Isolation and Characterization of *Rhizoctonia solani* and Binucleate *R. solani*-like Fungi from Aerial Stems and Subterranean Organs of Potato Plants

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ABSTRACT

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Lesion, hymenial, and sclerotial isolates of *Rhizoctonia solani* were collected from potato plants growing at various locations in Alaska. Most lesion and hymenial isolates were members of anastomosis group 3 (AG-3). Isolates assigned to AG-2-1 were obtained from lesions and hymenia collected at two locations and from sclerotia collected at a third. Lesion development was usually extensive on AG-3-infected plants but minor or undetectable on plants associated with other isolates. Three lesion and three hymenial isolates were identified as binucleate *R. solani*-like fungi. Eight multinucleate isolates failed to anastomose with *R. solani* tester isolates representing AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, or AG-BI, but they did anastomose with one another, constituting an anastomosis group not yet identified. In 57 cases, both hymenial and lesion isolations were made from the same plant. On 51 of these plants, the anastomosis group of lesion and hymenial isolates matched, whereas on six plants, lesion and hymenial isolates did not match. Anastomosis of matched pairs of AG-3 isolates generally resulted in perfect fusion without cell death, indicating pairs were members of the same clone. Anastomosis of matched pairs of AG-2-1 isolates generally resulted in perfect fusion with cell death, indicating dissimilarity of clones. AG-3 isolates from lesions, hymenia, and sclerotia were moderately to highly virulent on potato sprouts, but AG-2-1, undesignated multinucleate, and binucleate *R. solani*-like isolates were mildly virulent or avirulent. Comparative pathogenicity of isolates on germinating cauliflower seeds also was determined.

Rhizoctonia disease of potato (*Solanum tuberosum* L.), caused by *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (Frank) Donk) is commonly observed in commercial potato plantings in Alaska. Although yield reductions induced by *R. solani* reportedly are not significant or not worthy of control treatment in many locations (13,20), preliminary field studies in Alaska (D. Carling, *unpublished*) indicate that cultivar-dependent yield reductions of 7–64% may result if the seed source is contaminated with sclerotia.

Signs and symptoms of Rhizoctonia disease include sclerotia on the tubers and sunken necrotic lesions on roots, stolons, and subterranean portions of the main stems. Sclerotia are less common on potatoes grown at these northern latitudes than in regions with longer growing seasons, because the short local seasons do not permit the normally protracted senescence and death of vines. It is known that most sclerotial development occurs during plant senescence and after vine death (11). Vine killing with herbicides or other methods generally is not practiced in Alaska, because the early and abrupt end of most growing seasons does not permit the minimum wait (10–15 days) required for vine-killing benefits to be realized. In south central Alaska, hymenia of *T. cucumeris* commonly develop on the lower portions of stems during the latter part of the growing season (i.e., August). In heavily infested commercial fields, 50% or more of the plants may show this sign.

Recent reports indicate that most isolates of *R. solani* associated with potato tubers and other potato plant parts are members of AG-3; however, isolates of AG-1 (1,8), AG-2 types 1 and 2 (7,8), AG-4 (14), and AG-5 (1) also have been recovered from potato plants. In addition, AG-1, AG-2, AG-3, AG-4, and AG-5 as well as nonanastomosing multinucleate isolates and isolates of binucleate *R. solani*-like fungi were found in potato field soils (3). The remaining anastomosis groups, including AG-8 (16), have not been reported in association with potato. This research was conducted to determine the anastomosis group affinity and comparative

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pathogenicity of *R. solani* and *R. solani*-like fungi present on potatoes in Alaska. The role that each anastomosis group may play in the disease is discussed.

