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Comparative virulence of strains of *Rhizoctonia* spp. on leafy spurge (*Euphorbia esula*) and disease reactions of cultivated plants in the greenhouse[1]

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Abstract:

Six multinucleate and two binucleate strains of *Rhizoctonia* spp. pathogenic to the weed leafy spurge (*Euphorbia esula*) were compared in aggressiveness. Pathogenicity was tested by inoculating stems of leafy spurge or planting roots or seeds in soil infested with *Rhizoctonia* strains (8 cfu/g). Two multinucleate strains were significantly more virulent on roots of leafy spurge than the other strains. Eleven cultivated plant species were found to be susceptible to at least one of the eight *Rhizoctonia* strains, having mean disease ratings significantly different (P = 0.05) from those of control plants. Two or more strains caused significantly different mean disease ratings in eight of these host species, indicating that there was variation among strains. Four strains had equally broad host ranges of six plant species, but their respective host ranges were not identical. The two binucleate strains, which ranked lowest in overall aggressiveness to leafy spurge, also had relatively narrow host ranges of one and three species. The results indicate variation in aggressiveness to leafy spurge and in host range among strains of *Rhizoctonia* spp., from which optimum biocontrol strains may be selected for appropriate use.

Additional keywords:

Biological control.

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Leafy spurge (*Euphorbia esula* L.), a noxious perennial weed, infests approximately 1.4 million hectares of rangeland in the Northern Plains of the United States and western Prairie Provinces of Canada (3). Leafy spurge, introduced from Eurasia, is an aggressive, persistent species that is toxic to livestock and competes with native and desirable range species (11). Direct and secondary economic losses due to this weed were estimated to total $110 million in Montana, North Dakota, South Dakota, and Wyoming in 1990 (3). Research has indicated that chemical control measures are not economical (11), and therefore alternative means, such as biological control, are being sought. Pathogens of leafy spurge have been discovered, including strains of *Rhizoctonia solani* Kuhn anastomosis group 4 (AG-4) and a few binucleate *Rhizoctonia* species (5).

Samples collected in the summer of 1991 were tested for pathogenicity by inoculating leafy spurge stems and crowns and planting seed of leafy spurge in soil infested with the various strains of *Rhizoctonia* (5). In pathogenicity tests on leafy spurge, there were apparent differences in the extent of lesion expansion following stem inoculations, in the severity of root and crown rot of mature plants, and in rates of damping-off of seedlings (5). The objectives of the present study were 1) to compare aggressiveness to leafy spurge in six strains of *R. solani* and two strains of binucleate *Rhizoctonia* spp.; 2) to test strains of *Rhizoctonia* spp. pathogenic to leafy spurge, to determine their pathogenicity and comparative aggressiveness on several crops, including species that are of economic importance in the Northern Plains; and 3) to select strains that are the most potentially effective for biological control of leafy spurge and also have a narrow host range. *R. solani* AG-4 is considered to have a broad host range (2,10). It is possible, too, that strains of AG-4 may vary with regard to host range and aggressiveness (4).

Materials and methods

Inoculum preparation and storage of strains

Strains of *Rhizoctonia* spp. pathogenic to leafy spurge, described in an earlier study (5), were collected from several locations in the Northern Plains (Table 1). For storage and inoculum production for host range studies, cultures were grown on millet seed prepared as previously described (12). Sterile millet seed was inoculated with mycelial disks (9 mm in diameter) taken from the edge of 5-day-old cultures growing on acidified potato-dextrose agar (APDA). For storage, the inoculated millet was placed in small, sterile glass jars or sterile 26 × 150 mm test tubes (Sigma, St. Louis, MO) and kept at 20-25ºC. For pathogenicity tests, the prepared millet was placed in 30 × 61 cm autoclavable plastic bags (VWR Scientific, Seattle WA), autoclaved for 1 hour on two consecutive days, and inoculated with agar plugs as described above. The necks of the bags were sealed with foam plugs 35-45 mm in diameter (Curtin Matheson, Chicago, IL) and incubated at 25ºC for 4-7 days to allow mycelial colonization of the millet. The bag was frequently agitated to ensure that the millet seeds were thoroughly colonized.

