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Characterization and Pathogenicity of *Rhizoctonia* and *Rhizoctonia*-Like spp. From Pea Crops in the Columbia Basin of Oregon and Washington

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Abstract

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Isolates of *Rhizoctonia* and *Rhizoctonia*-like spp. (n = 179) were baited selectively from soil and plant samples collected from irrigated pea crops in the semiarid Columbia Basin of Oregon and Washington from 2011 to 2013, and characterized to species, subspecies, and anastomosis groups (AG) based on sequences of the internal transcribed spacer region of ribosomal DNA. *Rhizoctonia solani* comprised 76% of all isolates, and included isolates of AG 4 (31% of all isolates), AG 2-1 (18%), AG 3 (10%), AG 8 (8%), AG 5 (5%), AG 10 (3%), and AG 9 (1%). The isolates of *Ceratobasidium* spp. (20%) comprised four AGs: AG K (11%), AG A (6%), AG I (2%), and AG I-like (1%). *Waitea circinata* isolates (4%) comprised two subspecies: *W. circinata* var. *circinata* (approximately 4%) and *W. circinata* var. *zeae* (<1%). Repeated pathogenicity tests of isolates of the 10 most frequently detected AGs and subspecies on 'Serge' pea at 15°C revealed that *R. solani* AG 2-1 caused the greatest reduction in pea emergence, followed by *R. solani* AG 4. *R. solani* AG 4 caused the most severe root rot, stunting, and reduction in pea seedling biomass, followed by isolates of AG 2-1. *R. solani* AG 8 did not affect emergence, plant height, and total biomass compared with noninoculated control plants; however, root rot caused by isolates of AG 8 was ranked the third most severe among isolates of the 10 *Rhizoctonia* subgroups, after that caused by isolates of AG 4 and AG 2-1. Isolates of other AGs and subspecies were either weakly virulent or nonpathogenic on pea. The most common AGs (AG 4 and AG 2-1) detected in pea fields in the Columbia Basin were also the most virulent. In a growers' pea crop grown for seed ('Prevail') planted 5 days after herbicide application and incorporation of a preceding winter wheat crop, severe stunting caused by *Rhizoctonia* spp. resulted in an average 75% yield loss within patches of stunted plants. In contrast, the yield of processing pea from a green pea crop of Serge did not differ significantly for plants sampled within versus outside patches of stunted plants; however, plants within patches were significantly more mature. In the Prevail seed crop, a greater frequency of *R. solani* AG 8 was detected than AG 2-1 or AG 4 from within patches of stunted plants, indicating that isolates of AG 8 may be associated with the root rot complex in some pea crops in the Columbia Basin.

Pea (*Pisum sativum* L.) is cultivated either for fresh consumption as a green pea crop, as a processing pea crop from which the harvested pea seed are frozen or canned, or as a dry pea crop for split pea or pea seed production in the semiarid Columbia Basin of Oregon (54) and Washington (4). Pea also is cultivated as a rotational crop to break disease cycles, manage weeds, and maintain nitrogen fertility in the soil (40). Worldwide, many soilborne pathogens can cause pea root rot, seed rot, and damping-off (16,39), including in the Columbia Basin (1,4,54). Pea root rot is a disease complex (16) that is often associated with *Rhizoctonia* spp. (32), *Fusarium* spp. (40), *Pythium* spp. (1), and *Aphanomyces* spp. (14). *Rhizoctonia* spp. are important damping-off, seedling blight, and root rot pathogens of pea (18,23,52,57). The genus comprises a complex of genetically distinct species and anastomosis groups (AGs), with a wide host range or virulence preference for certain hosts (2). *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (A. B. Frank) Donk) is a multinucleate species that has been divided into 14 AGs (AG 1 to AG 13 and AG BI) (6,8,52). Binucleate *Rhizoctonia* spp. (teleomorph: *Ceratobasidium*) are divided into 19 AGs (AG A to AG S). *R. oryzae* and *R. zeae* are multinucleate with the teleomorphs *Waitea circinata* var. *circinata* and *W. circinata* var. *zeae*, respectively (52).

Different taxonomic groups of *Rhizoctonia* spp. can cause root rot of pea (18,23,30,52). Among the AGs and subspecies of *Rhizoctonia* or *Rhizoctonia*-like spp., *R. solani* AG 4 has been reported most frequently and is typically the most virulent of the *Rhizoctonia* isolates causing pea root rot worldwide (21,50), including in North Dakota (23) and the Canadian prairies (18). Isolates of other AGs have been reported occasionally to be associated with pea diseases: AG A in Yunnan Province, China (61); AG I in Washington State (44); AG 2-1 in the Canadian prairies (18,58), Japan (41), and North Dakota (23); AG 5 in the Canadian prairies (18,58), North Dakota (23), and New York (30); and *W. ciricinata* var. *circinata* in Idaho (34). From the Columbia Basin of Oregon, *Ceratobasidium* sp. AG I and *R. solani* AG 2-1, AG 3, AG 4, AG 8, and AG 9 were isolated from volunteer pea plants growing in onion (*Allium cepa* L.) crops (33).

Pea root rot was recognized as a significant disease in the Columbia Basin as early as 1938 (26). Evidence suggests that pea root rot caused by *Rhizoctonia* spp. has become common and widespread in the Columbia Basin (1). The disease can be severe in fields in which cereal crops are planted preceding pea crops, including winter cereal cover crops sprayed with herbicide and incorporated into the soil just prior to pea planting. Establishment of *Rhizoctonia* spp. is favored particularly in coarse, sandy soils common in the Columbia Basin (38). *Rhizoctonia* spp. rapidly colonize the cereal crop residues

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incorporated into the soil (53), which can lead to colonization of the roots of pea seedlings, causing root rot and, ultimately, stunting of pea plants (Fig. 1). Infected seedlings typically remain stunted, and the stunted plants usually occur in patches that can range from <1 m to >10 m in diameter. Patches may cover as much as 10% of the area of a pea crop. Furthermore, stunted pea plants tend to bloom earlier than healthy plants and, consequently, mature earlier than healthy plants. Patches of stunted plants in pea crops resemble the bare patches in cereal crops caused by *R. solani* AG 8 in the inland Pacific Northwest (37).

Isolates of *R. solani* AG 8 obtained from cereals have been demonstrated to cause root rot and stunting of pea under experimental conditions (9,10,51). In addition, *R. solani* AG 8 can cause severe onion stunting in the Columbia Basin (33). Because pea commonly is rotated with cereal and onion crops in the Columbia Basin of Oregon and Washington, *R. solani* AG 8 may also be associated with pea stunting in cereal-based pea cropping systems of this region. Therefore, this study was undertaken to (i) identify the AGs or subspecies of *Rhizoctonia* and *Rhizoctonia*-like spp. associated with patches of stunted plants in pea crops in the Columbia Basin, (ii) assess the pathogenicity of isolates of these AGs or subspecies of *Rhizoctonia* and *Rhizoctonia*-like spp. on pea, and (iii) assess potential yield loss caused by stunting of pea crops in the Columbia Basin.

