Phylogenetic Diversity of *Rhizoctonia solani* Associated with Canola and Wheat in Alberta, Manitoba, and Saskatchewan

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Abstract

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Rhizoctonia solani is a damaging soilborne pathogen, which affects most field crops in the Canadian provinces of Alberta, Manitoba, and Saskatchewan. The objective of this study was to conduct a phylogenetic comparison of isolates of *R. solani* collected from a previous survey in the major canola- and wheat-growing regions of western Canada. A total of 128 multinucleate isolates from a previous survey were identified by internal transcribed spacer (ITS) sequence and compared to anastomosis group (AG) results. The multinucleate isolates of *R. solani* were grouped into eight distinct clades. Each clade corresponded to a specific AG with the exception of two distinct clades that

were observed for isolates classified as AG 2-1 by anastomosis testing. While most isolates of AG 5 clustered together according to ITS sequences, three isolates classified by anastomosis grouping as AG 5 grouped with AG 2-1, AG 4, and a binucleate *Rhizoctonia* sp. in the phylogenetic analysis. In most instances, the results from AG tests were consistent with ITS sequence, but there were still several cases where isolates were inconsistently classified or failed to undergo anastomosis with any of the tester strains used in this study. This provides support for the use of the ITS region as a valuable tool for rapid identification of *R. solani* isolates to their respective AGs.

Globally, *Rhizoctonia solani* is an important soilborne pathogen of a number of agriculturally important crops causing damping-off, foliar blight, and root and stem rot. The species is highly genetically diverse and has been separated into independent anastomosis groups (AGs) based on the ability of strains to successfully fuse and exchange nuclear material with known tester strains. Several recent studies have attempted to use molecular approaches to separate AGs or lineages within *R. solani*. The genetic relatedness of *R. solani* AGs was evaluated by Kuninaga et al. (14), and they found that a percent similarity higher than 96% of the internal transcribed spacer (ITS) sequence was evident for isolates within an AG subgroup, 66 to 100% for isolates of different subgroups within an AG, and 55 to 96% for isolates among different AGs. AG 2 was found to be especially diverse and includes a number of subgroups, which are subdivided according to their morphology, host range, virulence, and hyphal anastomosis frequency. Currently, subgroups 2-1, 2-2 IIIB, 2-2 IV, 2-2 LP, 2-2 WB, 2-3, 2-4, and 2-BI are included in AG 2 (2,6), and ITS sequencing analysis was consistent with the above listed subgroups (2,26,27). In addition, the rDNA-ITS sequences accurately divided subgroups within AG 1 (14), AG 3 (14), AG 4 (1,14), and AG 6 (21).

The wide host range of *R. solani* extends from dicotyledonous plants, including canola, soybeans, dry beans and peas, to monocotyledonous plants, such as wheat and corn. The majority of reports of *R. solani* on cereals originate from Australia (16) and the northwest region of the United States (18), where AG 8 is the most commonly detected subgroup. In a survey of wheat in Texas, AG 4

was most frequently recovered in addition to several isolates of AG 2-2 and AG 5 (25). *R. solani* is also becoming an increasingly important pathogen on canola as production of this crop continues to expand beyond Canada and North Dakota into the northwestern United States and Australia, and extends into new areas such as South Africa. Most reports have found that AG 2-1 is the most frequently recovered *R. solani* AG from canola (9,12,15,20,37,38). However, several recent studies have isolated other AGs from canola, including AG 10 (28) and *Ceratobasidium* spp. in Washington (29), and AG 3, AG 4-HGII, AGA, AG I, and AG K in South Africa (35). Even though wheat and canola are the two major crops in western Canada, little is known about the diversity of *Rhizoctonia* spp. infecting them.

A recent survey by Melzer et al. (17) evaluated the diversity of *Rhizoctonia* species from barley, bean, canola, pea, soybean, and wheat crops in Canada. They found that *R. solani* isolated from canola and wheat included members of AG 2-1, 4, and 5 (17). However, 17 isolates were not successfully assigned an AG group. This was likely due to the fact that only four tester strains (AG 2-1, 2-2, 4, and 5) were available for anastomosis testing of all isolates, and the unassigned strains may belong to different AG groups than those four tested. Therefore, if isolates from AG groups other than those four, or from novel AG groups, were recovered, they would go unidentified. The alternative strategy currently being used for classification of *R. solani* isolates has relied on differences in the sequence of the ITS regions of their rDNA. Analysis of the rDNA-ITS region was shown to be a reliable method to identify *Rhizoctonia* spp. (32,33). In a recent survey of *Rhizoctonia* in Europe, Goll et al. (7) identified 288 isolates of *Rhizoctonia* by ITS sequences, and the determination of AG was based on the homology of the sequence from the unknown isolate to that of an appropriate set of reference sequences from the NCBI database. As the ITS region can vary substantially even among isolates of an AG, the authors set the positive identification level at 85% and found that, in 27% of the cases, homology was <95%. It is that 27% that may represent distinct groups within an AG, but without a proper phylogenetic analysis it is difficult to determine which AGs are highly conserved and which have a high level of variability both within and among AGs.

