Virulence of *Rhizoctonia oryzae* and *R. solani* AG-8 on Wheat and Detection of *R. oryzae* in Plant Tissue by PCR

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ABSTRACT

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Rhizoctonia oryzae and *R. solani* anastomosis group (AG)-8 both cause root rot of wheat and barley, but *R. oryzae* has been considered secondary in importance to *R. solani* AG-8 on these cereals in the U.S. Pacific Northwest. Of 19 isolates of *R. oryzae*, 12 caused both preemergence damping-off of wheat and a significant reduction in root biomass of 21-day-old seedlings in natural soil at 12°C, whereas 7 isolates induced minimal or no damage to wheat under these growth conditions. *R. solani* AG-8 had no effect on seedling emergence and seminal root development, but four of eight isolates tested caused severe root rot of wheat. Thus, *R. oryzae* and *R. solani* AG-8 may cause distinctive and different damage as pathogens of wheat, and their relative importance may vary among field sites and with the developmental stage of the host plant. The nucleotide sequence of the rDNA internal transcribed spacer (ITS) regions was divergent between the two species; therefore, the oligonucleotide primers RO1 and RO2 were developed from sequences within ITS1 and ITS2, respectively, that are unique to *R. oryzae*. These primers amplified a 511-bp fragment from DNA of *R. oryzae* but not DNA from any intraspecific group of *R. solani* or from binucleate *Rhizoctonia* spp. A polymerase chain reaction protocol with the RO1 and RO2 primer set was used to detect *R. oryzae* in wheat roots and is a suitable method to diagnose this fungus.

Rhizoctonia root rot of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) is an important disease in the U.S. Pacific Northwest, especially where these cereals are grown in no-till or direct-drill management systems (23,33). *Rhizoctonia solani* Kühn anastomosis group (AG)-8 and *Rhizoctonia oryzae* Ryker & Gooch are widely distributed in the Pacific Northwest, and both are capable of causing this disease (22,27). In a survey by Ogoshi et al. (22), *R. solani* AG-8 and *R. oryzae* were the dominant species, and intraspecific groups of *Rhizoctonia* recovered from wheat plants exhibiting symptoms of root rot, but 85% of the *R. solani* isolates recovered from wheat-field soils belonged to AG-3, -4, -5, -9, and -10. In Texas, *R. solani* AG-4 was the dominant *Rhizoctonia* species recovered from wheat, and isolates of AG-4 and -5 were capable of causing significant postemergence root rot of wheat (24).

Although *R. oryzae* and *R. solani* AG-8 are isolated at similar frequencies from wheat roots (22) and both incite root rot of wheat, comparative pathogenicity studies indicate that *R. solani* AG-8 is the relatively more important of the two pathogens under the growing conditions encountered in the Pacific Northwest (22,27). Isolates of *R. oryzae* required a higher optimal temperature than those of *R. solani* AG-8 for maximal growth, and they caused moderate root rot at 20°C but little or no disease at 10°C (22). In contrast, isolates of *R. solani* AG-8 caused mild or moderate disease at 20°C but severe root rot of wheat at 10°C (22). Smiley and Uddin (27) found that their isolates of *R. oryzae* did not cause significant root rot of wheat grown in natural soil at a day/night temperature of 23/11 or 19/6°C, but they did cause severe root necrosis at 27/16°C. *R. solani* AG-8 caused more severe disease at low than at high day/night temperatures, and root rot severity was equivalent to or greater than that caused by *R. oryzae* regardless of temperature. Soil temperatures during seedling emergence of direct-drilled cereals are typically in the range of 10 to 12°C, whether the crop is winter wheat seeded in late autumn or spring wheat planted in mid-spring. During the growing season, roots occupying the top 10 to 15 cm of the soil profile potentially will be exposed to temperatures that, on spring cereals, are advantageous first to *R. solani* AG-8 and then to *R. oryzae* as soil warms in the spring and, on winter wheat, are first advantageous to *R. oryzae* and then to *R. solani* AG-8 as soil cools in the fall. In the field, *R. solani* AG-8 caused severe rot of both seminal and coronal roots of winter wheat and, thereby, caused stunting, delayed maturity, and lowered yields, whereas *R. oryzae* never caused rotting of coronal roots and did not significantly limit yields of winter wheat regardless of seeding date (27). Based on these findings, *R. solani* AG-8 was thought to be the major cause of Rhizoctonia root rot of cereals in the Pacific Northwest.

Increased disease severity at higher temperatures also has been reported for barley stunt disease caused by *R. oryzae* (2) and root rot of corn (30) caused by *R. zeae*. However, the severity of root rot of corn caused by *R. solani* AG-2 did not vary between day/night temperature cycles of 21/8, 28/16, and 34/20°C (30). Previous reports have implicated *R. zeae* (13) and *R. solani* AG-4 (29) as causal agents of damping-off of wheat, but these studies were conducted in steamed or autoclaved soils and used unnaturally high inoculum concentrations (5% inoculum by weight of rooting medium). In Texas, *R. solani* AG-4 limited emergence of wheat seedlings (18), but in subsequent studies, isolates of *R. solani* AG-4 and -5 that caused root rot of wheat had no effect on seedling emergence (24). There are no reports of preemergence damping-off of wheat resulting from infection by either *R. solani* AG-8 or *R. oryzae*.

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A major impediment to the diagnosis of the causal agent(s) of Rhizoctonia root rot of cereals, recognized since the earliest work with this disease (6,20,25), is the difficulty in recovering *Rhizoctonia* spp. from infected tissues due to numerous secondary colonists, especially *Fusarium* spp. Semiselective media have been developed specifically for the isolation and enumeration of *Rhizoctonia* spp. (12,32), but these media have been no more effective than water agar in isolating *Rhizoctonia* spp. from naturally infected wheat roots (M. Mazzola, *unpublished data*). The polymerase chain reaction (PCR) has been employed recently as a highly sensitive method to rapidly detect fungi in soil and plant tissue by amplification of pathogen-specific DNA sequences. PCR-based protocols have been developed that enable identification of fungi at the species (19,31,35) and subspecies levels (1,7).

The objective of our research was to (i) assess the virulence of several isolates of *R. oryzae* and *R. solani* AG-8 on wheat and (ii) develop species-specific primers for the detection of *R. oryzae* in infected wheat tissues.

