 3), with optimal growth observed at 30°C. Both *W. circinata* var. *zeae* isolates, OCGC 1.1 and NVCC 9.1, appeared to have optimal growth at 30°C (Table 3, Fig. 2). These isolates continued to grow at 35°C, while the unknown were significantly inhibited, indicating a difference between these groups.

Nuclear number. All isolates had three to eight nuclei per vegetative hyphal cell as determined by DAPI staining (Table 4). In addition to the morphological and physiological features described above, this excluded these isolates as being *R. cerealis* or any other binucleate *Rhizoctonia* species. The multi-nuclear state of these isolates further identified them as possibly being a variety of *W. circinata*.

ITS-region sequence characterization. Sequence alignment and phylogenetic

analysis indicated that eight of the unknown isolates were *W. circinata* var. *circinata* and one of them was *W. circinata* var. *zeae* based on significant bootstrap (99 to 100%) support with known isolates (Fig. 3). None of the tested isolates belonged to *W. circinata* var. *oryzae*. For the eight *W. circinata* var. *circinata* isolates tested, the amplified ITS-region sequences were 100% similar to each other except for two isolates (EMCC 1.1 and BSCC 17.1) which had a heterozygous (A/G) position within the ITS1 region. The sequences were more than 99% similar (1- to 2-bp difference) to those *W. circinata* var. *circinata* sequences deposited in GenBank (isolates Z4, Z41, Z1 from Alaska [24]). Our *W. circinata* var. *zeae* isolate clustered with other deposited *W. circinata* var. *zeae* isolates, but there was more sequence variation within this group than within *W. circinata* var. *circinata*. Two heterozygous

positions (A/G), one in ITS1 and one in ITS2 region, were also found in our *W. circinata* var. *zeae* isolate.

Identification of isolates based on HapII digestion. Amplified ITS-region sequences from the eight isolates confirmed as *W. circinata* var. *circinata* by ITS-region analysis produced the diagnostic fragments approximately 170 and 475 bp in size (Fig. 4) after digestion with HapII. The remaining 14 isolates also produced the same two fragments after HapII digestion, indicating that these were also *W. circinata* var. *circinata*. Digestion of ITS regions from the four *W. circinata* var. *zeae* isolates tested (OCGC 1.1, OCGC 1.2, NVCC 9.1, NVCC 9.2) were not cut by the enzyme, which is diagnostic for this fungus (24).

Pathogenicity of *W. circinata* var. *circinata* to *P. annua* and *A. stolonifera*. For the four *W. circinata* var. *circinata* isolates

Table 3. Growth of *Rhizoctonia* isolates on potato dextrose agar^y

Temperature (°C)	Colony diameter (cm) after 48 h growth on PDA ^z										
	AVCC 12.1	BSCC 17.1	DMLV 1.1	EDHG 1.1	EMCC 1.1	KRCC 1.1	MGCC 2.1	TGCC 17.1	TRGC 1.1	NVCC 9.1	OCGC 1.1
5	1.2 e	1.0 c	1.0 d	1.1 d	1.1 c	1.1 d	1.1 d	1.0 d	1.1 d	1.1 e	1.0 e
10	1.5 e	1.0 c	1.0 d	1.3 d	1.4 c	1.4 de	1.2 d	1.2 d	1.1 d	1.2 e	1.0 e
15	3.3 d	1.6 c	1.3 d	2.6 c	2.0 c	2.8 c	2.2 cd	2.7 c	2.1 c	2.3 d	1.5 e
20	4.9 c	3.2 b	2.8 c	3.9 b	3.2 b	3.3 c	4.4 b	4.1 b	3.6 b	3.5 c	3.5 d
25	5.9 b	5.2 a	4.0 b	5.1 a	5.5 a	5.7 b	5.4 a	5.2 a	5.4 a	5.3 b	5.8 b
30	6.6 a	5.5 a	4.9 a	6.0 a	5.8 a	6.5 a	5.9 a	5.1 a	5.7 a	7.0 a	6.7 a
35	1.8 e	1.4 c	1.3 d	1.7 cd	1.7 c	1.8 d	1.7 cd	1.8 cd	1.7 cd	5.3 b	4.3 c

^y Selected isolates shown; data from the remaining isolates were similar and excluded from this table.

^z Means represent results from three replicated trials; means followed by the same letter are statistically equivalent (Fisher's protected LSD, $\alpha = 0.05$).