Materials And Methods

Sample collection and *Rhizoctonia* isolation. Twelve commercial pea fields in the Columbia Basin of Oregon and Washington State were surveyed from 2011 to 2013 to isolate *Rhizoctonia* and *Rhizoctonia*-like spp. Plant and soil samples were collected from four fields (field A, near Boardman, OR; field B-2, near Royal City, WA; field C, near Soap Lake, WA; and field D-11, near Paterson, WA) in 2011, five fields (fields E-9 and E-16, near Irrigon, OR; field F near Prosser, WA; and fields G-1 and G-2 near Royal City, WA) in 2012, and three fields (field H-220-2 near Walla Walla, WA; field I near Royal City, WA; and field J-106 near Basin City, WA) in 2013. Samples were collected from patches of stunted pea plants and adjacent healthy areas outside the patches, and randomly from across the fields. Putative *Rhizoctonia* spp. were baited from soil samples collected at an approximately 15-cm depth using the toothpick baiting method described by Paulitz and Schroeder (36). The epicotyl and roots of pea plants within the same soil samples were rinsed gently with tap water and then sterilized distilled water, and dried between pieces of sterilized filter paper. Sections (approximately 1 cm long) cut from the roots and epicotyls were transferred onto plates of 2% water agar amended with chloramphenicol (100 μg/ml). Plates were incubated for 12 to 48 h at ambient temperature (24 ± 2°C). Putative *Rhizoctonia* spp. were isolated and cultured for long-term storage.

DNA extraction and AG determination. Genomic DNA was extracted from mycelium of each putative *Rhizoctonia* isolate using a FastDNA kit (MP Biomedicals, Santa Ana, CA) and a FastPrep-24 homogenizer (MP Biomedicals) following the manufacturer's protocols. Genomic DNA was stored at −20°C until used for the polymerase chain reaction (PCR) assay. Eukaryotic universal primers UN-UP18S42 (5′-CGTAACAAGGTTTCCGTAGGTGAAC-3′) and UN-LO28S576B (5′-GTTTCTTTTCCTCCGCTTATTAATATG-3′) were used to amplify the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) consisting of the ITS1, 5.8S, and ITS2 regions, in a Bio-Rad T100 thermal cycler (Bio-Rad, Hercules, CA) (33). Following the protocol provided by Elim Biopharmaceuticals, Inc. (Hayward, CA) for sequencing, the amplified ITS rDNA of each isolate was subjected to forward sequencing. Sequences were edited manually in Chromas Lite (version 2.1; Technelysium Pty. Ltd., South Brisbane, Australia). ITS sequences that were ≥100 bp long (range of 102 to 688 bp for all isolates) were used to identify the fungal species or subgroup by comparison with the National Center for Biotechnology Information BLAST database (http://blast.ncbi.nlm.nih.gov), using a sequence identity ≥98% with reference accessions in GenBank.

Pathogenicity evaluation. Isolates obtained from the field surveys were tested for pathogenicity on pea plants. Four isolates of each of three AGs of *Ceratobasidium* spp. (AG A [isolates Rh051364, Rh1212154, Rh1212179, and Rh1212210], AG I [Rh0911047, Rh1112233, Rh1211008, and Rh1211009], and AG K [Rh051337, Rh1212129, Rh1212196, and Rh121277]); four isolates of each of

Fig. 1. Stunting and root rot symptoms of pea in the Columbia Basin of Washington. **A,** Patches of stunted pea plants in May 2013 in a pea seed crop near Basin City, WA, caused by *Rhizoctonia solani*. Herbicide (glyphosate) was applied to kill a winter wheat crop in the field 5 days before planting the pea seed crop. **B,** Rhizoctonia root rot on pea seedlings 35 days after planting. Symptoms include lesions on the hypocotyl, tap root, and secondary roots. The arrow indicates spear tipping or a pinched-off root tip. **C,** Patch of severely stunted pea plants at flowering in a field near Walla Walla, WA where winter wheat-summer fallow was practiced for 6 years prior to planting this dry pea crop.

six AGs of *R. solani* (AG 2-1 [Rh051307, Rh051324, Rh051350, and Rh1112117], AG 3 [Rh051332, Rh1112112, Rh111293, and Rh121247], AG 4 [Rh051339, Rh051344, Rh1112184, and Rh1112230], AG 5 [Rh061307, Rh0911048, Rh0911049, and Rh1011017], AG 8 [Rh051320, Rh061303, Rh1212213, and Rh1212232], and AG 10 [Rh0911028, Rh0911029, Rh111277, and Rh1211002]); and three isolates of *W. circinata* var. *circinata* (Rh1112237, Rh1211007, and Rh1211012) were selected randomly for pathogenicity evaluation. Inoculum of each isolate was grown on oat grains autoclaved twice in 1-liter Erlenmeyer flasks, and prepared for inoculation following the protocol of Paulitz and Schroeder (36). Pathogenicity tests were performed in a growth room set at 15 ± 1°C, using 'Serge' pea. Pea seed was disinfested in 0.6% sodium hypochlorite with manual agitation for 2 to 3 min, then rinsed three times in sterilized, distilled water. Rinsed seed were dried overnight on paper towels at ambient temperature (24 ± 2°C) in a laminar flow hood.

Sandy loam soil collected from an uncultivated portion of a field in the Columbia Basin was steam pasteurized twice (for 1 h at 60°C each time) at a 24-h interval, air dried on Kraft paper, sieved to a particle size ≤2 mm, and stored in buckets. Sterilized oat grains colonized with each isolate were ground separately just prior to inoculation, and mixed into the pasteurized soil (1% wt/wt). A cone-tainer (4 cm in diameter and 21 cm long; Stuewe and Sons, Inc., Tangent, OR) was filled with 150 g of soil and saturated with 50 ml of tap water. One pea seed was planted in each cone-tainer, and covered with a thin layer (10 g) of pasteurized soil. Cone-tainers were arranged in a randomized complete block design in plastic trays, and covered with Kraft paper for 4 days to conserve soil moisture for promoting pea germination and emergence. Cone-tainers without inoculum were used as a noninoculated control treatment. After the first sign of emergence of pea seedlings, the Kraft paper was removed and plants were irrigated with 25 ml of water and 25 ml of one-third strength Hoagland's solution (with macroelements only) every 3 to 4 days until completion of the trial. Each treatment was replicated five and four times in the first and second pathogenicity experiments, respectively.

Pea emergence was counted 1 and 2 weeks after planting. Four weeks after planting, plants were removed and the roots rinsed thoroughly with tap water. Plant height, root rot severity (measured on a 1-to-9 scale as described below), and dry weight of the shoot and root were measured. Root rot severity was rated as 1 = no lesions on the roots or hypocotyl; 3 = discrete, light- or dark-brown, superficial necrotic lesions; 5 = necrosis and decay of the adventitious roots or taproot; 7 = extensive root rot; and 9 = the plant was dead (27). The shoot and root of each plant were dried in a paper bag at 60°C for 72 h, and the dry biomass recorded.

Yield loss assessment. A survey was conducted to determine the yield loss caused by stunting in a processing green pea crop (Serge) planted near Paterson, WA in 2011 and in a green pea seed crop ('Prevail') planted near Basin City, WA in 2013 (Fig. 1). In both growers' fields, standard agronomic practices were employed for the Columbia Basin (1,4,54). Seed of Serge was planted on 11 April 2011. Ten stunted patches (each ≥9 m^2) were flagged on 23 June. Whole plants were harvested on 9 July from a 0.61-m^2 area inside each patch, and an equivalent area of asymptomatic plants adjacent to each patch. Pea pods were shelled and total green pea weight recorded for the plants sampled from inside and outside each patch. Two successive tenderometer readings were measured for the pea seed using a TU-12 tenderometer (Food Technology Corp., Sterling, VA), following standard practices for processing green pea crops. Seed of Prevail was planted on 20 March 2013 in a grower's field where, 5 days prior, a winter wheat crop was sprayed with herbicide (glyphosate) and incorporated into the soil. Patch sizes were estimated in four randomly assigned sections of the field, and 10 patches of severely stunted plants were flagged on 7 June. Plants were harvested from a 1-m^2 area inside each of the 10 patches on 4 July, and from 1 m^2 of an asymptomatic area of plants adjacent to each patch. The plants were then dried to <10% moisture, the pods shelled, and the pea seed cleaned and weighed to compare yields inside versus outside each stunted patch. Tenderometer readings were not relevant for the pea seed harvested from this trial because this was a seed crop, not a processing crop. The harvested seed was tested for germination using the blotter protocol of the Association of Official Seed Analysts (59).