Given the potential shortfalls of both identification methods mentioned above, the aims of this study were to: (i) conduct a phylogenetic analysis of isolates of *R. solani* collected from canola

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and wheat from western Canada; and (ii) determine if classical anastomosis grouping using tester strains is consistent with results from phylogenetic analyses.

Materials and Methods

Rhizoctonia isolates and determination of anastomosis groups. *Rhizoctonia* isolates used in this study were obtained through the survey of Melzer et al. (17) as a subset of isolates from western Canada. Details of these survey methods and results are presented elsewhere (17). In brief, during May, June, and July of 2009 to 2011, canola, pea, and wheat crops were surveyed in Alberta, Saskatchewan, and Manitoba. The majority of crops were sampled at the seedling stage, but some crops were sampled at later stages of plant development. When available, the cropping history

of sampled fields was recorded. A diamond-shaped pattern of 50 m per side was used for sampling. Twenty seedlings were collected per field, with one seedling sampled every 10 m around each sides of the diamond. If areas with symptoms of disease were observed in the crop during sampling, additional seedlings were collected from these diseased areas. Seedlings were stored at 4°C until being processed. Seedlings were rinsed in cool water, upper stems and leaves were removed, and remaining crowns and roots were then surface-disinfested for 30 s in 70% ethanol followed by 90 s in 0.6% sodium hypochlorite. Seedling crowns and roots were rinsed, blotted dry, then plated on water agar (WA). Isolates of *Rhizoctonia* spp. were identified using hyphal and colony characteristics, and transferred to acidified potato dextrose agar (Difco; PDA) medium (APDA). Isolates were stored on APDA slants at 7°C.

Table 1. Isolates of *Rhizoctonia solani* collected from canola, wheat, and pea in Alberta, Manitoba, and Saskatchewan, Canada^a

Isolate	Province	Host	AG group ^b	Accession number
10-C-F_5C	Alberta	Canola	AG 2-1	KF870886
10-C-F_16C	Alberta	Canola	AG 2-1	KF870887
10-C-F_20C	Alberta	Canola	AG 2-1	KF870888
10-C-T_7R	Alberta	Canola	AG 2-1	KF870889
10-C-T_13C	Alberta	Canola	AG 2-1	KF870890
10-D4_16R	Alberta	Pea	AG 2-1	KF870891
10-D5_3C	Alberta	Wheat	AG 2-1	KF870892
10-D5_12C	Alberta	Wheat	AG unk	KF870893
11-A2_16R	Alberta	Wheat	AG 2-1	KF870894
11-A4_10C	Alberta	Canola	AG 2-1	KF870895
11-A7_4C	Alberta	Wheat	AG 5	KF870896
11-A10_9C	Alberta	Wheat	AG 5	KF870897
11-A10_10C	Alberta	Wheat	AG 5	KF870898
11-A10_11C	Alberta	Wheat	AG 5	KF870899
11-A10_16C	Alberta	Wheat	AG 5	KF870900
D4_5R	Alberta	Canola	AG 2-1	KF870901
D4_9C	Alberta	Canola	AG 2-1	KF870902
D4_12_C	Alberta	Canola	AG 2-1	KF870903
D4_14R	Alberta	Canola	AG 2-1	KF870904
D5_12C	Alberta	Canola	AG 2-1	KF870905
D5_13C	Alberta	Canola	AG 2-1	KF870906
D5_19_C	Alberta	Canola	AG unk	KF870907
D5_19S	Alberta	Canola	AG 2-1	KF870908
D6_16C	Alberta	Canola	AG 2-1	KF870909
M1_1C	Alberta	Wheat	AG 4	KF870910
M1_2S	Alberta	Wheat	AG 4	KF870911
M1_6S	Alberta	Wheat	AG 4	KF870912
M1_8S	Alberta	Wheat	AG 4	KF870913
M1_9C	Alberta	Wheat	AG 4	KF870914
M1_12C	Alberta	Wheat	AG 4	KF870915
10-C-5_10R	Manitoba	Canola	AG 2-1	KF870916
10-C-5_19C	Manitoba	Canola	AG 2-1	KF870917
10-D-10_9C	Manitoba	Wheat	AG 2-1	KF870918
10-D-10_11C	Manitoba	Wheat	AG 5	KF870919
10-M13_8C	Manitoba	Canola	AG 2-1	KF870920
10-M13_19C	Manitoba	Canola	AG 2-1	KF870921
10-M13S_5C	Manitoba	Canola	AG 2-1	KF870922
10-M13S_6C	Manitoba	Canola	AG 2-1	KF870923
11-M11_14C	Manitoba	Canola	AG 2-1	KF870924
11-M11_20C	Manitoba	Canola	AG 2-1	KF870925
M6_14C	Manitoba	Wheat	AG 2-1	KF870926
M7_2C	Manitoba	Wheat	AG 2-1	KF870927
M7_3C	Manitoba	Wheat	AG 4	KF870928
M7_4C	Manitoba	Wheat	AG 2-1	KF870929
M7_7C	Manitoba	Wheat	AG 2-1	KF870930
M7_8C	Manitoba	Wheat	AG 2-1	KF870931
M7_10R	Manitoba	Wheat	AG 2-1	KF870932
M7_11R	Manitoba	Wheat	AG 2-1	KF870933
M7_12C	Manitoba	Wheat	AG 4	KF870934
M7_16R	Manitoba	Wheat	AG 4	KF870935
M9_16R	Manitoba	Wheat	AG unk	KF870936
M9_18C	Manitoba	Wheat	AG 5	KF870937
M9_19R	Manitoba	Wheat	AG 2-1	KF870938