MATERIALS AND METHODS

Collection and isolation. Isolates of *R. solani* and *R. solani*-like fungi were collected from six south central Alaska (Palmer 1–6) potato fields (Table 1) during August 1984. Five of the sites were commercial fields and one was an Agricultural Experiment Station Research Farm field. Potatoes had been grown in all fields at least once in the 4 yr prior to the 1984 growing season. In one of the commercial fields (Palmer 1), potatoes had been grown continuously for 5 yr. Alternate crops at the other commercial sites included *Lactuca sativa* L. (lettuce), *Daucus carota* L. (carrot), *Phleum pratense* L. (timothy), and fallow. The Agricultural Experiment Station Farm field (Palmer 3) had been fallowed for at least 3 yr prior to the 1983 growing season, when a crop of in vitro-produced, virus-tested potato plantlets (cultivar Bakeking) was planted. The seed source for the 1984 Palmer 3 crop consisted of formaldehyde-dipped tubers harvested from the 1983 Palmer 3 crop. It was presumed that the seed planted at Palmer 3 in 1984 was free of *R. solani*. The other five Palmer sites were seeded with tubers (cultivars Green Mountain, Bakeking, Superior, or Denali) obtained from certified or noncertified local sources.

Plants with gray to white mycelial mats developing near the bases of stems were selected randomly for analyses. The original intent was to collect hymenial, lesion, and sclerotial isolates from each of at least 20 plants at the six locations. However, sclerotia were extremely rare on tubers at the time hymenial and lesion isolates were collected, and it was necessary to postpone tuber (sclerotia) collection until after harvest.

Selected plants with a portion of the root system attached were removed from the soil and taken to the laboratory. Small pieces of hymenia including, in most instances, a thin layer of stem tissue, were separated from the stem with a scalpel or forceps and placed on rehydrated potato-dextrose agar (PDA) in petri dishes. The steps of washing and surface-sterilization were bypassed because of the delicate nature of the hymenia. After 48–72 hr at room temperature, reisolations from the perimeter of each colony were placed on PDA containing 50 mg/L streptomycin sulfate or on water agar. Reisolation was necessary in most instances to free the fungus of bacterial contaminants. After reisolation, each culture was placed on a PDA slant and stored at room temperature.

Root systems were washed and inspected; the extent of lesion development on stems, stolons, and roots of each harvested plant was noted. Small pieces of necrotic tissue were excised (most often from the subterranean portion of the main stem), surface-disinfected in 1% sodium hypochlorite for 30 sec, then placed on PDA. Reisolation and storage were performed as previously described. Lesions, when present, were very small on plants associated with isolates other than AG-3, but isolations were attempted from each root system, even in the absence of detectable lesions.

Tubers bearing sclerotia from the six Palmer fields were collected from storage in November 1984. In addition, tubers bearing sclerotia were acquired from four other locations. Tubers were washed and sclerotia were excised and surface-disinfected; this was followed by isolation, reisolation, and storage. Lesion, hymenial, and sclerotial sampling was confined to the six Palmer locations (Table 1); only sclerotial samples were collected from the other four locations. Attempts were made to isolate from at least 20 plants at each location. Fifty-seven plants were sampled at Palmer 3, because preliminary surveys indicated types other than AG-3 were present.

Pathogenicity testing. Comparative pathogenicity of selected isolates of AG-3 and all isolates of AG-2-1, nonanastomosing *R. solani*, and binucleate *R. solani*-like groups was determined on potato sprouts. Potato seed pieces (cultivar Bakeking) were surface-disinfected with 2% formaldehyde and planted in a sterilized sand-soil mixture. Agar disks cut from cultures of the various isolates growing on PDA were placed about 2 cm above the seed pieces. Pots were incubated at 10 C for 5 wk, during which time the developing sprouts passed through the inoculum layer. Plants were then removed from the soil, washed, and inspected for damage to sprouts. Attempts were made to recover the respective isolates from lesions, and to confirm anastomosis group identity by pairing recovered isolates on water agar with testers from the appropriate group.

Similarly, comparative pathogenicity was determined on germinating cauliflower (*Brassica oleraceae* L. cv. Arapaho) seeds. Cauliflower was chosen as a representative of cruciferous plants, a group susceptible in varying degrees to attack by isolates of *R. solani* AG-2-1. Surface-disinfected seeds were placed at the developing edges of colonies of *Rhizoctonia* growing on 2% water agar plates. Seeds were examined for decay and hypocotyl necrosis 5 and 8 days after placement on the agar.