MATERIALS AND METHODS

Isolates of *Rhizoctonia* spp. The isolates of *R. solani* AG-8 and *R. oryzae* used in these studies (Table 1) are part of a collection compiled and characterized by Ogoshi et al. (22). All isolates of *R. solani* AG-8 and *R. oryzae,* except S1-BSI, FAC1727, and C-505, were from the roots of winter wheat or spring barley from fields in the Pacific Northwest. The fungal collection was maintained on 1/5-strength potato dextrose agar (1/5 PDA) (Difco Laboratories, Detroit).

Growth rate. Agar disks were excised from the margin of actively growing cultures of *R. oryzae* growing on 1/5 PDA and placed in the center of fresh 1/5 PDA plates. Cultures were incubated at 5, 10, 15, and 25°C, with three replicate plates per isolate at each temperature. Radial growth was measured at 48, 72, and 96 h after inoculation.

Pathogenicity assays. The relative abilities of isolates of *R. oryzae* and *R. solani* to cause preemergence damping-off and root rot were assessed using wheat seedlings grown in controlled environment chambers. Oat-grain inoculum for each isolate of *R. solani* AG-8 and *R. oryzae* was prepared as previously described (36). Plastic tapered tubes (26 cm long × 2.5 cm diameter, Ray Leach Cone-Tainer, Canby, OR) were filled with a 30-cm^3 layer of vermiculite topped by a 15-cm^3 layer of natural or pasteurized (exposed to air-steam mixture at 96°C for 90 min) Puget silt loam from the Washington State University Research and Extension Unit at Mt. Vernon. Soil was passed through a sieve of 1-cm^2 mesh prior to use. Two pathogen-colonized oat grains (approximately 0.1 g) or two sterile oat grains (noninfested control) were placed in soil at a depth of 1 cm in each tube, 10 ml of distilled water was placed in each, and the tubes were incubated for 48 h. Two seeds of the wheat cultivar Penewawa were sown on the soil surface of each tube, and the seeds were covered with 5-cm^3 of vermiculite.

Experiments were conducted using a randomized complete block design with 10 tubes (potentially 20 plants) used for each of three replicates for a total of potentially 60 plants for each of 19 isolates of *R. oryzae* and 8 isolates of *R. solani* AG-8. Plants were grown at 12°C with a 12-h photoperiod. Seedlings were watered with 5 ml of 1/3-strength Hoagland's solution at 12 days after planting. Wheat seedlings were harvested at 21 days after planting, and roots were washed with a high-pressure stream of water. The number of emerged wheat seedlings, plant height, and number of seminal roots per plant were recorded. Plants were dried at 37°C for 72 h, and root and shoot dry weights were determined separately. In separate studies, four isolates of *R. solani* AG-8 and five isolates of *R. oryzae* were evaluated by the protocols described above, except plants were grown at 20°C. All experiments were conducted twice, and data were analyzed by analysis of variance and mean separation using Fisher's protected LSD.

Development of *R. oryzae*-specific primers. Internal transcribed spacer (ITS) regions have been used successfully to generate specific primers capable of differentiating closely related fungal species (1,19,31). Therefore, we focused on the ITS regions of ribosomal genes (Fig. 1) as a potential source of primers specific to *R. oryzae*. Fungal cultures were grown in complete medium (5 g of sucrose, 6 g of yeast extract, 6 g of casein enzymatic hydrolysate per liter) at room temperature on an orbital shaker for 4 to 5 days. Mycelia were filtered, ground in liquid nitrogen with a mortar and pestle, and DNA was extracted from 1 to 2 g of ground tissue by

TABLE 1. Isolates of *Rhizoctonia* spp. used in this study

Isolate	Anastomosis group	Origin	Host
R. oryzae			
1-6-1	WAG O	Lind, WA	Spring barley
2-3-2	WAG O	Hermiston, OR	Wheat
2-4-1	WAG O	Dixie, WA	Wheat
3-2-1	WAG O	Pendleton, OR	Spring barley
3-3-2	WAG O	Pendleton, OR	Spring barley
3-4-1	WAG O	Pendleton, OR	Spring barley
3-5b-1	WAG O	Pendleton, OR	Spring barley
3-7-1	WAG O	Adams, OR	Wheat
3-9b-1	WAG O	Pullman, WA	Spring barley
3-9-4	WAG O	Pullman, WA	Spring barley
4-3b-1	WAG O	Duffy, WA	Spring barley
5-2-1	WAG O	Duffy, WA	Wheat
5-3-7	WAG O	...[z]	...
5-4-1	WAG O	Friend, OR	Wheat
5-5-5	WAG O	Corvallis, OR	Wheat
5-8-1	WAG O	Goldendale, WA	Spring barley
C-505	WAG O	...	Rice
Lind V2	WAG O	Lind, WA	...
Yakima V1	WAG O	Yakima, WA	...
R. solani			
BV-7	AG-1	...	...
F-2	AG-1	...	...
D-1	AG-5	Hermiston, OR	Wheat
SO-2-1	AG-6	...	...
UBU-1-4	AG-6	...	...
3-1b-1	AG-8	Pendleton, OR	Spring barley
3-7-2	AG-8	Adams, OR	Wheat
4-5b-1	AG-8	Lewiston, ID	Wheat
5-6-1	AG-8	Moxee, WA	Wheat
7-1-3	AG-8	Lacrosse, WA	Spring barley
7-2-2	AG-8	Lind, WA	Spring barley
C-1	AG-8	Clyde, WA	Spring barley
FAC1727	AG-8	...	...
S1-BSI	AG-8	Scotland	Spring barley

[z] Origin or host is unknown.

