

Characterization and Pathogenicity of Anastomosis Groups of *Rhizoctonia solani* Isolated from *Beta vulgaris*

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ABSTRACT

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Of 361 cultures of *Rhizoctonia* isolated from sugar beet seedlings, 36 isolates were binucleate. The remaining 325 cultures were multinucleate with characteristics typical of *R. solani* and were identified to six anastomosis groups (AG): AG-4 predominated and infected 44.3% of the seedlings, followed by AG-5 (27.1%), AG-2-2 (19.7%), AG-1 (1.8%), AG-2-1 and AG-3 (0.3% each); 6.5% of the cultures did not anastomose with any of the tester cultures (AG-1 through AG-9 and AG-BI). About 96% of the 163 cultures isolated from rotted roots ≥ 8 wk old were AG-2-2, 3.7%

were AG-4, and 0.6% were AG-5. Pathogenicity tests on sugar beet seedlings in the greenhouse showed that AG-1, 2-2, and 4 severely reduced stands, whereas AG-2-1, 3, 5, and the unidentified isolates were less pathogenic; the binucleate isolates of *Rhizoctonia* were nonpathogenic. When the same cultures were inoculated on 8- to 9-wk-old sugar beet roots, only AG-2-2 isolates were highly virulent; AG-4 isolates produced superficial lesions near the point of inoculation, and isolates of the other anastomosis groups and binucleate isolates were nonpathogenic.

Additional keywords: root rot, seedling blight.

Sugar beet (*Beta vulgaris* L.) production in 1987 comprised nearly 187,000 ha in the Red River Valley of Minnesota and North Dakota and in west central Minnesota. Soilborne diseases are a problem in sugar beet fields throughout the area. *Aphanomyces cochlioides* Drechs., *Pythium* spp., and *Rhizoctonia solani* Kühn have been isolated from diseased seedlings; *A. cochlioides* and *R. solani* also have been isolated from rotted roots of older plants (C. E. Windels, unpublished). However, no studies have been done to determine the anastomosis groups (AG) of *R. solani* on sugar beet in the region.

A review by Ogoshi (17) states that there are at least nine anastomosis groups of *R. solani* (teleomorph: *Thanatephorus cucumeris* (Frank) Donk), and one of these (AG-2) is further divided into two subgroups (AG-1, 2-1, 2-2, 3, 4, 5, 6, 7, 8, and BI). Recently, Carling et al (3) described a new anastomosis group, AG-9, in Alaska.

R. solani causes damping-off, root rot, crown rot, and foliar blight of sugar beet (7,10,20,26). In Colorado, Ruppel (20) reported that isolates obtained from sugar beet crowns and foliage were AG-4, whereas root isolates were AG-2. Herr (7) found hymenia of *T. cucumeris* on diseased petioles of crowns and root rot diseased sugar beets in Ohio, and most of these isolates were AG-2; AG-4 and binucleate isolates ("Rhizoctonia-like") also were isolated from diseased petioles. Reports from Japan indicate a greater diversity of AGs of *R. solani* associated with sugar beet compared with those found in the United States. *R. solani* AG-1, 2-1, 2-2, 3, 4, and 5 have been isolated from dying seedlings (13); AG-1, 2-2, 4, and 5 from petioles (15); AG-2-2 from crowns and roots (15); and AG-1 and 2-2 from blighted leaves (14).

The objectives of this study were to characterize anastomosis groups of *R. solani* associated with diseased sugar beet seedlings and root rot of older plants and determine the relative virulence of isolates of each anastomosis group on seedlings and roots of older sugar beet plants. A brief report on this research has been published (27).

MATERIALS AND METHODS

Collection and isolation. Isolates of *Rhizoctonia* were collected from sugar beet seedlings, older plants, and from debris in a sugar beet field. During 1985-1987, diseased sugar beet seedlings were collected from 40 fields in the Red River Valley. During 1984-1987, older sugar beet plants (≥ 8 wk old) with root rot were collected from an additional 40 fields in the Red River Valley.

