Characterization and Pathogenicity of *Rhizoctonia solani* Isolates Affecting *Pisum sativum* in North Dakota

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Abstract

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Acreage of dry field pea (*Pisum sativum*) in North Dakota has increased approximately eightfold from the late 1990s to the late 2000s to over 200,000 ha annually. A coincidental increase in losses to root rots has also been observed. Root rot in dry field pea is commonly caused by a complex of pathogens which included *Fusarium* spp. and *Rhizoctonia solani*. *R. solani* isolates were obtained from roots sampled at the three- to five-node growth stage from North Dakota pea fields and from symptomatic samples received at the Plant Diagnostic Lab at North Dakota State University in 2008 and 2009. Using Bayesian inference and maximum likelihood analysis of the internal tran-

scribed spacer (ITS) region of the ribosomal DNA (rDNA), 17 *R. solani* pea isolates were determined to belong to anastomosis group (AG)-4 homogenous group (HG)-II and two isolates to AG-5. Pathogenicity of select pea isolates was determined on field pea and two rotation hosts, soybean and dry bean. All isolates caused disease on all hosts; however, the median disease ratings were higher on green pea, dry bean, and soybean cultivars when inoculated with pea isolate AG-4 HG-II. Identification of *R. solani* AGs and subgroups on field pea and determination of relative pathogenicity on rotational hosts is important for effective resistance breeding and appropriate rotation strategies.

Dry field pea (*Pisum sativum* L.) acreage in North Dakota increased from approximately 27,000 ha in 1999 to over 210,000 ha annually between 2005 and 2009 (United States Department of Agriculture National Agriculture Statistics Service [USDA-NASS]). Acreage is heavily concentrated in the northwest part of the state, with approximately 75% of planted area in eight northwestern counties. Incorporation of dry field pea in North Dakota crop production systems is likely due to a good rotational fit and competitive prices (20), and there is no indication that acreage will decrease in the near future. The increase in dry field pea acreage has coincided with an increase in losses to root rots; this is currently viewed as the most important disease issue facing pulse production in North Dakota.

Root rot in pea is frequently attributed to multiple pathogens comprise a root rot complex (RRC) (18,19), including *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk) and multiple *Fusarium* spp. (26). *R. solani* is thought to be less important than *Fusarium* spp. (18) in the pea RRC but economic losses in the Prairie Provinces of Canada from *R. solani* have been documented (17). In North Dakota, *R. solani* was recovered infrequently as part of an RRC disease survey conducted by Gregoire and Bradley (14) and was reported to be the primary etiological agent reducing stand loss in one pea trial (24). Additionally, *R. solani* can cause yield loss on crops such as soybean (*Glycine max* (L.) Merr.) and dry bean (*Phaseolus vulgaris* L.) (26), which are frequently rotated with pea in North Dakota. Under favorable environments, *R. solani* can cause seed and seedling rot, damping-off, hypocotyl rot, and root rot on numerous hosts worldwide (11), resulting in poor stand, plant stunting and yellowing, delayed plant development, and reduced yield (22,48).

Anastomosis groups (AGs) of *R. solani* are based on the characterization of hyphal interactions of the isolates via somatic incom-

patibility and, to date, 14 AGs (AG-1 to AG-13 and AG-BI) have been described, 8 of which contain subgroups (9,10,21,34,36). Recently, the internal transcribed spacer (ITS) region of the ribosomal DNA (rDNA) has been used to characterize AGs and subgroups of *Rhizoctonia* spp. and relate AG to pathogenicity (7,13,27,33). AG-4 is one of the most widely recognized *R. solani* in the north-central United States and has a broad host range (2). Three homogeneous groups (HGs), HG-I, HG-II, and HG-III, are proposed for AG-4 based on fatty acid methyl ester analysis and morphological features (35). AG-4 was reported to be most prevalent on field pea and lentil in the Canadian Prairie Provinces neighboring North Dakota (17). In North Dakota, the frequency and pathogenicity of AGs isolated from pea has not been investigated. Characterization of *R. solani* AGs and subgroups is necessary for effective breeding and rotational recommendations (43), particularly as pea acreage continues to progress into cropping systems where soybean, dry edible bean, and other crops susceptible to *R. solani* are grown. The objectives of this research were to (i) identify the AGs and subgroups of *R. solani* isolates obtained from symptomatic samples in North Dakota using the ITS sequence and hyphal interaction and (ii) assess the pathogenicity of selected *R. solani* isolates on field pea, soybean, and dry bean.

