Characterization of *Rhizoctonia* spp. Causing Disease of Leafy Spurge in the Northern Plains

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ABSTRACT

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Diseases of leafy spurge (*Euphorbia esula*) occurred widely in Montana and were also found in Colorado and North Dakota during the spring and summer of 1991. *Rhizoctonia solani* AG-4 was isolated from mature leafy spurge plants showing crown rot and necrotic root buds, whereas binucleate *Rhizoctonia*-like fungi were isolated from stem cankers and blights. Pathogenicity of the *Rhizoctonia* strain to leafy spurge was tested by inoculating cut stems or by planting mature crowns or seed in a greenhouse soil mix artificially infested with the fungi. Five of six *R. solani* AG-4 strains, but only two of eight binucleate strains, were pathogenic on stems. All strains of *R. solani* AG-4 caused root and crown rot and preemergence damping-off of spurge, and four of eight binucleate strains induced damping-off. All *R. solani* AG-4 strains were from Montana.

Leafy spurge (*Euphorbia esula* L.) is an aggressive and persistent noxious weed that has infested millions of hectares in the Northern Plains of the United States and the Prairie Provinces of Canada since it was first reported in the United States in 1827 (19). The weed is native to Eurasia, where it is typically found along roadsides and in disturbed areas. Leafy spurge displaces native grasses and other desirable plants in rangelands and produces a milky latex that is a gastric irritant to cattle. Economic losses resulting from decreased land values and reduced crop yields in states with heavy infestations of the weed are estimated to be in the range of $76 million per year (4). The urgency of the leafy spurge problem, as well as the expense and relative ineffectiveness of chemical controls, has led to investigations of the biological control of this weed with plant pathogens.

Coincident with heavy rains in the spring and summer of 1991 in Montana and other states in the Northern Plains were widespread occurrences of disease on leafy spurge. Symptoms ranged from stem cankers and tip dieback to crown rots and acceleration of ongoing stand declines in certain locations. The most prevalent microorganisms consistently associated with diseased tissues were *Rhizoctonia*-like fungi.

Our objective is to describe the range of occurrence, pathogenicity, growth rate in vitro, and nuclear condition and identity of *Rhizoctonia* species isolated from tissues of leafy spurge plants collected in the Northern Plains.

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MATERIALS AND METHODS

Isolation and characterization of *Rhizoctonia* spp. We obtained the *Rhizoctonia* spp. by regular visits to areas infested with leafy spurge and by requesting county, state, and federal weed control personnel to make random surveys for diseased leafy spurge. Twelve samples were obtained this way, and *Rhizoctonia* was isolated from diseased plants of leafy spurge from eight different locations in Montana, Colorado, and North Dakota. At each of the eight sites, leafy spurge showed symptoms of either a stem rot at the soil line or dead root buds. At five of the eight locations, shoots of leafy spurge with dark-brown, water-soaked lesions or necrotic cankers were also collected.

Affected portions of diseased plants from all locations were selected and washed thoroughly in running tap water for 20–30 min and then wiped vigorously with a piece of sterile cotton saturated with 0.5% NaOCl. Diseased tissue was excised with a sterile scalpel, placed on acidified potato-dextrose agar (APDA), Nash and Snyder medium (15) to isolate *Fusarium* spp., PVP (12) and PARP (9) media to isolate pythiaceous fungi, and a medium consisting of 2% water agar amended with 100 μg/ml each of streptomycin and chloramphenicol to isolate *Rhizoctonia* spp. (1). Culture plates were placed in the dark at 20–28 C.

Cultures were examined microscopically, and those with branching at right angles and constrictions at the branch points or at the septa were further characterized. Cultures of apparent *Rhizoctonia* spp. were hyphal-tipped and transferred to APDA.

