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Moisture requirements and host specificity of *Rhizoctonia solani* from leafy spurge (*Euphorbia esula*) in Nebraska[1]

GARY Y. YUEN and ROBERT A. MASTERS

Asst. Prof., Dept. of Plant Pathol., and Range Scientist, USDA-ARS, Dept. of Agron., Univ. of Nebraska, Lincoln, NE 68583.

Abstract:

R230, an isolate of *Rhizoctonia solani* anastomosis group 5 from leafy spurge, caused root and crown rots on leafy spurge when inoculated via soil in greenhouse experiments. The fungus grew through soil over a wide range of matric potentials (-0.01 to -1 MPa). When applied to leafy spurge foliage, the fungus blighted young adventitious shoots, but not mature stems. Foliar infection required high relative humidity. Mycelial growth on plant surfaces stopped at below 92% relative humidity. Pathogenicity of R230 was not limited to leafy spurge. The fungus caused stem and foliar lesions on all crops tested (alfalfa, smooth bromegrass, corn, Kentucky bluegrass, soybean, tall fescue, and wheat) when plants were kept under high humidity. It reduced seedling emergence and survival in soybean, Kentucky bluegrass, and tall fescue. R230 also caused a foliar blight on spotted spurge. The potential for R230 to damage crops will limit application of the fungus to noncultivated areas and to sites where it is endemic.

Nomenclature:

Rhizoctonia solani Kühn; leafy spurge, *Euphorbia esula* L. #[2] EPHES; spotted spurge, *Euphorbia maculata* L. # EPHMA; alfalfa, *Medicago sativa* L.; corn, *Zea mays* L; Kentucky bluegrass, *Poa pratensis* L.; smooth bromegrass, *Bromus inermis* Leyss.; soybean, *Glycine max* (L.) Merr.; tall fescue, *Festuca arundinacea* Schreb.; wheat, *Triticum aestivum* L.

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[2] Letters following this symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 1508 West University Ave., Champaign, IL 61821-3133.

Additional index words:

Biological control, host range, humidity, mycoherbicide, soil moisture, *Euphorbia maculata*, EPHES, EPHMA.

Introduction

Leafy spurge is a very serious perennial weed of northern Great Plains rangelands (10). Biological approaches to weed control have been sought because infestations often occur in areas where herbicides cannot be used because of risks to native flora or potential for contamination of ground and surface waters. Herbicides also may not be profitable in some situations. Researchers have obtained leafy spurge pathogens from its native range in Eurasia (2), but development of introduced pathogens requires years of testing under quarantine conditions before field evaluation. In contrast, endemic pathogens may be more rapidly deployed. *Rhizoctonia solani* has been reported on leafy spurge in the northern Plains (5, 18). Caesar et al. (5) demonstrated that isolates of *R. solani* anastomosis group (AG[3]) 4 (a subspecies based on hyphal fusion) and binucleate *Rhizoctonia* spp. from Montana, Colorado, and North Dakota can cause root and stem rots on leafy spurge and suggested that these fungi may have potential as biocontrol agents. *R. solani* would represent a unique biological control agent as it can infect entire plants. Foliar blights are caused by some AGs of *R. solani* on tropical legumes (9, 20) and turfgrasses (3), but foliar infection requires prolonged leaf wetness and high humidity (1, 3, 21). *R. solani* more commonly infects subterranean and soil-line plant parts (13). As a soilborne pathogen, it would have an advantage over strictly foliar pathogens of leafy spurge, such as *Alternaria augustiovoidea* E. Simmons (19) because *R. solani* can destroy roots and crowns, and may reduce regrowth from below-ground adventitious buds. Virulence on leafy spurge and host specificity were found to differ among AG 4 isolates (4), indicating that these characteristics must be evaluated for each isolate.

An isolate of *R. solani* AG 5 (R230) from leafy spurge in Nebraska was pathogenic to this weed in preliminary tests. AG 5 has not been reported previously as a leafy spurge pathogen. R230 was further investigated in this study to obtain information for use in designing field application strategies. One objective was to ascertain the ability of R230 to cause foliar and below-ground damage to leafy spurge. Another objective was to determine the effects of relative humidity (RH[4]) and soil moisture on infection. A third objective was to determine pathogenicity of the isolate on several crops commonly grown in Nebraska.