(continued on next page)

^a Isolates were assigned to an AG group based on successful anastomosis with known tester strains AG 2-1, AG 2-2, AG 4, and AG 5.

^b Indicates the isolate was able to successfully anastomose with a known tester strain of the AG. Those isolates that did not anastomose with tester strains used in this study are labeled AG unk (unknown).

Hyphae of isolates of *Rhizoctonia* spp. were stained with trypan blue (0.5% in lactophenol) and examined microscopically for the number of nuclei per cell to determine if the isolates were binucleate or multinucleate. Multinucleate isolates were considered to be *R. solani*. To determine the anastomosis groups of the recovered multinucleate *R. solani* isolates, an agar plug was taken from the actively growing margin of an isolate of unknown AG and plated on WA 2 to 3 cm apart from an isolate of known AG. Isolates were considered to be in the same AG when anastomosis was observed a minimum of five times.

DNA extraction, polymerase chain reaction (PCR) amplification and sequencing. DNA was extracted from mycelia cultured on PDA lined with cellophane (Flexel Sales Inc.), using the Power

Plant Pro DNA Isolation Kit (MoBio Laboratories Inc.). Samples were stored at -20°C . PCR was performed in 50- μl reaction volume containing 10 μl of 5 $\times$ Green GoTaq reaction buffer (Promega Corp.), 5 μl of 25 mM MgCl_2 , 1 μl containing 10 mM each dNTP, 0.25 μl of GoTaq Taq DNA polymerase, 5 μl of each primer ITS4 and ITS5 (36) at 5 pmol μl^{-1} , 2 μl of DNA, and 21.75 μl of sterile distilled water. The PCR amplification conditions were initial denaturation at 95°C for 2 min, followed by 40 cycles of 15 s at 95°C , 1 min at 57°C , and 30 s at 72°C , with a final 10 min at 72°C . Amplified products were purified using Qiaquick spin columns (Qiagen Inc.). DNA was quantified using a NanoDrop 1000 spectrophotometer (Thermo Scientific). Amplified products were sequenced by including 14 ng of purified DNA with the BigDye

Table 1. (continued from previous page)