Table 1. Origin and identification of strains of *Rhizoctonia* spp. pathogenic to leafy spurge

Strain designation	Geographic origin	Species and anastomosis group (AG)[z]
BB5E	Sidney, MT	*Rhizoctonia solani* AG-4
CC#2 5L	Cabin Creek, MT	*R. solani* AG-4
FB6J	Fort Benton, MT	*R. solani* AG-4
LS-CO	Meeker, CO	*Rhizoctonia* sp. (binucleate)
LYMCRK	Bozeman, MT	*R. solani* AG-4
MISS-M	Missoula, MT	*R. solani* AG-4
WSS-M	White Sulphur Springs, MT	*R. solani* AG-4
WSS sr	White Sulphur Springs, MT	*Rhizoctonia* sp. (binucleate)

[z]Field isolates paired with tester strains on water agar were assessed microscopically for anastomosis (12).

Reactions of 22 plant species to strains of *Rhizoctonia* spp. pathogenic to leafy spurge.

For host range studies, seeds of the various plant species were planted in individual pots (10.2 cm in diameter) in a steamed greenhouse soil mix composed of sphagnum peat, sand, and Bozeman silt loam (1:1:1, v/v), pH 6.6. Three to four weeks after planting, the plants were thinned to three per pot, and each treatment was applied to three pots.

The plants were grown in the greenhouse at 20-28ºC and watered uniformly at 3-day intervals. They were inoculated by carefully excavating a 3-cm^3 volume of soil adjacent to the crown and roots of the plants, filling the excavated volume with 3 cm^3 of colonized millet seed inoculum, and covering the inoculum with the soil mix (15). In the control treatment, 3 cm^3 of sterilized millet seed was placed adjacent to the crown and roots. Preliminary tests indicated that snap bean is uniformly susceptible to the AG-4 strains used in the study, and therefore it was used as a standard to assess the consistency of disease development throughout the host range studies.

Species inoculated were alfalfa (*Medicago sativa* L. cv. Nitro), artichoke (*Cynara scolymus* L. cv. Green Globe), barley (*Hordeum vulgare* L.), field corn (*Zea mays* L.), sweet corn (*Z. mays* cv. Golden Bantam), cowpea (*Vigna unguiculata* (L.) Walp. cv. California Blackeye), flax (*Linum usitatissimum* L.), mung bean (*Phaseolus aureus* Roxb. cv. Berken, syn. *Vigna radiata* (L.) R. Wilcz.), oats (*Avena sativa* L.), okra (*Hibiscus esculentus* L. cv. Clemson Spineless), peanut (*Arachis hypogaea* L. cv. Virginia Jumbo), rice (*Oryza sativa* L.), rye (*Secale cereale* L.), safflower (*Carthamus tinctorius* L.), snap bean (*Phaseolus vulgaris* L. cv. Blue Lake 274), sorghum (*Sorghum bicolor* (L.) Moench, soybean (*Glycine max* (L.) Merr., sugarbeet *(Beta vulgaris* L.), garden beet (*B. vulgaris* cv. Detroit Red), sunflower (*Helianthus annuus* L. cv. D131), wheat (*Triticum aestivum* L.), and zinnia (*Zinnia violacea* Cav. cv. Zenith Mixed).

Inoculated plants were placed in a dew chamber and incubated at 20-28ºC. Three to four weeks later, the plants were carefully removed from the pots, and roots and hypocotyls were rated for disease severity on a scale of 1-5, in which 1 = less than 2%, 2 = 2-10%, 3 = 11-50%, and 4 = more than 50% discolored and decayed tissue, and 5 = dead or dying plant. In the present study, a crop species was considered a host if the mean disease rating after planting in infested soil was significantly greater than that of the control (P =

0.05). The experiments were repeated twice. The pots were arranged in a completely randomized design. Data from the three experiments, from a total of 27 plants per strain, were pooled for analysis of variance ($P = 0.05$), and Waller and Duncan's exact Bayesian k ratio LSD rule was used to separate means. Results of pathogenicity tests on individual crops were analyzed to determine whether there were significant differences in the aggressiveness of the strains on each crop. The analyses of all data from the study were used to identify crops susceptible to the set of *Rhizoctonia* strains in our investigation and to determine the comparative aggressiveness of these strains on 1) individual crops, 2) crops grouped as members of Fabaceae, other dicots, and members of Gramineae, and 3) all crops tested.