Data analysis. The total number and frequency of isolates of each species, subspecies, and AG of binucleate and multinucleate *Rhizoctonia* were calculated based on the sequencing results. The number of fungal isolates was categorized further based on the source of isolation (plant material or soil) and sampling location in the field (within a patch, outside a patch, or collected randomly from the field). A statistical model was calculated to account for the combined effects of all AGs and subspecies on pea emergence. Separate statistical analyses were conducted to determine the effect of AGs or subspecies, rather than the effect of individual isolates within AGs or subspecies, on pea germination, root rot severity, and plant height and biomass. The effect of isolates of different AGs of *Ceratobasidium* and *Rhizoctonia*, and of *W. circinata* var. *circinata* on pea emergence was determined using a χ^2 test (45) with JMP (version 11 Pro; SAS Institute Inc., Cary, NC). For the χ^2 test, any plot (cone-tainer) with an emerged pea seedling was categorized as a successful event and assigned a value of 1. Cone-tainers without an emerged seedling were each defined as a failed event and assigned a value of 0. Because the root rot ratings (1 to 9 scale) are ordinal data, nonparametric tests described by Shah and Madden (46) were used to calculate the median, mean rank ($\bar{R}_{iJ}$), and relative treatment effect ($\hat{p}_{ij}$) with 95% confidence intervals (CIs) for root rot severity caused by each AG or subspecies of *Rhizoctonia* or *Rhizoctonia*-like spp. PROC RANK was used to calculate the median and mean rank. PROC MIXED was used to calculate relative treatment effects (46), and CIs were calculated using the LD_CI macro (5) in SAS (version 9.2; SAS Institute, Inc.). Mean tenderometer readings for pea seed harvested from the 2011 processing green pea crop, pea yield measured inside versus outside each of 10 patches in both the 2011 processing pea crop and the 2013 pea seed crop, and seed germination results for the 2013 pea seed crop were compared using Student's *t* test with JMP.

Results

Binucleate or multinucleate *Rhizoctonia* spp. In total, 179 isolates obtained from three pea fields in Oregon and nine fields in Washington were assigned to binucleate or multinucleate *Rhizoctonia* spp. and AGs based on the ITS rDNA sequences using BLAST. Isolates of *R. solani* included seven AGs and comprised 76% of all isolates, followed by *Ceratobasidium* spp. (20% of all isolates) with three AGs and *W. circinata* (4%) with two subspecies (Fig. 2). The seven *R. solani* AGs included AG 4 (31% of all isolates), AG 2-1 (18%), AG 3 (10%), AG 8 (8%), AG 5 (5%), AG 10 (3%), and AG 9 (1%). Isolates of AG 4 and AG 2-1 were detected at greater frequencies than any other AG or subspecies. Among the *Ceratobasidium* spp., isolates of AG K were most prevalent (11% of all isolates), followed by AG A (6%) and AG I (2%). In addition, two AG I-like isolates (95% ITS sequence identity to that of AG I isolates) were also detected. Members of *W. circinata* were detected at relatively low frequency compared with *R. solani* and *Ceratobasidium* spp. Only eight isolates of *W. circinata* were detected, of which seven were *W. circinata* var. *circinata* (4% of all isolates), and only one was *W. circinata* var. *zeae* (Fig. 2).

A greater number of isolates was baited from pea plants (57% of all isolates) compared with isolates obtained from soil as a baiting medium (43%) (Fig. 3). Among the locations of sampling within pea fields, more isolates were obtained from inside the patches of stunted pea plants (47% of all isolates), followed by samples collected at random (28%), and samples collected from healthy areas adjacent to the stunted patches (25%) (Fig. 3). From inside the patches, isolates of AG 4, AG 3, AG 2-1, AG K, AG 8, AG A, AG 10, *W. circinata* var. *circinata*, AG 9, AG I, *W. circinata* var. *zeae*, and AG 5 (in decreasing order) were obtained (Fig. 3A). A greater total number

of *Rhizoctonia, Ceratobasidium*, and *Waitea* subgroups was isolated from soil sampled inside the patches (12 versus 9 subgroups) compared with areas sampled adjacent to the patches (Fig. 3A and B). Inside the patches, a greater number of isolates of AG A, AG K, AG 2-1, AG 4, and AG 9 was isolated from pea plants compared with soil samples (Fig. 3A). In contrast, isolates of AG I, AG 5, AG 10, *W. circinata* var. *circinata*, and *W. circinata* var. *zeae* were obtained only from the soil samples collected inside the patches, whereas AG 9 was only isolated from pea plants in the patches, not from soil (Fig. 3).

From healthy areas adjacent to the stunted patches, isolates of AG 4, AG 5, AG K, AG 3, AG 2-1, *W. circinata* var. *circinata*, AG A, AG I-like, and AG I (in decreasing order) were obtained (Fig. 3B). Equal numbers of *Rhizoctonia, Ceratobasidium*, and *Waitea* subgroups (seven) were isolated from soil and pea plants outside the patches. AG A, AG I, AG K, AG 3, AG 4, and AG 5 isolates were obtained in greater numbers from soil than from plant samples collected adjacent to the patches. AG I and *W. circinata* var. *circinata* were isolated only from soil, whereas AG I-like and AG 2-1 were obtained only from pea plants collected outside the patches (Fig. 3B).

Among the isolates obtained from soil and pea plants collected randomly in the pea fields, AG 4, AG 2-1, AG 8, and *W. circinata* var. *circinata* (in decreasing order) were detected (Fig. 3C). All isolates of AG 2-1, AG 8, and *W. circinata* var. *circinata*, and the majority of AG 4 isolates were obtained from pea plants. Common *Rhizoctonia* subgroups present in stunted patches, adjacent to the patches, and at random sites in the fields included AG 2-1, AG 4, and *W. circinata* var. *circinata*. Details of the fields from which individual isolates of the AGs of *Ceratobasidium* and *Rhizoctonia* and subspecies of *Waitea circinata* were obtained are provided in Supplementary Table S1.

Pathogenicity of binucleate and multinucleate *Rhizoctonia* spp.

Pea emergence. The statistical model that accounted for the effects of isolates of all AGs and subspecies of *Rhizoctonia* evaluated for pathogenicity on pea showed a significant effect of the isolates on pea emergence by both the first and second weeks after planting in experiment 1 ($P = 0.001$ and 0.004, respectively) but only by the second week after planting in experiment 2 ($P = 0.097$ and 0.001 for weeks 1 and 2, respectively) (Table 1). The four isolates of *R. solani* AG 2-1 significantly reduced emergence in both experiments ($P = 0.001$ and 0.029 in weeks 1 and 2, respectively, of experiment 1; and $P = 0.038$ and 0.050 in weeks 1 and 2, respectively, of experiment 2). *R. solani* AG 4 isolates significantly reduced emergence in the first experiment ($P = 0.041$ and 0.017 in weeks 1 and 2, respectively) but not in the second experiment ($P = 0.085$ for both weeks 1 and 2 in the latter trial). This reflected the greater χ^2 values calculated for the isolates of AG 2-1 (i.e., AG 2-1 isolates reduced pea emergence most significantly, followed by the AG 4 isolates). AG 5 isolates of *R. solani* affected pea emergence only at week 1 in experiment 1 ($P = 0.041$) (Table 1). Isolates of the remaining AGs and *W. circinata* var. *circinata* did not reduce pea emergence significantly in either repeat of the experiment (Table 1).