Anastomosis group typing. Field isolates were matched against tester isolates obtained from various sources. Tester isolates representing AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, or AG-BI were included. Each field isolate was required to anastomose with two tester isolates representing a common group before assignment to an anastomosis group. Anastomosis group identities were determined according to Castro's (6) modification of the procedure of Parmeter et al (19). Field isolates were paired with testers on cellophane rectangles 3× 1.5 cm placed on 1.5% water agar in petri dishes. Cellophane rectangles had been dipped in soft PDA (13 g/L) before placement on water agar. A mycelial disk from a tester isolate was placed on one end of the cellophane rectangle, and a disk from a field isolate was placed on the other. The pair was incubated at room temperature 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 (400×) for nuclear number, septal configuration, and hyphal anastomosis. Perfect fusion, the fusion of cell wall and cytoplasm (15,19,21) was sought, and at least five perfect fusion sites were required for each positive anastomosis reading.

Anastomosis with AG-3 tester isolates was attempted with all field isolates. The multinucleate field isolates failing to anastomose with AG-3 testers were paired with testers representing AG-1, AG-2-1, AG-2-2, AG-4, AG-5, AG-6, AG-7, AG-8, or AG-BI until anastomosis affiliation had been established.

Clonal identification. Isolates from hymenia and lesions collected from one plant and representing a common anastomosis group (Table 2) were evaluated for clonal similarity. Each pair was placed on cellophane (6), and anastomosis reactions were categorized as either *K* or *S* reactions (17). The *K* reaction, described as hyphal anastomosis accompanied by cell death,

TABLE 1. Summary of sampling locations in Alaska where *Rhizoctonia solani* and *R. solani*-like fungi were isolated

Location	Plants sampled (no.)	Successful isolations from			
		Lesions	Hymenia	Pairs[a]	Sclerotial isolations[b]
Palmer 1	20	19	19	18 (11)	10
Palmer 2	20	21[c]	18	18 (11)	12
Palmer 3	57	18	27	9 (9)	0
Palmer 4	20	19	20	19 (10)	16
Palmer 5	20	9	11	6 (6)	3
Palmer 6	20	19	15	15 (10)	6
Delta Junction	...	...	...	...	2
Fairbanks	...	...	...	...	10
Soldotna	...	...	...	...	8
Unalakleet	...	...	...	...	6
Total	157	105	110	85 (57)	73

[a]Indicates the number of times at each location that both lesion and hymenial isolates were recovered from the same plant. Parenthetical numbers indicate the number of pairs subjected to anastomosis group classification. Note that not all pairs from Palmer 1, 2, 4, and 6 were typed.
[b]Tubers with sclerotia were collected from storage from each location.
[c]One lesion yielded two isolates.

indicates dissimilarity of clones. The *S* reaction, hyphal anastomosis without cell death, indicates similarity of clones.

RESULTS

R. solani was isolated from lesions and hymenia with relative ease from samples collected at some locations and with difficulty from samples collected at others. At the four locations (Palmer 1, 2, 4, and 6), where AG-3 was the dominant isolate, 96% of lesion isolates and 90% of hymenial isolates yielded pure cultures of *R. solani*. At locations where a combination of AG-3, AG-2-1, binucleate, and undesignated multinucleate isolates were collected (Palmer 3 and 5), successful isolations were achieved from 36% of the lesions and from 50% of the hymenia.

R. solani was isolated from all tubers bearing sclerotia. Seventy-one of the sclerotial isolates anastomosed with AG-3 tester isolates, and two anastomosed with AG-2-1 testers. No sclerotia were found on tubers harvested at the Palmer 3 location, although AG-2-1 was found on the aerial and subterranean portions of stems. Attempts to isolate *R. solani* from blemished or undisturbed surfaces of Palmer 3 tubers yielded no *R. solani*-like fungi.