Comparative aggressiveness of strains of *Rhizoctonia* spp. on leafy spurge

For root and crown inoculations, 6-month-old spurge roots were sterilized by soaking them in 0.5% sodium hypochlorite for 1 hour and then rinsing them for 1 hour in running tap water. The plants were left in the water overnight to dissipate the NaOCl and were either used immediately or stored in plastic bags at 4ºC for 1-5 days. Inocula of eight *Rhizoctonia* strains (FB6J, WSS-M, WSS sr, MISS-M, BB5E, CC#2 5L, LS-CO, and LYMCRK) were produced in a broth medium (9) consisting of 250 ml of peptone-sucrose-yeast extract (PSY) supplemented with frozen bean pods (14) for a total volume of 400 ml. The sterile medium was inoculated with mycelial disks (9 mm in diameter) taken from the edge of 5-day-old cultures growing on APDA and then incubated at 20 ± 5ºC as a stationary culture, with occasional shaking, for 10-14 days, at which time sclerotia and microsclerotia had formed. Mycelial mats were triturated in a blender for 30 seconds and thoroughly mixed into the greenhouse soil mix described earlier. After incubation for 6-10 days at 20-25ºC, populations of *Rhizoctonia* were determined by plating fourfold dilutions of soil on water agar amended with streptomycin and chloramphenicol (each at 100 µg/ml) (1) and processing the data to determine the most probable number (7) of colony-forming units (cfu) per gram of air-dried soil. Populations were adjusted by the addition of greenhouse soil mix to obtain average populations of 8 cfu of *Rhizoctonia* per gram of air-dried soil. *Rhizoctonia*-infested soil was placed in 10 2.5-L pots per strain and control treatment, with one healthy 6-mo-old spurge root planted in each pot. In the control treatment, leafy spurge roots were planted in pasteurized greenhouse soil mix amended with an amount of autoclaved beans and PSY medium equivalent to that used to culture the inocula of the *Rhizoctonia* strains. The treatments were completely randomized. The pots were watered every 3 days. The plants were harvested after 6 weeks in the greenhouse at 23-27ºC, and root, root bud, and hypocotyl disease severity were assessed on the disease rating scale described above. The experiment was repeated twice. Data from all three experiments, from a total of 30 plants per strain, were pooled and subjected to analysis using Waller and Duncan's exact Bayesian k ratio LSD rule.

For seedling inoculations, field-collected seed of leafy spurge was planted in flats of greenhouse soil, each of which was infested with 8 cfu of each of the *Rhizoctonia* strains per gram of air-dried soil. Three flats of infested soil were planted per treatment, with 50 seeds per flat. Three flats of greenhouse soil mix planted with 50 seeds per flat were used as the control treatment. The flats were incubated in the greenhouse at 23-27ºC for 3-4

weeks, at which time damping-off symptoms and the final stand per flat were assessed; the proportion of healthy seedlings was determined for each treatment. Infection was confirmed by isolation from seedlings and from seeds that failed to emerge, after they had been recovered by wet-sieving the soil 3-4 weeks after planting. The experiment was repeated twice, and data from all three experiments were pooled for statistical analysis using Waller and Duncan's procedure described previously.

For stem inoculations, mycelial disks (0.8 cm in diameter) from the margin of a colony of *Rhizoctonia* growing on APDA were inserted in incisions made near the bases of stems. Three shoots per pot were inoculated or used as controls, with three pots for each treatment. Inoculated shoots were covered with a plastic bag and incubated in the greenhouse at 23-27ºC for 10-14 days. The plants were then assessed for chlorosis, decay, and collapse of inoculated stems and rated on a scale of 1-5, in which 1 = less than 20%, 2 = less than 40%, 3 = less than 60%, and 4 = less than 80% affected tissue, and 5 = entire stem dead or dying. The controls consisted of wounded plants without further treatment and wounded plants with sterile agar disks placed in the incision. The pots were completely randomized. The experiment was repeated twice, and data from the three experiments were analyzed collectively by the Waller and Duncan procedure described earlier.