Isolate	Province	Host	AG group ^b	Accession number
M10_1C	Manitoba	Wheat	AG 2-1	KF870939
M10_2C	Manitoba	Wheat	AG 5	KF870940
M10_3C	Manitoba	Wheat	AG 4	KF870941
M10_9C	Manitoba	Wheat	AG 4	KF870942
M10_13C	Manitoba	Wheat	AG 4	KF870943
M10_18C	Manitoba	Wheat	AG 4	KF870944
M10_21C	Manitoba	Wheat	AG 4	KF870945
M10_23C	Manitoba	Wheat	AG 5	KF870946
M11_1R	Manitoba	Wheat	AG 2-1	KF870947
M11_4R	Manitoba	Wheat	AG 5	KF870948
M11_9R	Manitoba	Wheat	AG 2-1	KF870949
M11_16C	Manitoba	Wheat	AG 5	KF870950
M11_18C	Manitoba	Wheat	AG 5	KF870951
M11_24R	Manitoba	Wheat	AG 5	KF870952
10-C-P1_4C	Saskatchewan	Canola	AG 2-1	KF870953
10-C-P1_5C	Saskatchewan	Canola	AG unk	KF870954
10-C-P1_6C	Saskatchewan	Canola	AG 2-1	KF870955
10-C-P1_7R	Saskatchewan	Canola	AG 2-1	KF870956
10-C-P1_8C	Saskatchewan	Canola	AG 2-1	KF870957
10-C-P1_11C	Saskatchewan	Canola	AG 2-1	KF870958
10-C-P1_12C	Saskatchewan	Canola	AG 2-1	KF870959
10-C-P1_13C	Saskatchewan	Canola	AG 2-1	KF870960
10-C-P1_15C	Saskatchewan	Canola	AG 2-1	KF870961
10-C-P1_16C	Saskatchewan	Canola	AG 2-1	KF870962
10-C-P1_17R	Saskatchewan	Canola	AG 2-1	KF870963
10-C-P1_18C	Saskatchewan	Canola	AG 2-1	KF870964
10-C-P1_20C	Saskatchewan	Canola	AG 2-1	KF870965
10-C-P3_10C	Saskatchewan	Canola	AG 2-1	KF870966
10-C-P3_15C	Saskatchewan	Canola	AG 2-1	KF870967
10-D7-13_12_C	Saskatchewan	Canola	AG 2-1	KF870968
11-S3_4C	Saskatchewan	Canola	AG 2-1	KF870969
11-S3_10C	Saskatchewan	Canola	AG 2-1	KF870970
11-S3_14C	Saskatchewan	Canola	AG 2-1	KF870971
11-S4_14C	Saskatchewan	Canola	AG 2-1	KF870972
11-S4_15C	Saskatchewan	Canola	AG 2-1	KF870973
11-S4_16C	Saskatchewan	Canola	AG 2-1	KF870974
11-S4_20C	Saskatchewan	Canola	AG 2-1	KF870975
11-S5_1C	Saskatchewan	Wheat	AG 2-1	KF870976
11-S5_2C	Saskatchewan	Wheat	AG 2-1	KF870977
11-S5_4C	Saskatchewan	Wheat	AG 2-1	KF870978
11-S5_6C	Saskatchewan	Wheat	AG 2-1	KF870979
11-S5_7C	Saskatchewan	Wheat	AG 2-1	KF870980
11-S5_8C	Saskatchewan	Wheat	AG 2-1	KF870981
11-S5_9C	Saskatchewan	Wheat	AG unk	KF870982
11-S5_10C	Saskatchewan	Wheat	AG 2-1	KF870983
11-S5_11C	Saskatchewan	Wheat	AG 2-1	KF870984
11-S5_13R	Saskatchewan	Wheat	AG 2-1	KF870985
11-S5_14C	Saskatchewan	Wheat	AG 2-1	KF870986
11-S5_15C	Saskatchewan	Wheat	AG 2-1	KF870987
11-S6_6C	Saskatchewan	Canola	AG 2-1	KF870988
11-S6_7C	Saskatchewan	Canola	AG 2-1	KF870989
11-S6_11C	Saskatchewan	Canola	AG 2-1	KF870990
11-S6_15C	Saskatchewan	Canola	AG 2-1	KF870991
11-S6_16C	Saskatchewan	Canola	AG 2-1	KF870992
11-S6_18C	Saskatchewan	Canola	AG 2-1	KF870993
11-S6_19C	Saskatchewan	Canola	AG 2-1	KF870994
CMC2_9C	Saskatchewan	Canola	AG unk	KF870995
D7_11C	Saskatchewan	Canola	AG 2-1	KF870996
D8_13R	Saskatchewan	Canola	AG 2-1	KF870997

Fig. 1. Maximum likelihood tree of the internal transcribed spacer (ITS) sequence of 112 isolates of *Rhizoctonia* recovered from canola (c), pea (p), and wheat (w) from Alberta (AB), Manitoba (MB), and Saskatchewan (SK). Isolate name consists of isolate number, AG group, from anastomosis test, province (AB, MB, or SK), and crop (C, P, or W). Isolates imported from GenBank are followed by an accession number. Posterior probabilities >60% are displayed at the nodes.

version 3.1 Ready Reaction Kit (Applied BioSystems) on an ABI 3730 DNA Analyzer (Applied BioSystems) at the University of Guelph Genomics Facility. Forward and reverse sequences were assembled and aligned using Geneious Pro software (v.5.4.3). All sequences were deposited in the NCBI GenBank, and accession numbers were included in Table 1.

Phylogenetic analysis. To infer species, AGs, and outgroups, reference sequences from studies by Sharon et al. (31,33), Gonzalez et al. (8), or from vouchered cultures in the Centraalbureau voor Schimmelcultures (CBS) fungal biodiversity center were imported from GenBank. The phylogenetic and molecular analyses were completed using *MEGA* version 4.0 (13). Alignments were completed using ClustalW, and trees were inferred using the neighbor joining (NJ) method (Kimura two-parameter distance calculation). Gaps were treated as missing data with missing or ambiguous sites ignored for the affected pairwise comparison. All positions were included in the analyses, and relative support for the branches was estimated with 1,000 bootstrap replications (5) for NJ analysis. Analysis of polymorphic sites was completed using DnaSP (24).

Phylogenetic trees were also inferred using Bayesian inference as implemented in MrBayes v. 3.1.2 (10). A Bayesian analysis using the general time reversible (GTR) model was selected for the entire unpartitioned alignment, with likelihood parameter settings (lset) number of substitution types (nst) = 6, with a proportion of sites invariable and the rest drawn from the gamma distribution (rate = invgamma). Four independent analyses, each starting from a random tree, were run under the same conditions for the combined gene alignment. Three hot and one cold chain Markov Chain Monte Carlo with 1,000,000 generations with sampling every 100 generations was used for the analysis. The first 250,000 generations were discarded as the chains were converging (burn-in period).