Pea root rot. The isolates of all AGs as well as those of *W. circinata* var. *circinata* resulted in median root rot severity ratings ranging from 3 to 8 in experiment 1, which was significantly greater than the median root rot rating of 1 for pea plants growing in noninoculated control soil, based on the 95% CI for $\hat{p}_{ij}$ (Table 2). Similarly, in experiment 2, the median severity of root rot for isolates of all AGs and *W. circinata* var. *circinata* ranged from 2 to 8 versus 1 for the roots of pea plants in control soil. However, in this trial, the 95% CI for $\hat{p}_{ij}$ was only significantly greater than that of the control treatment for isolates of AG 2-1, AG 4, and AG 8 (Table 2).

In both experiments, isolates of *R. solani* AG 4 consistently caused severe root rot (median root rot severity of 8 in both experiments), with the greatest mean rank in severity of root rot ($\bar{R}_{iJ} = 150$ and 125 in experiments 1 and 2, respectively) and mean relative effect on root rot (95% CI for $\hat{p}_{ij}$ was 0.75 to 0.90 in experiment 1 and 0.80 to 0.93 in experiment 2) (Table 2). In experiment 1, AG 2-1 and AG 8 isolates incited similarly severe root rot (median severity of 7) but, in experiment 2, only the AG 2-1 isolates caused root rot as severe as that caused by the AG 4 isolates (median root rot severity of 8 for AG 2-1 and AG 4 isolates versus 5 for AG 8 isolates) (Fig. 4). The mean rank ($\bar{R}_{iJ}$) and relative treatment effect ($\hat{p}_{ij}$) of AG 2-1 isolates were similar to those of the AG 8 and AG 4 isolates in both experiments (the 95% CI for $\hat{p}_{ij}$ for AG 2-1 overlapped with that

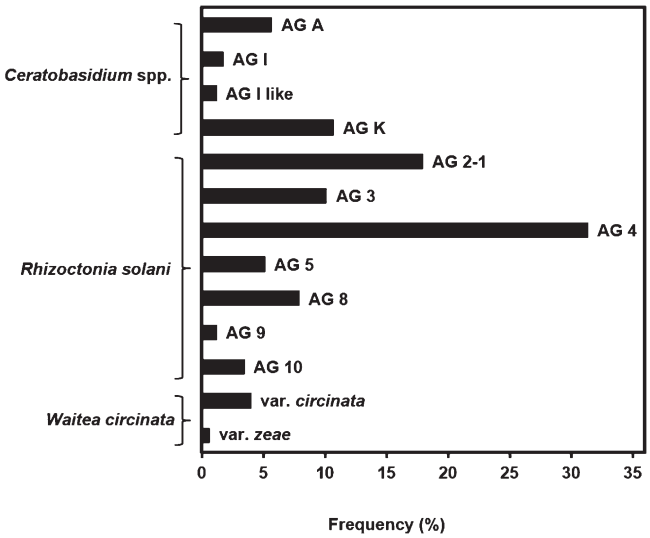

Fig. 2. Frequency of *Ceratobasidium* spp., *Rhizoctonia solani* anastomosis groups (AGs), and *Waitea circinata* subspecies ($n = 179$) obtained from 12 pea fields surveyed in the Columbia Basin of Oregon State and Washington State in 2011 to 2013.

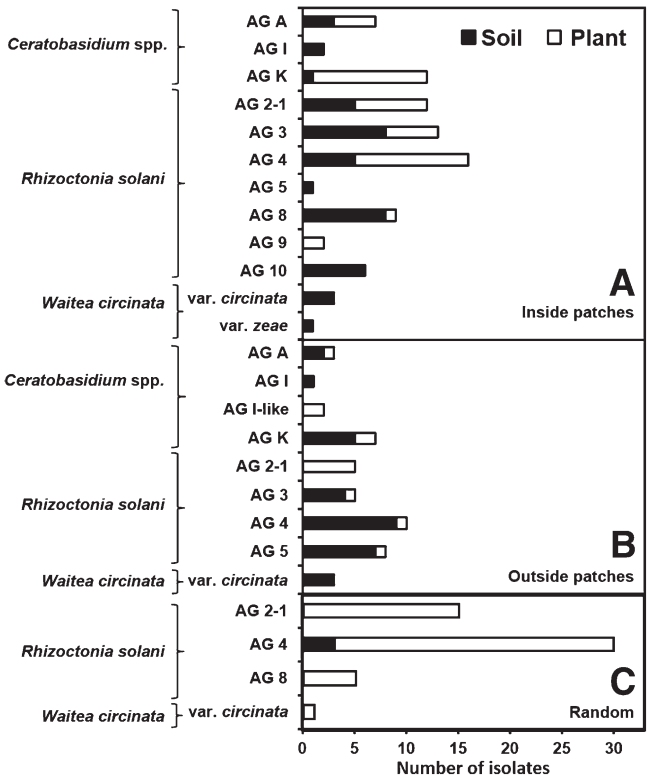

Fig. 3. Number of isolates of *Ceratobasidium* spp., *Rhizoctonia solani* anastomosis groups (AGs), and *Waitea circinata* subspecies obtained from plant and soil samples collected from **A,** within and **B,** outside patches of stunted pea plants and **C,** sampled randomly across pea fields in the Columbia Basin of Oregon and Washington States in 2011 to 2013 (total number of isolates = 179).

of both AG 4 and AG 8) but the 95% CI for the AG 8 isolates was less than that of the AG 4 isolates in experiment 2 (Table 2). Although root rot severity caused by the AG 8 isolates (median severity of 5) was less than that of AG I isolates (median severity of 6) in experiment 2, the mean rank (131 and 104 in experiments 1 and 2, respectively) and relative treatment effect (0.74 and 0.73, respectively) of the AG 8 isolates for root rot severity were greater than those of the AG I isolates (mean rank of 100 and 83 and relative treatment effect of 0.56 and 0.59 in experiments 1 and 2, respectively). The variation in symptoms caused by isolates of AG 2-1, AG 4, and AG 8 are shown in Figure 4. Isolates of AG A, AG K, AG 3, AG 5, AG 10, and *W. circinata* var. *circinata* caused less severe root rot (severity ratings of 2 to 3), with low mean ranks in both experiments. Also, in both experiments, the relative treatment effects associated with these subgroups were <0.5, indicating less likelihood of causing severe root rot than the weighted mean root rot severity rating for all the *Rhizoctonia* subgroups.