Two hundred twenty-four of the 288 isolates collected were paired with testers and assigned to anastomosis groups (Table 3). More than 73% of the isolates belonged to AG-3 and 20.1% belonged to AG-2-1. AG-3 isolates were recovered from lesions, hymenia, and sclerotia, as were isolates of AG-2-1. Six binucleate isolates and eight multinucleate isolates that failed to anastomose with known tester strains were collected from lesion or hymenial samples. The eight undesignated multinucleate isolates anastomosed perfectly with one another. Additional undesignated multinucleate isolates collected from potato plants and soils from other field locations in this region (D. Carling, *unpublished*) indicated the existence of an anastomosis group different from those described in the literature.

Lesion and hymenial isolations were attempted on 157 plants (Table 1); 46 yielded *R. solani* or binucleate *R. solani*-like fungi from either lesions or hymenia and 85 yielded isolates from both lesions and hymenia. Isolations attempted from the remaining 26 plants failed. Of the 85 plants yielding isolates of *Rhizoctonia* from lesions and hymenia (a total of 170 isolates), 114 (57 pairs) were categorized by determining nuclear number and anastomosis group affiliation (Table 2). Of the 57 pairs, 44 were AG-3, seven were AG-2-1, and six had nonmatching designations. In one of the six nonmatching cases (Palmer 3), a binucleate hymenial isolate and a nonanastomosing multinucleate isolate from a stem lesion were collected from the same plant. In another case (Palmer 2), an AG-3 isolate was obtained from a hymenium and a lesion from the same plant yielded a binucleate isolate and an AG-3 isolate. In the remaining four cases, AG-3 or AG-2-1 isolates were collected from plants also yielding undesignated multinucleate isolates. At no time did we recover AG-3 and AG-2-1 from the same plant.

The 51 plants mentioned before yielded hymenial and lesion isolates with similar anastomosis group designations, but clonal identities of these paired isolates were sometimes dissimilar. When anastomosed, all AG-3 matched pairs displayed perfect fusion, but cell death accompanied perfect fusion only 20% of the time, indicating most pairs were of the same clone. When AG-2-1 matched pairs were anastomosed, perfect fusion was accompanied by cell death 86% of the time. Thus, most AG-2-1 pairs were of dissimilar clones, because cell death observed in conjunction with anastomosis indicates clonal dissimilarity.

Pathogenicity tests (Table 4) on potato indicated that all AG-3 isolates were capable of damaging potato sprouts. Sclerotial isolates appeared to be somewhat less aggressive than either lesion or mycelial isolates. Isolates from AG-2-1, undesignated multinucleate, and binucleate groups were associated with minimal damage. Lesions were initiated by all eight isolates of the undesignated group, but 38% of the AG-2-1 isolates and 67% of the binucleate isolates produced no symptoms.

All AG-2-1 isolates damaged or killed germinating cauliflower seeds (Table 5). Undesignated multinucleate isolates induced limited hypocotyl necrosis but no seedling death. AG-3 and binucleate isolates did not damage germinating cauliflower seed.

TABLE 2. Anastomosis group (AG) affiliation of paired isolates (lesion and hymenial) of *Rhizoctonia solani* and *R. solani*-like fungi collected from the same potato plant in Palmer, AK

Collection location	Matched pairs (no.)	Unmatched pairs (no.)	AG type of isolate from	
			Lesion	Hymenium
Palmer 1	11	0	AG-3	AG-3
Palmer 2	10	0	AG-3	AG-3
	1[a]	1	AG-3 + Bi[b]	AG-3
Palmer 3	1	1	AG-NO[b]	AG-2-1
	1	1	AG-NO	Bi
	1	1	AG-2-1	AG-NO
	6	0	AG-2-1	AG-2-1
Palmer 4	10	0	AG-3	AG-3
Palmer 5	1	1	AG-3	AG-NO
	1	1	AG-2-1	AG-NO
	3	0	AG-3	AG-3
	1	0	AG-2-1	AG-2-1
Palmer 6	10	0	AG-3	AG-3
Total	57	6		

[a]This "pair" consisted of three isolates; AG-3 and binucleate from the lesion and AG-3 from the hymenium.

[b]Bi = binucleate *R. solani*-like fungus. AG-NO = multinucleate isolates that failed to anastomose with tester isolates representing AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, or AG-BI.