Materials and Methods

Survey and *R. solani* isolation. A survey of North Dakota dry pea fields was conducted in July 2008 and 2009 to determine the impact of the RRC in North Dakota pea-growing counties (23). In each field, 10 plants were uprooted at each of five equally spaced sites along the arms of a W pattern. Symptomatic plants with red to brown to black discoloration of the roots or stem base, yellowing of the basal foliage, and stunted growth were saved for cultures. Symptomatic roots were washed in tap water and surface sterilized, rinsed in sterile distilled water four times, and blotted dry between sterile filter papers. Five pieces, approximately 1 cm long, of each root were placed on 1% water agar (WA) amended with 0.02% streptomycin sulphate. Plates were incubated at 25°C under continuous dark conditions for 7 days. *R. solani* colonies were subcultured and each isolate was purified by making a hyphal tip transfer to potato-dextrose agar (PDA; Difco Laboratories, Detroit). *R. solani* isolates were identified morphologically by examining the hyphal branching after 3 days of growth on WA followed by stain-

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ing with aniline blue. *R. solani* cultures were also derived from samples received at the Plant Diagnostic Lab at North Dakota State University.

Suspected *Rhizoctonia* isolates were grown on PDA for 7 days at 25°C for molecular identification. DNA was extracted from the lyophilized mycelium of the individual isolates and resuspended in 50 µl of rehydration solution (1% Tris-EDTA buffer) using a Wizard Genomic DNA Purification Kit (Promega Corp., Madison, WI). A single fragment approximately 650 to 700 bp in length was amplified using polymerase chain reaction (PCR) from the rDNA-ITS region consisting of ITS1, 5.8S, and ITS2 with primers ITS4 and ITS5 (45). Reactions for PCR amplifications in a 25-µl mixture contained template DNA at 20 to 30 ng/reaction, 10 µM each primer, 10 mM each dNTP, Taq DNA Polymerase (Qiagen, Valencia, CA) at 5 units/µl, and 10× Qiagen PCR Buffer containing 15 mM MgCl₂ (Qiagen). Cycle parameters were an initial denaturation at 94°C for 3 min; followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min; and a final extension at 72°C for 10 min. A 5-µl aliquot of each PCR product was run electrophoretically on a 1% agarose gel to confirm amplification. All DNA samples were sequenced (McLab, South San Francisco, CA) using the ITS4 and ITS5 primers. Sequences were trimmed at the ends for quality and ITS sequence analysis was performed using BLASTN via the National Center for Biotechnology Information (NCBI) database (www.ncbi.nlm.nih.gov) to confirm *R. solani* prior to the phylogenetic analyses.

Phylogenetic analysis and AG determination. To identify AGs and subgroups by phylogenetic analyses, the dataset used by Ohkura et al. (27), consisting of 81 rDNA-ITS sequences, was obtained from the NCBI database and used for comparison. Sequences of *R. solani* isolates from North Dakota and reference sequences were aligned using ClustalX (41) and its default parameters. Sequence alignment was adjusted manually by visual examination using the Molecular Evolutionary Genetics Analysis (MEGA version 4) software (39). For phylogenetic analyses, the general time reversible with a γ -shaped parameter (GTR+G) model (40) was chosen as the appropriate evolutionary model by Modeltest 3.7 (29) using the Akaike Information Criterion (1). These models were implemented in the maximum likelihood (ML) analysis (38) in PAUP* version 4.0b10 (37) for tree construction.