Results

Reactions of 22 cultivated species to strains of *Rhizoctonia* spp.

Eleven crops were found to be susceptible to at least one of the eight *Rhizoctonia* strains tested. These were alfalfa, artichoke, garden beet, mung bean, oats, okra, peanut, safflower, snap bean, soybean, and sugarbeet. Cowpea and sunflower exhibited disease symptoms, but the analyses of variance failed to indicate significant differences from the controls (data not shown). Additionally, there were significant differences in aggressiveness of two or more of the *Rhizoctonia* strains on eight of these crops (Table 2). The two binucleate strains, LS-CO and WSS sr, had narrow host ranges, causing significant disease on one and three species, respectively. Four of the strains had host ranges of equal breadth, causing significantly greater mean disease ratings in six crops than in the control. However, the host ranges of these strains were not identical.

Data pooled from all tests indicate that the most virulent strain was CC#2 5L, which caused a mean disease severity rating (over all 22 species) of 2.1 (Table 3). This was followed, in descending order, by strains MISS-M, BB5E, FB6J, LYMCRK, and WSS-M. Strain WSSM was significantly less virulent than CC#2 5L. The two binucleate strains, LSCO and WSS sr, were the least virulent. The mean disease ratings for crops grouped as members of Fabaceae, members of Gramineae, and other dicots were 1.6, 0.7, and 1.9, respectively. The mean disease ratings for each crop group were significantly different ($P = 0.05$).

Table 2. Disease ratings of various crop species due to strains of *Rhizoctonia* spp. from leafy spurge[y,z]

Rhizoctonia strain	Alfalfa	Artichoke	Garden beet	Mung bean	Oats	Okra	Peanut	Safflower	Snap bean	Soybean	Sugar beet
CC#2 5L	2.1 a	4.5 a	2.0 bc	2.8 ab	0.3 b	4.0 a	3.3 abc	3.0 ab	2.4 a	4.0 a	2.5 ab
FB6J	2.0 a	...	2.5 ab	2.8 ab	0.7 b	3.1 ab	4.0 ab	1.5 abc	2.1 ab	3.2 ab	3.0 a
LYMCRK	1.7 a	5.0 a	2.0 bc	2.7 ab	0.7 b	4.3 a	1.5 bc	3.1 ab	2.0 ab	1.5 bcd	2.3 ab
MISS-M	1.7 a	2.3 ab	4.0 a	3.0 ab	3.7 a	4.0 a	3.0 abc	2.3 abc	2.8 a	2.0 bcd	3.0 a
WSS-M	1.7 a	1.0 b	1.7 bcd	2.3 ab	0.7 b	3.0 ab	1.3 bc	3.3 a	2.0 ab	2.3 abcd	1.4 ab
BB5E	1.7 a	3.0 ab	3.0 ab	4.3 a	0.7 b	3.7 a	3.0 abc	2.9 abc	2.4 a	2.5 abc	2.5 ab
LS-CO	1.8 a	2.0 ab	0.0 d	1.1 b	0.0 b	2.0 ab	4.0 ab	1.1 bc	2.0 ab	2.0 bcd	2.3 ab
WSS sr	1.3 ab	2.0 ab	0.0 d	3.0 ab	0.0 b	3.0 ab	5.0 a	1.1 bc	1.0 bc	1.0 cd	1.0 b
Control	0.0 b	1.0 b	0.4 cd	1.0 b	0.0 b	0.9 b	0.3 c	0.8 c	0.5 c	0.6 d	1.0 b

[y]Disease ratings based on a scale of 1-5, in which 1 = less than 2%, 2 = 2-10%, 3 = 11-50%, and 4 = more than 50% discolored and decayed tissue, and 5 = dead or dying plant. Readings were taken 3-4 weeks after inoculation and incubation at 20-28°C in a dew chamber. Means are based on 27 plants inoculated with each strain over three experiments.
[z]For each crop species, means followed by the same letter are not significantly different at $P = 0.05$, by Waller and Duncan's exact Bayesian *k* ratio LSD rule. Multiple comparisons were among strains on a single species.

Table 3. Comparative virulence of strains of *Rhizoctonia* spp. from leafy spurge on various crop species.