Pea height, root length, and dry weight. The most severe stunting of pea plants was caused by isolates of *R. solani* AG 4, followed by isolates of *R. solani* AG 2-1, in both experiments (Table 3; Fig. 4A and B). The four AG 4 isolates significantly ($P = 0.0001$ for experiment 1 and $P = 0.0002$ for experiment 2) reduced seedling height compared with the noninoculated control plants in both experiments (Table 3). AG 2-1 isolates caused a significant ($P = 0.0001$) reduction in plant height in experiment 1 but not in experiment 2. However, seedling height did not differ significantly between plants inoculated with the AG 4 versus AG 2-1 isolates in either experiment. Isolates of none of the remaining AGs or the *W. circinata* var. *circinata* isolates reduced the height of pea seedlings significantly in either experiment (Table 3) compared with noninoculated plants. Similarly, none of the AGs or subspecies significantly reduced root length of pea plants in experiment 1. In fact, plants growing in soil inoculated with isolates of four AGs (AG A, AG 3, AG 5, and AG 10) had significantly longer roots than the noninoculated control plants. In contrast, in experiment 2, pea plants growing in soil inoculated with AG 4 or AG 2-1 isolates had shorter roots compared with plants in noninoculated soil, and none of the AGs resulted in an increase in root length. Shoot dry weight was reduced significantly by AG 4 isolates in experiment 2 only, and not by any other AG in either experiment. In contrast, root dry weight and total dry weight of pea seedlings were reduced significantly by AG 4 and AG 2-1 isolates in both experiments (Table 3), and root dry weight also was significantly reduced by isolates of AG I and AG 8 in experiment 2. The other AGs, including *W. circinata* var. *circinata*, did not significantly reduce plant height, root length, or plant dry weights compared with the control plants.

Table 1. Effect of isolates of different anastomosis groups (AGs) of *Ceratobasidium* spp., *Rhizoctonia* spp., and *Waitea circinata* var. *circinata* on emergence of plants of the pea cultivar Serge evaluated 1 and 2 weeks after seeding in each of two experiments in a growth chamber set at 15 ± 1°C[x]

		Experiment 1				Experiment 2			
		Week 1		Week 2		Week 1		Week 2	
AG, species[y]	df[z]	χ^2 value	*P* value	χ^2 value	*P* value	χ^2 value	*P* value	χ^2 value	*P* value
Model	10	31.15	**0.001**	25.55	**0.004**	16.08	0.097	39.36	**0.001**
AG A	3	3	0.392	2.94	0.402	3.06	0.383	NA	…
AG I	3	3	0.392	2.94	0.402	3.45	0.327	2.98	0.394
AG K	3	0.75	0.861	3	0.392	6.38	0.095	NA	…
AG 2-1	3	15.79	**0.001**	9.06	**0.029**	8.43	**0.038**	7.64	**0.05**
AG 3	3	NA	…	NA	…	3.45	0.327	3.06	0.383
AG 4	3	8.28	**0.041**	10.18	**0.017**	6.63	0.085	6.63	0.085
AG 5	3	8.28	**0.041**	2.94	0.402	3.06	0.383	2.98	0.394
AG 8	3	2.94	0.402	2.94	0.402	3.45	0.327	NA	…
AG 10	3	2.94	0.402	2.94	0.402	2.98	0.394	NA	…
W. circinata	2	3.94	0.14	2.34	0.31	1.82	0.403	2.39	0.303

[x] For both experiments, χ^2 value = value of a χ^2 test (45) for a significant treatment effect with ordinal data, and *P* = probability of a nonsignificant χ^2 test. Significant *P* values (<0.05) are highlighted in bold. NA = the model is not applicable as all pea seed germinated for that AG or subspecies.

[y] Model = combined effect of all isolates of AGs and subspecies on emergence of pea seedlings.

[z] Degrees of freedom (df): four isolates were tested for pathogenicity on pea for each AG or subspecies (df = 3), except for three isolates of *W. circinata var. circinata* (df = 2).

Table 2. Median, mean rank ($\bar{R}_{iJ}$), and relative treatment effect ($\hat{p}_{ij}$) with 95% confidence intervals (CI) for root rot severity (measured on a scale of 1 to 9) caused by isolates of different anastomosis groups (AGs) of *Ceratobasidium* spp., *Rhizoctonia* spp., and *Waitea circinata* var. *circinata* when inoculated onto plants of the pea cultivar Serge in each of two experiments in a growth chamber set at 15 ± 1°C[y]

	Experiment 1				Experiment 2			
AG, species	Median[z]	$\bar{R}_{iJ}$	$\hat{p}_{ij}$	95% CI for $\hat{p}_{ij}$	Median	$\bar{R}_{iJ}$	$\hat{p}_{ij}$	95% CI for $\hat{p}_{ij}$
AG A	3	82	0.46	(0.34, 0.58)	2	49	0.35	(0.25, 0.46)
AG I	5	100	0.56	(0.44, 0.68)	6	83	0.59	(0.43, 0.73)
AG K	3	77	0.43	(0.32, 0.55)	3	67	0.47	(0.38, 0.56)
AG 2-1	7	133	0.75	(0.64, 0.83)	8	123	0.87	(0.76, 0.92)
AG 3	3	55	0.31	(0.24, 0.39)	2	49	0.34	(0.26, 0.44)
AG 4	8	150	0.84	(0.75, 0.90)	8	125	0.88	(0.80, 0.93)
AG 5	3	81	0.45	(0.36, 0.55)	2	47	0.33	(0.24, 0.44)
AG 8	7	131	0.74	(0.67, 0.79)	5	104	0.73	(0.66, 0.79)
AG 10	3	53	0.30	(0.23, 0.37)	2	40	0.28	(0.20, 0.39)
W. circinata	3	66	0.37	(0.27, 0.49)	3	66	0.46	(0.35, 0.58)
NTC	1	18	0.10	(0.07, 0.13)	1	15	0.10	(0.07, 0.13)

[y] $\bar{R}_{iJ}$ = mean rank in root rot severity rating for AGs and *W. circinata* tested for pathogenicity on pea, calculated as described by Shah and Madden (46). The greater the number, the higher the ranking (i.e., the more virulent the group of isolates at causing pea root rot). $\hat{p}_{ij}$ = relative effect of the group of isolates at causing root rot of pea as described by Shah and Madden (46). 95% CI for $\hat{p}_{ij}$[d] = 95% confidence interval, calculated as described by Brunner et al. (5).

[z] Median root rot rating/plant on a scale of 1 to 9, as described in the text.

Yield loss assessment. Mean yield of the processing green pea Serge in 2011 harvested from inside versus outside patches of stunted plants did not differ significantly (Table 4). However, tenderometer readings were significantly greater for pea seed harvested from inside versus outside the stunted patches. In the 2013 pea seed crop of Prevail, stunted areas constituted approximately 11% of the field based on aerial infrared images provided by the grower. Pea yield was four times greater in the nonstunted areas sampled compared with the patches of stunted plants (i.e., an average 75% yield loss was incurred in the patches). Seed harvested from nonstunted plants adjacent to the patches had a greater germination rate (92%) than seed harvested from inside the patches (85%). The incidence of rotted seed did not differ inside versus outside the patches. Patches of stunted pea plants and root symptoms associated with the stunting are shown in Figure 1.