[3] Abbreviations: AG, anastomosis group; PDA, potato dextrose agar; RH, relative humidity.

Materials and methods

R230 was isolated from diseased leafy spurge collected from Valley County, NE in 1991. Stunted plants which had root and crown rot were obtained from a population in which severe die-back occurred one year earlier. Isolations were made from diseased and healthy tissues on water agar after surface sterilization with 10% (v/v) commercial bleach for 5 min. The only potential pathogens cultured were isolates of *R. solani*, and they grew exclusively from diseased tissues. These isolates were of the same clone in AG 5 based on anastomosis testing (15).

R230 was maintained and propagated on potato dextrose agar (PDA[4]). Water agar was used to reisolate R230 from plant parts. The fungus was identified in water agar cultures by hyphal morphology, and its identity was confirmed by co-culturing with known R230 on PDA.

Pathogenicity tests on leafy spurge. Leafy spurge plants were propagated from a single plant collected from Lancaster County, NE. They were grown in a greenhouse in 15- to 20-cm-diam clay pots containing a pasteurized soil mix of equal volumes of Sharpsburg silty clay loam, sand, and vermiculite.

Infection of leafy spurge shoots was investigated on 6-month-old plants having mature flowering stems, younger axillary shoots and adventitious shoots growing from root buds. Inoculum consisted of 0.5-cm-diam plugs cut from the margins of 1-week-old PDA cultures. Four mature shoots on each plant were inoculated by placing an agar plug of R230 on a leaf axil at the mid-point of each stem. Four other mature stems on the same plant were severed above a node at stem midpoint and inoculated with agar plugs placed in the leaf axils below the cuts. Four axillary shoots were inoculated at the upper-most leaf axils. Finally, four agar pieces were placed on the soil surface next to adventitious shoots.

The soil was moistened to field capacity, and each plant was enclosed in a plastic bag to maintain RH above 95% and grown in a growth chamber under 12 hour light at 25º C. After 4 days, the bags were removed and plants were kept for an additional week at 35 to 60% RH. Three plants were inoculated with R230, and two plants were treated with sterile PDA plugs to serve as controls. Plants were examined for mycelial growth and symptom development every 2 to 3 days. The distance that stem necrosis developed from inoculation points was measured. Diseased tissues were placed on water agar to culture the pathogen. A completely randomized design was used. This inoculation was performed twice.

The ability of R230 to infect subterranean parts of leafy spurge was examined by first removing one third of the soil mix from 15-cm-diam pots containing 3-month-old plants. The removed material was replaced with soil mix amended (1 % w/v) with autoclaved proso millet seed on which R230 was cultured for 3 weeks (15). Plants treated with fungus-free soil mix served as controls. After 2 months in a greenhouse at 18 to 32º C and 50 to 100% RH, plants were examined for stunting and necrosis on roots and crowns. The roots and crowns from inoculated and control plants were placed on water agar to isolate the pathogen. A completely randomized design with three plants per treatment was used. The experiment was conducted twice.

Moisture experiments. The effects of humidity on R230 growth on the surface of leafy spurge stems was determined in this experiment. Ten-cm-long stem segments, measured from the terminal bud, were cut from 4-month-old plants and the leaves were removed. The stems were taped onto plastic mesh suspended in large jars. Each stem was inoculated with an agar plug of R230 at the terminal end and three stems were sealed in each jar. The jars contained distilled water or saturated solutions of K_2SO_4, KNO_3, or, $(NH_4)_2SO_4$, which provided 100, 97, 92, and 80% RH, respectively, at 25 ± 1º C (16).There were three jars for each solution. Vapor pressures in the jars equilibrated within 10 minutes upon sealing as measured with a humidity/temperature probe. Each day, one jar for each solution was removed from the incubator and the stems examined for R230 mycelial growth and necrosis. Hyphal growth and necrosis down each stem was measured using a dissecting microscope. This experiment was conducted twice. Homogeneity of error variances was tested and the results from the two experiments were pooled for analysis of variance.

