Anastomosis Group Identity and Virulence of *Rhizoctonia solani* Isolates Collected from Potato Plants in Alberta, Canada

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ABSTRACT

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Of 64 isolates of *Rhizoctonia solani* collected from potato plants grown in central and southern Alberta, 49 belonged to AG-3, and seven each to AG-4 and AG-5. One isolate did not anastomose with any of the tester cultures (AG-2-1, AG-3, AG-4, AG-5, and AG-9) used. In vitro radial growth rate of AG-3 isolates, as a group, was significantly slower than that of AG-4 and AG-5 isolates. Growth rates of AG-4 and AG-5 were similar. Members of AG-3, AG-4, and AG-5 were not restricted to any particular geographical region of the province, and occurred on infected plants growing in the same field. As a group, AG-3 isolates were significantly more virulent than AG-4 and AG-5 isolates, whereas virulence of AG-4 and AG-5 isolates was similar. The high incidence and virulence of AG-3 isolates indicate that AG-3 is the major cause of Rhizoctonia disease of potato in Alberta, whereas AG-4 and AG-5 are of minor importance.

Rhizoctonia disease of potato caused by *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk) is a serious disease of potato (*Solanum tuberosum* L.) in cooler regions of the world (8,14,21). *Rhizoctonia solani* comprises a collection of noninterbreeding populations that are recognized through the anastomosis group concept (2). Since virulence and host range of these groups differ, knowledge of anastomosis group affiliation of an isolate involved in a particular disease has become very useful (2,7,20). Although members of AG-3 are the major causal agents of Rhizoctonia disease of potato (6,9,11,22), members of other AGs (AG-1, AG-2-1, AG-4, AG-5, AG-9) have also been reported to cause this disease (1,3,6,9,13,15,16). This study was initiated to determine the anastomosis group of *R. solani* isolates recovered from potato plants grown in Alberta and to determine the cultural characteristics and virulence of these isolates on potato plants. Preliminary results of this study have been reported (4,5).

MATERIALS AND METHODS

Fungal isolation and identification. In the summer of 1988, *R. solani* isolates were obtained from infected potato plants grown in central and southern Alberta. Stem, stolon, and root pieces (2–3 cm long) with lesion margins were washed in running tap water for 1 hr. The pieces were surface sterilized for 5 min in 1% sodium hypochlorite and rinsed three times with sterile distilled water. Sterilized pieces were cut longitudinally and placed with their cut surfaces down on acidified potato-dextrose agar (PDA) medium. After 4 days of incubation at room temperature, *R. solani*–like colonies were transferred to 2% water agar. Hyphal tip sections from colonies developed on water agar medium were then grown on PDA. The isolates were identified as *R. solani* if they met the vegetative characteristics described for the species (20,23).

Cultural characteristics. To determine rates of radial growth of the isolates, a 5-mm-diameter mycelial disk from the edge of an actively growing colony was placed in the centre of a 9-cm-diameter petri plate containing PDA. Four replicate plates were used for each isolate. The colonies were measured after 24, 48, 72, and 96 hr of incubation in the dark at room temperature (22±2 C). Observations were also made for type of mycelium and development of zonation in the mycelium, and formation of sclerotia. The experiment was repeated once.

Anastomosis group identification. *Rhizoctonia solani* isolates were tested for their ability to anastomose with known AG tester cultures (AG-2-1, AG-3, AG-4, AG-5, and AG-9) using the clean slide technique of Kronland and Stanghellini (19). A 5-mm-diameter disk from the edge of a 3-day-old colony of an unknown isolate was transferred to a glass slide cleaned by dipping in 95% ethanol and wiped dry. A mycelial disk from a similarly grown AG tester culture was placed on the slide at a distance of 2 cm from the first disk. Two other slides were prepared on which the distance between mycelial disks was 2.5 and 3 cm. This was replicated three times for each unknown isolate. Since a large number of isolates were expected to belong to AG-3, initially, all isolates were paired against the AG-3 tester culture. The isolates that did not anastomose with the AG-3 tester culture were paired against the remaining tester cultures. Each pairing was repeated twice.

Pathogenicity determination. Pathogenicity of *R. solani* isolates was determined on potato (cv. Russet Burbank) plants. The test used was similar to that of Carling and Leiner (9). The tubers were surface disinfested with 2% formaldehyde and kept at room temperature for sprouting. After sprouting was initiated, four tubers were planted at a depth of about 5 cm in a 15-cm plastic pot containing sterilized soilless mixture (Metro-Mix, Terra-Lite 2000, W. R. Grace & Co., Ajax, Ontario). Each tuber was inoculated by placing a 6-mm-diameter mycelial disk about 2 cm above the tuber. The disks were cut from an actively growing edge of a 3-day-old colony of an isolate growing on PDA. The mycelial disks were covered with 2 cm of the mixture. Each isolate was tested using three replications. The plants were grown in a greenhouse at 18–24 C. Twenty-eight days after planting, the plants were harvested, washed, and examined for lesions on the stems. Disease severity was calculated as described by Hide and Firmager (17).