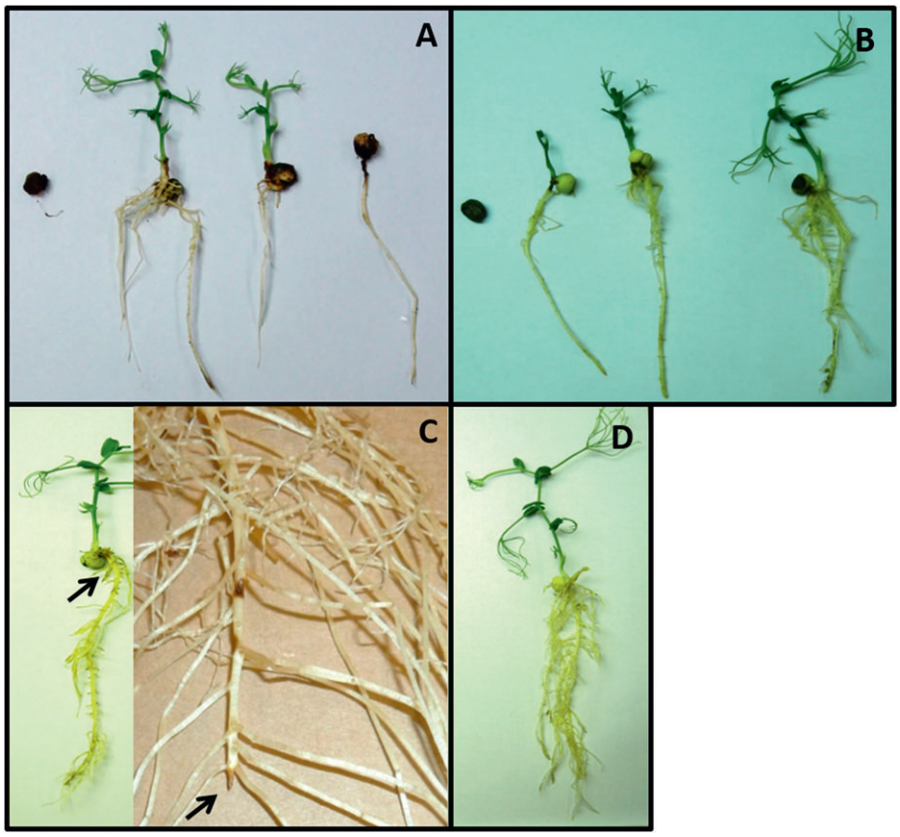

Fig. 4. Rhizoctonia root rot symptoms four weeks after inoculation of pea plants with isolates of *Rhizoctonia solani*. **A,** Symptoms caused by an isolate of *R. solani* AG 2-1 included seed rot, lesions on the epicotyl and hypocotyl (mostly at the collar region), and severe stunting. **B,** Symptoms caused by an isolate of *R. solani* AG 4 also included seed rot, and lesions on the epicotyl and hypocotyl (mostly at the collar region). **C,** Similar symptoms were caused by an isolate of *R. solani* AG 8. Note the lesions on primary as well as secondary roots. Pinched-off or spear tipping of primary and secondary roots is typical of infections caused by *R. solani* AG 8 (indicated by arrows). **D,** Noninoculated control plant.

Table 3. Mean seedling height, root length, and dry weight of shoots, roots, and whole plants measured 4 weeks after seed of the pea cultivar Serge was planted into soil inoculated with isolates of different anastomosis groups (AGs) of *Ceratobasidium* spp., *Rhizoctonia* spp., and *Waitea circinata* var. *circinata* in each of two experiments in a growth chamber set at 15 ± 1°C[z]

	Experiment 1					Experiment 2				
AG, species	Seedling height (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)	Total dry weight (g)	Seedling height (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)	Total dry weight (g)
AG A	6.21 a–c	13.47 a	0.038 b–d	0.048 ab	0.087 ab	9.39 a	14.83 def	0.068 a	0.074 a–c	0.143 a
AG I	6.41 a–c	12.09 bc	0.045 ab	0.049 ab	0.094 a	7.36 c	13.64 ef	0.060 ab	0.055 c	0.119 a
AG K	6.69 a–c	11.35 c	0.036 b–d	0.051 ab	0.087 ab	8.37 a–c	13.86 d–f	0.061 ab	0.062 a–c	0.123 a
AG 2-1	4.49 de	11.30 cd	0.028 de	0.036 bc	0.064 bc	6.79 cd	8.96 f	0.047 bc	0.024 d	0.075 bc
AG 3	7.22 a	14.42 ab	0.041 a–d	0.061 a	0.102 a	8.68 a–c	17.06 ab	0.062 a	0.082 ab	0.143 a
AG 4	4.02 e	7.84 d	0.025 e	0.029 c	0.055 c	4.72 d	8.59 f	0.032 c	0.025 d	0.057 c
AG 5	6.74 ab	15.50 a	0.043 a–c	0.058 a	0.101 a	8.99 ab	17.25 a	0.068 a	0.08 ab	0.148 a
AG 8	5.82 c–e	11.77 c	0.037 b–d	0.045 a–c	0.082 a–c	7.89 bc	15.41 b–d	0.056 ab	0.058 bc	0.114 ab
AG 10	7.18 a	15.08 a	0.048 a	0.059 a	0.107 a	8.59 a–c	16.57 a–c	0.064 a	0.082 ab	0.145 a
W. circinata	5.85 b–d	11.47 cd	0.033 c–e	0.054 ab	0.087 ab	8.83 ab	14.27 c–f	0.074 a	0.081 ab	0.155 a
NTC	6.90 a–c	9.74 cd	0.041 a–e	0.061 a	0.102 a	8.85 a–c	16.3 a–e	0.066 ab	0.086 a	0.152 a
LSD (P = 0.05)	Rank	Rank	Rank	0.0182	0.0276	Rank	Rank	Rank	0.0249	0.0432
ANOVA P value	<0.0001	<0.0001	0.0006	0.0002	0.0039	0.0002	<0.0001	0.003	<0.0001	<0.0001

[z] Data were subjected to analysis of variance (ANOVA), and means were compared among isolates using Fisher's protected least significant difference (LSD) at P = 0.05. Numbers within a column followed by the same letter are not significantly different. Rank transformation was done when assumptions of parametric analysis failed. NTC = non-treated control plants.

Discussion

This study characterized *Rhizoctonia* and *Rhizoctonia*-like spp. baited from pea crops in the Columbia Basin of Oregon and Washington from 2011 to 2013 by identifying the AGs and subspecies of isolates from soil and plant samples. The study also evaluated the pathogenicity of isolates of each of 10 AGs or subspecies obtained from these fields. A wide diversity of taxonomic groups was detected from the pea fields sampled, including *Ceratobasidium* sp. AG A, AG I, AG I-like, and AG K; *R. solani* AG 2-1, AG 3, AG 4, AG 5, AG 8, AG 9, and AG 10; and *W. circinata* var. *circinata*, and *W. circinata* var. *zeae*. This might reflect the diversity of plant species rotated with pea crops in the semiarid, irrigated Columbia Basin (30,33). Pea is rotated commonly with alfalfa (*Medicago sativa* L.), barley (*Hordeum vulgare* L.), carrot (*Daucus carota* L.), onion, potato (*Solanum tuberosum* L.), sweet corn (*Zea mays* L.), and wheat (*Triticum aestivum* L.) in the Columbia Basin (38). The finding of polyphagous soilborne plant pathogens of different AGs and subspecies of *Rhizoctonia* in these pea crops may reflect maintenance of inoculum of these fungi in soils between crops as a result of rotations with susceptible plant species. The results confirm those of similar studies on onion crops in the Columbia Basin (33,49). Patzek et al. (33) detected isolates of 11 unique groups (AG A, E, I, 2-1, 3, 4, 5, 8, 9, *W. circinata* var. *circinata*, and *W. circinata* var. *zeae*), and Sharma-Poudyal et al. (50) detected isolates of 13 groups (AG A, K, G, 1-1B, 2-1, 3, 4, 5, 8, 10, and 11, as well as *W. circinata* var. *circinata*, and *W. circinata* var. *zeae*) in onion bulb crops in this region. Results of those surveys and this study indicate that the Columbia Basin has a very diverse composition of *Rhizoctonia* and *Rhizoctonia*-like spp. on the various crops grown in this temperate, semiarid region of the United States.