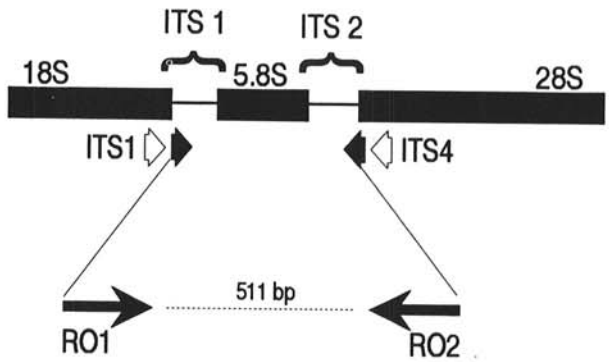

Fig. 1. Oligonucleotide primers specific for *Rhizoctonia oryzae* were developed from the internal transcribed spacer (ITS) region. Genomic DNA from isolates of *R. oryzae* and from several intraspecific groups of *R. solani* was amplified with the universal primers ITS1 and ITS4, and the resulting products were cloned and sequenced. Primers RO1 and RO2 were selected from regions within the ITS1 and ITS2 spacers that were different between the two species.

a cetyltrimethylammoniumbromide-based procedure (21). Genomic DNAs of *R. oryzae* isolate 5-8-2, *R. solani* AG-1 isolates B-7 and F-2, *R. solani* AG-5 isolate D-1, *R. solani* AG-6 isolates SO-2-1 and UBU-1-4, and *R. solani* AG-8 isolates C-1, 3-7-2, 4-5b-1, 5-6-1, and 7-2-2 were amplified with the universal primers ITS1 and ITS4 described by White et al. (34). These primers amplify a region that spans from the 3′-end of the 18S rRNA gene to the 5′-end of the 28S rRNA gene and includes the 5.8S rRNA gene and the two ITS regions (ITS1 and ITS2). Individual PCR-reaction samples contained 2.5 units of Ampli*Taq* DNA polymerase (Perkin-Elmer Corp., Norwalk, CT), 1× GeneAmp PCR buffer (supplied by Perkin-Elmer), 100 μM of each dNTP, 100 pmol of each primer, 100 to 200 ng of purified fungal DNA, and sterile water to obtain a final volume of 50 μl in a 0.5-ml microfuge tube. A single drop of mineral oil was added to each tube. Amplification of the 18-28S intragenic region was performed in a DNA Thermal Cycler 480 (Perkin-Elmer) by 25 PCR cycles of denaturation at 94°C for 60 s, primer annealing at 49°C for 120 s, and DNA extension at 74°C for 180 s. Aliquots (10 μl) of the amplification products were separated on a 1.0% agarose gel in Tris-borate-EDTA buffer at 50 V for 6 h, the gel was stained with ethidium bromide for 30 min, and the PCR products were viewed using a UV transilluminator.

The PCR products generated with the ITS1 and ITS4 primers were ligated into the TA cloning vector pCRII (TA cloning kit, Invitrogen, San Diego, CA), and ligation products were used for the transformation of *Escherichia coli* INVαF′ by the protocol provided by the manufacturer. The cloned ITS region from *R. oryzae* and those of isolates from several intraspecific groups of *R. solani* were sequenced at the Nucleic Acids Research Facility (Iowa State University, Ames). Comparisons of ITS nucleotide sequences were made using the SeqAid II program (version 3.81) (D. D. Rhodes and D. J. Roufa, Kansas State University, Manhattan) and the Genetic Computing Group (5) package. Sequences specific to *R. oryzae* and not *R. solani* were identified in the noncoding regions between the 18S and 28S ribosomal RNA genes and were used to generate the *R. oryzae*-specific primers RO1 and RO2. The primers used were synthesized by Operon Technologies, Alameda, CA.

The specificity of the RO1 and RO2 primer set was assessed in amplification reactions with purified genomic DNA from isolates of several intraspecific groups of *R. solani,* binucleate *Rhizoctonia* spp., *Fusarium solani*, *F. sambucinum*, *Pythium graminicola*, *Gaeumannomyces graminis* var. *tritici*, and *G. graminis* var. *avenae*. These primers also were tested for their ability to amplify the appropriate-size fragment from several isolates of *R. oryzae.* PCR reactions were conducted as described above, except amplifications were preceded with 1 cycle of 94°C for 90 s, reactions were run for 30 cycles, and primer annealing was at 62°C for 120 s.

The same plants that were used in assays to determine the ability of isolates of *R. oryzae* to cause disease were used to develop a PCR protocol for the detection of the pathogen in wheat roots with the RO1 and RO2 primer set. A 20-mg (wet weight) root sample was obtained from individual wheat plants harvested at 21 days after planting. The root sample was placed in 250 μl of sterile water and ground with a sterile mortar and pestle. The ground root tissue and extract were placed in a 1.5-ml microfuge tube, and the sample was immersed for 15 min in a boiling water bath. The root tissue was pelleted by centrifugation for 5 min at 16,000 × *g*. The extract was diluted with 200 μl of sterile distilled water, and a 1-μl aliquot of the root extract was used in PCR reactions consisting of 30 cycles of denaturation at 94°C for 60 s, primer annealing at 60°C for 120 s, and DNA extension at 74°C for 180 s. Extract from roots of uninfected plants and genomic DNA from *R. solani* AG-8 isolate C-1 served as negative controls. Purified

TABLE 2. Effect of isolates of *Rhizoctonia oryzae* and *R. solani* anastomosis group (AG)-8 on growth and development of wheat grown in natural and pasteurized (steamed) soil (NS and SS, respectively) at 12°C for 21 days