Data analysis. Data for mycelial growth rate and virulence of the isolates were analyzed by orthogonal contrasts using SAS (SAS Institute, Cary, NC).

RESULTS

Sixty-four isolates from 29 fields conformed to specific descriptions of *R. solani*. Of these, 49 belonged to AG-3, seven each to AG-4 and AG-5, and one isolate did not anastomose with any of the testers used in this study (Table 1).

On PDA, many isolates reached the edge of the petri plate after 96 hr of incubation. Growth rate of the isolates, therefore, was determined for the interval between 24 and 72 hr. Isolates of AG-3 grew slower than isolates of AG-4 and AG-5 (Table 1). Growth rates of AG-4 and AG-5 isolates were similar. All 64 isolates on PDA medium developed mycelium that was aerial or appressed to the agar surface and all but two showed zonation on the medium. AG-3 isolates developed light brown to dark brown colonies and all formed sclerotia. AG-4 isolates were light brown and all but one formed sclerotia. AG-5 isolates developed light tan mycelium and two of the seven formed sclerotia.

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Table 1. Growth rate and virulence of potato isolates of *Rhizoctonia solani* from Alberta

Anastomosis group	No. of isolates	Growth rate[w] (mm/day)				Disease index[x]			
				Probability > F^y				Probability > F^y	
		Mean	Range	AG-3	AG-4	Mean	Range	AG-3	AG-4
AG-3	49	17.2	2.2–25.3	...	...	29.0	0.3–62.2	...	...
AG-4	7	19.6	15.0–25.7	0.0001	...	18.4	0.0–61.3	0.0010	...
AG-5	7	19.9	16.8–24.8	0.0001	0.6034	15.7	1.5–31.4	0.0001	0.5012
Unidentified	1[z]	13.3				7.7			

[w] Isolates were grown from a 5-mm-diameter mycelial disk on potato-dextrose agar at 22±2 C. Values represent means for radial growth per 24 hr for the interval between 24 and 72 hr of incubation.
[x] Virulence of isolates was determined by monitoring disease index (17) on potato (cv. Russet Burbank) plants grown in greenhouse.
[y] Determined by orthogonal contrast analysis.
[z] The isolate did not anastomose with any AG tester cultures used, and was not included in statistical analyses.

Sixty-two of the 63 isolates characterized to AG were pathogenic to potato plants, and isolates within each AG differed significantly in their virulence. Isolates of AG-3 were more virulent than isolates of AG-4 and AG-5 (Table 1) and virulence of isolates of AG-4 and AG-5 was similar. The isolate that did not anastomose with any of the testers was also pathogenic to potato plants.

DISCUSSION

Rhizoctonia solani isolates collected from potato plants in Alberta belonged to AG-3 (76.6%), AG-4 (10.9%), and AG-5 (10.9%). One isolate (1.6%) was unidentified. This is the first report of the association of AG-4 and AG-5 isolates of *R. solani* with Rhizoctonia disease of potato in Alberta and in Canada. Distribution of AG-3, AG-4, and AG-5 was not restricted to any particular geographical region of the province. Isolates of AG-3 and AG-4 or AG-5 were obtained from infected plants growing in the same field. Isolates of *R. solani* other than AG-3—including AG-1 (16), AG-2-1 (9,15), AG-4 (3,10,16), AG-5 (1,6,10), and AG-9 (13)—have been reported in association with potato in different parts of the world. With the exception of AG-4 in China and Peru (3,16), isolates other than AG-3 constitute a low percentage of the total number of *R. solani* isolates obtained from infected potato plants or tubers.

In this study, AG-3 was more virulent than AG-4 and AG-5, and virulence of AG-4 and AG-5 was similar. Isolates from AG other than AG-3, including AG-2-1, AG-5, and AG-9, have been reported to exhibit low virulences on potato (6,9,15). In two instances, AG-4 isolates induced higher disease incidence than AG-3 isolates (3,16). Isolates of the AGs tested showed wide variation in their relative virulence on potatoes. Carling and Leiner (11) have suggested that AG-3 isolates range from highly virulent to nonpathogenic. AG-3 isolates obtained from a specific part of potato plant showed wide variation in virulence among themselves (18), the average virulence of the isolates, however, was generally similar to that of isolates collected from another part of the plant (11,18). A number of other reports have also indicated similar variation in virulence among AG-3 isolates (6,9,12). Since the majority of Alberta isolates of *R. solani* from potato belonged to AG-3, and AG-3 isolates as a group were significantly more virulent than AG-4 and AG-5, the results agreed with the previous studies (6,9,23) that members of AG-3 are the major cause of Rhizoctonia disease of potato in Alberta. Low incidence and virulence of AG-4 and AG-5 isolates of *R. solani* suggest that they are of some minor importance.

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