Soil was collected from 39 fields in the Red River Valley and 18 fields in west central Minnesota during the summer or fall of 1984-1987 from areas that had been planted to sugar beet that season or in a previous season, and a seedling assay was done to isolate *R. solani*. Soil collected from these fields was passed through a 5-mm-mesh screen, thoroughly mixed, dispensed into 9-cm² plastic pots or 26-cm² × 6-cm-deep plastic trays, and seeded (25 seeds/pot or 25 seeds/row in trays) at a 2-cm depth (four to six containers/field). Seed used in this and subsequent studies was the cultivar Maribo Ultramono, provided by American Crystal Sugar Co., Moorhead, MN. Seed was treated with 0.3 g a.i. of metalaxyl plus 2.1 g a.i. of thiram per kilogram, or with 1.3 g a.i. of fenaminosulf per kilogram. Containers were placed in a greenhouse or incubator at 18 C for 7 days, and then the temperature was increased to 24-27 C to favor postemergence damping-off for 3 wk, when the study was terminated. Soil was moistened as needed. As seedlings died, they were collected for assay.

For diseased seedlings collected in the field and greenhouse, the portion of the root that was decayed or lesioned was selected. On older, field-collected beets, two to six cores (1 cm diameter) were removed per beet with a cork borer, or root pieces (1 × 0.5 cm) were removed with a knife or razor blade. Root pieces were surface-treated in 0.5% NaOCl for 15 sec, rinsed twice in sterile distilled water, placed in 9-cm-diameter quadrant plates (one root segment/sector) containing 5 ml of sterile distilled water and incubated at 20-24 C for 2-5 days. Roots in water culture were examined microscopically to distinguish mycelium and spores of several possible seedling pathogens, including *A. cochlioides*, *Pythium* spp., and *Rhizoctonia* (26). Characteristic mycelium of *Rhizoctonia* grew over the surface of the water and was

distinguished from other pathogens. Root pieces then were drained on paper towels, placed on potato-dextrose agar (PDA) or on Ko and Hora's medium (9), and incubated at 20–24 C for 5–7 days.

Soil from a sugar beet research plot at the Northwest Experiment Station was sampled in June 1985 by randomly collecting 15 cores to a 15-cm depth with a standard 2.5-cm soil probe in a 2 × 30-m area in four portions of the plot. Soil samples for each area were pooled and assayed following a soil-debris isolation method (19), except that a modified Ko and Hora's medium (12) was used.

Cultures of *Rhizoctonia* (isolated from sugar beet seedlings, older roots, and from debris particles in soil) were transferred to water agar (WA) containing streptomycin sulfate (30 mg/L). After 1 or 2 days, each culture was hyphal-tipped to PDA and incubated at 20–24 C.

Identification and characterization. Characteristics of the septal pore apparatus and numbers of nuclei in vegetative cells were determined for each culture. Mycelia (2 or 3 days old) were stained with 0.5% safranin O and 3% KOH (28). All binucleate cultures also were stained with DAPI (4', 6'-diamidino-2-phenylindole) to verify nuclear condition (11). Stained mycelia were examined microscopically at 400–1,000×.

Anastomosis grouping. Cultures of *R. solani* were paired with known AG test cultures provided by Dr. Neil Anderson, Department of Plant Pathology, University of Minnesota, St. Paul (AG-1, 2-1, 2-2, 3, 4, 5), or by Dr. Akira Ogoshi, Hokkaido University, Sapporo, Japan (AG-1, 2-1, 2-2, 3, 4, 5, 6, 7, 8, BI). Cultures that did not anastomosis with these tester isolates also were tested against AG-9 provided by Dr. Donald Carling, University of Alaska, Palmer.

Pairings were made on sterile microscope slides coated with 2% WA, incubated at 20 C for 24–48 hr or until the hyphae started to overlap and intermingle, stained with lacto phenol-cotton blue, and microscopically examined for hyphal anastomosis (8,18). At least two slides were examined to verify each identification.

Storage. Cultures of *R. solani* and the binucleate cultures isolated during 1984–1985 were stored for 1 yr on PDA slants in an incubator at 5 ± 0.5 C. These cultures, and all those collected thereafter, subsequently were stored on sterilized barley grain at 5 ± 0.5 C (21) in tubes sealed with cigarette paper (25) and covered with stainless steel closures. Every year, cultures were transferred to a fresh tube of barley grain to retain viability.