Fig. 2. Growth of *Rhizoctonia* isolates on potato dextrose agar. Colony diameters were measured after 48 h incubation at 5 to 35°C. Points reflect the means of three replicated tests, and error bars reflect standard deviation. Isolates OCGC 1.1 and NVCC 9.1 were morphologically identified as *Waitea circinata* var. *zeae* (*R. zeae*). Data from the other isolates reflect growth responses typical for the unknown isolates; data from the other isolates were excluded from the graph for clarity.

used, chlorosis on both *P. annua* and *A. stolonifera* was observed in as little as 3 days, with necrosis and blighting of crowns, stems, and leaves occurring by 7 days (Table 5). Plants inoculated with the *W. circinata* var. *zeae* isolate (OCGC 1.1) reacted similarly. Overall, under our experimental conditions, the disease was more severe on *A. stolonifera* versus *P. annua* (ANOVA, $P < 0.001$). Among the isolates used to inoculate *A. stolonifera*, there were some significant differences with regard to virulence, but no differences were observed for the inoculated *P. annua*.

DISCUSSION

In this work, we have described the presence of a variety of *W. circinata* causing disease on annual bluegrass (*P. annua*) in the United States based upon morphological and genetic characterization. The isolates from California, Washington, and Nevada were identified as *W. circinata* var. *circinata* based upon similar morphological characteristics with regard to (i) sclerotial size, shape, and color, (ii) cellular nuclear number, (iii) optimal growth temperature range, and (iv) similar genetic characteristics with regard to rDNA ITS-region sequences.

This is significant in the sense that the disease caused by this pathogen appears to have a wide temperature range at which it has been observed to affect turf and symptomatically can be confused with yellow patch, a less serious disease caused by *R. cerealis*. For the isolates collected in this study identified as *W. circinata* var. *circinata*, daytime high temperatures ranged from 14 to 37°C for the 7-day period before symptoms were seen on annual bluegrass. Tani and Beard (22) reported that in Japan, this fungus could be active between 10 and 35°C, with an optimal temperature of 25 to 30°C for causing disease on creeping bentgrass. Generally, we observed a wider temperature range for disease development on annual bluegrass and noticed that the highest number of diagnostic samples suspected of being infected by *W. circinata* var. *circinata* were submitted when maximum daytime temperatures occurred between 20 to 30°C.

For the diagnosis of *W. circinata* var. *circinata* from turfgrass samples, the anamorph is easily distinguished from *R. solani* based upon hyphal size and the production of irregular white to orange sclerotia in culture. Likewise, sclerotia formed by *W. circinata* var. *circinata* are easily distinguished from *W. circinata* var. *zeae* (*R. zeae*). The sclerotia of most of the *W. circinata* var. *circinata* isolates collected turned brown to dark brown over time, but not in all cases; some remained orange. At the present time, after 2 years of sampling, no *W. circinata* var. *oryzae* has been found in more than 50 diagnostic samples from cool-season turfgrass; per-

haps this is due to host preference toward warm season grasses (4,9).

It should be noted that in this study, some of the isolates recovered from diagnostic samples and characterized in this study were *W. circinata* var. *zeae*. This pathogen is known to exist on turf in California, but had not been widely documented as a major pathogen on annual bluegrass in the West. Interestingly, an isolate of this fungus identified as being from California by Hsiang and Dean (10) was collected in California in 1981 by A. H. McCain at University of California, Berkeley (T. Hsiang, *personal communication*). Also from one of the locations where the pathogen was found, maximum daytime temperatures were only 19 to 33°C for the week preceding the development of symptoms. Similarly, *W. circinata* var. *zeae* was recently reported from Ontario, Canada, only at temperatures above 25°C (11). Both observations suggest that golf course superintendents should be aware that permissive temperatures for this disease can be below the optimal 28 to 33°C range associated with the disease (4), and there may be some overlap between *W. circinata* var. *zeae* and *W. circinata* var. *circinata* activity.

It had been assumed that *R. cerealis* was the dominant pathogen on annual bluegrass in the West, causing symptoms of yellow rings, since these typically appeared under cooler temperature conditions in the spring, but this pathogen has only been isolated once from diagnostic samples from our laboratory since 2003. This suggests that *W. circinata* var. *circinata*, and to a lesser extent *W. circinata* var. *zeae*, have long been the causal agents of what was assumed to be “yellow patch” in the West.

Although *W. circinata* var. *circinata* was reported from soils in Alaska (12), as a pathogen of rice in California (8) and wheat and barley in Turkey (5), there has been no formal report of this pathogen from turf in the United States. However, Adams and Grogan (1) had likely discovered this pathogen from annual bluegrass greens in California in 1983, but did not characterize it as *W. circinata* var. *circinata*. They described a *Rhizoctonia oryzae*-like fungus with the same morphological and physical characteristics we have described for *W. circinata* var. *circinata* isolates. Oddly enough, some of the isolates he characterized in 1983 came from the same golf course in El Macero, CA (G. Adams, *personal communication*) that we used in the current study.