The ML tree was bootstrapped 1,000 times using the GTR+G model on PAUP and a heuristic search was performed in the ML analyses to find the best tree using random stepwise addition, the tree bisection–reconnection (TBR), and the branch-swapping algorithm. The concatenated sequences were also used for Bayesian inference (BI) analysis with MrBayes 3.1.2 (30). The GTR+G model was implemented in MrBayes so that BI posterior probabilities could be directly compared with bootstrap values in ML from PAUP. For BI, the following parameters for likelihood analysis (number of substitution types = 6; among-site rate variation = γ ; assumed nucleotide frequencies using maximum-likelihood estimates [A, C, G, T] = 0.29, 0.18, 0.19, 0.34; proportion of invariable sites = 0; and γ distribution parameter = 0.38) were entered into MrBayes. Markov chain Monte Carlo (MCMC) length from a random starting tree was initiated in the BI and run for 10⁶ generations with trees sampled every 100 generations; the first 319 trees were discarded as burn-in by plotting likelihood scores against generations. Posterior probabilities (PPs) for each node were estimated from the resulting 50% majority-rule consensus tree and nodes with 95% or greater PPs were considered significant (46). Each Bayesian MCMC analysis was run at least twice to confirm the consistency of the results. Both ML and BI analyses included 958 characters and all trees were generated using *Athelia* (anamorph = *Sclerotium*) *rolfsii* (Curzi) C.C. Tu & Kimbr. as the outgroup.

Six isolates of AG-4 and one isolate of AG-5 (Table 1) were randomly selected to confirm AGs by an anastomosis test with tester strains using the clean-slide technique (28). A 5-mm-diameter disk from the edge of a 3-day-old colony of a *R. solani* isolate was

placed on 2% WA in a 9-cm-diameter glass petri dish and a mycelial disk from similarly grown AG tester isolate was placed in the petri dish at a distance of 2 cm from the first disk, and incubated at 22°C for 24 to 48 h. The overlapping hyphae were stained with 0.5% safranin O in distilled water and 3% KOH following Bandoni (3). Hyphal branches of the stained area were microscopically observed for the presence of fusion at a minimum of three points with the tester isolates. All pairings were tested at least twice.

Pathogenicity evaluation. Pathogenicity of the six randomly selected isolates of AG-4 and one isolate of AG-5 was determined in a greenhouse evaluation on commonly grown North Dakota cultivars of yellow pea ('Admiral'), green pea ('Striker'), soybean ('Barnes'), and dry bean ('Montcalm'). A potted-plant assay using a modified inoculum-layer technique (4) was used to assess the ability of these isolates to cause root and hypocotyl rot. The inoculum was prepared by growing each isolate on autoclaved wheat seed for a week at 20 to 22°C under dark conditions. Two pre-germinated, nontreated seeds of each cultivar were planted in 3.5-by-3.5-in. pots containing two-thirds pasteurized potting mix (60°C for 30 min). The seed were covered with a thin layer of soil, followed by 10 g of *R. solani* (either AG-4 or AG-5) inoculum and 10 g of soil. Pots were watered to saturation after planting in the greenhouse and lightly watered every alternate day. Each individual pot represented a replication and each experiment consisted of five replications per isolate. Pathogenicity tests were repeated at least twice. Noninoculated plants and those inoculated with wheat grains colonized with an AG-4 and AG-5 tester isolate served as negative and positive controls, respectively, for all the hosts. Fourteen days after planting, plants were removed from the soil and the roots were washed. Seedlings were evaluated for root rot on a scale from 1 to 9, where 1 = no lesions; 3 = discrete, light- or dark-brown, superficial necrotic lesions; 5 = adventitious root or taproot necrosis and decay; 7 = extensive root rot; and 9 = plant dead (25). After harvest, pieces of symptomatic and healthy tissue (negative control) from different hosts were placed on PDA to recover *R. solani* used in the inoculation and the identity of *R. solani* was confirmed by morphology.