Pathogenicity tests. Forty-three cultures of *Rhizoctonia* were selected from different geographic areas and seasons to represent each anastomosis group. These cultures were tested in the greenhouse for pathogenicity on sugar beet seedlings and on 8- to 9-wk-old plants. Five isolates of *R. solani* AG-1 were selected (three from seedlings and two from soil), one isolate of AG-2-1 (from a seedling), 10 isolates of AG-2-2 (five from seedlings, five from older plants), one isolate of AG-3 (from a seedling), 10 isolates of AG-4 (five from seedlings, five from older plants), six isolates of AG-5 (five from seedlings, one from an older plant), and five isolates of multinucleate *R. solani* that did not anastomosis with any of the known tester strains (from seedlings). Because cultures of binucleate *Rhizoctonia* also were isolated, five isolates from seedlings were included for comparison with cultures of *R. solani*.

Pathogenicity of cultures on sugar beet seedlings was tested by an inoculum layer technique (8,22). The soil mix consisted of field soil, sand, and peat moss (3:1:1, v/v/v) that was moistened and autoclaved for 1 hr on each of two consecutive days. Plastic pots (10 cm diameter) were lined with paper toweling, and 250 cm³ of soil was added and gently packed. A 6- to 7-day-old culture growing on 2% WA in a 9-cm-diameter petri dish was added and covered with 50 cm³ of soil. Twenty-five sugar beet seeds that had been dipped in 70% ethanol, placed in 1% NaOCl for 2–3 min, rinsed twice with sterile distilled water, and dried under a hood, were placed equidistantly on the soil surface. Then, 50 cm³ of soil was placed over the seed and gently packed. The control consisted of uncolonized WA added to soil, following the same procedures. A randomized complete block design was used with four replicates of each isolate. Seedling assays were conducted in a greenhouse at

26 ± 6 C, and photosynthetically active radiation measured 400 μmole·m⁻²·s⁻¹ for 16 hr daily. Stand counts were made 3 wk after planting, when the experiment was terminated. All isolates were tested in two greenhouse trials.

Pathogenicity of cultures of *Rhizoctonia* also was evaluated on older plants in the greenhouse. Plastic pots (15 cm diameter) were lined with paper toweling, and 850 cm³ of autoclaved soil mix (prepared as for the seedling test) was added and gently packed. Four sugar beet seeds (surface-treated as previously described) were placed in the center of the pot. An additional 250 cm³ of soil was placed over the seeds, and pots were watered. About 2 wk after planting, all but the most vigorous seedling were removed from each pot. Sugar beets were inoculated (8-wk-old plants in the first trial and 9-wk-old plants in the second trial) with cultures of *Rhizoctonia* that had grown on sterilized barley grain for 1 mo, following a procedure modified from Gaskill (4). Soil was scraped away from the sugar beet root to a depth of 2–2.5 cm with a sterilized spatula, and 2.5 cm³ of barley grain inoculum was placed in the hole in contact with the taproot. Soil was pushed back to the root surface, and an additional 250 cm³ of soil was added to cover the crown, a practice that favors disease development (23). A randomized complete block design was used with six replicates of each isolate (one plant/isolate/replicate). The control consisted of roots treated with sterilized barley grain. Greenhouse temperatures were 26 ± 6 C, and photosynthetically active radiation measured 400 μmole·m⁻²·s⁻¹ for 16 hr daily. Three weeks after the beets were inoculated, they were removed from pots, washed, and rated on a 0–7 scale (0 = healthy, 7 = 100% rot).

The same 43 cultures were tested on seedlings in the two greenhouse trials and on older plants in the first greenhouse trial. When the trial on older plants was repeated 6 mo later, two of the nonanastomosing multinucleate cultures had died and were not replaced with other cultures; five other cultures had died and substitutions were made (two cultures of AG-1, two of AG-2-2, and one binucleate).

For each culture tested, at least one dying seedling was collected, and for older plants, a 1-cm² piece was cut from each of three roots (with rotted or scurfy surfaces at point of inoculation). Seedling and root samples were surface-treated with 0.5% NaOCl for 15 sec, rinsed twice in sterile distilled water, drained on a paper towel, and placed on PDA. The resultant cultures were retested against the corresponding AG tester culture to verify the identity of the AG introduced into the soil or onto the beet root. For binucleate isolates, mycelia were stained and examined for number of nuclei.

Statistical analysis. Statistical analyses (analysis of variance and mean separations) were performed with the Statistical Analysis System (SAS Institute, Inc., Cary, NC). Arcsine transformations were performed on percent data. A significance level of *P* = 0.05 was used in all statistical tests.

