Anastomosis Group 3 Is the Major Cause of Rhizoctonia Disease of Potato in Maine

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ABSTRACT

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Three hundred seven isolates of *Rhizoctonia solani* associated with stem and stolon canker and black scurf of potato in Maine were characterized with regard to anastomosis group (AG). The results confirmed that members of AG-3 are the major cause of Rhizoctonia disease of potato in fields surveyed. One hundred percent of the isolates from tuberborne sclerotia and 82.1% of the isolates from stem and stolon lesions were members of AG-3. The most important non-AG-3 isolates were members of AG-5, which comprised 13.4% of the cultures isolated from stem and stolon lesions. Anastomosis group 2, type 1, associated with potato in other geographic areas, was not isolated from sclerotia, nor from infected plants in Maine.

Rhizoctonia canker disease of potato, also commonly called black scurf, is caused by *R. solani* Kühn and occurs in all areas where potatoes are grown, although it is favored by low soil temperatures and high soil moisture (7). A number of reports in the literature (1,3,6,10) have shown that members of anastomosis group (AG) 3 of *R. solani* are the major pathogens associated with both lesions on the potato plants and sclerotial infestation of progeny tubers. The role of other AGs in the development of disease, however, is less clear and seems to vary from one geographical region to another.

Maine soils cropped intensively with potatoes support a diverse population of *R. solani*, including members of five different AGs and isolates that do not anastomose with testers from AG-1 to AG-5, as well as binucleate Rhizoctonialike fungi (2). This study was undertaken to determine which AGs are the major cause of Rhizoctonia canker and black scurf of potatoes in Maine.

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MATERIALS AND METHODS

Isolation of *R. solani* from plant material. Potato plants showing symptoms of Rhizoctonia disease were collected from 24 different fields in major potato growing areas in southern, central, and northern Maine. Pieces of tissue from the margins of lesions on stems and stolons were surface-sterilized with 0.5% sodium hypochlorite for 1–3 min and plated on acidified potato-dextrose agar (APDA). Pieces of roots from infected plants were surface-

sterilized and plated. Hyphal-tipped isolates tentatively identified as *R. solani* were transferred to potato-dextrose agar (PDA) slants and stored at 18 C.

Isolation of *R. solani* from sclerotia on tubers. Samples of tubers harvested after vine-killing were collected from the same areas as the plant samples described above, and in all, represented 11 different potato varieties. Additional tubers were obtained from the University of Maine Agricultural Research Farm in Presque Isle.

Tubers were thoroughly washed with tap water immediately before individual sclerotia were cut from the surface with a scalpel and plated onto APDA. After the plates were incubated approximately 48 hr at room temperature, hyphal-tip isolations were made from colonies resembling *R. solani* and were cultured on PDA.

Identification of isolates. To distinguish between binucleate fungi resembling *Rhizoctonia* and multinucleate *R. solani*, nuclei in young (less than 7 days old) vegetative cells from cultures grown on PDA were stained with 0.5% aniline blue in lactophenol combined with an acidified wetting agent (8). Multinucleate isolates were then tested to determine their anastomosis grouping by pairing each isolate with tester strains from AG-1 to AG-5, according to established procedures (9,11). In addition, 150 isolates were tested by growing them on a differential medium (Stewart's medium) for identification of AG-3 (4).

Pathogenicity tests. Twenty of the sclerotial isolates were tested for their pathogenicity on potato sprouts. Dormant Katahdin tubers that were free of visible sclerotia were surface-sterilized in 2% formaldehyde for 5 min and then air-dried at room temperature for several days to allow the residual formaldehyde to evaporate. After sprouting was initiated, tubers were planted in perlite, moistened as needed with a 5 mM Ca $(NO_3)_2$ solution (to prevent necrosis of sprout tips), and incubated at 20–25 C for about 2 wk or until sprouts had grown approximately 5–10 cm and had formed roots. The sprouts with roots were then carefully excised from the surface of the tuber and planted in moist vermiculite. Each sprout was inoculated with a 9-mm-diameter plug of mycelium plus agar cut from 6-day-old cultures of *R. solani* on PDA. The inoculum plug was placed adjacent to the sprout, about 2 cm below the growing tip. The sprout plus inoculum was then covered to a depth of 3 cm with moist vermiculite. The sprouts were kept in the dark at approximately 22 C and watered with Hoagland's solution once a week. After 2 wk, virulence ratings were determined based on the percentage of sprout tissue decayed.

RESULTS AND DISCUSSION

A total of 69 isolates were obtained from stems, stolons, and roots of infected plants. Their distribution among anastomosis groups is shown in Table 1. Two of the isolates obtained from infected stems were binucleate Rhizoctonialike fungi and two were nonanastomosing multinucleate isolates. Of the remaining 65 isolates that did anastomose with the tester strains, nine of them (13.8%) were members of AG-5. The nine AG-5 isolates came from four different fields, one field in southern Maine, one field in central Maine, and two widely separated fields in northern Maine. Fifty-five of the remaining 56 isolates were members of AG-3, derived from stems, stolons, and roots. One member of AG-1 was obtained from roots.

A total of 88 isolates were obtained from sclerotia on tubers collected from the same areas as the plants described above. All of these isolates were *R. solani* AG-3.

An additional 150 isolates were taken from sclerotia on tubers produced on the university's research farm in Presque Isle. All of the 150 isolates were shown to be multinucleate and all were identified as members of AG-3 (Table 2). The results of the tests on Stewart's medium are also shown in Table 2. Of 150 isolates tested, 130 produced the expected brown color typical of AG-3 on the differential medium (5). The remaining 20 isolates (13.3%) produced a white color on Stewart's medium and, therefore, could not be positively identified by this test.