Rhizoctonia strain	Mean disease ratings by crop category[x,y]			Cumulative mean disease rating[s]
	Legumes	Other dicots	Gramineae	
CC#2 5L	3.1 a	2.7 a	1.0 ab	2.1 a
MISS-M	2.4 abc	2.5 ab	1.1 a	2.0 a
BB5E	2.8 b	2.3 ab	0.9 abc	1.9 ab
FB6J	2.4 abc	2.3 ab	1.0 a	1.9 ab
LYMCRK	2.4 abc	2.8 a	0.9 abc	1.9 ab
WSS-M	1.9 bc	1.9 bc	0.8 abcd	1.5 bc
LS-CO	2.2 abc	1.6 c	0.4 bcd	1.3 c
WSS sr	1.8 c	1.5 c	0.3 d	1.2 c
Control	0.6 d	0.7 d	0.4 cd	0.6 d

[x]Disease ratings based on a scale of 1-5, in which 1 = less than 2%, 2 = 2-10%, 3 = 11-50%, and 4 = more than 50% discolored and decayed tissue, and 5 = dead or dying plant. Readings were taken 3-4 wk after inoculation and incubation at 20-28ºC in a dew chamber. In each crop category, means followed by the same letter are not significantly different at $P = 0.05$, by Waller and Duncan's exact Bayesian *k* ratio LSD rule.
[y]Average of disease ratings for crops classed as legumes (alfalfa, cowpea, mung bean, peanut, snap bean, and soybean), other dicotyledonous crops (artichoke, okra, garden beet, sugarbeet, and zinnia), and Gramineae (field corn, sweet corn, oats, rice, rye, sorghum, and wheat), inoculated with strains of *Rhizoctonia* spp. Means for individual crops are based on three separate trials.
[z]Cumulative mean disease ratings for all 22 crop species included in the host range study. Means followed by the same letter are not significantly different at $P = 0.05$, by Waller and Duncan's exact *k* ratio LSD rule.

Comparative aggressiveness to leafy spurge

Only one *Rhizoctonia* strain was significantly different from the others in aggressiveness to leafy spurge (Table 4). The AG-4 strain BB5E was the most virulent on stems, giving a mean disease rating of 3.3, which was significantly greater than that due to the binucleate strain LS-CO. Two strains (LYMCRK and BB5E) were more virulent to roots of leafy spurge than the other strains, causing mean disease ratings of 3.9 and 3.8, respectively (Table 4). Six strains (LS-CO, BB5E, CC#2 5L, WSS sr, and MISS-M) caused significantly more severe seedling disease than was observed in the control, with proportional final stands ranging from 0.020 to 0.065, compared to 0.140 for the control.

Table 4. Comparative virulence of *Rhizoctonia* strains in stem, root, and seedling disease of leafy spurge.

	Disease ratings[u]		
Treatment	Root and crown[v]	Stem[w]	Proportional seed stand[x]
LYMCRK	3.9 a	2.6 ab	0.080 abcd
BB5E	3.8 a	3.3 a	0.044 ed
CC#2 5L	3.1 b	2.8 ab	0.044 cd
WSS-M	3.0 b	2.4 ab	0.065 bcd
FB6J	3.0 b	2.4 ab	0.095 abc
MISS-M	2.9 b	2.9 ab	0.060 bcd
WSS sr	… [y]	2.3 ab	0.046 cd
LS-CO	... [y]	2.1 b	0.020 d
Control (bean-amended greenhouse soil mix)	1.0 c	…	0.110 ab
Control	0.3 d	0.1[z] c	0.140 a