Diversity within AGs was also assessed by collapsing sequences down into haplotypes using SNAP workbench 2.0 (22), to enable analysis of the clone-corrected data set. In the case of isolates belonging to AG 5, a set of 95% plausible haplotype networks connecting the haplotypes by mutational steps was constructed using statistical parsimony in the program TCS (3). Due to the genetic distance between isolates belonging to AG 2-1 and AG-9, rather than completing a haplotype network, a phylogenetic analysis similar to the one described above was completed. In addition to the analysis of the AG 2-1 haplotypes, isolates listed as AG 2-1 in NCBI GenBank were imported to evaluate the similarity of AG 2-1 isolated from canola and wheat in Canada to other isolates of AG 2-1 from around the world.

Results

For the majority of multinucleate isolates of *R. solani* recovered from Alberta, Manitoba, and Saskatchewan, phylogenetic identification against known sequences from GenBank corresponded consistently to the results of AG tester strains. While anastomosis testing grouped the isolates into four AG groups, analysis of the ITS sequences grouped the isolates into six distinct clades corresponding with AG 2-1, AG 4, AG 5, AG 9, AG 11, and *Ceratobasidium* sp. AG B (Fig. 1), and seven isolates that formed hyphal fusions with the AG 2-1 tester strain but formed a distinct seventh clade that did not have a close sequence match to any of the known AG groups in GenBank (Fig. 1). The separation of these two groups was supported with strong posterior probability support (92%) and may represent two distinct lineages that retain the ability to exchange genetic material but perhaps only rarely. AG 2-1 was the most frequently recovered anastomosis group in all three provinces surveyed in this study by both anastomosis testing and ITS sequences.

There were three instances where isolates successfully fused with an AG tester strain but were found to be more closely related to another AG group based on ITS sequence data. In one instance, an isolate (M7 8C) successfully fused with an AG 2-1 tester strain, but sequence data indicated it was a member of AG 5. The second

and third instances were isolates M10 23C and M10 2C, respectively, that successfully fused with AG 5, but sequences indicated these isolates were more closely related to AG 4 and *Ceratobasidium* sp. AG B, respectively. There were six isolates that did not successfully fuse with the tester strains AG 2-1, AG 2-2, AG 4, and AG 5. In two of these instances, the isolate belonged to an AG group based on ITS sequence data but did not fuse with the corresponding tester strain. One of the isolates clustered with members of AG 2-1 (D5 19C), another isolate (M9 16R) clustered with members of AG 5. After the identity of these isolates was determined, they were re-evaluated with their respective tester strains to ensure that their inability to anastomose was not an error. The results from the repeated tests proved that neither isolate was able to successfully fuse with the corresponding tester strain. Of the remaining four isolates that did not fuse with tester strains used in this study, three clustered with strong bootstrap support to an isolate of AG 9 used by Gonzalez et al. (8), and one isolate was found to be most similar to an isolate of AG 11 also used in the study by Gonzalez et al. (8). Tester strains of these two AG groups were not available to evaluate the ability of unknowns to anastomose with members of their respective groups.

No genetic diversity was observed among isolates classified as AG 4, as all 15 isolates formed a single haplotype. In contrast, multiple haplotypes of AG 2-1, AG 5, and AG 9 were observed. In the case of AG 5, the 13 isolates were separated into seven haplotypes with seven isolates grouping together in haplotype H5, and the remaining haplotypes were represented by a single isolate each (Fig. 2). The haplotype map provides a similar result to the phylogenetic tree, which has the seven isolates of H5 separated from the other members of AG 5 with strong bootstrap support (97%). In the case of AG 2-1, the 83 isolates were grouped into 15 haplotypes. In the phylogenetic analysis, these 15 haplotypes grouped into three distinct clades with strong posterior probability (80%) (Fig. 3). The BLAST search found that several isolates of AG 2-1 have been previously recovered from canola in Alberta. In addition, the isolates of AG 2-1 from the prairies of Canada have close similarity to isolates recovered from potato in France and Finland, cabbage in New York, ginseng in China, cauliflower in Belgium, and *Brassica oleracea* in the United Kingdom, demonstrating the global distribution and host range of this important soilborne pathogen. Finally, the three isolates of AG 9 were grouped into two haplotypes and had the greatest similarity to isolates of AG 9 from *B. oleracea* from the United Kingdom.

Wheat plants hosted the greatest diversity of *R. solani* AGs including strains from AG 2-1, AG 4, AG 5, AG 9, AG 11, and an unclassified AG (Fig. 4). All isolates of AG 4 and AG 5 from this study were recovered from wheat. In contrast, all isolates recovered from canola belonged to AG 2-1, with four exceptions. A single isolate grouped with AG 9, while three other isolates clustered with the unclassified AG group. There was no geographical clustering of any of the AG groups across the three provinces. Representatives from six clades were found in Alberta and Manitoba (Fig. 5). No isolates from AG 4 or AG 5 clades were recovered in Saskatchewan, but the only isolate of AG 11 was found there (Fig. 5).