Although *W. circinata* var. *circinata* is documented solely as a pathogen of creeping bentgrass in Japan, it had not yet been diagnosed from this turf species in the western United States. The pathogenicity tests indicated that isolates collected from annual bluegrass are pathogenic to both species. Perhaps environmental conditions

in the western United States favor its development exclusively on annual bluegrass. Anecdotally, brown patch, caused by *R. solani*, is common on creeping bentgrass greens in many parts of the United States, but it is rare on this turf species in California. Epidemiological models (6,19) suggest that conditions do not favor this disease in drier parts of the West. Thus, this pathogen may be present in the western United States on creeping bentgrass, but environmental conditions do not favor its development, while annual bluegrass may be more sensitive to *W. circinata* var. *circinata* under these same environmental conditions. The presence of *W. circinata* var. *circinata* in other cool- or warm-season turfgrass species is unknown at this time.

The nomenclature for this pathogen with respect to both the common disease name and taxonomic name is somewhat controversial. The common name “brown ring patch” was used by Toda et al. (24) for the disease on creeping bentgrass; on annual bluegrass, the most striking feature of the disease is the development of thin bright yellow rings or crescents on putting greens. These rings may turn necrotic and brown, fade away under dry conditions, or remain as yellow rings. Thus, the description of the common name for the disease may not be applicable in this case. If the disease was renamed “yellow ring patch” based upon symptoms on annual bluegrass, it would be more descriptive. If the disease became widespread on both turf species, there would be two common names for a

Table 4. Nuclear number of *Rhizoctonia* isolates as determined by DAPI staining

Isolate	Mean ^z	Minimum	Maximum
AVCC 12.1	3.6 ± 0.19	3	4
AVCC 12.3	3.6 ± 0.19	3	4
BSCC 17.1	5.6 ± 0.51	4	7
BSCC 17.3	5.9 ± 0.19	4	7
DMLV 1.1	5.0 ± 0.0	4	6
DMLV 2.1	4.7 ± 0.33	4	6
EDHG 1.1	4.1 ± 0.51	3	5
EDHG 2.1	3.4 ± 0.19	3	4
EMCC 1.1	4.6 ± 0.77	3	5
EMCC 3.1	4.2 ± 0.51	3	5
EMCC 4.1	4.1 ± 0.19	3	5
EMCC 5.3	4.0 ± 0.58	3	5
KRCC 1.1	3.3 ± 0.0	3	4
KRCC 1.2	3.7 ± 0.0	3	4
MGCC 2.1	4.8 ± 0.19	4	5
MGCC 4.1	4.6 ± 0.19	4	5
TGCC 17.1	6.6 ± 0.38	6	8
TGCC 17.2	6.9 ± 0.51	6	8
TRGC 1.1	6.3 ± 0.33	6	7
TRGC 2.1	5.9 ± 0.19	5	7
TRGC 3.1	6.1 ± 0.51	5	7
TRGC 4.1	6.3 ± 0.58	5	7
OCGC 1.1	3.4 ± 0.19	3	4
OCGC 2.1	3.6 ± 0.38	3	4
NVCC 9.1	3.2 ± 0.19	3	4
NVCC 9.2	3.6 ± 0.19	3	4

^z Average of three replicated tests examining nuclei in three DAPI-stained cells per test; means followed by standard deviation of averages for the three tests.

Fig. 3. Phylogenetic tree showing the relatedness of *Waitea circinata* isolates collected in this study and known isolates based upon internal transcribed spacer (ITS) region sequence using amplification of rDNA with ITS1 and ITS4.

disease caused by the same pathogen, which is not acceptable. At this time, there is sufficient justification to use “brown ring patch”, as it does describe symptoms that are seen on annual bluegrass under some conditions; yet we would propose a different name be designated to cover the range of symptoms seen on both turf species, and perhaps symptoms on other turf species that have not been recognized as hosts of *W. circinata* var. *circinata*. It would be premature to make any changes at this time. However, a new American Phytopathological Society–approved common disease name should be considered if the pathogen and disease from additional locations and hosts in the United States are identified and characterized.