[u]In each column, means followed by the same letter are not significantly different (P = 0.05), by Waller and Duncan's exact Bayesian *k* ratio LSD rule. Disease severity was assessed in 27-30 plants per strain, over three experiments.
[v]Disease ratings recorded 6 weeks after leafy spurge crowns were planted in greenhouse sod mix amended with 8 cfu per gram of soil, based on a scale of 1-5, in which 1 = less than 2%, 2 = 2-10%, 3 = 11-50%, and 4 = more than 50% discolored and decayed tissue, and 5 = dead or dying plant.
[w]Chlorosis, decay, and collapse of inoculated stems rated on a scale of 1-5, in which 1 = less than 20%, 2 = less than 40%, 3 = less than 60%, and 4 = less than 80% affected tissue, and 5 = entire stem dead or dying.
[x]Proportion of seedlings that had emerged 4 wk after seeds were planted in greenhouse soil mix infested with *Rhizoctonia spp.* Means followed by the same letter are not significantly different (P = 0.05), by Waller and Duncan's exact Bayesian *k* ratio LSD rule.
[y]Strains WSS sr and LS-CO were not included in these tests because a previous study showed that they are not pathogenic to roots or crowns of leafy spurge (5).
[z]Includes disease ratings pooled from two control treatments used in stem inoculation studies. Controls were plants with longitudinal stem incisions without further treatment and plants with incised stems into which sterile agar disks were placed.

Discussion

The results of this study show that there is considerable variation in the aggressiveness of eight strains of *Rhizoctonia* spp. on leafy spurge and other hosts. Strains highly virulent to roots of leafy spurge caused significantly greater mean disease ratings in six crop species. However, the range of susceptible crop species was different for each strain.

The strains of *Rhizoctonia* in this study can be grouped in three classes based on host range and aggressiveness to leafy spurge. One class of strains (WSS-M, LS-CO, and WSS sr) caused the lowest mean disease ratings in all 22 crop species and had host ranges of three, three, and one species, respectively. Another class (MISS-M and CC#2 5L) caused high overall disease ratings in crops and exhibited low aggressiveness to leafy spurge. A third class of strains (BB5E and LYMCRK) caused high disease ratings in several crops and exhibited high aggressiveness to roots of leafy spurge.

In weighing risks to important crop species and potential benefits to weed control by *Rhizoctonia*, desirable biological control strains might best be chosen from the first and third classes. Strains that cause disease on major crops are to be avoided as biological control agents. According to such criteria derived from these findings, some *Rhizoctonia* strains would be discarded as potential biological control agents of weeds based on single traits. An example of such a strain is MISS-M, which was the sole strain causing significant disease in a graminaceous crop (oats). Obviously, such a strain should not be utilized for the biological control of leafy spurge in areas adjacent to oat fields.

The variation in the host ranges of *Rhizoctonia* strains indicates that there is a choice in selecting suitable strains when and where risks to specific crops exist. Most crops found to be relatively susceptible to *Rhizoctonia* spp. in the present study, including artichoke, mung bean, okra, and peanut, are not grown in the Northern Plains. Major susceptible crops grown in the Northern Plains include safflower, soybean, snap bean, and sugarbeet. Sugarbeet (15) and several other susceptible crops grown in the Northern Plains, e.g., potato, canola, and corn, are equally or more seriously affected by anastomosis groups other than AG-4 (6,8,13). Studies in Montana on leafy spurge (5) and on barley by others (J. Hudak, personal communication) suggest that AG-4 is endemic in soils in that state. Thus, the application of *Rhizoctonia* as a biological control agent would not necessarily introduce a new pathogen. Furthermore, in several states in the Northern Plains, spurge infestations occur in rangelands that are distant from cropped areas. Nonetheless, there is a need to determine whether strains of *Rhizoctonia* would have more than a negligible effect on pathogen populations where cropped fields are adjacent to areas infested with leafy spurge.

The severity of disease caused by strains of *Rhizoctonia* spp. observed on leafy spurge in the field did not always correlate with aggressiveness rankings in greenhouse studies (A. Caesar, unpublished data). This suggests that other factors modify disease severity due to *Rhizoctonia* on leafy spurge, such as the presence of root-feeding insect biological control agents such as *Aphthona flava* Guill. (11), plant-deleterious microorganisms acting as synergists, and microbial antagonists. As an example of microbial antagonism, strain BB5E was ranked high in aggressiveness to leafy spurge in this study, but the consistent presence of *Erwinia herbicola* in the vascular tissues and rhizosphere of leafy spurge apparently suppresses *Rhizoctonia* in the field (A. Caesar, unpublished data). Such aspects of *Rhizoctonia*-leafy spurge ecology are the subject of current research by the author, and knowledge attained thereby could lead to the development of methods and strategies for optimizing the use of *Rhizoctonia* spp. in the biological control of leafy spurge.

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