TABLE 3. Results of anastomosis group (AG) typing of 224 *Rhizoctonia solani* and *R. solani*-like isolates collected from potato plants in Alaska

Source of isolate	*R. solani* AG			Binucleate isolates[d]
	AG-2-1[a]	AG-3[b]	AG-NO[c]	
Lesion	18	48	2	3
Hymenial	25	46	6	3
Sclerotial	2	71	0	0
Total	45	165	8	6
% of Total	20.1	73.7	3.6	2.7

[a]All AG-2-1 lesion and hymenial isolates were collected from plants in fields 3 and 5 at Palmer, AK. The sclerotial AG-2-1 isolates came from Unalakleet, AK.

[b]AG-3 isolates were collected at all sites except field 3 at Palmer, AK.

[c]AG-NO (undesignated multinucleate isolates) were collected from plants in fields 3 and 5 at Palmer, AK. These isolates would not anastomose with tester isolates representing AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, or AG-BI.

[d]Binucleate isolates were collected from plants in fields 1, 2, and 3 at Palmer, AK.

TABLE 4. Disease reactions of potato sprouts exposed to isolates of *Rhizoctonia solani* or *R. solani*-like fungi collected from potato plants in Alaska

Isolate group	Isolate origin	No. of isolates	Disease index[a]	
			Mean	Range
AG-3	Lesion	10	3.29	1.5–4.0
	Mycelium	10	3.25	2.5–4.0
	Sclerotium	5	2.60	2.0–3.5
AG-2-1	Lesion	18	0.42	0.0–1.0
	Mycelium	25	0.32	0.0–1.0
	Sclerotium	2	0.25	0.0–0.5
AG-NO[b]	Lesion	2	0.65	0.5–0.8
	Mycelium	6	0.85	0.5–1.3
Binucleate	Lesion	3	0.00	0.0–0.0
	Mycelium	3	0.02	0.0–0.3

[a]Disease index: 0 = no lesions, 1 = one to several lesions less than 1 mm in diameter, 2 = several lesions 1–3 mm in diameter, 3 = lesions larger than 3 mm in diameter and sprout girdling, and 4 = sprout girdling and sprout death.

[b]Multinucleate isolates that failed to anastomose with tester isolates representing AG-1, AG-2-1, AG-2-2, AG-3, AG-4, AG-5, AG-6, AG-7, AG-8, and AG-BI.

DISCUSSION

Isolation success (93% for sites yielding predominantly AG-3 compared with 43% for sites yielding a combination of AG-3, AG-2-1, undesignated multinucleate, and binucleate isolates) may be explained in part by the relative vigor of the various groups on the potato host. For example, hymenia from which AG-3 isolates were collected were dense, generally opaque mats that coated the circumference of the stem and extended up from the soil line to heights of 5 cm or more. The AG-3 hymenia frequently were sufficiently resilient to be grasped with a forceps and removed in pieces from the stem. Conversely, hymenia yielding AG-2-1, undesignated multinucleate, or binucleate *R. solani*-like fungi were less well developed, semitransparent gray to white blotches frequently confined to the shaded sides of the stems. Rarely did they encircle the stems or extend higher than 1–2 cm above the soil line.

Generally, AG-3 isolates were associated with plants bearing large lesions on stolons, roots, or subterranean portions of the main stems. This was in sharp contrast to the small lesions yielding AG-2-1, undesignated multinucleates, or binucleates. The comparative development of lesions and hymenia formed by AG-3 and non-AG-3 isolates may account in part for the difference in isolation success. Moreover, the comparative lack of aggressiveness by the non-AG-3 isolates, as indicated by sizes of associated lesions, suggests that they may be of little importance as pathogens of potato, at least under the boreal conditions encountered at these latitudes.

Additional research is being conducted to characterize the undesignated multinucleate group (AG-NO). Attempts are being made to induce the perfect state to form in culture. Studies on pathogenicity and measurement of hyphal dimension, nuclear number, and colony growth rates are being made. Representatives of this group will be placed in a type culture collection if these studies confirm their identity as a new anastomosis group.