	Emergence (%)		Seminal roots/plant		Root dry weight (mg)		Shoot dry weight (mg)		Plant height (cm)	
Isolate	NS	SS	NS	SS	NS	SS	NS	SS	NS	SS
Control	95 a[y]	93 a	4.3 a–c	4.6 ab	16.5 a	14.5 a	22.3 a	18.3 a	7.6 ab	7.2 a
R. oryzae										
1-6-1	60 c–e	68 c	3.7 c–e	3.8 b	9.3 c–e	8.8 b	16.2 c–e	14.7 ab	5.7 cd	6.1 ab
2-3-2	34 e–g	5 fg	1.6 i	0.7 d–f	3.9 g	1.4 c–e	8.6 g	5.4 f–h	3.6 fg	1.6 e–g
2-4-1	82 a–c	73 bc	4.6 a–c	3.9 b	13.5 ab	7.9 b	18.8 ab	9.9 c–e	7.5 ab	5.8 b
3-2-1	65 b–d	5 fg	3.7 c–e	1.3 cd	10.1 b–d	0.6 e	16.2 c–e	3.8 gh	6.6 bc	1.9 d–g
3-3-2	82 a–c	31 d	4.1 a–d	1.3 cd	11.9 bc	0.9 de	16.3 b–d	5.4 f–h	6.5 bc	5.8 b
3-4-1	84 a–c	18 d–f	3.6 d–f	1.0 c–f	9.2 c–e	2.0 c–e	16.0 c–e	6.3 e–h	6.0 cd	2.3 c–g
3-5b-1	19 fg	2 g	1.4 i	0.3 f	4.5 fg	0.1 e	8.8 g	2.6 h	2.9 g	1.1 g
3-7-1	13 g	2 g	1.5 i	1.2 c–e	4.3 fg	1.4 c–e	9.5 g	5.4 f–h	3.4 g	2.5 c–f
3-9b-1	58 c–e	19 d–f	2.6 gh	0.6 d–f	7.8 d–g	2.8 c–e	12.2 d–g	6.7 e–h	5.0 d–f	2.8 c–e
3-9-4	42 d–f	10 fg	2.9 e–h	1.3 cd	6.0 e–g	1.5 c–e	10.6 fg	4.5 f–h	5.8 cd	1.9 d–g
4-3b-1	45 d–f	4 fg	2.3 g–i	0.5 d–f	5.8 e–g	1.0 de	10.6 fg	5.2 f–h	5.3 c–e	2.3 c–g
5-2-1	98 a	25 de	4.8 ab	1.5 c	12.9 a–c	4.5 c	19.5 ab	10.2 c–e	7.5 ab	3.5 c
5-3-7	81 a–c	33 d	3.7 c–e	1.3 cd	11.7 bc	4.0 cd	16.7 b–d	8.4 c–f	6.4 bc	3.1 cd
5-4-1	98 a	60 c	4.6 a–c	3.9 ab	13.7 ab	11.7 ab	20.9 a	18.4 a	8.0 bc	6.3 ab
5-5-5	55 de	8 fg	2.9 e–h	0.3 f	10.7 b–d	1.1 de	16.1 c–e	3.6 gh	5.6 cd	1.2 fg
5-8-1	64 b–d	8 fg	3.1 d–g	0.4 ef	7.9 d–f	4.4 c	14.6 d–f	11.4 b–d	6.3 b–d	1.9 d–g
Lind V2	33 e–g	3 g	1.9 hi	0.2 f	6.3 e–g	0.4 e	11.5 e–g	6.4 e–h	4.1 e–g	1.6 e–g
Yakima V1	59 c–e	2 g	2.1 hi	0.7 d–f	6.2 e–g	0.9 de	12.6 d–g	4.2 f–h	5.8 cd	1.2 fg
C-505	90 ab	18 d–f	4.1 a–d	1.9 c	10.1 b–d	2.7 c–e	15.4 c–e	7.4 d–g	6.7 a–c	2.8 c–e
R. solani										
3-1b-1	…[z]	90 a	…	4.8 a	…	12.4 a	…	18.9 a	…	7.1 a
4-5b-1	…	87 ab	…	5.0 a	…	12.3 a	…	18.9 a	…	7.0 a
5-6-1	…	83 ab	…	5.0 a	…	14.3 a	…	17.8 a	…	7.2 a
7-1-3	…	98 a	…	4.9 a	…	3.4 c–e	…	12.6 bc	…	5.9 b
7-2-2	…	85 ab	…	5.0 a	…	13.5 a	…	14.0 ab	…	7.2 a
C-1	85 a–c	87 ab	5.0 a	4.7 a	4.0 g	2.8 c–e	9.3 g	12.0 bc	5.0 d–f	5.9 b
FAC1727	…	87 ab	…	4.9 a	…	5.8 bc	…	12.3 bc	…	5.8 b
S1-BSI	…	83 ab	…	5.0 a	…	1.5 c–e	…	12.6 bc	…	5.8 b

[y] Means in the same column followed by the same letter are not significantly different ($P = 0.05$) based on Fisher's LSD.
[z] Not determined.

DNA from *R. oryzae* 5-8-2 was used as the positive control. Reaction products were separated on agarose gels and visualized as described above.

RESULTS

Disease severity. The ability of *R. oryzae* and *R. solani* to cause disease on wheat was characterized by various measures of plant growth and development. The relative damage to wheat seedlings caused by individual isolates of *R. oryzae* and *R. solani* was similar for the two experiments; isolates that caused the most damage in experiment 1 did so in experiment 2, and isolates that were nonpathogenic in experiment 1 were nonpathogenic in experiment 2. However, growth and development of wheat plants were slower and disease was less severe in experiment 1 relative to those obtained in experiment 2. The more rapid growth and development of wheat seedlings in experiment 2 (exemplified by the healthy checks) facilitated the detection of differences in disease severity caused by isolates of *R. oryzae* and *R. solani*. Therefore, the data reported are from experiment 2.

Effect of *R. oryzae* and *R. solani* AG-8 on seedling emergence. Four of eight isolates of *R. solani* AG-8 caused severe root rot (root pruning and seedling stunting) of wheat, four caused no damage, and none of the isolates affected seedling emergence in pasteurized soil (Table 2). In contrast, all 19 isolates of *R. oryzae* caused significant amounts of preemergence damping-off of wheat in pasteurized soil at 12°C, and 12 of 19 isolates reduced wheat emergence in natural soil at 12°C (Table 2). For example, seedling emergence was less than 40% in soil infested with *R. oryzae* isolate 2-3-2, 3-5b-1, 3-7-1, or Lind V2 but was 95% in natural noninfested soil (Table 2).

Root disease symptoms. Distinctly different symptoms were observed on wheat roots infected with *R. solani* AG-8 compared with those infected with *R. oryzae*. At 12°C, the four pathogenic isolates of *R. solani* AG-8 caused brown to red-brown lesions on roots and induced the typical spear-tipped roots (35), i.e., a complete girdling and severing of the root by the pathogen. In contrast, at the same temperature, *R. oryzae* caused yellow-brown lesions and cortical rot of wheat roots but did not girdle or sever roots. *R. oryzae* isolates 3-4-1, 3-5b-1, and Lind V2 induced typical dark-brown lesions and spear-tipped roots on plants grown at 20°C in pasteurized soil. More significantly, many isolates of *R. oryzae,* but not those of *R. solani* AG-8, caused a delay or complete inhibition of seminal root development. At 21 days after planting, wheat seedlings grown in noninfested soil possessed four to five seminal roots, but seedlings grown in natural soil infested with *R. oryzae* isolate 2-3-2, 3-5b-1, 3-7-1, or Lind V2 averaged less than two seminal roots per plant (Table 2). In contrast, the number of seminal roots at 21 days for plants grown in pasteurized or natural soil infested with *R. solani* was not significantly different from that of healthy wheat seedlings (Table 2), although otherwise normal seminal roots often were severed (spear-tipped) as a result of infection by *R. solani* AG-8.