Fourteen *Rhizoctonia* isolates from diseased leafy spurge were assessed for ability to anastomose with 12 strains representing anastomosis groups IA, IB, 2-1, 2-2, 3, 4, 5, 6, 7, 8, 9, and BI of *Rhizoctonia solani* Kühn. Field isolates and tester strains were paired on plates of 2% water agar by placing agar plugs removed from the margins of actively growing colonies about 2.5 cm apart and assessing them for anastomosis (17). Intermingled hyphae of anastomosis pairings were stained with methyl blue/lactophenol to aid in visualizing anastomosis. Isolates that failed to anastomose with any of the tester strains were stained for 5–10 min with a 1 μg/ml solution of 4′,6-diamidino-2-phenylindole (DAPI) (20) to determine the number of nuclei per vegetative cell.

To aid identification of binucleate *Rhizoctonia* spp. and confirm anastomosis tests, induction of the perfect stage of *Rhizoctonia* spp. was attempted. Two methods were utilized. In the first, strains were cultured on potato-yeast extract-marmite agar (14) for 3 days and then transferred to tap-water agar for 12 days at 25 C. In the second method, the strains were cultured on a modified potato-yeast extract agar medium until the colonies reached the edge of the plates. The plates were then covered with air-dried field soil (20) and incubated at 20–25 C for 10–14 days. The respective native soil was used for each strain of *Rhizoctonia* spp.

Pathogenicity tests. For pathogenicity tests with infested soil, inocula of the 14 *Rhizoctonia* strains were produced on a medium based on two previously described methods. Frozen bean pods (22) were added to 250 ml of a peptone-sucrose-yeast extract (PSY) broth (11) to establish a total volume of 400 ml. The sterile medium was inoculated with 9-mm-diameter disks of mycelia transferred from the edge of 5-day-old cultures growing on APDA and then incubated at 20 ± 5 C for 10–14 days to allow sclerotia and microsclerotia to form.

Mycelial mats were triturated with an electric blender for 30 sec and thoroughly mixed into pasteurized greenhouse soil mix (about 33% each of sphagnum peat, sand, and Bozeman silt loam), pH 6.6. The infested soil was incubated at 20–25 C for 6–10 days, then populations of *Rhizoctonia* were determined. Data obtained by fourfold dilutions were processed to estimate the most probable number (7) of colony-forming units per gram of air-dried soil. Populations were adjusted by the addition of greenhouse soil mix to establish average populations of 8 cfu of *Rhizoctonia* per gram of air-dried soil. *Rhizoctonia*-infested soil was used to fill 10 2.5-L pots per strain, and one healthy 6-mo-old spurge root was planted per pot. Controls were spurge roots planted in either pasteurized greenhouse soil mix amended with sterile, uninoculated bean/PSY media or unamended soil. Plants were harvested and assessed for root lesions and necrotic root buds after 6 wk in the greenhouse at 23–27 C.

In a second pathogenicity test, greenhouse soil infested with each of the strains was used to fill three flats per treatment, and 50 untreated, field-collected seeds of leafy spurge were planted per flat. Seed was collected from mature seed heads of healthy spurge plants. Controls were flats of greenhouse soil mix amended with sterile, uninoculated bean/PSY medium or unamended soil mix planted with 50 seeds per flat, three flats per treatment. Flats were incubated at 23–27 C for 3–4 wk in the greenhouse. Treatments were assessed for damping-off symptoms and by isolation from seeds that failed to emerge after recovering seed by wet-sieving the greenhouse soil mix 3–4 wk after planting.

In a third pathogenicity test, incisions were made near the base of 9-wk-old stems of leafy spurge, and an 0.8-cm-diameter disk of mycelium from the margin of a colony of *Rhizoctonia* growing on APDA was inserted into the incision. Inoculated shoots were covered with a plastic bag and incubated in the greenhouse at 23–27 C for 10 days. Plants were then assessed for chlorosis, decay, and collapse of inoculated stems. Controls consisted of wounded plants without further treatment or wounded plants with sterile agar disks placed in the incisions.

Tissue of leafy spurge from pathogenicity tests as well as asymptomatic tissue from appropriate controls were plated on either the antibiotic-amended water agar or Ko and Hora medium (10). All pathogenicity tests were repeated at least once.