Pathogenicity tests on pea revealed the most common group detected in pea fields, *R. solani* AG 4 (31% of all isolates), can adversely affect pea emergence. Isolates of AG 4 also caused severe root rot and, consequently, reduced plant height as well as root and total plant dry weight of pea seedlings in the pathogenicity experiments. The greater frequency of isolation of AG 4 from pea plants than from soil samples collected in pea fields in this study (39 versus 17 isolates, respectively) demonstrated the close association of AG 4 isolates with pea roots and hypocotyls. Therefore, *R. solani* AG 4 appears to be the most important of the *Rhizoctonia* spp. causing pea root rot in the Columbia Basin, as demonstrated in other parts of the world (18,21,23). *R. solani* AG 4 isolates also were the most frequently detected and the most pathogenic to pea in a survey of pea crops in North Dakota (23), and in a survey in the Canadian prairies (18). Similarly, a survey of onion crops in the Columbia Basin revealed AG 4 to be the most widespread and among the most frequently isolated *Rhizoctonia* groups (33,49). AG 4 isolates of *R. solani* can cause damping-off, root rot, crown rot, root canker, stem canker, and stem blight on a number of hosts (2,8,13), including dry bean (*Phaseolus vulgaris* L.) (12), canola (*Brassica napus* L.) (62), and potato (52), all of which are grown in the Columbia Basin. However, *R. solani* AG 4 isolates have been demonstrated to be weakly virulent or avirulent on wheat and barley (29). Such susceptible hosts grown in rotation with pea crops, as well as volunteer plants from these crops, may help maintain soilborne inoculum levels of *R. solani* AG 4 in fields in the Columbia Basin, which may cause losses in highly susceptible crops such as pea.

R. solani AG 2-1, the second most common AG group detected in pea crops in this study (18% of all isolates), consistently caused the most severe reduction in pea emergence, and root rot as severe as that caused by isolates of AG 4. In addition, AG 2-1 isolates caused reductions in pea plant height, root length, and dry root and total biomass similar to those caused by isolates of *R. solani* AG 4. Therefore, *R. solani* AG 2-1 potentially can cause preemergence damping-off and stunting in pea fields. Similarly to AG 4 isolates, a greater number of isolates of *R. solani* AG 2-1 were obtained from pea plants than from soil samples in the fields surveyed (27 versus 5 isolates, respectively). In the Canadian prairies, *R. solani* AG 2-1 was second in frequency of isolation to *R. solani* AG 4 in pea fields (18), but AG 2-1 was not detected in a survey of pea crops in North Dakota (23). AG 2-1 isolates of *R. solani* cause preemergence and postemergence damping-off on a wide range of crops (52). AG 2-1 is cosmopolitan, and readily detected in wheat production areas of the inland Pacific Northwest, including the Columbia Basin (19), even in the absence of crucifer hosts of this AG (13,33,49). AG 2-1 is a significant pathogen of canola crops in the Pacific Northwest, in which isolates infect roots and cause pre- and postemergence damping-off, lesions on hypocotyls, and severe stunting (35). Therefore, *R. solani* AG 2-1 may be a primary cause of preemergence damping-off and stunting in pea crops rotated with cereals, crucifers, or other crops in the Columbia Basin that are susceptible to this AG.

R. solani AG 8, which comprised 8% of all isolates in this pea crop survey, was obtained from soil samples and pea plants (8 versus 6 isolates, respectively). A majority of these isolates ($n = 8$) were baited from soil samples collected inside patches of stunted pea plants. Five isolates were from pea plants collected randomly in the fields, and one was obtained from a pea plant inside a stunted patch. The greater frequency of detection of *R. solani* AG 8 isolates from within versus outside patches of stunted pea plants was similar to a survey of *Rhizoctonia* spp. detected in patches of stunted plants in onion crops in the Columbia Basin (49). *R. solani* AG 8 is a primary cause of onion stunting in the Columbia Basin when onion crops are planted on coarse, sandy soils soon after cereal crop residues are incorporated into the soil (33,49). This is similar to cereal bare patch, a widespread disease of cereals in the inland Pacific Northwest that is associated primarily with *R. solani* AG 8 (37,42). The limited recovery of *R. solani* AG 8 isolates from patches of stunted pea plants compared with other AGs might be confounded by the difficulty of isolating AG 8 from soil or plant samples (15,36). *R. solani* AG 8 isolates tend to be slow growing compared with isolates of AG 2-1, AG 3, AG 4, and *R. oryzae*; therefore, a bias in such surveys might be introduced as a result of faster-growing fungi being isolated more readily than slower-growing fungi such as those of *R. solani* AG 8 (20,29).

Isolates of *R. solani* AG 8 did not affect pea emergence, seedling height, root length, or total dry weight of plants of Serge pea plants in growth chamber trials at 15°C in this study, but reduced pea dry root weight significantly in one of the two experiments. AG 8 isolates from cereal bare patches near Pendleton, OR significantly reduced plant height and weight of the pea cv. Dark Skin Perfection in inoculated trials (51). For the pea cv. Columbia, AG 8 isolates did not reduce emergence but reduced root dry weight under greenhouse conditions (10). The lack of significant differences in plant height between AG 8-inoculated plants and control plants of Serge in this study may reflect a cultivar response to AG 8. However, pea plants inoculated with AG 8 isolates always developed a rot of the cortex that left a pointed "spear tip" on infected taproots and secondary roots

Table 4. Effect of pea stunting caused by *Rhizoctonia* spp. on yield of a processing pea crop of the cv. Serge in 2011, and yield of a pea seed crop of the cv. Prevail in 2013 in grower-cooperator fields in the Columbia Basin of Washington[z]

Yield variable	Inside patches	Outside patches	Student's *t* test (*P* value)
Processing pea crop			
Pea yield (g/m^2)	667 ± 157	569 ± 165	0.238
Tenderometer reading	77 ± 6	66 ± 2	<0.001
Pea seed crop			
Dry pea seed yield (g/m^2)	127 ± 59	514 ± 40	<0.001
Seed germination (% normal seed)	85 ± 10	92 ± 6	<0.001
Nongerminated seed (%)	11 ± 8	5 ± 5	<0.001
Rotten seed (%)	4 ± 4	3 ± 4	0.638

[z] Pea yield was measured inside and outside each of 10 patches of stunted plants in each field (and tenderometer readings were measured for the processing pea crop) as described in the text. Incidence (%) of seed germination, nongerminated seed, and rotten seed was determined according to the blotter assay of the Association of Official Seed Analysts (57). Each value is the mean ± standard deviation for 10 replicate samples.

(Fig. 4C). The spear-tipped root symptoms are similar to those found in cereal and onion plants in stunted patches in the Columbia Basin (33). In the pathogenicity experiments, median root rot severity ratings of 7 and 5 in the first and second experiments, respectively, with relative treatment effects ≥0.73, indicated that the *R. solani* AG 8 isolates caused root rot of pea seedlings. Therefore, even if pea seedlings are not stunted by *R. solani* AG 8 isolates, pruning of the tips of secondary roots and tap roots may affect absorption of water and minerals from the soil, potentially leading to stunted growth at flowering and differential maturity of pea plants in stunted patches versus healthy areas of a field. This can affect tenderometer readings, which are important for timing the harvest of processing pea crops because tenderometer readings measure firmness of the pea seed (54).