Concerning use of the anamorphic name of this pathogen, the situation is also

somewhat complicated. In this work, the teleomorphic names of *W. circinata* were used exclusively. Leiner and Carling (12) had proposed that the anamorphs of *W. circinata* be given the species name *Rhizoctonia circinata*, followed by a variety designation (e.g., var. *circinata*, *oryzae*, or *zeae*), in order to allow for the expansion of new *W. circinata* varieties. To date, two publications have followed suit (16,17). However, there is a strong precedent already set for using the anamorphic names of *R. oryzae* and *R. zeae* to describe diseases of numerous graminaceous hosts. From a practical standpoint, the teleomorphic state of *W. circinata* is not found readily on symptomatic turfgrass, so there should be an emphasis on the use of the anamorphic names. Each anamorphic state of the varieties of *W. circinata* have dis-

tinct morphologies (12,24), fatty acid composition (16,17), some differences in temperature optima for causing disease (3,22), differences in host range (4,22,25), and distinct rDNA ITS-region sequences. Therefore, it could be readily argued that instead of the proposed use of *R. circinata* var. *circinata*, *oryzae*, or *zeae*, the older names, e.g., *R. oryzae* and *R. zeae*, could be maintained with the addition of *R. circinata* as an anamorphic name for *W. circinata* var. *circinata*. Further research and evidence would elucidate this issue, and the formal justification for a name change would be proposed after a detailed examination and review of the species *W. circinata*.

Regardless of the nomenclature used, the most important point is that a *Rhizoctonia*-anamorph of *W. circinata* var. *circi-*

Fig. 4. *HapII* digestion of amplified rDNA internal transcribed spacer (ITS) regions for selected isolates. Digestion of amplified ITS regions with *HapII* (1 unit per 100 ng DNA) followed by gel electrophoresis in 2% agarose-TBE gels yielded two fragments of sizes ~170 and 475 bp from isolates previously characterized as *Waitea circinata* var. *circinata* by ITS region sequencing. Digestion with *HapII* would not produce these fragments from *W. circinata* var. *oryzae* or *W. circinata* var. *zaeae* isolates. A single isolate of *W. circinata* var. *circinata* previously characterized by ITS-region sequence is shown for comparison to other *W. circinata* var. *circinata* isolates not characterized by sequencing. Four isolates of *W. circinata* var. *zaeae* are also included for comparison.

Table 5. Pathogenicity of *Waitea circinata* isolates to creeping bentgrass (*Agrostis stolonifera*) and annual bluegrass (*Poa annua*)

Isolate ^a	Disease rating (0 to 10) ^b	
	Creeping bentgrass	Annual bluegrass
DMLV 1.1	8.3 ab	5.9 a
EDHG 1.1	5.4 c	4.7 a
KRCC 1.1	9.1 a	6.3 a
MGCC 2.1	6.9 bc	4.3 a
OCGC 1.1	9.3 a	5.7 a
Check (none)	0.0 d	0.0 b

^bRating on scale of 0 to 10, where 10 = 100% diseased plants. Five-week-old plants were used for the bioassays and were rated 7 days after inoculation and incubation at 28°C and 80% RH. Means followed by the same letter are statistically equivalent based upon ANOVA and multiple comparisons (Fisher's LSD, $\alpha = 0.05$) using arcsine-transformed data.

^aOCGC 1.1 is *W. circinata* var. *zaeae*, the remaining isolates are *W. circinata* var. *circinata*.

nata has been identified as a pathogen of annual bluegrass in the United States, and it appears as if there are some difficulties in diagnosing and controlling it. Symptoms similar to those caused by *W. circinata* var. *circinata* have been recently reported affecting annual bluegrass in the Midwest and northeastern United States. The identity of the pathogen affecting annual bluegrass collected from these locations is being confirmed, although morphologically, many of these isolates appear to be *W. circinata* var. *circinata*. Further research on the epidemiology, etiology, distribution, and biology of this pathogen on turf will help in the development of better management strategies. Perhaps the most interesting aspect of this will be the determination of the population structure of *W. circinata* var. *circinata* for isolates from the West and other parts of the United States to help determine the origin and mode of spread of the pathogen. Until now, this pathogen did not appear to be a major problem on annual bluegrass, and what triggered its emergence is yet to be determined.

ACKNOWLEDGMENTS

The authors would like to thank Dr. Larry Stowell for his help and insight, Dr. Takeshi Toda and

Professor Mitsuro Hyakumachi for their suggestions and advice, Dr. Pam Gunnell and Professor Gerard Adams for their invaluable historical information, J. Rios, C. Nielsen, K. Huryn, G. Perez, and W. Kong for their assistance, and the numerous golf course superintendents who provided us with their insight on the disease caused by this pathogen.

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