Data from pathogenicity tests on field pea were analyzed using SAS software (version 9.2; SAS Institute Inc., Cary, NC). The ordinal data did not have a normal distribution based on a Shapiro-Wilk 'W' test (32); therefore, they were analyzed using the non-parametric methodology of Brunner et al. (8) as described by Shah and Madden (31). One-way analyses was performed to separately

Table 1. Origin, GenBank accession number, and anastomosis group (AG) of *Rhizoctonia solani* isolates derived from field pea (*Pisum sativum*) in North Dakota characterized in this study

Isolate	GenBank accession number	County	Year of collection	AG ^a
ND01	HQ629858	Bottineau	2009	AG-4
ND02	HQ629859	Bottineau	2009	AG-4
ND03	HQ629860	Bottineau	2009	AG-4
ND04	HQ629861	Bottineau	2009	AG-4
ND05	HQ629862	Bottineau	2009	AG-4
ND06	HQ629863	Bottineau	2009	AG-5
ND07	HQ629864	Cass	2008	AG-4
ND08	HQ629865	Cass	2008	AG-4
ND09	HQ629866	Foster	2009	AG-4
ND10	HQ629867	Foster	2009	AG-4
ND11	HQ629868	McKenzie ^b	2009	AG-4
ND12	HQ629869	McLean	2009	AG-4
ND13	HQ629870	Rolette ^b	2009	AG-4
ND14	HQ629871	Ward	2009	AG-4
ND15	HQ629872	Ward	2009	AG-4
ND16	HQ629873	Ward	2008	AG-4
ND17	HQ629874	Ward ^b	2007	AG-5
ND18	HQ629875	Ward	2008	AG-4
ND19	HQ629876	Ward	2008	AG-4

^a Identified by phylogenetic analysis.

^b Received by the Plant Diagnostic Lab, North Dakota State University; all others obtained from 2008–2009 field disease survey.

assess disease severity as influenced by individual AG-4 isolates on field pea for the first analysis and by AGs on different hosts for the second analysis. PROC RANK was used to obtain mid-ranks followed by PROC MIXED to calculate test statistics and significance levels. Confidence intervals were calculated using the LD_CI macro (31).

Environmental data. Environmental data were obtained from one North Dakota Agricultural Weather Network weather station in each county surveyed (Tables 2 and 3). Mean monthly air temperature and total precipitation for 2008, 2009, and the 30-year average were obtained for April to August, approximately the growing season for field pea in North Dakota.

Results

Survey and *R. solani* isolation. In all, 77 fields in 10 counties were surveyed in 2008 and 38 fields in 7 counties were surveyed in 2009 (Table 4). Symptoms of root rots were observed in every county but variation of incidence among counties was high and incidence of plants with visible root rot symptoms ranged from less than 10% in McClean and Cass Counties in 2008 to 66% of the plants in Divide County in 2009. In general, root rot symptoms were more frequently observed in 2009.

Cultures of *R. solani* were recovered from infected roots in 21 North Dakota pea fields over the course of the study (Table 4).

Table 2. Total and normal rainfall accumulation for each county and year survey was conducted