Effect of soil temperature and pasteurization. At a given temperature, isolates of *R. oryzae* exhibited similar growth rates on PDA (data not shown). After incubation for 96 h, radial growth at 5°C had extended only 3 to 6 mm, whereas mycelial growth for all isolates had completely covered the agar surface when grown at 25°C.

As a group, isolates of *R. oryzae* caused more preemergence damping-off and root rot in steamed soil than in natural soil (Table 2). Only isolate 5-4-1 had no effect on root dry weight when assays were conducted in pasteurized soil, and this isolate as well as isolates 2-4-1 and 5-2-1 had no effect on root dry weights of plants grown in natural soil. In contrast, the root dry weights of plants grown in natural soil infested with isolate 2-3-2, 3-5b-1, or 3-7-1 were approximately 25% of those of plants grown in noninfested natural soil. Likewise, as a group, suppression of plant height and shoot dry weight by *R. oryzae* was more pronounced in pasteurized than in natural soil (Table 2). Only isolates 1-6-1 and 5-4-1 of *R. oryzae* had no effect on shoot dry weight and height of 21-day-old wheat plants grown in pasteurized soil. *R. oryzae* isolate 2-3-2, 3-5b-1, or 3-7-1 limited shoot biomass and plant height by more than 50%, but again, isolate 2-4-1, 5-2-1, or 5-4-1 had no effect on these growth parameters in natural soil.

All five isolates of *R. oryzae* examined caused root rot of wheat when assays were conducted at 20°C in pasteurized soil (Table 3). Even isolate 5-4-1, which had no measurable effect on wheat biomass production or root development at 12°C, significantly limited both shoot and root dry weight of wheat seedlings at 20°C. Isolates of *R. solani* AG-8 were nonpathogenic or caused mild suppression of root growth (e.g., isolate C-1) at 20°C .

Primer selection. Amplification of *R. solani*, *R. oryzae,* or binucleate *Rhizoctonia* spp. genomic DNA with the ITS1 and ITS4 universal primers resulted in a single amplification product. All isolates of *R. oryzae* yielded a single 651-bp fragment, and isolates of *R. solani* belonging to nine anastomosis groups yielded a single fragment of 670 to 680 bp. The size of the product obtained with the ITS1 and ITS4 primers also varied among strains within *R. solani* AG-8, with isolates 5-6-1, 7-2-2, and S1-BSI yielding a 679-bp fragment and amplification of C-1, FAC1727, 7-1-3, and 3-7-2, each resulting in a 674-bp fragment. Among isolates of *R. solani* AG-8, the nucleotide sequences for the amplified fragment from isolates C-1 and 3-7-2 (both pathogenic) were greater than 97% identical, as were those of isolates 5-6-1 and 7-2-2 (both nonpathogenic in our assays). However, between these two groups, nucleotide sequence identity in ITS1 and ITS2 was 87 and 94%, respectively. The ITS1 nucleotide sequence of 5-6-1 and 7-2-2 was identical to that of *R. solani* AG-6 isolates SO2-1 and TSH-1-1, and these two groups possessed 96% sequence identity in ITS2.

Species-specific primers were constructed based on a comparison of the ITS1 and ITS2 sequences from *R. oryzae* and *R. solani* AG-1, -5, -6, and -8. *R. oryzae* and the AGs of *R. solani* possessed approximately 50 and 35 to 40% sequence identity for ITS1 and ITS2, respectively (Fig. 2). Primer RO1 (5′-GATTGGTGG-CTGTTGCTGGC-3′) was selected from within the ITS1 sequence, and primer RO2 (5′-GGTACTTCAAGGCGTAG-3′) was selected from a region within ITS2 (Fig. 1). The RO1 and RO2 primers amplified a 511-bp fragment from purified genomic DNA for each of the five isolates of *R. oryzae* tested (Fig. 3). These primers did not amplify purified genomic DNA from any intraspecific group of *R. solani*, binucleate *Rhizoctonia* spp., or several species of fungi commonly associated with wheat roots (Fig. 3). Amplification of a dilution series of genomic DNA isolated from a pure culture of *R. oryzae* isolate 5-8-2 with primers RO1 and RO2 was

TABLE 3. Effect of *Rhizoctonia oryzae* and *R. solani* anastomosis group (AG)-8 on growth and development of wheat grown at 20°C in pasteurized soil for 21 days

Isolate	% Emergence	Seminal roots/plant	Plant height (cm)	Root dry weight (mg)	Shoot dry weight (mg)
Control	90 a[z]	4.9 a	14.5 a	24.0 a	23.7 a
R. oryzae					
1-6-1	73 bc	3.3 b	10.9 bc	10.6 c	19.7 bc
3-4-1	20 d	1.4 c	4.5 d	1.5 d	5.7 d
3-5b-1	8 d	0.2 d	1.4 e	1.0 d	4.1 d
5-4-1	70 c	3.4 b	10.3 c	15.5 bc	17.9 c
Lind V2	15 d	1.3 c	4.7 d	1.2 d	2.6 d
R. solani					
5-6-1	85 ab	4.5 a	14.1 a	24.3 a	22.5 ab
7-2-2	93 a	4.7 a	14.2 a	21.8 a	20.5 a–c
C-1	88 a	4.6 a	12.7 ab	14.2 bc	18.9 bc
FAC1727	95 a	4.7 a	13.9 a	19.7 ab	21.6 a–c

[z] Means in the same column followed by the same letter are not significantly different ($P = 0.05$) based on Fisher's LSD.

conducted to estimate the sensitivity of this PCR method. The amplification product was detected visually in agarose gels in two of four and four of four samples containing 1 and 10 pg of genomic DNA, respectively.

