Virulence of Isolates of *Rhizoctonia solani* AG-3 Collected from Potato Plant Organs and Soil

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ABSTRACT

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Isolates of *Rhizoctonia solani* were collected directly from hymenia and lesions on potato stems and sclerotia on tubers and by direct and indirect methods from soil. Isolates were collected from soil planted to barley, bluegrass, and carrot and from fallow soil. Nearly 95% of isolates from plants and tubers were determined to be members of anastomosis group 3 (AG-3), whereas only 52% of isolates from soil were AG-3. Most isolates of *R. solani* AG-3 were moderately to highly virulent on potato sprouts at 10 C. On average, isolates from hymenia were significantly more virulent than isolates from lesions, but neither was significantly different in virulence from isolates collected from sclerotia or soil. Crop, year, and growth rate of isolates at the time of collection also had no significant effect on relative virulence.

Soils remain cool throughout the short growing season in Alaska, and under these conditions Rhizoctonia disease, or black scurf, is capable of causing significant reductions in the quality and yield of potato (*Solanum tuberosum* L.) (10). Yield reductions due to Rhizoctonia disease also have been reported at other locations, such as Quebec (4), Great Britain (16), and Northern Ireland (20), where cool soil temperatures may be a factor. In warmer, drier areas such as Idaho (12), losses due to this disease are inconsistent and may not occur. In areas where Rhizoctonia disease does damage potatoes, losses can be minimized by using seed tubers that are free of the fungus, planting seed near the soil surface, and following a rotation scheme with two or more years between potato crops.

Rhizoctonia disease of potato is caused by the fungus *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk). *R. solani* is divided into subgroups based on anastomosis behavior. Hyphae of isolates representing the same anastomosis group (AG) are attracted to and connect, or fuse, with one another, whereas isolates from different AGs do not exhibit this behavior (21). Isolates of many of the 11 AGs currently known (9,21,22) have been associated with potato. Of these, one (AG-3) is acknowledged by most to be the principal cause of Rhizoctonia disease of potato (3,6,23). Exceptions include reports by Anguiz and Martin (1) and Gudmestad et al (14) describing the root- and stem-rotting potential of isolates of *R. solani* AG-4 on potato.

Isolates of *R. solani* AG-3 range from highly virulent to nonpathogenic. More than 98% of the isolates of AG-3 that we have collected from potato, other plants, and soil have been moderately to highly virulent (D. E. Carling and R. H. Leiner, *unpublished*). Opinions differ, however, regarding the factors responsible for this variation in virulence. Some investigators have reported generally low levels of virulence from tuberborne sclerotia (18,26,28,29) and have indicated that isolates from stem lesions are generally more virulent on potato. Conversely, Hill and Anderson (17) reported that isolates of *R. solani* AG-3 from stem lesions are significantly less virulent than isolates from stolons, tuberborne sclerotia, and hymenia. Isolates from soil display a wide range of virulence, although in many instances the AG of isolates from soil associated with potato has not been reported (26,29).

To clarify the influence of variables such as source, host, and method of collection on the virulence of isolates of *R. solani* AG-3, we assessed the virulence of isolates collected by several methods, from different sources, and from soil in which a variety of crops were grown over a 2-yr period.

MATERIALS AND METHODS

Collection and isolation. Isolates of *R. solani* were collected from a silt loam soil left fallow or planted to one of the following five crops: barley (*Hordeum vulgare* L.), Kentucky bluegrass (*Poa pratensis* L.), carrot (*Daucus carota* L.), potato planted with seed tubers free of *R. solani,* and potato planted with seed tubers contaminated with *R. solani.* Single plots of each crop measuring 6.1 × 4.3 m were located at the Alaska Agricultural and Forestry Experiment Station Farm near Palmer. The soil planted to all crops was infested as uniformly as possible with *R. solani* AG-3 in 1984, 2 yr before the start of this experiment, by growing a crop of potatoes from seed naturally contaminated with the fungus.