Year, county	Rainfall (mm)									
	April		May		June		July		August	
	Total	Avg ^a	Total	Avg ^a	Total	Avg ^a	Total	Avg ^a	Total	Avg ^a
2008										
Bottineau	4.3	33.8	15.2	52.6	106.4	84.3	43.7	68.8	117.1	50.8
Foster	12.4	36.6	29.6	63.2	127.3	96.3	47.1	79	38.9	63
Cass	58.4	34.8	55.6	66.3	162	89.2	42.4	73.2	114.9	64
Hettinger	20.1	46.5	47.2	65.8	65.8	80.5	72.1	65.1	32	42.9
McClean	23.5	36.6	19.1	55.6	121.4	84.3	41	67.8	45	49.8
Mountrail	7.9	35.1	59.9	56.6	78.5	82	44.7	65.8	64.5	43.9
Renville	5.6	31.5	62.7	55.1	106.7	75.7	22.4	72.6	65.8	55.1
Sheridan	45	19.8	17.2	50	85	71.1	50.8	58.2	172.8	58.2
Ward	11.6	39.6	68	57.9	136.4	76.5	60.1	64	80	51.1
Williams	5.1	28.7	35.1	53.1	54.1	69.1	19.8	62.2	33	41.4
	19.39	34.3	40.96	57.62	104.36	80.9	44.41	67.67	76.4	52.02
	...	-14.9	...	-16.7	...	23.5	...	-23.3	...	24.4
2009										
Bottineau	35.6	33.8	41.1	52.6	30.5	84.3	77.5	68.8	29.2	50.8
Divide	41.9	25.4	6.4	51.8	25.7	65	57.4	71.9	76.5	39.6
Foster	42.6	36.6	34.4	63.2	41	96.3	38.1	79	50.5	63
McLean	17.5	36.6	80.2	55.6	62.5	84.3	68.3	67.8	32.1	49.8
Mountrail	20.6	35.1	75.2	56.6	78.2	82	62.7	65.8	24.5	43.9
Ward	32.3	39.6	38.1	57.9	47.7	76.5	42.3	64	37.1	51.1
Williams	39.4	28.7	15.5	53.1	37.8	69.1	64.8	62.2	70.4	41.4
	32.8	33.7	41.6	55.8	46.2	79.6	58.7	68.5	45.8	48.5
	...	-0.8	...	-14.3	...	-33.4	...	-9.8	...	-2.8

^a Avg = 30-year average.

Table 3. Mean and average temperature for each county and year survey was conducted

Year, county	Mean temperature (°C)									
	April		May		June		July		August	
	Total	Avg ^a	Total	Avg ^a	Total	Avg ^a	Total	Avg ^a	Total	Avg ^a
2008										
Bottineau	3	5	10	12	15	17	19	19	20	19
Foster	4	6	10	14	16	18	20	21	20	20
Cass	5	6	12	14	18	19	22	21	21	21
Hettinger	4	6	11	12	15	18	21	21	21	20
McClean	5	5	11	12	16	17	21	20	21	20
Mountrail	4	5	11	12	15	17	20	20	20	20
Renville	4	5	10	12	15	17	19	19	19	19
Sheridan	4	6	10	13	16	18	20	21	20	20
Ward	5	5	11	13	16	18	20	20	21	19
Williams	6	7	12	13	17	18	22	21	21	21
	4.4	5.6	10.8	12.7	15.9	17.7	20.4	20.3	20.4	19.9
	...	-1.2	...	-1.9	...	-1.8	...	0.1	...	0.5
2009										
Bottineau	4	5	10	12	15	17	17	19	17	19
Divide	4	5	10	12	15	17	17	19	17	18
Foster	4	6	11	14	16	18	18	21	18	20
McLean	4	5	11	12	16	17	18	20	18	20
Mountrail	4	5	11	12	15	17	17	20	17	20
Ward	4	5	11	13	16	18	18	20	18	19
Williams	6	7	12	13	17	18	19	21	19	21
	4.3	5.4	10.9	12.6	15.7	17.4	17.7	20.0	17.7	19.6
	...	-1.1	...	-1.7	...	-1.7	...	-2.3	...	-1.9

^a Avg = 30-year average.

However, *R. solani* was infrequently recovered even in locations where it was identified, and was not found to be more than 5% of total fungi recovered in any county. Isolates of the genera *Fusarium*, *Ascochyta*, *Diaporthe*, and *Pythium* accounted for the vast majority of fungal recovery from roots (*data not presented*). Severity of root rot symptoms in fields where *R. solani* was recovered was similar to severity in fields where *R. solani* was not recovered (Table 4).