RESULTS

Characterization of isolates. Of 361 cultures of *Rhizoctonia* isolated from sugar beet seedlings, 36 (10%) isolates were binucleate. These binucleate fungi were especially common in fields infested with *A. cochlioides*. In fields in west central Minnesota, where *A. cochlioides* is a severe pathogen, binucleates made up 32% of the cultures of *Rhizoctonia* isolated.

The remaining 325 cultures were multinucleate, showed characteristics typical of *R. solani*, and were identified to six anastomosis groups (Table 1). Of cultures of *R. solani* collected from seedlings in Red River Valley fields, AG-4 and AG-5 predominated, but from diseased seedlings grown in greenhouse and incubator assays of soil, AG-2-2, AG-4, and AG-5 predominated. Isolates of *R. solani* AG-5 commonly were obtained from fields in west central Minnesota that also were infested with *A. cochlioides*, but AG-5 isolates also were present in many fields in the Red River Valley where *A. cochlioides* was not detected. Cultures of AG-1 occasionally were isolated, and cultural characteristics corresponded to the IC population (17,24). Both AG-2-1 and AG-3 were isolated only once from seedlings. There were some typical isolates of *R. solani* that did not anastomose,

TABLE 1. Occurrence of anastomosis groups of *Rhizoctonia solani* isolated from diseased sugar beet seedlings collected from field soil assayed in the greenhouse or incubator, diseased seedlings collected in the field from 1985 to 1987, and from older plants collected in the field from 1984 to 1987

<i>Rhizoctonia</i>	Seedlings						Older plants ^d	
	Greenhouse & incubator				Field ^c		Field	
	WC-MN ^a		RRV ^b		RRV		RRV	
	No. cultures	%	No. cultures	%	No. cultures	%	No. cultures	%
AG-1	0	0	5	2.6	1	0.9	0	0
AG-2-1	1	3.6	0	0	0	0	0	0
AG-2-2	3	10.7	55	28.9	6	5.6	156	95.7
AG-3	0	0	1	0.5	0	0	0	0
AG-4	3	10.7	71	37.4	70	65	6	3.6
AG-5	16	57.1	49	25.8	23	21.5	1	0.6
Unidentified	5	17.9	9	4.7	7	6.5	0	0
Total No. Cultures	28		190		107		163	

^aCultures from diseased seedlings collected from field soils in west central Minnesota (WC-MN) that were assayed in the greenhouse or incubator.

^bCultures from diseased seedlings collected from field soils in the Red River Valley (RRV) that were assayed in the greenhouse or incubator.

^cCultures from field-collected diseased seedlings in fields in RRV.

^dCultures from field-collected diseased plants ≥ 8 wk old in fields in the RRV.

despite multiple pairings with the tester cultures (AG-1 through AG-9 and AG-BI).

Cultures isolated from roots of older plants were predominantly *R. solani* AG-2-2, but occasionally a culture of AG-4 was isolated (Table 1). An isolate of AG-5 was obtained once from a root that also was infected by *A. cochlidioides*.

Assay of soil collected in the sugar beet research plot yielded cultures of *R. solani* AG-1 IC. Population densities of these AG-1 isolates were one propagule per 100 g of air-dried field soil.

Pathogenicity tests on seedlings in greenhouse. Data for each anastomosis group and the binucleate group were analyzed separately. Bartlett's test determined that for each group, error variances for the two trials were homogeneous, so data were pooled for a combined analysis of variance. Percent stands presented in Figures 1 and 2 represent number of surviving plants 3 wk after planting/number of seeds planted.

All cultures of *R. solani* AG-4 (Fig. 1A) and AG-2-2 (Fig. 1B) were very pathogenic to sugar beet seedlings 3 wk after planting, regardless of origin (diseased seedlings or roots of older plants), and averaged 5 and 9% stand, respectively. Although data are not shown, it was observed that the overall percent emergence (number of seedlings emerged/number of seeds planted) about 1 wk after planting in soil infested with cultures of AG-4 averaged 8%, and in soil infested with cultures of AG-2-2 averaged 43%, compared with the untreated control soil, which averaged 91% emergence.

Cultures of *R. solani* AG-1 varied in severity of reducing sugar beet seedling stands 3 wk after planting, but all isolates were pathogenic compared with the control (Fig. 2A). Although data are not shown, the overall emergence of sugar beet seedlings about 1 wk after planting into soil infested with cultures of AG-1 averaged 62% (50% stand for the most pathogenic isolate and 76% stand for the least pathogenic isolate), and all isolates resulted in emergence significantly different from the control.