Lesions were absent from many plants yielding hymenial isolates of AG-2-1, undesignated multinucleates, or binucleates, indicating that hymenial development on a potato plant does not necessarily indicate a pathogenic relationship between that plant and fungal isolate. Herr (12) recovered hymenial isolates of AG-2, AG-4, and binucleate *R. solani*-like fungi from diseased *Beta vulgaris* L. (sugar beets) and found all except the binucleates were pathogenic on sugar beet. Grisham and Anderson (10) recovered AG-2-1 isolates fom hymenia on carrot stems that proved to be pathogenic on *Raphanus sativus* L. (radish) but not on carrot. They indicated that carrot stems in these instances were sites of sexual recombination only. Similarly, the potato plant may be little more than a sexual recombination site for many of the non-AG-3 isolates we observed.

TABLE 5. Reactions of germinating cauliflower seeds to isolates of *Rhizoctonia solani* and *R. solani*-like fungi collected from potato plants in Alaska[a]

Isolate group	Seeding damage	Seeds exposed to fungus for	
		5 Days	8 Days
AG-3	No damage	25	25
	Hypocotyl necrosis	0	0
	Dead	0	0
AG-2-1	No damage	16	11
	Hypocotyl necrosis	26	7
	Dead	3	27
AG-NO	No damage	0	0
	Hypocotyl necrosis	8	8
	Dead	0	0
Binucleate	No damage	6	6
	Hypocotyl necrosis	0	0
	Dead	0	0

[a]Surface-disinfected seeds were placed on water agar inoculated with various isolates of *R. solani* and *R. solani*-like fungi. Damage assessments were made 5 and 8 days after placement of seeds.

Anastomosis of the AG-3 field isolates with various AG-3 tester isolates resulted in the production of five or more perfect fusions (21) or none at all. Imperfect fusion (15,19) was rarely observed among AG-3 isolates. Conversely, anastomosis of AG-2-1 with tester isolates was less definitive, indicating the general lack of homogeneity that exists within AG-2 (2). An AG-2-1 field isolate would anastomose with differing degrees of success with different tester isolates. Perfect fusion, imperfect fusion, and hyphal contact without fusion were observed on each slide. In many instances, it was not possible to locate the five perfect fusions we required for a positive reading.

More than 97% of our tuber sclerotial isolates were members of AG-3. This is consistent with other reports (1,7,18), where 95.6% or more of sclerotial isolates were identified as AG-3. Other investigators report anastomosis groups other than AG-3 from potato plants. Chang and Tu (8) reported recovering AG-1, AG-3, and AG-4 from stems and tubers but AG-2 from stems only. In Peru, only AG-4 was recovered, although the plant part sampled was not stated (14). One may wonder why AG-3 was not detected in the ancestral home of *S. tuberosum* and related species.

Although most tuberborne sclerotial isolates prove to be members of AG-3, isolates recovered from potato field soils in Maine (3) represented several anastomosis groups as well as binucleate *R. solani*-like fungi. Bandy et al (3) demonstrated that AG-5 isolates were pathogenic on potato and they discussed possible disease roles played by the other isolates of *Rhizoctonia*. However, isolation from potato field soil does not necessarily indicate an intimate association with *S. tuberosum*. Soil isolates may be pathogens or associates of weed hosts or alternate crops, or they may be present in a purely saprophytic mode. This report clearly links AG-2-1, undesignated multinucleate, and binucleate *R. solani*-like fungi with *S. tuberosum*. Each type was isolated from aerial stems and subterranean organs, indicating at least an epiphytic relationship with the plant. Field symptoms suggest a pathogenic relationship, but lesions yielding representatives of these groups were generally less than 2 mm in diameter, indicating at most a mild pathogenesis. Pathogenicity tests in sterile soil confirmed that non-AG-3 isolates were weakly virulent on potato sprouts.