Growth rates in vitro. Radial growth rate of 14 strains of *Rhizoctonia* were determined at 20, 25, and 28 C. A 0.4-cm-diameter mycelial plug was transferred to APDA from the margin of a 7-day-old colony. Colony radius was measured at 24-hr intervals until the

Table 1. Origin, field symptoms, results of greenhouse pathogenicity tests, and identity of *Rhizoctonia* spp. isolated from leafy spurge

	Field symptoms			Pathogenicity in greenhouse tests			
Strain, origin	Dead root buds[t]	Crown rot[u]	Stem rot[v]	Root and crown rot[w]	Stem lesions[x]	Damping-off[y]	Identity of *Rhizoctonia* spp.[z]
Cabin Creek No. 2 5L, Montana	+	−	−	+	+	+	AG-4
Ft. Benton 6J, Montana	+	+	−	+	+	+	AG-4
Lyman Creek, Montana	−	+	−	+	−	+	AG-4
Missoula, Montana	+	+	−	+	+	+	AG-4
Sidney, Montana	+	+	−	+	+	+	AG-4
White Sulphur Springs, Montana	+	+	−	+	+	+	AG-4
Cabin Creek, AN1-2, Montana	−	−	+	−	−	−	Binucleate
Cabin Creek, CC1, Montana	−	−	+	−	−	+	Binucleate
Cabin Creek site 1, Montana	−	−	+	−	−	−	Binucleate
Colorado	−	+	+	−	+	+	Binucleate
Ft. Benton 5K, Montana	−	−	+	−	−	−	Binucleate
Missoula-2, Montana	−	−	+	−	−	+	Binucleate
North Dakota	−	−	+	−	−	−	Binucleate
White Sulphur Springs sr, Montana	−	−	+	−	+	+	Binucleate

[t] Blackened or browned with internal necrosis extending a few millimeters from the tips of the root buds.
[u] Girdling near the soil line or internal vascular necrosis extending from the crown area.
[v] Blackened, dry, necrotic cankers or water-soaked, brown stem lesions.
[w] Symptoms observed 6 wk after healthy 6-mo-old spurge roots were planted in *Rhizoctonia*-infested greenhouse soil mix. Plants were harvested and assessed for root lesions and necrotic root buds after incubation at 23–27 C.
[x] Chlorosis, decay, and collapse of stems 10 days after inoculation near bases of 9-wk-old stems. Disks of mycelium from the margin of a colony of *Rhizoctonia* growing on APDA were inserted into stem incisions, and shoots were covered with a plastic bag and incubated at 23–27 C.
[y] Pre- and postemergence damping-off was determined after untreated, field-collected seeds of leafy spurge were planted in flats of greenhouse soil infested with each strain. Flats were incubated at 23–27 C for 3–4 wk in the greenhouse, then damped-off seedlings and unemerged seeds were recovered and plated on water amended with 100 μg/ml each of streptomycin and chloramphenicol (1).
[z] AG-4 = *Rhizoctonia solani* AG-4; binucleate = *Rhizoctonia*-like species.

colonies reached the edge of the plate. Three plates per strain at each of the temperatures were assessed, and the experiment was repeated twice. Data were analyzed using the Waller-Duncan exact Bayesian *k*-ratio LSD rule to compare mean radial growth for each strain at each temperature.

RESULTS

Several fungi were isolated from diseased leafy spurge, including *Pythium* spp., *Phoma* spp., and *Fusarium* spp. The species most consistently isolated from diseased leafy spurge during the summer of 1991 in the Northern Plains were *Rhizoctonia* spp.

All multinucleate strains were from Montana and anastomosed with the AG-4 tester strain (Table 1). Five of six AG-4 strains (Ft. Benton 6J, White Sulphur Springs, Missoula, Sidney, and Cabin Creek No. 2 5L) infected and caused girdling of stems. Symptoms and signs of infection by pathogenic *Rhizoctonia* spp. were usually readily evident within 48 hr after inoculation. Symptoms were chlorosis and necrosis proximal and distal to the point of inoculation, resulting in complete girdling and collapse of stems (Fig. 1A). Signs were strands of pigmented mycelia visible at the point of inoculation and later over all necrotic plant tissue. Inoculation of stems with nonpathogenic *Rhizoctonia* spp. failed to result in more than limited growth of the fungus on cut surfaces (Fig. 1B).