In pathogenicity tests, 19 of the 20 AG-3 tuberborne isolates tested were found to be pathogenic to potato sprouts, and one isolate appeared to be nearly avirulent (Table 3). The hypovirulent isolate and seven of the most virulent ones were white on Stewart's medium, indicating that there was no correlation between pathogenicity and the color reaction.

The results of our research confirm those of other similar studies (1,3,6,10) that show that members of AG-3 are the major pathogens that cause Rhizoctonia disease of potato. All of the 238 isolates obtained from sclerotia on tubers in this study were members of AG-3, in spite of the fact that the soils from which the tubers were taken were populated by at least five anastomosis groups (2). Other studies have shown that a small percentage (less than 5%) of sclerotial isolates from tubers may be members of groups other than AG-3. In Japan, Abe and Tsuboki (1) found members of AG-5 among sclerotial isolates from tubers. Chand and Logan in Ireland (6) found that 4.4% of the sclerotia from tubers were members of AG-2-1, while 95.6% belonged to AG-3. Otrysko et al (10) demonstrated that 715 of 720 sclerotial isolates (99.3%) taken from tubers of six cultivars from five Canadian provinces were in AG-3. The remaining five isolates failed to anastomose with testers from AG-1 to AG-5. Carling and Leiner (3) found that 97% of the sclerotial isolates from tubers grown in Alaska were members of AG-3, and about 3% were from AG-2-1. From these reports, it appears that the formation of sclerotia on tubers by non-AG-3 *R. solani* occurs only incidentally, perhaps due to local biotic and abiotic environmental factors. Therefore, it is not surprising that our relatively large sample size of 238 sclerotial isolates included only AG-3.

The results with Stewart's medium indicate that the selective medium may be useful for screening large numbers of isolates for the presence of AG-3, but it may not be reliable as the sole method for quantitative assays because over 13% of the isolates produced white mycelium instead of the brown color required for identification of AG-3 (5).

Nineteen of the 20 sclerotial isolates

Table 1. Anastomosis grouping of isolates of *Rhizoctonia solani* obtained from lesions on infected potato plants

Anastomosis group[a]	Number of isolates			
	Stem	Stolon	Root	Total
1	0	0	1	1
2	0	0	0	0
3	36	16	3	55
4	0	0	0	0
5	8	1	0	9
Unknown	2	0	0	2
Total *R. solani*	46	17	4	67

[a] Two binucleate Rhizoctonialike isolates were obtained from stems.

Table 2. Identification of *Rhizoctonia solani* isolates from tuberborne sclerotia based upon anastomosis tests and color reaction on Stewart's medium

Potato variety	Number of isolates			
			Results on Stewart's medium	
	Total	AG-3[a]	Brown	White
Katahdin	50	50	43	7
Atlantic	50	50	49	1
Superior	50	50	38	12
Total	150	150	130	20

[a] These isolates were positively identified as members of AG-3 by anastomosis tests with tester strains of known AG.

tested for pathogenicity in this study were moderately to highly virulent on potato sprouts (Table 3). This agrees with the findings of others (3,6,10) that AG-3 isolates of *R. solani* from tuberborne sclerotia are pathogenic on potato.

Of the 67 *R. solani* isolates obtained from infected potato plants in this study, nine (13.4%) were members of AG-5. Four of the AG-5 isolates were collected from a field in southern Maine, one isolate (from a stolon) was from a field in central Maine, and two isolates were collected from each of two widely separated fields in northern Maine, indicating that the AG-5-potato association is widespread in Maine. It was shown in a previous study that members of AG-5 were widely distributed in potato soils in Maine, and were capable of infecting potato sprouts in greenhouse tests (2). Abe and Tsuboki in Japan (1) reported that members of AG-5 were obtained from lesions on stems, as well as from sclerotia on tubers. In North America, the only other extensive survey of *R. solani* associated with naturally infected potato plants in the field is that by Carling and Leiner in Alaska (3). The authors of that study did not find members of AG-5 among the 288 isolates they collected from lesions, hymenia, and tuberborne sclerotia, presumably because AG-5 is absent from the soils of that region (4).

The Maine and Alaska studies also differ with respect to the occurrence of AG-2. In Maine, none of the isolates from infected potato plants were from AG-2, although that group was well represented in the soil population (2). In Alaska, however, Carling and Leiner (3) reported that 26.5% of the *R. solani* isolates recovered from lesions on stems and stolons were members of AG-2-1. The authors indicated that isolates of AG-2-1 caused minimal damage on potato sprouts in pathogenicity tests and were associated with relatively small lesions on infected plants from the field, suggesting that AG-2-1 is probably less important in the development of Rhizoctonia disease on potatoes than its frequency of isolation would suggest. The same situation may be true for members of AG-5 in that they may infect potato plants only incidentally, depending upon local environmental conditions or upon the local occurrence of strains that have evolved to be potato pathogens. The time of sample collection may also be a critical factor since Abe and Tsuboki (1) noted that isolates of AG-5 were obtained from lesions on stems in the later stages of growth. Detailed studies involving repeated sampling during each growing season for a period of years would probably be required to fully elucidate the role of non-AG-3 groups in Rhizoctonia disease of potato.

Table 3. Distribution of 20 sclerotial isolates (AG-3) of *Rhizoctonia solani* with respect to virulence on potato sprouts

Virulence ratings[a]	Number of isolates
0–1.9	1
2–2.9	0
3–3.9	1
4–4.9	3
5–6.0	15

[a] Virulence ratings were based on the percentage of sprout tissue decayed: 0 = no decay; 1 = 1–5% decay; 2 = 5–25%; 3 = 25–50%; 4 = 50–75%; 5 = 75–95%; 6 = 95–100%. Virulence ratings for each isolate were based on the mean of eight replicates (one sprout = one replicate).

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