Seedlings infected individually with 1 of 10 isolates of *R. oryzae* were used to determine the utility of this PCR protocol for the detection of *R. oryzae* in wheat roots. A 20-mg sample of fresh root tissue was ground in 250 µl of sterile distilled water and diluted with 200 µl of sterile distilled water prior to use in amplification reactions. In three independent experiments, 8 of 10, 7 of 10, and 10 of 10 isolates of *R. oryzae* were detected, respectively, using 1 µl of the root extract and the RO1 and RO2 primers in the sample reaction (Fig. 4). The primers did not amplify DNA from roots of wheat plants grown in a sterile system. On occasion, PCR of DNA extracted from wheat roots grown in infested and noninfested natural soil resulted in multiple amplification products. However, these products were always smaller (100 to 300 bp) than the 511-bp fragment that was diagnostic for *R. oryzae,* and the *R. oryzae*-specific fragment was never amplified from extracts of noninfected wheat roots.

DISCUSSION

R. solani AG-8 and *R. oryzae* both have been implicated as pathogens of wheat and barley in the Pacific Northwest, with *R. solani* AG-8 thought to be the primary incitant of Rhizoctonia root rot of wheat in this region (27,33). Smiley and Uddin (27) observed that *R. oryzae* caused only minimal damage to wheat in natural soil and caused severe root rot of wheat seedlings only in pasteurized soil at relatively high temperatures but did not delay plant growth and development. Ogoshi et al. (22) obtained similar results with these two taxa of fungi. In our study, all isolates of *R. oryzae* examined caused disease on wheat in pasteurized soil at 12 and/or 20°C, and 7 of 19 isolates of *R. oryzae* caused as much damage to wheat at 12°C in natural soil as did *R. solani* AG-8, based on shoot and root dry weight. In addition, infection by *R. oryzae*, but not *R. solani* AG-8, resulted in the delay or complete inhibition of seminal root development.

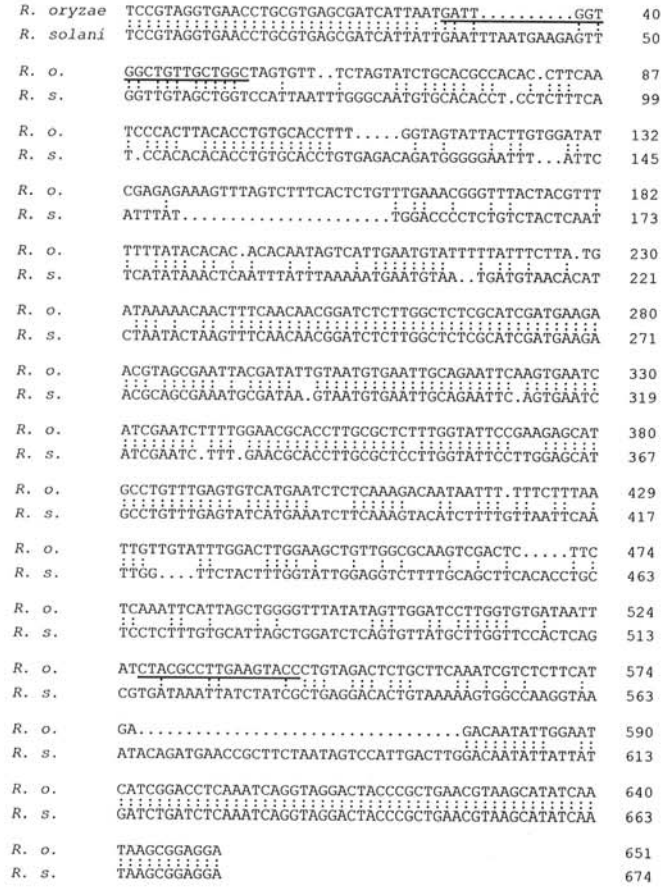

Fig. 2. Comparison of the nucleotide sequence of the internal transcribed spacer (ITS) region and the 5.8S rRNA gene from *Rhizoctonia oryzae* isolate 5-8-2 and *R. solani* anastomosis group (AG)-8 isolate C-1. Underlined sequences indicate primers RO1 and RO2 specific for *R. oryzae*. Numbering begins at the 5′ end of the ITS1 primer and ends at the 3′ terminus of the ITS4 primer.

The difference between our findings and those of previous investigations may be the result of the isolates of *R. oryzae* used in the experiments. Ogoshi et al. (22) and Smiley and Uddin (27) based their conclusions on results obtained with up to three isolates each of *R. oryzae* and *R. solani* AG-8. In our study, the severity of disease caused by *R. oryzae* varied considerably among isolates, even isolates obtained from the same field. The differences are not likely to be attributable to variation in growth rate because all 19 isolates of *R. oryzae* tested in our study exhibited similar rates of growth at a temperature range of 5 to 25°C. Among the 19 isolates we surveyed, some (e.g., isolates 5-2-1 and 5-3-7) incited disease in natural and pasteurized soil in a manner that supports previous reports, i.e., moderate levels of root rot of wheat in pasteurized soil but no disease in natural soil at low temperatures. However, other patterns of disease-causing capabilities were observed in assays conducted at 12°C, including one isolate of *R. oryzae* (isolate 5-4-1) that caused no discernible disease in either pasteurized or natural soil, and several isolates (e.g., isolates 2-3-2, 3-5b-1, 3-7-1, Lind V2, and Yakima V1) that caused severe disease in both pasteurized and natural soil. The severity of root rot incited by isolates of *Rhizoctonia* (W-AK), a *Rhizoctonia* similar in morphology to *R. oryzae*, on barley also varied in a manner analogous to what we have demonstrated for *R. oryzae* (15).

Rush et al. (24) observed that isolates of *R. solani* AG-4 and -5 that caused significant root rot did not reduce emergence of wheat. In our study, four isolates of *R. solani* AG-8 similarly caused root rot but did not affect wheat emergence, whereas isolates of *R. oryzae* reduced wheat emergence as much as 87 and 98% in natural and pasteurized soil, respectively. Ingram and Cook (9) found that *P. ultimum* var. *sporangiiferum* reduced emergence 20 to 30% at 10 to 15°C in pasteurized soil, but *P. heterothallicum, P. irregulare*, and *P. torulosum* had no effect on wheat emergence. Our findings suggest that *R. oryzae* is potentially as important as *P. ultimum* var. *sporangiiferum* in limiting stand establishment of wheat in the Pacific Northwest.