Fig. 2. Most parsimonious haplotype network for 7 haplotypes of *Rhizoctonia solani* AG-5 recovered from wheat in Alberta and Manitoba, Canada, derived from the internal transcribed spacer (ITS) gene region. Sizes of haplotype circles indicate frequencies of the haplotypes. Each line represents a single nucleotide mutation, and small empty circles represent unsampled haplotypes.

Discussion

Molecular characterization of *R. solani* in the provinces of Alberta, Manitoba, and Saskatchewan showed the presence of a diverse assemblage of AG groups including AG 2-1, AG 4, AG 5, AG 9, AG 11, and one isolate of *Ceratobasidium* (Fig. 1). The dominant group was AG 2-1, and this was likely due to the fact that nearly half of the isolates used in this study were recovered from canola. The high frequency of AG 2-1 associated with canola is consistent with previous reports from this crop (11,12,15,39). In contrast to canola, wheat harbored a diverse group of isolates belonging to six AG groups and one isolate of *Ceratobasidium* (Fig. 2). Of these groups, AG 2-1, AG 4, and AG 5 were the most frequently recovered. Both AG 4 and AG 5 were shown to consistently cause disease on wheat (17). However, while AG 2-1 was consistently isolated from wheat roots, the isolates of this AG do not typically cause disease on wheat (17). Wheat appears to be harboring isolates of AG 2-1, allowing inoculum levels to remain high enough to cause disease on successive canola crops. This represents the first documentation of the diversity of *R. solani* recovered from wheat in Canada, which is unexpected given the importance of the crop to central Canada and previous reports of *R. solani* on canola in the same region. Interestingly, *R. solani* AG 8 was not recovered in our study area, which is the most common AG on wheat grown in the Pacific Northwest of the United States (18) and in Australia (16). However, in a more recent survey of *Rhizoctonia* spp. from wheat soils across 14 states in the United States, AG 2-1 was the most frequently recovered strain in addition to AG 3, AG 4, AG 10, and AG 11 (30). These results are consistent with this study with the exception that AG 3 and AG 10 were not found in our study sites, and AG 5 and AG 9 were not recovered in the United States. However, AG 5 as well as AG 2-1 and AG 4

Fig. 3. Maximum likelihood tree of the internal transcribed spacer (ITS) sequence of 15 haplotypes of the *Rhizoctonia solani* AG2-1 complex and 2 haplotypes AG-9, recovered from canola, pea, and wheat from Alberta, Manitoba, and Saskatchewan. Isolates imported from GenBank include accession number, crop, and location the isolate was recovered from. Posterior probabilities >70% are displayed at the nodes.

were reported from wheat in Turkey (4). In a recent survey conducted across several European countries, *Rhizoctonia* species were baited from agricultural soils and AG 9 and AG 4 were the most abundant AGs found, but a number of other AGs were also reported, including AG 1, AG 2-1, AG 3, AG 5, AG 8, and AG 11 (7). However, as these isolates were baited from the soil, it only provides information on which AGs are most abundant and not which AGs are pathogenic on specific crops that may be planted in that soil. While this is the only other recent survey to report the presence of AG 9 in wheat-producing areas, none of the isolates were recovered from wheat plants, and therefore it is difficult to determine the importance of this AG as a potential pathogen of wheat.

In the study by Melzer et al. (17), isolates of *R. solani* recovered from western Canada were tested for pathogenicity on canola, lentil, pea, soybean, and wheat in growth room studies. All of the

isolates in AG 2-1 were pathogenic on canola, but only 37, 21, and 5% of the 94 isolates tested were pathogenic on lentil, pea, and wheat, respectively. There were eight isolates identified as AG 2-1 that were pathogenic on canola, lentil, and pea, and seven of these isolates clustered together to form the second AG 2-1 clade (Fig. 1), providing further support for a distinct subgroup in AG 2-1 that may be based on host range. Isolates of unknown AG in the clade with known isolates of AG 9 were not pathogenic on hosts tested or had low virulence (17). Isolates of AG 4 were pathogenic on all hosts tested, and isolates of AG 5 were pathogenic on wheat, lentil, and canola. AG 5 was not as virulent on these hosts as isolates from AG 4.

While AG tests were consistent with ITS sequence most of the time, approximately 10% of isolates were incorrectly classified or failed to undergo anastomosis with tester strains used in this study, and the ITS gene proved effective at rapidly classifying these isolates. This provides further evidence of the utility of the ITS gene in identifying a greater number of isolates, including rare AGs, in large surveys. This also supports several other previous studies that have demonstrated that grouping of *Rhizoctonia* isolates by molecular analysis based on the ITS sequences supports the AG grouping of *Rhizoctonia* isolates based on classical hyphal anastomosis reactions (19,23,33,34).

While ITS remains the preferred method for rapid assignment of *R. solani* isolates to their respective AGs, there remains a number of drawbacks to using this region. As *R. solani* is multinucleate, there is the potential for a strain to possess multiple ITS sequences that vary by one or more nucleotides (26,27). This generally results in hard-to-interpret chromatograms. The authors have observed this complication in the case of isolates of *R. solani* recovered from soybean and dry bean that belong to AG 2-2. Preliminary data indicate that many strains have at least four independent ITS sequences. This study also provides evidence that there are likely a number of distinct lineages or species that make up the *R. solani* complex, with strong posterior probability for separation of lineages. However, further study with a greater number of nuclear and mitochondrial genes is needed.