Phylogenetic analysis and AG determination. *R. solani* samples from multiple fields were lost during storage but three additional cultures were obtained from symptomatic samples received at the North Dakota State University Plant Diagnostic Lab in Fargo and characterized. In sum, 19 isolates from nine distinct locations were characterized for AG determination (Table 1). ML and BI trees enabled characterization of isolates at the AG level and subgroup level for AG-4. Based on 95% PP, the BI analysis inferred the identification of 19 isolates, of which 17 were AG-4 (PP 98%) and 2 were AG-5 (PP 97%) (Fig. 1). Using the same model, the ML tree supported the BI analysis and inferred 17 isolates to AG-4 HG-II (BS 99.9%) and 2 isolates to AG-5 (BS 99.3%) (Fig. 1). AG and AG subgroup of the same *R. solani* was confirmed by anastomosis tests with the tester strains.

Pathogenicity evaluation. All six AG-4 HG-II and one AG-5 *R. solani* isolates were pathogenic on the yellow pea Admiral; however, the disease rating of isolate ND12 was lower than several other isolates (Table 5), including the AG-4 tester and AG-5 isolates. Leaf yellowing from AG-4 HG-II isolates was earlier than AG-5 and noticeable at the base of the plants 10 days after inoculation; however, the degree of wilting was not measured.

When the disease rating of other hosts were assessed to AG-4 HG-II (isolate ND7) and AG-5 (isolate ND17) isolates, infection occurring on all hosts was statistically different than the control (Table 6). No differences in disease rating between AG-4 and AG-5 isolates were found on yellow pea (Admiral) but disease ratings on green pea (Striker), soybean (Barnes), and dry bean (Montcalm) were higher when infected with AG-4.

Discussion

In this study, 17 *R. solani* isolates recovered from dry field pea roots in this study were identified to be AG-4 HG-II and 2 isolates were determined to be AG-5 (Fig. 1; Table 4). To the best of our knowledge, this is the first time that *R. solani* AG-5 has been found on field pea and the subgroup of AG-4 isolates from pea determined in the northern Great Plains. Representative isolates from

each AG were found to be pathogenic on multiple hosts rotated with field pea in North Dakota (Table 6), suggesting that both *R. solani* AGs may be an important part of the RRC in pea and other rotational crops. If pea acreage continues to increase and overlap with soybean or other hosts of *R. solani*, root rot damage from *R. solani* could be exacerbated in the future and improvement or better utilization of management strategies may be needed.

R. solani was recovered from only approximately 18% of fields surveyed in 2008 and 2009 and, even in locations where it was found, it was recovered less frequently than other pathogens (Table 4), particularly *Fusarium* spp. (*data not presented*). This is in agreement with data from other areas of pea production in North America (5,16,42). However, temperature and moisture impact the development and impact of *R. solani* and, subsequently, the ability to find and isolate the pathogen. Hwang et al. (17) found that a temperature of 17.5°C or higher was optimal for development of the pathogen on pea seedlings. In this study, the mean monthly air temperatures were between -1.1 and -1.9°C below the 30-year average in April, May, and June 2008 and were at least -1.1°C below the 30-year average in each month from April to August 2009 (Table 3). Below-normal temperatures may have negatively impacted the development of *R. solani* throughout this study. However, the effect that soil moisture may have had on the *R. solani* in field pea is less clear. In this study, rainfall was below the 30-year average in April, May, and July 2008 and 2009 and also in June 2009 (Table 2). Bolton et al. (6) determined that root disease from *R. solani* on sugar beet was statistically highest at 75 and 100% moisture holding capacity (MHC) and lowest at 25%, while Van Bruggen et al. (44) reported highest infection levels in kidney bean at 20% MHC. Given environmental conditions throughout this survey, it is possible that the amount of recovery of *R. solani* may have been different if the years surveyed had different environmental conditions.