Soil samples were collected and isolations were made from the same plots during the 1986 and 1987 growing seasons. Soil was collected with a core sampler to 15-cm depths from each plot at biweekly intervals throughout the growing seasons. Ten cores collected from each plot at each sampling date were combined and mixed into single samples. A 95-g subsample was taken from each bulk sample, and *R. solani* was isolated directly from soil pellets (15) placed onto petri dishes containing the Ko-Hora medium (19) modified with 5 μl of prochloraz per liter (11). Before pelletizing, 5 ml of an aqueous solution of prochloraz (1 ml/L) was atomized onto the 95-g subsample of soil (11). This step simplified the final stages of isolation and improved rates of recovery by reducing the growth of fungi that rapidly produce aerial mycelium.

Petri dishes with pellets were incubated at 20 C for 72 hr. All hyphae suspected of being *Rhizoctonia,* based on the angle of hyphal branching, were then transferred to petri dishes containing rehydrated potato-dextrose agar (PDA). The vigor of isolates, based on the relative size of the colony, was noted at the time of transfer. If isolates resembled *Rhizoctonia,* they were again transferred, then stored at room temperature for determination of AG affiliation and virulence.

The relative virulence of 60 isolates of *R. solani* AG-3 collected in 1986 and 45 collected in 1987 was determined. These 105 isolates were randomly selected from the 776 isolates of *R. solani* AG-3 collected in the 2 yr by growth rate at the time of isolation, year, and crop.

Isolates of *R. solani* also were collected in 1986 from five sources on three farms, including two commercial potato farms and the Alaska Agricultural and Forestry Experiment Station Farm near Palmer. Attempts were made to collect 20 isolates from each source at each farm. The five sources included three from plants

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(sclerotia, hymenia, and lesions) and two from soil (isolated by direct or indirect methods).

To recover isolates from sclerotia and lesions, small pieces of the appropriate fungus and/or host tissue were excised, surface-disinfested in 1% NaOCl for 30 sec, rinsed in distilled water, and placed in petri dishes on 2% water agar or PDA. After hyphal tip transfers, AG affinity and virulence were determined. Hymenial isolates were handled in the same way, except that no surface disinfestant was used (9). In all, 141 plant isolates were evaluated, including 46 from hymenia, 46 from lesions, and 49 from sclerotia.

Isolates from the soil adjacent to each plant were collected indirectly as well as by the direct method described earlier. The indirect method involved trapping isolates on sterile beet (*Beta vulgaris* L.) seed (9,24). A total of 52 soil isolates were evaluated, including 30 recovered by the direct method and 22 recovered by the indirect method.

AG typing. AG identities were determined following a modification (9,11) of the method of Parmeter et al (25). Field isolates and tester isolates were paired on opposite ends of cellophane rectangles measuring 3 × 1.5 cm and incubated at 20 C until hyphae overlapped (usually 48–72 hr). The area of cellophane on which hyphae overlapped was then removed from the agar, placed on a slide, stained with 0.05% trypan blue in lactophenol, and examined microscopically (×100 and ×400) for hyphal anastomosis. Connection of cell walls accompanied by death of anastomosing and adjoining cells (category 2 reaction [7]) was usually observed at the point of anastomosis. Occasionally, fusion of the cell walls and plasmalemma of anastomosing hyphae (category 3 reaction [7]) was observed. At least five anastomosis points demonstrating either category were required to characterize the reaction as positive.

Pathogenicity determination. Pathogenicity was determined on potato sprouts growing from seed tuber pieces (cultivar Russet Burbank) in a sand-soil mix. Before they were cut, seed tubers were surface-disinfested in a 1.85% aqueous solution of formaldehyde for 2 min at room temperature to eliminate any *R. solani* on the tuber surface (10). Washed builders' sand was blended with a silt loam soil in a 2:1 ratio. The moistened soil mixture was heated to approximately 80 C for two 30-min periods separated by a cooling period, which has proved to be a very reliable method for eliminating *Rhizoctonia* from soil (D. E. Carling and R. H. Leiner, *unpublished*). Seed pieces weighing 45–60 g were cut immediately before planting, placed in a shallow layer of soil near the bottom of 1,000-ml plastic pots, and covered with 2–3 cm of soil. Inoculum, consisting of 12 agar disks 7 mm in diameter cut from the growing edge of the appropriate fungal culture growing on PDA, was then placed on the soil 2–3 cm above the seed piece and covered with 7–10 cm of soil. Control pots were prepared with sterile disks of PDA.