All six AG-4 strains stunted and killed root buds and caused damping-off (Table 1). Root bud infection was evident by blackened or brown tips of buds, with necrosis distal to the tips. Infections occasionally expanded into the root cortex. Root buds of leafy spurge plants grown in unamended greenhouse soil or greenhouse soil to which nonpathogenic strains were added were a healthy pink. Two of eight binucleate strains (White Sulphur Springs sr and Colorado) caused disease on leafy spurge plants with inoculated stems, and four (Missoula-2, Colorado, White Sulphur Springs sr, and Cabin Creek CC1) caused damping-off (Table 1). Each strain of *Rhizoctonia* that caused disease in greenhouse pathogenicity tests was reisolated from diseased tissue.

The growth rates of leafy spurge strains at 20, 25, and 28 C fell into three separate response categories (Table 2). Two strains, North Dakota and Cabin Creek AN1-2, had higher growth rates at 20 C, with progressively lower growth rates at the higher temperatures. Five strains—Missoula, Ft. Benton 5K, White Sulphur Springs sr, Colorado, and Cabin Creek No. 2 5L—had optimum growth rates at 25 C. Growth rates of the Sidney strain were equal at 25 and 28 C. Strains in which growth rates increased progressively with temperature were Cabin Creek CC1, White Sulphur Springs, Lyman Creek, Missoula-2, Cabin Creek site 1, and Ft. Benton 6J.

Attempts to produce the perfect stages of the various isolates failed.

DISCUSSION

To our knowledge, this is the first detailed description of a disease of *E. esula* caused by *Rhizoctonia* spp. Our identification of the pathogenic strain as *R. solani* AG-4 agrees with a previous study where AG-4 caused disease of *E. pulcherrima* Willd. ex Klotzsch (18). A survey of fungi isolated from leafy spurge in Canada listed two strains of *R. solani* as pathogenic to leafy spurge, but no further details were given (13).

We observed, but did not measure, differences among strains in the rapidity of lesion progression and modes of pathogenicity under identical conditions. Those observations indicate the need for more detailed studies of comparative virulence of the multinucleate strains.