Finally, a previously undocumented AG group was identified, and the AG 5 tester strain was found to form hyphal fusions with several unrelated groups of multinucleate and binucleate *Rhizoctonia* spp., proving there are still a number of questions regarding species boundaries and potential for genetic exchange through hyphal fusion in *R. solani*.

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Fig. 4. Maximum likelihood tree from the analysis of the internal transcribed spacer (ITS) sequences of 55 isolates of *Rhizoctonia solani* recovered from wheat (w) from Alberta (AB), Manitoba (MB), and Saskatchewan (SK). Isolate name consists of isolate number, AG group from anastomosis test, province (AB, MB, or SK), and crop (W). Posterior probability >70% included at nodes.

Fig. 5. Distribution of *Rhizoctonia solani* isolates belonging to seven anastomosis groups present in Alberta, Manitoba, and Saskatchewan, Canada. Isolates identified as AG (2-1) represent those strains which successfully anastomosed with tester strain AG 2-1, but formed a distinct group in the phylogenetic analysis.

Literature Cited

- Boysen, M., Borja, M., delMoral, C., Salazar, O., and Rubio, V. 1996. Identification at strain level of *Rhizoctonia solani* AG4 isolates by direct sequence of asymmetric PCR products of the ITS regions. *Curr. Genet.* 29:174-181.
- Carling, D. E., Kuninaga, S., and Brainard, K. A. 2002. Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of *Rhizoctonia solani* anastomosis group-2 (AG-2) and AG-BI. *Phytopathology* 92:43-50.
- Clement, M., Posada, D., and Crandall, K. A. 2000. TCS: A computer program to estimate gene genealogies. *Mol. Ecol.* 9:1657-2659.
- Demirci, E. 1998. *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. *Plant Pathol.* 47:10-15.
- Felsenstein, J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* 6:227-242.
- Godoy-Lutz, G., Kuninaga, S., Steadman, J. R., and Powers, K. 2008. Phylogenetic analysis of *Rhizoctonia solani* subgroups associated with web blight symptoms on common bean based on ITS-5.8S rDNA. *J. Gen. Plant Pathol.* 74:32-40.
- Goll, M. B., Schade-Schuetze, A., Swart, G., Oostendorp, M., Schott, J. J., Jaser, B., and Felsenstein, F. G. 2014. Survey of the prevalence of *Rhizoctonia* spp. in European soils and determination of the baseline sensitivity towards sedaxane. *Plant Pathol.* 63:148-154.
- Gonzalez, D., Carling, D. E., Kuninaga, S., Vilgalys, R., and Cubeta, M. A. 2001. Ribosomal DNA systematics of *Ceratobasidium* and *Thanatephorus* with *Rhizoctonia* anamorphs. *Mycologia* 93:1138-1150.
- Gugel, R. K., Yitbarek, S. M., Verma, P. R., Morrall, R. A. A., and Sadasivaiah, R. S. 1987. Etiology of the *Rhizoctonia* root rot complex of canola in the peace river region of Alberta. *Can. J. Plant Pathol.* 9:119-128.
- Huelsbeck, J. P., and Ronquist, F. 2001. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19:1572-1574.
- Hwang, S. F., Swanson, T. A., and Evans, I. R. 1986. Characterization of *Rhizoctonia solani* isolates from canola in west central Alberta. *Plant Dis.* 70:681-683.
- Khangura, R. K., Barbetti, M. J., and Sweetingham, M. W. 1999. Characterization and pathogenicity of *Rhizoctonia* species on canola. *Plant Dis.* 83:714-721.
- Kumar, S., Tamura, K., and Nei, M. 2004. MEGA3: Integrated software for molecular evolutionary analysis and sequence alignment. *Briefings Bioinform.* 5:150-163.
- Kuninaga, S., Natsuaki, T., Takeuchi, T., and Yokosawa, R. 1997. Sequence variation of the rDNA ITS regions within and between anastomosis groups in *Rhizoctonia solani*. *Curr. Genet.* 32:237-243.
- Lamprecht, S. C., Tewoldemedhin, Y. T., Hardy, M., Calitz, F. J., and Mazzola, M. 2011. Effect of cropping system on composition of the *Rhizoctonia* populations recovered from canola and lupin in a winter rainfall region of South Africa. *Eur. J. Plant Pathol.* 131:305-316.
- MacNish, G. C., and Neate, S. M. 1996. *Rhizoctonia* bare patch of cereals - An Australian perspective. *Plant Dis.* 80:965-971.
- Melzer, M. S., Dickson, A., Labun, T., and Boland, G. J. Characterization and pathogenicity of *Rhizoctonia* spp. from field crops in Canada. *Can. J. Plant Pathol.* In press.