Pots were placed in a dark room at 10 C and watered whenever the soil surface appeared dry. The experiment was harvested about 6 wk after planting, when the control plants had emerged. Plants were washed free of soil, and the amount of damage on sprouts was assessed. Damage was rated numerically as follows: 0 = no damage, no lesions; 1 = minor damage, one to several lesions less than 5 mm long; 2 = moderate damage, lesions more than 5 mm long, and girdling of some sprouts; 3 = major damage, large lesions, and girdling and death of most sprouts; 4 = all sprouts killed. Data were analyzed by analysis of variance, using Duncan's multiple range test to separate means. A completely randomized design was used, and four observations were made of each treatment. The experiment was repeated once with similar results.

RESULTS

Of the 776 isolates of *R. solani* AG-3 collected in 1986 and 1987 from soil planted to the five crops listed earlier or left fallow, 105 were tested for pathogenicity on developing potato sprouts (Table 1). Besides *R. solani* AG-3, isolates of *R. solani* AG-2-1 and AG-9, binucleate *Rhizoctonia,* and a type of multinucleate *Rhizoctonia* similar to *R. oryzae* Ryker & Gooch (27) were recovered. In this study, virulence was determined only for isolates of *R. solani* AG-3.

Isolates collected from soil in 1986 and 1987 did not differ significantly in virulence, expressed as the amount of damage to sprouts (Table 1). Also, there was no significant difference in the virulence of isolates collected from soil planted to the five crops or left fallow (Table 1). All of these isolates were recovered directly from soil via the soil pellet method, and their growth rates at the time of collection ranged from low to high (Table 1). Pathogenicity tests indicated no difference in the average virulence of these isolates based on growth rate at the time of isolation.

The 71 isolates of *R. solani* AG-3 collected from field 2, a commercial field with a history of continuous potato and vegetable production, were significantly more virulent (P=0.05) than those collected from fields 1 and 3 (Table 1), where potatoes had been grown in rotation with small grains and forage grasses. When isolates were grouped by source, including the three plant and two soil sources (Table 1), hymenial isolates as a group were significantly more virulent than the lesion isolates, but no other significant differences appeared among the five sources.

Table 2 shows the AG identity of isolates as a percentage of all isolates of *R. solani* collected from each source. Ninety-five percent of the isolates collected from plant sources were members

Table 1. Damage to potato sprouts caused by isolates of *Rhizoctonia solani* AG-3 by year of collection from soil, by field of origin (isolates from soil and from potato plants), by rate of growth at the time of inoculation, by crop, and by source

Criterion	No. of isolates	Damage to sprouts[x]
Year of collection		
1987	45	2.83 a
1986	60	2.78 a
Field		
2	71	2.93 a
1	78	2.74 b
3	44	2.67 b
Growth rate		
Slow	40	2.83 a
Medium	34	2.80 a
Fast	31	2.77 a
Crop		
Fallow	13	2.98 a
Bluegrass	5	2.95 a
Potato (+)[y]	27	2.82 a
Barley	16	2.80 a
Potato (−)[y]	30	2.73 a
Carrot	14	2.71 a
Source		
Hymenium	46	2.91 a
Sclerotium	49	2.86 ab
Soil (beet seed)	22	2.76 ab
Soil (KHP)[z]	30	2.75 ab
Lesion	46	2.66 b

[x]Damage was rated on a scale of 0 to 4, where 0 = no damage and no lesions and 4 = all sprouts killed. Means for each criterion (year of collection, field, growth rate, crop, and source) followed by the same letter do not differ significantly (P = 0.05) according to Duncan's multiple range test.

[y]Seed tubers contaminated with *R. solani* (+) or free of *R. solani* (−).

[z]Ko-Hora medium (19) modified with 5 μl of prochloraz per liter.