As expected, AG-3 isolates predominated at most sampling sites. This group was more dominant overall than the percentages in Table 3 indicate, considering most non-AG-3 isolates were recovered from two sites (Palmer 3 and 5). AG-2-1 was the only described anastomosis group recovered at the Palmer 3 site, but it was not recovered at all at four sampling sites. Failure to recover AG-2-1 does not necessarily indicate its absence at a site. AG-2-1, undesignated multinucleate, and binucleate hymenial isolates may have been present at all sample sites but were not observed or isolated because of extensive development of AG-3 hymenia.

Isolates of AG-3 were recovered from all sites where potatoes were grown commercially. These isolates may have been introduced into the fields as surface contaminants on the current season's seed, or they may have resulted from development of existing soil populations introduced on potato seed in previous years. Isolates of AG-3 were not recovered from the Agricultural Experiment Station field (Palmer 3). Our inability to isolate AG-3 from the Palmer 3 site, an indication of the absence or very low populations of AG-3, was due in part to the use of surface-disinfected seed, and in part to the absence of a potato crop from the site for more than 3 yr. In south central Alaska (D. Carling, *unpublished*), isolates of AG-3 apparently are able to survive in the soil from 3–4 yr in the absence of potatoes, but fungal populations decrease as time away from potatoes increases.

Lesions induced by *R. solani* are frequently observed on the subterranean portions of plants that bear hymenia on the aerial stem (9), implying that the lesion and hymenium are linked to the same isolate of *Rhizoctonia*. These data demonstrate this may be true in most instances, because lesion and hymenial isolates (paired isolates) were members of the same anastomosis group on 51 of 57 plants yielding both (Table 2). Exceptions do exist, however, because the remaining six paired isolates had nonmatching anastomosis group designations.

Identification of isolates of *R. solani* with similar designations may be carried one step beyond anastomosis grouping by observing cytological differences among anastomosis reactions. Ogoshi and Ui (17), in a study of clonal variation within anastomosis groups, assigned isolates that fused perfectly without cell death (*S* reaction) to the same clone and isolates where perfect fusion was accompanied by cell death (*K* reaction) to different clones. When we anastomosed AG-3 paired isolates, 80% displayed the *S* reaction and the remaining 20% displayed the *K* reaction. Thus, a small percentage of the AG-3 pairs were of dissimilar clones. When the AG-2-1 paired isolates were anastomosed, 86% displayed the *K* reaction, thus most paired isolates were of dissimilar clones. These data indicate AG-2-1 hymenia on potato stems do not arise, in most instances, as a direct result of AG-2-1 activity on the subterranean portion of the plant. A more likely explanation may be that the potato stem is functioning as a sexual recombination site for saprophytic AG-2-1 isolates present in the soil.

What the presence of nonmatching isolates of *Rhizoctonia* from individual potato plants may imply is not obvious at this time. The establishment of one anastomosis group or clone of *R. solani* on a potato plant does not result in the exclusion of all others, although it is worth noting that we did not recover AG-3 and AG-2-1 from the same plant. It is possible that complementary or antagonistic relationships exist among isolates of *R. solani* present on potato plants. Cardoso and Echandi (5) reported a reduction in *R. solani*-induced root rot of *Phaseolus vulgaris* L. (bean) when selected binucleate *Rhizoctonia*-like fungi were introduced into sterilized or field soil. Similarly, Burpee and Goulty (4) reported suppression of brown patch disease of *Agrostis palustris* Huds. (creeping bentgrass) by isolates of binucleate *Rhizoctonia*-like fungi, indicating binucleates have the capacity to interact with *R. solani*. Further investigations of relationships among the many multinucleate and binucleate isolates of *R. solani* present on potato are warranted.

Tolerance levels for *Rhizoctonia* disease have been established as part of the State of Alaska Potato Seed Certification Program. Seed lots can be rejected if *R. solani* AG-3 is detected above tolerance levels. Because many non-AG-3 isolates are capable of producing hymenia and lesions on potato, it may be difficult, by visual observation of plants in the field, to separate AG-3-related symptoms and signs from those induced by other isolates of *Rhizoctonia*. Anastomosis group identification by laboratory testing may be required in questionable cases.

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