Cultures of AG-5 (Fig. 2B) and unidentified *R. solani* isolates (Fig. 2C) were not as pathogenic as AG-4, 2-2, or -1, but did reduce stands significantly (averaging 63 and 59%, respectively) 3 wk after planting compared with the control.

Only one isolate each of *R. solani* AG-2-1 and AG-3 were tested, so results are not given in a figure. The culture of AG-2-1 resulted in a stand of 61%, and AG-3 resulted in a stand of 75%, and both of these stands were statistically less than the control 3 wk after planting.

The binucleate isolates of *Rhizoctonia* resulted in stands that were less than, but not statistically different from, seedling stands in noninfested soil (Fig. 2D).

One to three dying seedlings were collected for each isolate tested. Cultures of *R. solani* that grew from these seedlings anastomosed with the corresponding known tester culture, and the unidentified isolates failed to anastomose with any of the known

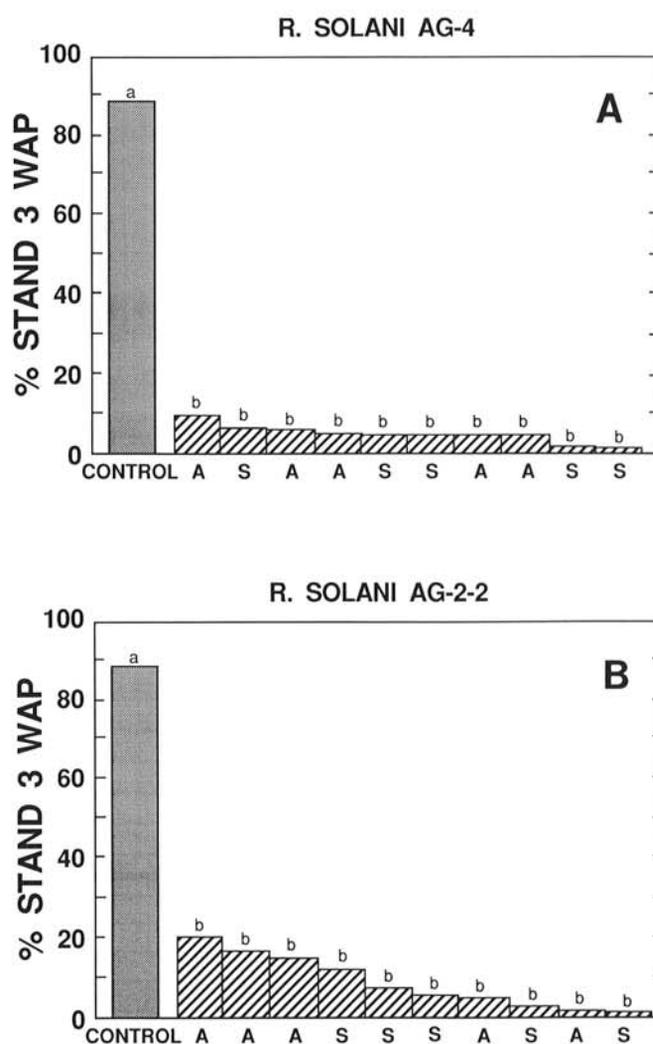


Fig. 1. Percent seedling stand of *Beta vulgaris* 'Maribo Ultramono' 3 wk after planting (WAP) seed into uninfested autoclaved soil (control) compared with autoclaved soil infested with cultures of A, *Rhizoctonia solani* AG-4 or B, *R. solani* AG-2-2. Each bar represents a culture that originally was isolated from a sugar beet seedling (S) or an adult plant (A). Bars with the same letter are not statistically different, $P = 0.05$, Student-Newman-Keuls' test.