The ability of R230 to grow through soil under different matric potentials was investigated by measuring the extent to which the fungus grew from pieces of buried millet seed inoculum toward 'baits.' Two R230-colonized seed were placed in the center of petri dishes (9-cm-diam) partially filled with pasteurized soil mix. Bait was autoclaved millet seed which were arranged on perpendicular transects at 1-cm increments (up to 4 cm) from the inoculum pieces. Four bait seeds were placed at each distance and were covered with a thin layer of soil. Water was applied to each dish with an atomizer to wet soils to 5, 10, and 20% (v/w) water content, which corresponded roughly to matric potentials of -1, -0.1, and -0.01 MPa, respectively. The dishes were covered and incubated at 25º C for 3 days, at which time bait seeds were cultured for R230 on water agar. The distance to which R230 grew from inoculum pieces along each transect was determined by the farthest bait seed from which R230 was isolated. The four distances measured in each replicate dish were averaged before performing analysis of variance and the LSD test. There were three replicate dishes for each moisture level, and similar dishes without R230-colonized seeds served as controls. The experiment was conducted twice. Error variances were tested for homogeneity before results from the two experiments were pooled for analysis of variance.

Host range studies. The effects of R230 on germination and seedling survival of alfalfa, corn, Kentucky bluegrass, smooth bromegrass, soybean, tall fescue, and wheat were determined under greenhouse conditions. Ten seeds of corn and soybean, 20 of wheat, and 50 of alfalfa, Kentucky bluegrass, smooth bromegrass, and tall fescue were sown into 15-cm-diam pots containing pasteurized soil mix and then covered with 30-ml volumes of soil mix containing 1 % (w/v) R230-colonized millet seed. Numbers of healthy seedlings were counted after 20 days. There were five inoculated and five untreated pots of each plant species in each of two independent experiments. The percentage of surviving seedlings was subjected to analysis of variance after arcsine transformation.

Pathogenicity tests for R230 were conducted on more established plants of the same crops and spotted spurge. All plants were grown from seed in 15-cm-diam pots for 2 to 4 months in pasteurized soil mix. Five to eight plants of Kentucky bluegrass, smooth bromegrass, and tall fescue and one to three plants of the other species were grown per pot. The soil mix was watered to field capacity, and then 1 g of R230-colonized millet

seeds was distributed on the soil mix surface. Pots were enclosed in plastic bags for 4 days, and then uncovered and maintained in the greenhouse for an additional month. Plants were examined for symptoms weekly. Disease severity in the grass species was determined as the percentage of foliage showing necrosis, and in soybean and alfalfa as the percentage of damaged stems. There were three R230-inoculated pots and two noninfested pots as controls for each plant species in each of two experiments.

Results and discussion

Pathogenicity of R230 on leafy spurge. R230 infected both above- and below-ground parts of leafy spurge (data not shown). Damage to above-ground parts was affected by tissue age and relative humidity. Within 2 days under high relative humidity in plastic bags, profuse mycelial growth developed from agar plugs placed on leaves, stems, and soil surfaces. The fungus blighted leaves and caused stem necrosis on young axillary stems. Stem dieback extended 44 ± 9 mm (mean ± s.d.) from the points of inoculation by 4 days after inoculation. Adventitious shoots with stems less than 2 mm in diameter were blighted or killed by the fungus growing from agar plugs placed on the soil surface. Intact mature stems did not become necrotic following stem or soil inoculation, although mycelium, grew on stem surfaces and attached leaves were blighted. Infection of severed, mature stems occurred with necrosis extending 19 ± 5 mm from cut ends in 4 days. Cultures of diseased leaves and stems yielded R230. No symptoms or mycelia were present in the controls.

The extent of necrosis caused by R230 on infected stems depended on the distance mycelia grew on the stem surface. Mycelial growth on plant surfaces stopped when plants were removed from a high humidity environment. Consequently, necrosis on stems stopped expanding while necrosis continued to intensify only on leaves already infected during the high humidity period. Similar findings have been reported for other foliar diseases caused by *R. solani* (1, 3, 23).