The disease caused by *R. oryzae* on wheat appears to be distinct from that caused by *R. solani* AG-8. Isolates of *R. oryzae* caused significant preemergence damping-off and delayed or prevented the emergence of seminal roots at 12°C in natural and pasteurized soil (depending on the isolate). This delay or failure of seminal root emergence could be a manifestation of infection of the germinating seed, more likely the embryo of the germinating seed as reported by Hering et al. (8) for *Pythium*. In contrast, *R. solani* AG-8 failed to incite either of these disease symptoms on wheat. Both species of *Rhizoctonia* were capable of causing rot of seminal roots, but only *R. solani* AG-8 caused significant rot of coronal roots (27). The lower root dry weight associated with infection by *R. oryzae* was the result of either root rot (loss of tissue), failure of roots to develop, or both, whereas the low root dry weights associated with infection by *R. solani* AG-8 resulted entirely from rotting and pruning (loss) of infected roots. These distinct characteristics of disease development are not unique to wheat-*Rhizoctonia* spp. interactions. Burton et al. (2) reported that *R. oryzae*, but not *R. solani* AG-8, caused preemergence damping-off of barley at 26°C, and neither fungus significantly reduced seedling emergence at 12 or 18°C. *R. oryzae* caused stunting of barley at 18 and 26°C but not at 12°C, whereas *R. solani* AG-8 caused stunting of barley at each temperature (2). *R. solani* AG-2 caused severe root rot of corn at day/night temperatures of both 21/8 and 28/16°C, whereas *R. zeae* caused significant root rot of corn only at 34/20°C (30).

Parasitic and saprophytic fitness describe, respectively, the ability of a pathogen to parasitize and cause disease on a susceptible host and the ability of facultative parasites to persist saprophytically on plant residue or other organic substrates in competition with other saprophytic microorganisms (26). The ability of a soilborne fungal pathogen to incite disease includes its ability to attain the advantage of position at the infection court in competition with other microorganisms prior to host penetration (4). We include this ability of the pathogen to colonize the rhizosphere in competition with other rhizosphere microorganisms during prepenetration phases of pathogenesis as a component of saprophytic fitness.

The amount of disease incited by a pathogen when the susceptible host is grown in pasteurized soil, i.e., with minimal competition, can be used as a measure of parasitic fitness, and a comparison of the amount of disease incited in natural versus pasteurized soil could be used as one measure of saprophytic fitness of the pathogen. In the example of *Rhizoctonia* spp. tested in our work, saprophytic fitness includes the ability to grow saprophytically from colonized oat grains through soil to the infection court. All isolates of *R. oryzae* caused disease on wheat in pasteurized soil, though for some isolates the ability to cause root rot and preemergence damping-off was temperature dependent. Some isolates, including isolate 5-2-1, caused severe root rot in pasteurized soil but only slight or no disease in natural soil. Similar results were reported previously (17,22,27). These findings suggest that some isolates of *R. oryzae* are less capable than others in competing with the resident microflora in soil or the rhizosphere of wheat and that saprophytic fitness, at least as it pertains to prepenetration growth from a food base, varies significantly among isolates of this pathogen.

Parasitic and saprophytic fitness are complex traits likely to be controlled by several genes and may involve the same or different genes. Disease severity induced by isolates of *R. oryzae* on wheat in pasteurized soil at 12°C varied from no disease to severe root rot and almost complete inhibition of seedling emergence. All four isolates of *R. oryzae* (3-2-1, 3-3-2, 3-4-1, and 3-5b-1) from a field near Pendleton, OR, caused severe root rot and preemergence damping-off when wheat was grown in pasteurized soil at 12°C, but only isolate 3-5b-1 caused severe disease in natural soil, and isolates 3-3-2 and 3-4-1 had no effect on seedling emergence in either soil type. This suggests, not surprisingly, that the ability of isolates of *R. oryzae* to compete with the resident soil and rhizosphere microbiota during the prepenetration phase of pathogenesis can vary even among isolates from the same field.

The variation in parasitic and saprophytic fitness observed among isolates of *R. oryzae* from the Pacific Northwest indicates that this fungal population is very diverse genetically. The accurate identification of the causal agent of Rhizoctonia root rot of wheat is important, both for diagnosis and the development of control measures, because of the variability among inter- and intraspecific groups of *Rhizoctonia* in sensitivity to fungicides (11,28) and antibiotics produced by biocontrol agents (10; M. Mazzola, *unpublished data*). In addition, the relative importance of *R. oryzae* and *R. solani* as pathogens of wheat also may vary with the stage of plant development. *R. oryzae* attacks seeds, and seed treatments should provide an effective control. In contrast, *R. solani* AG-8 infects roots of seedlings and older plants and is unlikely to be controlled with seed treatments unless the chemical or biocontrol agent is capable of movement along the expanding root system. Identification of *Rhizoctonia* spp. from wheat roots is a difficult and labor-intensive process involving the isolation of the pathogen and coculturing with tester strains to determine the appropriate anastomosis grouping.

We have developed an oligonucleotide primer set that allows for rapid identification of *R. oryzae* by PCR. The primers RO1 and RO2 should be useful for differentiating among *Rhizoctonia* spp.