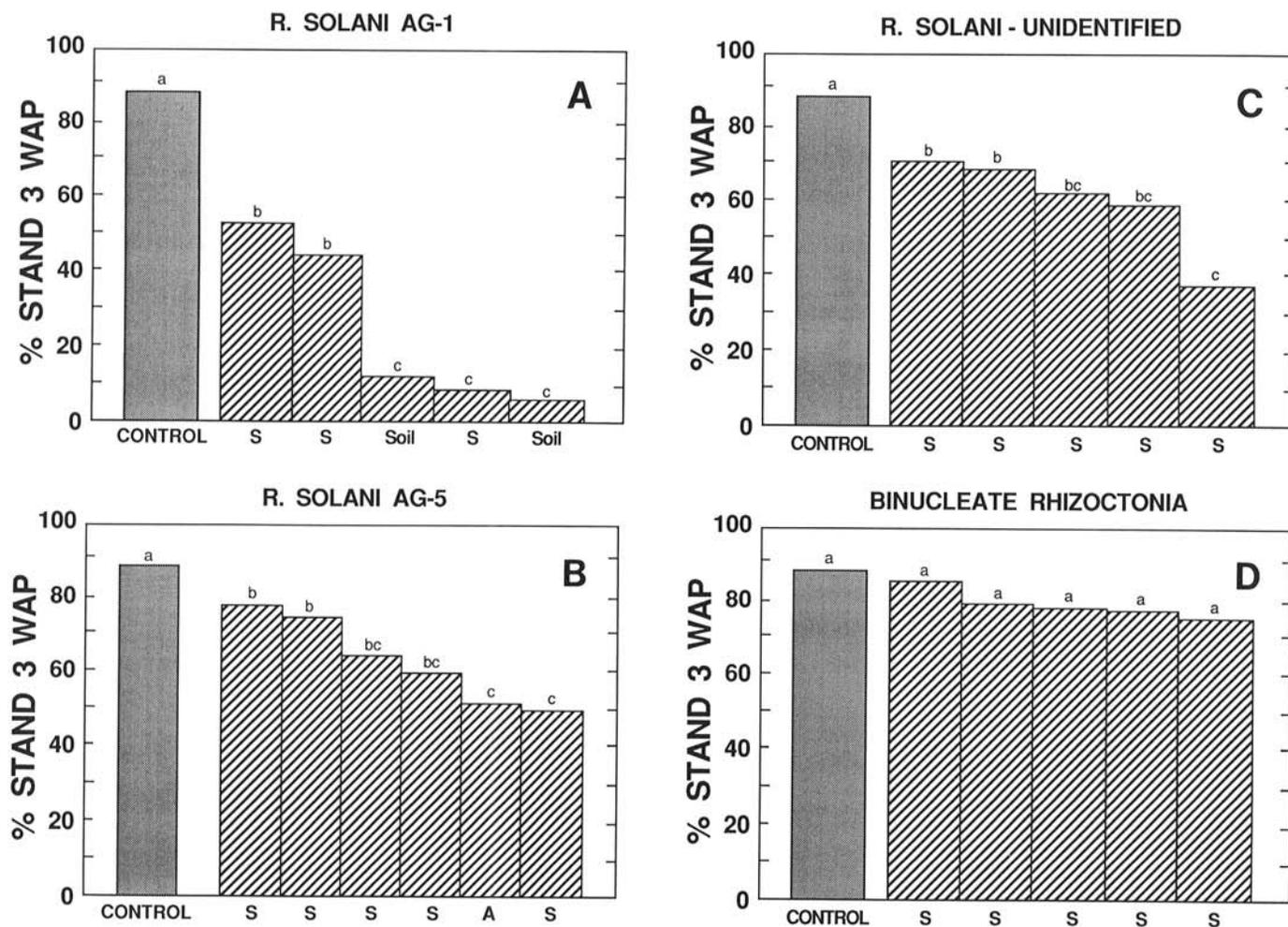


Fig. 2. Percent seedling stand of *Beta vulgaris* 'Maribo Ultramono' 3 wk after planting (WAP) seed into uninfested autoclaved soil (control) compared with autoclaved soil infested with cultures of A, *Rhizoctonia solani* AG-1, B, *R. solani* AG-5, C, unidentified *R. solani*, and D, binucleate *Rhizoctonia*. Each bar represents a culture that originally was isolated from a sugar beet seedling (S), an adult plant (A), or from soil. Bars with the same letter are not statistically different, $P = 0.05$, Student-Newman-Keuls' test.

tester cultures. The binucleate isolates of *Rhizoctonia* were confirmed as having two nuclei per vegetative cell. A few seedlings died in the uninfested control soil and yielded species of *Penicillium* and *Alternaria*, which occasionally are found on seed despite surface disinfection (C. E. Windels, unpublished).