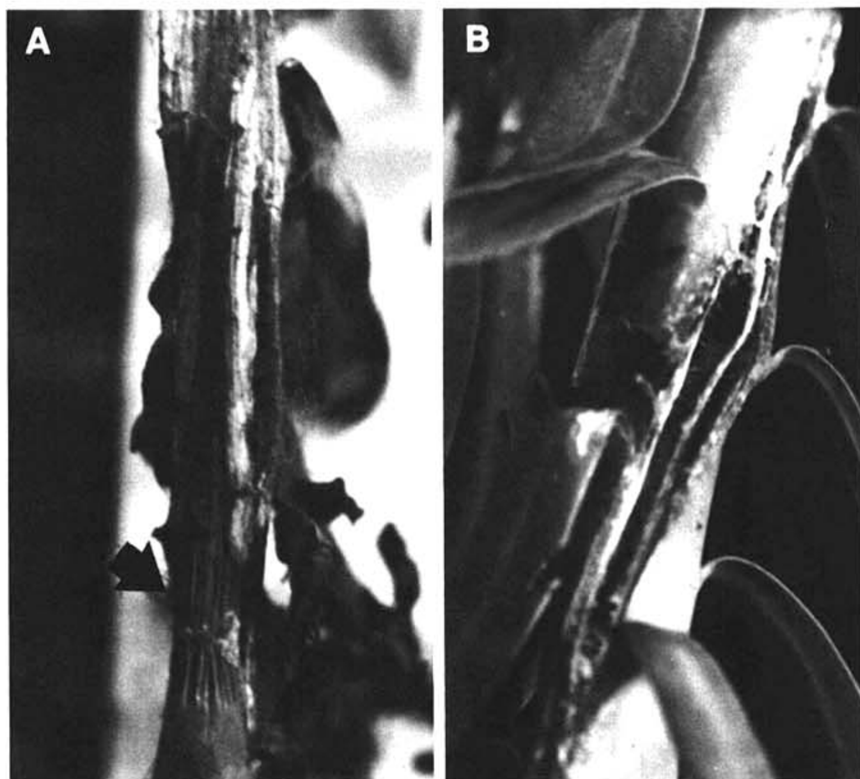

Fig. 1. Stems of *Euphorbia esula* inoculated with **(A)** a strain of *Rhizoctonia solani* AG-4 that caused girdling of stems (arrow) and profuse mycelial growth and **(B)** a nonpathogenic strain of *Rhizoctonia* sp. that produced no symptoms. Stems of *E. esula* were inoculated by placing agar disks taken from the edge of actively growing cultures into incisions. Mycelia of nonpathogenic strains grew on the cut surfaces of these incision sites.

Table 2. Radial growth rate of *Rhizoctonia* sp. on potato-dextrose agar at three temperatures

	Radial growth (cm)[y]		
Strains	**20 C**	**25 C**	**28 C**
Binucleate			
Cabin Creek AN1-2	0.18 e[z]	0.12 ef	0.13 fg
Cabin Creek CC1	0.51 a	0.57 d	0.80 b
Cabin Creek site 1	0.09 f	0.23 e	0.32 e
Colorado	0.09 f	0.21 e	0.10 g
Missoula-2	0.36 d	1.06 a	1.11 a
North Dakota	0.19 e	0.04 f	0.03 h
White Sulphur Springs sr	0.10 f	0.14 ef	0.05 h
Ft. Benton 5K	0.11 f	0.17 e	0.15 fg
Multinucleate (AG-4)			
Cabin Creek No. 2 5L	0.43 bc	0.80 b	0.67 c
Ft. Benton 6J	0.46 ab	0.75 b	0.75 bc
Lyman Creek	0.37 cd	0.62 cd	0.73 bc
Missoula	0.36 cd	0.71 bc	0.62 cd
Sidney	0.46 ab	0.80 b	0.80 b
White Sulphur Springs	0.43 bc	0.57 d	0.80 b

[y] Colony radius was measured at 24-hr intervals after incubation at the respective temperatures.
[z] Means with different letters are significantly different ($P = 0.05$) according to the Waller-Duncan exact Bayesian *k*-ratio LSD rule. Mean comparisons were done for growth rates of strains only within each temperature treatment.

Such a study is currently under way by the first author.

Our findings that AG-4 strains had optimum growth rates at 25–28 C are similar to results from a study on strains of AG-4 isolated from potato in Peru (3), which indicated that the optimum growth rates for several strains were 25–28 C. The average growth rate of the strains described in the our study was less than 50% of that of the strains of AG-4 from Peru, however.

Our results indicate the endemic and widespread occurrence of *R. solani* and other *Rhizoctonia* spp. in uncultivated soils of Montana and other states in the Northern Plains. The capability of the multinucleate strains found in this area to kill root buds of leafy spurge indicates their potential for use in the biological control of leafy spurge. It is widely held that strains of AG-4 have broad host ranges (2,16). However, studies are needed to determine whether individual strains might have narrower host ranges than attributed to AG-4 as a whole. Data presented in some studies have indicated that this is a possibility (5), and given the severity of symptoms caused by AG-4 on leafy spurge in the field (A. Caesar, *unpublished*), there is great potential benefit in such research.

Previous studies have indicated that for canola, potato, and sugar beets, AG-4 is typically less virulent, less prevalent, or both, relative to the anastomosis groups that are the principal cause of disease on these crops, viz., AG-2, AG-3, and AG-2-2, respectively (6,8,23). This trend also was reported on other crops and locations (21), where, for example, AG-4 was more commonly isolated from soil in carnation fields than other anastomosis groups but was weakly pathogenic compared with AG-2-2. Collectively, these results provide evidence that the use of AG-4 as a biological control agent of leafy spurge would be unlikely to present a threat to major crops grown in the Northern Plains, compared with some other anastomosis groups of *R. solani*.

Studies are continuing to assess both comparative virulence and host ranges of the strains of AG-4 described in the present report.

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