Table 2. Percentage of isolates of *Rhizoctonia solani* from different sources and fields that belong to anastomosis group (AG) 3[a]

	Field			
Source	1	2	3	Mean
Plant				
Lesion	100	89	92	94
Hymenium	95	95	83	92
Sclerotium	100	100	92	98
Mean				95
Soil				
Beet seed	47	37	50	44
KHP[b]	74	53	50	60
Mean				52

[a]Where percentages are less than 100, the balance is composed of isolates of *R. solani* AG-2-1 and AG-9.

[b]Ko-Hora medium (19) modified with 5 μl of prochloraz per liter.

of AG-3, whereas nearly half (48%) of the isolates collected from soil associated with potato plants were not members of AG-3.

DISCUSSION

The virulence of 105 isolates of *R. solani* AG-3 collected over a 2-yr period directly from soil planted to five crops or left fallow did not differ either by crop or by year. Fewer isolates of *R. solani* AG-3 were recovered from soil planted to barley, bluegrass, or carrot or left fallow than were recovered from soil planted to potatoes grown from either contaminated or uncontaminated seed (D. E. Carling and R. H. Leiner, *unpublished*), but on average the isolates caused similar damage to potato sprouts. Specht and Leach (30) suggested that buckwheat (and perhaps other crops) may select for *Rhizoctonia* that are less virulent or nonpathogenic on potato. However, our data indicate that isolates of AG-3 that persist in the absence of potatoes (for at least the 2 yr in this study) can be moderately to highly virulent.

The relative growth rate of isolates collected directly from soil was not related to virulence (Table 1). Baker (2) indicated that the potential vigor of an isolate is a function of genetic capacity and stored energy. Because the propagules in our soil may have been small or large sclerotia or pieces of mycelium of various sizes, we believe that their growth onto PDA from the soil pellets was more likely dictated by the amount of stored energy in each propagule than by their genetic inheritance. Genetically determined vigor also may have been involved, but we did not test for this.

The relative virulence of isolates of *R. solani* pathogenic on potato has been reported to depend on the source of the isolate. Some investigators report relatively high virulence of isolates from lesions and relatively low virulence of isolates from sclerotia (26,28,29). More recent studies (5,13,17) indicate that the average virulence of isolates from sclerotia can be similar to that of isolates collected from other sources on the plant. Our data agree with these more recent reports. In addition, we found that the average virulence of isolates collected from soil, by direct or indirect means, is similar to that of isolates collected from the three plant sources. Unfortunately, most of the other studies (5,13,26,28,29) did not identify *R. solani* isolates by AG.

The relatively high level of virulence observed in isolates from soil illustrates that inoculum of *R. solani* AG-3 capable of damaging potato plants can survive for extended periods in the absence of potato plants, at least in the cool soil environment found in south-central Alaska. The low percentage of *R. solani* isolates from soil that are AG-3 (52%) compared to the high percentage from plants (95%) is not surprising, since the isolates associated with potato plants have been screened on the basis of their affinity for that plant species. Isolates of *R. solani* from soil have not been screened in this way and therefore will include representatives of all types present, pathogenic and nonpathogenic alike.

When judging the virulence of isolates of *R. solani* from soil, it is important to remember that isolates from most AGs of *R. solani* other than AG-3 generally possess low virulence against potato. Possible exceptions include AG-8, AG-5, AG-4, and others, especially at higher temperatures (8). If we had determined the virulence of all isolates of *R. solani* we recovered from soil (AG-3, AG-2-1, and AG-9) and had included them all in the calculations of average virulence, the resulting figures would have been misleadingly low and would have implied that soil isolates are generally less virulent on potatoes than are isolates from potato plants. As the data in Table 1 illustrate, isolates of *R. solani* AG-3 from soil are as virulent on potato as isolates of *R. solani* AG-3 collected from plants.

R. solani is often described as a saprophytic survivor. This description is probably less true for *R. solani* AG-3 than it is for many other AGs, because populations of AG-3 decline rapidly in the absence of potato (D. E. Carling and R. H. Leiner, *unpublished*). Nevertheless, isolates of *R. solani* AG-3 that do survive in the soil in the absence of potato are capable of retaining high levels of virulence. Thus, at locations like south-central Alaska where soils remain cool, extended rotations without potatoes (7–10 yr) will reduce but may not eliminate the recurrence of Rhizoctonia disease when potatoes are grown again.

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