Pathogenicity on older plants. Within each AG and the binucleate group there was very little variation among isolates or between the two trials. Therefore, overall means are presented in Figure 3. Cultures of *R. solani* AG-1, 2-1, 3, 5, unidentified isolates, and the binucleate isolates resulted in root rot index values ≤ 1 in both trials, and these roots were considered essentially healthy (6). A rating of 1 equals roots with scurfy, superficial, arrested lesions at the point of inoculation with no cankers, cracks, or rot. Control roots also resulted in low root rot index values, averaging 0.3 in the first trial and 0.7 in the second trial. The control showed some scurfiness on the root where soil had been scraped away from the surface and sterilized barley grain had been introduced. Isolation from control roots yielded species of *Alternaria*, *Penicillium*, and *Fusarium*, which likely were seed- or airborne secondary invaders.

All of the cultures of *R. solani* AG-2-2 were very pathogenic to sugar beet and resulted in an average root rot index value of 6.9 in both trials. Cultures isolated from sugar beet seedlings and adult plants were equally pathogenic.

Cultures of *R. solani* AG-4 resulted in an average root rot index value of 1.7 in both trials (Fig. 3). Separate analysis of variance for each trial (the 10 isolates of AG-4 compared with the control) resulted in a significant F test. Bartlett's test showed that the variances were homogeneous for both trials, so the data were

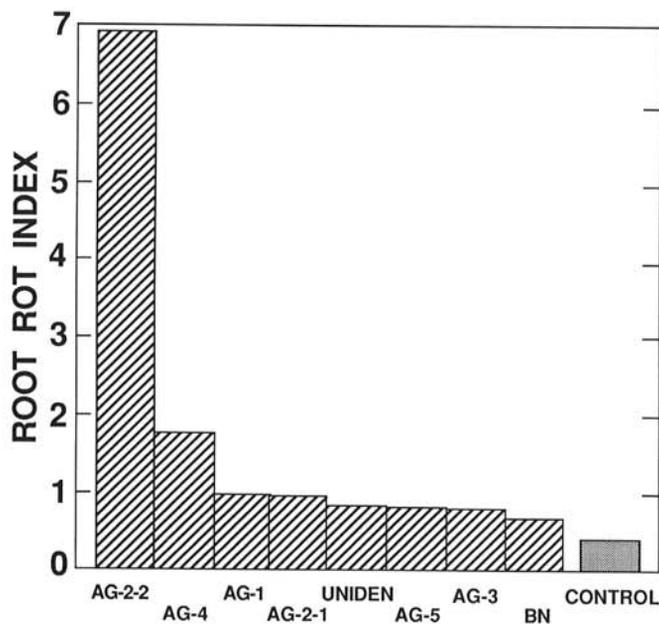


Fig. 3. Average root rot index values for the collective means of cultures of *Rhizoctonia solani* AGs, unidentified *R. solani* (uniden), and binucleate *Rhizoctonia* (BN) inoculated onto 8- to 9-wk-old roots of *Beta vulgaris* 'Maribo Ultramono' in greenhouse tests. Roots were evaluated 3 wk later on a 0-7 scale (0 = healthy, 7 = 100% rot).

pooled in the overall analysis. Although data are not shown, there were no statistical differences among the isolates of AG-4, but all isolates gave root rot index values (range:1.4-1.9) significantly greater than the control.

For each culture, isolations from roots at the end of the experiment were confirmed as belonging to the same group originally inoculated onto the root (with the exception of two isolates, where plates were contaminated with *Rhizopus* sp.).

DISCUSSION

The anastomosis groups of *R. solani* isolated from diseased seedlings in the Red River Valley and west central Minnesota expands the list of anastomosis groups previously reported on sugar beet in the United States from two (AG-2 and 4) to six (AG-1, 2-1, 2-2, 3, 4, and 5). Naito et al (13) reported isolating the same six populations of *R. solani* from diseased sugar beet seedlings in Japan. In both situations, isolations were made early in the season or in the greenhouse, which may favor isolation of a greater diversity of anastomosis groups. Also, as plants mature they may become less susceptible to infection by some anastomosis groups, as suggested by evidence in this study where plants ≥ 8 wk old usually were infected by *R. solani* AG 2-2. Some anastomosis groups may not be present in a field or geographic region, since *R. solani* is strongly influenced by other crops that are grown in rotations (1,17) or by soil texture (8).