- Ogoshi, A., Cook, R. J., and Bassett, E. N. 1990. *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. *Phytopathology* 80:784-788.
- Ohkura, M., Abawi, G. S., Smart, C. D., and Hodge, K. T. 2009. Diversity and aggressiveness of *Rhizoctonia solani* and *Rhizoctonia*-like fungi on vegetables in New York. *Plant Dis.* 93:615-624.
- Paulitz, T. C., Okubara, P. A., and Schillinger, W. F. 2006. First report of damping-off of canola caused by *Rhizoctonia solani* AG 2-1 in Washington State. *Plant Dis.* 90:829.
- Pope, E. J., and Carter, D. A. 2001. Phylogenetic placement and host specificity of mycorrhizal isolates belonging to AG-6 and AG-12 in the *Rhizoctonia solani* species complex. *Mycologia* 93:712-719.
- Price, E. W., and Carbone, I. 2005. SNAP: Workbench management tool for evolutionary population genetic analysis. *Bioinformatics* 21:402-404.
- Rinehart, T. A., Copes, W. E., Toda, T., and Cubeta, M. A. 2007. Genetic characterization of binucleate *Rhizoctonia* species causing web blight on azalea in Mississippi and Alabama. *Plant Dis.* 91:616-623.
- Rozas, J., Sanchez-DelBarrio, J. C., Messenguer, X., and Rozas, R. 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics* 19:2496-2497.
- Rush, C. M., Carling, D. E., Harverson, R. M., and Mathieson, J. T. 1994. Prevalence and pathogenicity of anastomosis groups of *Rhizoctonia solani* from wheat and sugarbeet in Texas. *Plant Dis.* 78:349-352.
- Salazar, O., Julian, M. C., Hyakumachi, M., and Rubio, V. 2000. Phylogenetic grouping of cultural types of *Rhizoctonia solani* AG 2-2 based on ribosomal ITS sequences. *Mycologia* 92:505-509.
- Salazar, O., Schneider, J. H. M., Julian, M. C., Keijer, J., and Rubio, V. 1999. Phylogenetic subgrouping of *Rhizoctonia solani* AG 2 isolates based on ribosomal ITS sequences. *Mycologia* 91:459-467.
- Schroeder, K. L., and Paulitz, T. C. 2012. First report of root rot caused by *Rhizoctonia solani* AG-10 on canola in Washington state. *Plant Dis.* 96:584.
- Schroeder, K. L., and Paulitz, T. C. 2012. First report of a *Ceratobasidium* sp. causing root rot on canola in Washington state. *Plant Dis.* 96:591.
- Schroeder, K. L., Shetty, K. K., and Paulitz, T. C. 2011. Survey of *Rhizoctonia* spp. from wheat soils in the US and determination of pathogenicity on wheat and barley. (Abstr.) *Phytopathology* 101:S161.
- Sharon, M., Kuninaga, S., Hyakumachi, M., and Sneh, B. 2006. The advancing identification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. *Mycoscience* 47:299-316.
- Sharon, M., Freeman, S., Kuninaga, S., and Sneh, B. 2007. Genetic diversity, anastomosis groups and virulence of *Rhizoctonia* spp. from strawberry. *Eur. J. Plant Pathol.* 117:247-265.
- Sharon, M., Kuninaga, S., Hyakumachi, M., Naito, S., and Sneh, B. 2008. Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. *Mycoscience* 49:93-114.
- Stodart, B. J., Harvey, P. R., Neate, S. M., Melanson, D. L., and Scott, E. S. 2007. Genetic variation and pathogenicity of anastomosis group 2 isolates of *Rhizoctonia solani* in Australia. *Mycol. Res.* 111:891-900.
- Tewoldemedhin, Y. T., Lamprecht, S. C., McLeod, A., and Mazzola, M. 2006. Characterization of *Rhizoctonia* spp. recovered from crop plants used in rotational cropping systems in the Western Cape Province of South Africa. *Plant Dis.* 90:1399-1406.
- White, T. J., Bruns, T., Lee, S., and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: *PCR Protocols: A Guide to Methods and Applications*. M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, eds. Academic Press, San Diego, CA.
- Yang, J., and Verma, P. R. 1992. Screening genotypes for resistance to preemergence damping off and post emergence seedling root rot of oilseed rape and canola caused by *Rhizoctonia solani* AG2-1. *Crop Prot.* 11:443-448.
- Yitbarek, S. M., Verma, P. R., and Morrall, R. A. A. 1987. Anastomosis groups, pathogenicity, and specificity of *Rhizoctonia solani* isolates from seedling and adult rapeseed canola plant and soils in Saskatchewan. *Can. J. Plant Pathol.* 9:6-13.
- Zhou, Q.-X., Chang, K.-F., Hwang, S.-F., Strelkov, S. E., Gossen, B. D., and Chen, Y.-Y. 2009. Pathogenicity and genetic diversity of *Rhizoctonia solani* isolates from lupin and other crops in Alberta, Canada. *Can. J. Plant Pathol.* 31:340-347.