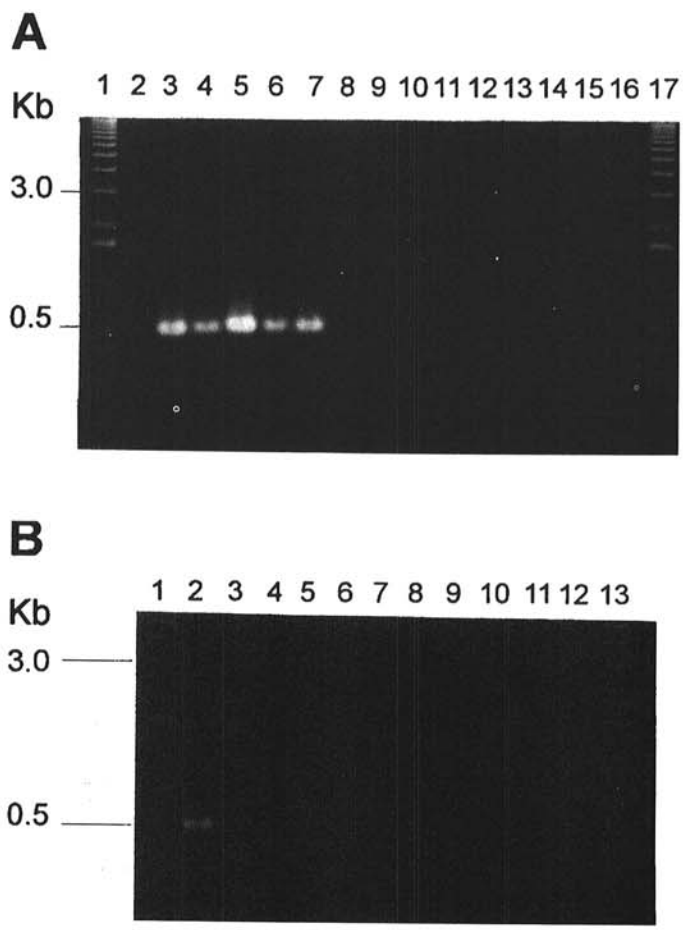

Fig. 3. Amplification of purified genomic DNA from different fungal species with primers RO1 and RO2. **A,** Lane 1, 1-kb ladder; lane 2, no DNA template; lane 3, *Rhizoctonia oryzae* 1-6-1; lane 4, *R. oryzae* 2-3-2; lane 5, *R. oryzae* 2-4-1; lane 6, *R. oryzae* 5-4-1; lane 7, *R. oryzae* 5-8-2; lane 8, *R. solani* anastomosis group (AG)1-1C; lane 9, *R. solani* AG-2-2; lane 10, *R. solani* AG-3; lane 11, *R. solani* AG-4; lane 12, *R. solani* AG-5; lane 13 *R. solani* AG-6; lane 14, *R. solani* AG-8; lane 15, *R. solani* AG-10; lane 16, *R. solani* AG-BI; and lane 17, 1-kb ladder. **B,** Lane 1, 1-kb ladder; lane 2, *R. oryzae* 5-8-2; lane 3, *Gaeumannomyces graminis* var. *tritici*; lane 4, *G. graminis* var. *avenae*; lane 5, *Fusarium sambucinum*; lane 6, *F. solani*; lane 7, *Pythium graminicola*; lane 8, *Rhizoctonia* sp. AG-A; lane 9, *Rhizoctonia* sp. AG-Bb; lane 10, *Rhizoctonia* sp. AG-D; lane 11, *Rhizoctonia* sp. AG-G, lane 12, *Rhizoctonia* sp. AG-I; and lane 13, 1-kb ladder.

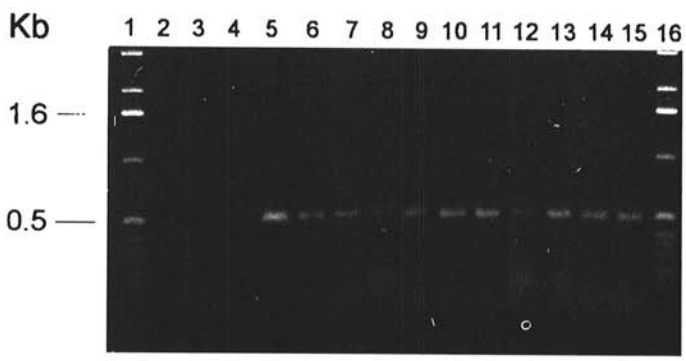

Fig. 4. Amplification of DNA extracted from roots of noninfected wheat plants or plants infected with *Rhizoctonia oryzae* or *R. solani* anastomosis group (AG)-8 with primers RO1 and RO2. Extraction of DNA from root tissue and polymerase chain reaction amplification conditions were as described in text. Lane 1, 1-kb ladder; lane 2, primers without DNA template; lane 3, DNA extracted from noninfected wheat; land 4, DNA extracted from wheat roots infected with *R. solani* AG-8 isolate C-1; lane 5, genomic DNA of *R. oryzae* isolate 5-8-2; lanes 6 through 15, DNA extracted from wheat roots infected with *R. oryzae* isolates 2-3-2, 3-5b-1, 3-7-1, 3-9b-1, 4-3b-1, 5-5-5, 5-8-2, Lind V2, Yakima V1, and C505, respectively; and lane 16, 1-kb ladder.

because they consistently and selectively amplify a 511-bp fragment in the ITS region from purified genomic DNA of *R. oryzae* but do not amplify DNA from binucleate *Rhizoctonia* spp. or any intraspecific group of *R. solani*. Based on methods used in previous studies (7,31), a protocol was developed for detection of *R. oryzae* in plant tissue by PCR. Sufficient *R. oryzae* DNA for PCR amplification of the target sequence was recovered from infected plants by grinding 20 mg of fresh root tissue in water and boiling the root extract for 15 min. The RO1 and RO2 primers amplified DNA from root extracts of wheat seedlings grown in soil infested with any 1 of 10 isolates of *R. oryzae*. In each case, a 511-bp fragment was amplified from infected wheat roots, and the primers did not amplify a similar sized DNA fragment from root extracts of healthy wheat seedlings. This detection protocol will be a suitable method for examining the relationship between saprophytic fitness and the variation in virulence among isolates of *R. oryzae* that was observed in this study.

The eight isolates of *R. solani* AG-8 examined in our study could be placed in two distinct groups: those that caused no disease, and those that caused severe root rot of wheat. Although this range is similar to that exhibited by isolates of *R. oryzae*, severity of disease caused by isolates of *R. oryzae* was more of a continuum. The polarity in the disease-causing capabilities of these two groups of *R. solani* AG-8 suggests that the two groups might be genetically distinct. Ribosomal DNA sequence data have been used to examine phylogenetic relationships among a variety of organisms, including plant pathogenic fungi (3,14,16). Preliminary studies based on the sequence of the ITS of the rDNA region confirm that isolates grouped together based on ability to anastomose can be quite different and that isolates of *R. solani* AG-8 can be placed into at least two evolutionary groups (8-1 and 8-2). Indeed, the nonpathogenic isolates of *R. solani* AG-8 (8-2) may be biologically and perhaps phylogenetically more similar to isolates of *R. solani* AG-6 than the pathogenic isolates of *R. solani* AG-8 (8-1).

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