The frequency of detection of *Ceratobasidium* sp. AG K (11% of all isolates detected in this survey) ranked third after that of AG 4 and AG 2-1 isolates of *R. solani*. AG K isolates were detected mostly from pea plants rather than baited from soil (13 versus 9 isolates, respectively). AG K isolates caused limited root rot (mean severity of 3) and did not reduce pea emergence, plant height, root length, and dry weight significantly compared with noninoculated control plants. Therefore, AG K isolates in this study were weakly virulent on pea. Similarly, AG K isolates have been reported as nonpathogenic on other crops, including sugar beet (*Beta vulgaris* L. subsp. *vulgaris*), radish (*Raphanus sativus* L.), tomato (*S. lycopersicon* Mill.), carrot, and onion (52,55), but have caused root rot on strawberry (*Fragaria* × *ananassa* D.) in Australia (11).

Five isolates of *Ceratobasidium* sp. AG A were obtained from soil and pea plants in this survey (6% of all isolates). Similar to AG K isolates, the AG A isolates did not reduce emergence, plant height, or shoot and root biomass of the pea cv. Serge. In fact, pea plants growing in soil infested with AG A isolates were significantly taller than control plants in noninfested soil in one experiment, although plant height was not affected significantly by AG A isolates in the repeat experiment. AG A isolates form mycorrhizal associations with many orchids (56). However, AG A isolates collected from Yunnan Province, China were pathogenic on pea (61), and some isolates of AG A have been pathogenic on crops such as strawberry (17), sugar beet (60), peanut (*Arachis hypogaea* L.) (31), and potato (28). Sugar beet and potato are grown on significant acreage in the Columbia Basin (43), and could contribute inoculum of *Ceratobasidium* AG A.

Isolates of *Ceratobasidium* sp. AG I caused a median root rot severity of 5 to 6 with a relative treatment effect ≥0.56, indicating that these isolates can cause root rot of pea. However, the AG I isolates had no significant effect on emergence, plant height, root length, and plant dry weight. An AG I-like isolate collected from roots and rhizosphere soil of canola plants near Ritzville, WA reduced pea plant height and dry weight by 12 and 17%, respectively, compared with noninoculated control plants in a study by Schroeder and Paulitz (44).

Four isolates each of *R. solani* AG 3, AG 5, and AG 10 and three isolates of *W. circinata* var. *circinata* were weakly virulent on pea in this study because the isolates did not adversely affect emergence, height, root length, or plant weight. However, isolates of these groups are pathogenic on many crops grown in the Columbia Basin. For example, *R. solani* AG 3 is pathogenic on potato (7). *R. solani* AG 5 has been reported as pathogenic on potato (3), bean (52), and apple (*Malus domestica* L.) (24). *R. solani* AG 10 has a wide host range (22) and is also reported from cereal-growing regions in the Pacific Northwest (29). However, most AG 10 isolates are considered saprophytic (8). In a previous study (34), *W. circinata* var. *circinata* isolated from pea plants near Lewiston, ID did not affect emergence of 'Little Marvel' pea plants but caused root necrosis, browning of root tips, and reduction in lateral root formation. Under greenhouse conditions, *W. circinata* var. *circinata* isolates reduced emergence and root dry weight of Columbia pea plants (10). Therefore, variations in reactions among pea genotypes to *W. circinata* var. *circinata* may exist, or isolates used in this study may be less virulent than isolates evaluated in previous studies. Because *W. circinata* var. *circinata* isolates are widespread and among the most frequent *Rhizoctonia* groups detected in cereal (19,29) and onion crops (49) in the inland Pacific Northwest, further work on variation in virulence among isolates of *W. circinata* var. *circinata* on pea genotypes will be important to understand the significance of this pathogen in causing pea root rot.

The most virulent AGs on pea, those of *R. solani* AG 4 and AG 2-1, were also the most widespread and common in the pea fields surveyed in this study. Therefore, isolates of these AGs are likely associated with pea root rot in the Columbia Basin. Isolations of combinations of AG 2-1 and AG 4, AG 2-1 and AG 8, or AG 4 and AG 8 from soil and plant samples collected from patches of stunted pea plants also indicate that pea stunting could be caused by a complex of *R. solani* AGs. Therefore, stunted patch characteristics and pea root rot symptoms may vary depending on the AGs causing root rot in a particular field. Based on symptoms observed in the pathogenicity tests in this study, if severe preemergence damping-off occurs in a field, the damage is more likely caused by isolates of *R. solani* AG 2-1 than AG 4. However, AG 4 isolates also can reduce pea emergence and may cause more severe stunting than AG 2-1 isolates based on the pathogenicity tests. Although isolates of *R. solani* AG 8 did not reduce the height of pea plants, a single pea cultivar was used in this study for pathogenicity tests. Screening additional pea cultivars is warranted to determine relative susceptibility of cultivars to isolates of different AGs. *R. solani* AG 8 isolates are probably more important pea pathogens in fields in which cereal crop residues are incorporated into the soil just prior to planting pea seed, as occurred in the pea seed crop of Prevail in 2013, and similar to what has been demonstrated for onion stunting caused by *R. solani* (33,49). For example, isolates of *R. solani* AG 8 were detected more frequently (50%) than isolates of AG 4 (42%) and AG 2-1 (8%) in the pea seed crop of Prevail in 2013 that displayed severe stunting. Isolates of AG 8 may cause stunting in a pea field that has significant cereal crop residues incorporated in close temporal proximity to planting the pea crop, without a reduction in pea stand. Similarly, AG 8 isolates cause stunting of wheat (25) and onion (33) without affecting plant stands. Understanding the saprophytic competitiveness of isolates of *R. solani* AG 2-1, AG 4, and AG 8 for colonizing cereal residues may help elucidate the relative role of these AGs in the pea root rot complex in the Columbia Basin. AG A, AG 3, AG 5, and AG 10 isolates caused less severe root rot on Serge. In addition, isolates of these AGs did not affect root length adversely.

Assessment of the yield loss caused by pea stunting in a processing pea crop and a pea seed crop in the Columbia Basin in this study demonstrated that stunting can reduce pea seed yield significantly, as observed in the seed crop of Prevail in 2013. However, the insignificant yield difference inside versus outside stunted patches of a processing crop of Serge probably reflects greater tenderometer readings of pea seed harvested from inside versus outside the stunted patches as a result of earlier maturity of stunted plants. Pea seed harvested from plants within stunted patches were more mature and weighed more than the less mature seed harvested from nonstunted plants. The difference in effect of stunting on pea yield in the 2011 processing crop versus the 2013 seed crop was also due, in part, to differences in severity of stunting in the two fields. Stunting was much less severe in the processing crop surveyed in 2011 compared with the 2013 seed crop. In addition, stunting in the pea seed crop adversely affected quality (germination) of the seed harvested, further affecting seed yield.

In conclusion, detection of isolates of 10 AGs or subspecies of *Rhizoctonia* or *Rhizoctonia*-like spp. in pea fields illustrated the diversity of these fungi in the Columbia Basin of Oregon and Washington. Isolates of *R. solani* AG 2-1, AG 4, and AG 8 were among the most virulent at causing pea stunting. These results could be utilized in pea breeding programs to identify and select tolerant or resistant pea germplasm. Simultaneous detection of these AGs in a pea seed crop with severe yield loss poses challenges to finding tolerant or resistant germplasm to the presence of multiple soilborne pathogens. Losses to onion stunting caused by *Rhizoctonia* spp. in the Columbia Basin have been reduced effectively using preplant incorporation of

azoxystrobin into soil (47), and increasing the interval between herbicide application to a cereal cover crop and planting onion seed (48). Similar disease control strategies could be utilized to manage stunting in pea crops, in addition to alternative winter cover crops that are not susceptible to these fungi, and the use of fungicide seed treatments to minimize the impact of *Rhizoctonia* spp. on pea production.

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