AG characterization for *R. solani* isolates was determined molecularly by ITS sequence data from the NCBI database. The ITS region is currently considered most appropriate in characterizing unknown isolates of *R. solani* and *Rhizoctonia*-like fungi to AGs (7,13,27,33). In addition to ITS sequence analysis, hyphal anastomosis reactions and nuclear conditions have been studied to better understand *Rhizoctonia* spp. systematics, and segregation of these fungi was not well defined for AG-2 subgroups in both our Bayesian and ML analyses (*data not shown*). Our study found that BI is as robust as ML to identify isolates to AGs and HGs that were

Table 4. Percent plants with roots symptomatic of root rot infection and frequency of *Rhizoctonia solani* isolation in North Dakota by county and year

Year, county	Number of fields ^a	Total		Fields with <i>R. solani</i>	
		Symptomatic (%) ^b	Frequency isolated (%) ^c	<i>N</i>	Symptomatic (%) ^b
2008					
Bottineau	7	38.57	1.19	1	10
Foster	1	40.00	0.00	0	0
Cass	3	6.67	2.38	2	20
Hettinger	2	55.00	0.00	0	0
McClean	15	7.33	4.76	4	10
Mountrail	2	40.00	0.00	0	0
Renville	7	17.14	2.38	2	35
Sheridan	2	15.00	0.00	0	0
Ward	25	26.00	3.57	4	37.5
Williams	13	29.23	1.19	1	50
2009					
Bottineau	1	60.00	3.30	1	60
Divide	3	66.67	0.00	0	0
Foster	1	50.00	1.10	1	50
McLean	11	61.82	0.55	1	50
Mountrail	4	42.50	0.00	0	0
Ward	10	52.00	1.10	2	45
Williams	8	57.50	1.10	2	35

^a Number of fields surveyed.

^b Percent plants with root rot symptoms.

^c Frequency of *R. solani* isolation = percentage of pathogens isolated.

AG-4 is reported to cause damping-off or seed rot on several crops grown in North Dakota including, soybean, dry bean, potato (*Solanum tuberosum* L.), and sugar beet (*Beta vulgaris* L.) (12,15,26,47). Although AG-4 subgroups on all crops have not been determined in North Dakota, *R. solani* AG-4 isolates from soybean were characterized as HG-II and HG-III and isolates from sugar beet characterized as HG-II by Stevens Johnk and Jones (35). Identification of exclusively AG-4 HG-II on field pea in North Dakota suggests that this subgroup within AG-4 may be important if field pea is rotated with soybean, dry edible bean, or sugar beet. AG-5 was not identified on field pea during a recent Canadian *Rhizoctonia* survey of 37 fields (17) but was identified in North Dakota. This could be due, in part, to a difference in number of

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counties that planted over 30,000 ha of pea in 2008 and 2009 (NASS).

In our study, only one representative AG-4 and AG-5 isolate was tested for pathogenicity on alternative hosts but variation in pathogenicity of *R. solani* isolates within and among AGs has been observed on other hosts (25,26). Similarly, variation in pathogenicity among AG-4 HG-II was observed in our study on the yellow pea Admiral, and variation in resistance to *R. solani* in pea cultivars is known (17). The determination of pea-derived *R. solani* AGs and their associated pathogenicity in this study is important for breeding for resistance and determining appropriate crop rotations for disease management; however, evaluation of the pathogenicity of additional *R. solani* isolates to multiple hosts and subsequent evaluations of pea varieties with the appropriate isolates may be prudent. This is particularly true in North Dakota, which now leads the United States in pea production and is one of the few (if not only) places in North America where pea production overlaps with that of dry bean, soybean, and sugar beet.