The effect of AGs of *R. solani* on seedling stand establishment varied. Cultures of AG-4 were the most aggressive in causing preemergence damping-off compared with the other AGs. Isolates of both AG-1 and AG-2-2 caused considerable postemergence damping-off. These results (with the exception of the AG-1 results) agree with Ruppel (20) who found that significant seedling damping-off was caused by isolates of both AG-2 and AG-4. On the other hand, Herr and Roberts (8) concluded that AG-4 isolates (from soil and weeds) generally were more virulent to sugar beet seedlings than AG-2 isolates (from soil) collected in healthy and diseased portions of sugar beet fields.

The ability of AG-2-2 to cause severe root rot is supported by other studies (8,15,17). Our study found that none of the AG-4 isolates were as aggressive as AG-2-2 isolates. Studies in Ohio (8) showed that although AG-2 isolates generally were more virulent than AG-4 isolates in causing root rot of older plants, disease ratings of some isolates of each group overlapped. The most virulent isolates of AG-4 in the Ohio study came from well-aerated fine sandy loam soils where AG-4 populations predominated.

Incidence of *R. solani* AG-2-2 was relatively low in isolations made from seedlings in the field, but was more common from seedling assays of soil in the greenhouse or incubator. This difference may have occurred because soils assayed in the greenhouse or incubator often were collected from fields planted to beets where a problem was occurring. Thus, "biased sampling" of fields with stand problems likely occurred after the inoculum potential of *R. solani* AG-2-2 increased. Naito et al (13) reported that *R. solani* AG-2-2 rarely was isolated from sugar beet seedlings collected in the field.

R. solani AG-1 occasionally was isolated from sugar beet seedlings in this study, but in Japan it has been isolated from 20% of diseased seedlings (13). Moreover, AG-1 causes the early phase of foliage blight, which later is caused mainly by AG-2-2 in Hokkaido, Japan (14). In Ohio, AG-2-2 was the predominant population isolated from blighted sugar beet foliage (7), but AG-4 also has been reported to cause foliage blight (20). To date, symptoms of foliar blight have not been observed in this region, but the presence of AG-1, 2-2, and 4 suggest that under prolonged periods of high humidity, the potential exists.

The role of *R. solani* AG-5 as a pathogen of sugar beet seedlings is not understood. The fungus commonly was isolated from lesions on sugar beet seedlings and, in Japan, is reported to be isolated from about 50% of diseased seedlings (13). *R. solani* AG-5 is less aggressive than other soilborne pathogens of sugar beet, but it may be favored in fields where plants are stressed by other diseases or unfavorable environmental conditions. The fungus also has been

isolated from subcrown internodes of *Triticum aestivum* in the Red River Valley (a crop commonly rotated with sugar beets), but pathogenicity on wheat has not been determined (C. E. Windels, unpublished). In the majority of cases, AG-5 has been isolated from soil and species of Leguminosae (16,17). Isolates of AG-5 were not pathogenic on carrot and radish (5), but caused small, brown, sunken lesions on potato stems (2).

Failure to anastomose is frequent among isolates in AG-2 (18). However, the nonanastomosing, multinucleate isolates of *R. solani* in this study did not always have cultural characteristics typical of AG-2, nor were they as pathogenic as the AG-2-2 isolates. Isolates of *R. solani* that do not fuse with tester cultures, or with other identified isolates collected from the same field or region as the unknown, commonly are reported (8,16-18). According to Parmeter et al (18), multinucleate isolates that have all the typical characteristics of *R. solani* may represent isolates that are unable to anastomose, anastomosis may be extremely rare in some isolates, additional anastomosis groups may exist, or these isolates may be other species with mycelial characteristics similar to *R. solani*.

Binucleate fungi made up about 25% of the cultures of *Rhizoctonia* from diseased sugar beet seedlings in Japan (13), 5.5% of the isolates collected from sugar beet field soils in Ohio (8), and 10% of the cultures collected from seedlings in this study. Pathogenicity tests indicate that they can be nonpathogenic to mildly pathogenic to seedlings (8,13), and that they are not pathogenic on older roots (8).

In conclusion, there is a greater diversity of AGs of *R. solani* associated with diseased sugar beet seedlings than from rotted roots of older plants. Of these groups, AG-1,2-2, and 4 are aggressive pathogens on seedlings, whereas AG-2-2 is very pathogenic to roots of older plants.

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