The recently observed increase in damage from root rots in North Dakota field pea suggests that improvement of awareness and utilization of management strategies for the RRC in field production systems is needed. Although a 4-year crop rotation is recommended to growers, limited information about the potential risk of specific crops used in that rotation is available. Similarly, susceptibility of specific varieties grown in North Dakota to *R. solani* is, at best, limited. The use of fungicidal seed treatments on pea in North Dakota increased from 14% (18,806 ha) of the

planted acreage in 2004 (49) to 35.3% (74,422 ha) of the planted acreage in 2008 (50). A statistical increase in yield with fungicide seed treatments has been demonstrated in *R. solani*-inoculated field trials in Alberta (17) and was observed in a noninoculated field trial in Carrington, ND in 2008, where *R. solani* was recovered from infected roots (*data not published*). However, it is possible that *Fusarium* spp. or other pathogens also played a role in both studies. Better understanding of susceptibility of rotational crops and pea varieties and increased use of fungicide seed treatments would likely reduce the impact of *R. solani* on field pea in North Dakota.

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Table 5. Median diseases rating and estimated relative treatment effects of *Rhizoctonia solani* anastomosis group (AG)-4 homogenous group (HG)-II isolates on yellow pea 'Admiral'

Isolate	Disease rating ^b	Estimated relative effect (<i>P</i>) ^a	CI (95%) for <i>p</i> ^a	
			Lower limit	Upper limit
Negative control	1.0	0.060	0.053	0.053
ND19	9.0	0.615	0.585	0.643
ND8	9.0	0.615	0.585	0.643
ND18	9.0	0.572	0.491	0.648
ND17	9.0	0.490	0.379	0.602
ND7	9.0	0.615	0.585	0.643
ND16	9.0	0.615	0.585	0.643
ND12	5.5	0.360	0.229	0.522
Tester, AG-4	9.0	0.615	0.585	0.643
Tester, AG-5	9.0	0.443	0.325	0.569

^a CI = confidence interval. Relative treatment effects were calculated by performing one-way analyses using the nonparametric method for ordinal data described by Shah and Madden (31).

^b Median disease rating. Disease severity ratings on all hosts were determined using a scale of 1 to 9 (25).

Table 6. Median and estimated relative treatment effects for the aggressiveness of *Rhizoctonia solani* anastomosis group (AG)-4 homogenous group (HG)-II and AG-5 isolated from field pea on 'Admiral' (yellow) and 'Striker' (green), soybean 'Barnes', and dry bean 'Montcalm' in a greenhouse assay

Isolate	AG	Test plant	Median disease rating ^b	Estimated relative effect (<i>P</i>) ^a	CI (95%) for <i>p</i> ^a	
					Lower limit	Upper limit
Negative control	...	Yellow pea, Admiral	1.0	0.167	0.167	0.167
ND17	AG-5	Yellow pea, Admiral	9.0	0.847	0.750	0.847
ND7	AG-4	Yellow pea, Admiral	9.0	0.826	0.720	0.826
Negative control	...	Green pea, Striker	1.0	0.167	0.167	0.167
ND17	AG-5	Green pea, Striker	4.0	0.654	0.526	0.654
ND7	AG-4	Green pea, Striker	9.0	0.848	0.811	0.848
Negative control	...	Dry bean, Montcalm	1.0	0.167	0.167	0.167
ND17	AG-5	Dry bean, Montcalm	4.0	0.610	0.439	0.519
ND7	AG-4	Dry bean, Montcalm	3.0	0.519	0.585	0.643
Negative control	...	Soybean, Barnes	1.0	0.167	0.167	0.167
ND17	AG-5	Soybean, Barnes	3.0	0.564	0.424	0.564
ND7	AG-4	Soybean, Barnes	9.0	0.835	0.768	0.835

^a CI = confidence interval. Relative treatment effects was calculated by performing nonparametric one-way analyses as described by Shah and Madden (31).

^b Disease severity ratings on all hosts were determined using a scale of 1 to 9 (25).

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