R230 incorporated into soil mix caused root and crown rots in two experiments (data not shown). No aboveground symptoms were observed 1 month after leafy spurge plants were transplanted into infested soil. After 2 months, one of three and two of three inoculated plants in each experiment died from root and crown rot. In each experiment, the surviving inoculated plants were stunted as compared to the controls. Roots growing in inoculated soil were necrotic and R230 was reisolated only from necrotic roots and crowns. There were no symptoms or presence of R230 in the controls.

Effects of moisture on growth of R230. High humidity was required for R230 growth on the surface of leafy spurge stems (Figure 1). R230 grew most extensively at 100% RH and its growth decreased significantly ($p = 0.05$) at 92% RH. There was no growth at 80% RH. Restriction of its growth to near-saturated humidity levels confirms other studies on *R. solani* (1, 23). This characteristic will limit effectiveness of R230 as a foliar mycoherbicide in the field. In the arid Northern Plains, prolonged periods of near-saturated atmospheres are infrequent. Use of invert emulsions (7) and polyacrylamide gels (12) may increase the efficiency of foliar inoculation, but the fungus would probably

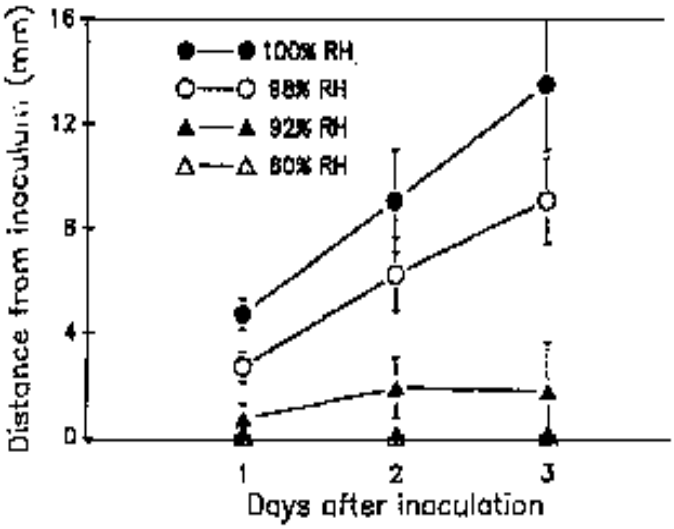

Figure 1. Growth of *Rhizoctonia solani* R230 from agar inoculum on the surface of leafy spurge stems at 25 C under various relative humidity (RH) levels controlled by saturated salt solutions. Values for day 1 are means from one experiment, whereas values for days 2 and 3 are means from two experiments. Error bars indicate standard deviation.

not limit regrowth from axillary and adventitious buds. It does not form a disseminative propagule, and therefore, lacks the capability to increase infection levels via a secondary disease cycle. Secondary disease cycles contribute to the effectiveness of the foliar mycoherbicide *Colletotrichum gloeosporiodes* (Penz.) Sacc. f. sp. *aeschynomene* (22).

In contrast to epiphytic growth of R230, its growth through soil was less affected by moisture. R230 grew 2.6, 2.8, and 2.3 cm from inoculum at -0.01, -0.1, and -1 MPa, respectively. Although growth after 3 days at -1 MPa was significantly less than at the high matric potentials ($p = 0.05$), most bait seeds placed 3 cm from R230 inoculum were colonized by the fungus at the three moisture levels tested.

It may be most effective to inoculate R230 in the field by applying inoculum under the soil surface because soil moisture levels would support fungal growth. As reported in other studies (1, 6), our *R. solani* isolate grew through soil at matric potentials that will sustain plant growth. Even if the soil should dry to the permanent wilting point, -3 MPa, near-saturated atmospheres would still exist in soil pores (6) and could sustain growth of R230. Placing inoculum beneath the surface would also position the pathogen closer to roots, crowns and adventitious buds.

Host range of R230. R230 caused damping-off of soybean seedlings. The percentage of healthy seedlings was 27% in R230-inoculated soil mix compared to 79% in the non-inoculated control after 20 days ($p < 0.05$) (Table 1). R230 also reduced germination and survival of Kentucky bluegrass and tall fescue ($p = 0.10$), but not as severely as in soybean. Survival of alfalfa, corn, smooth bromegrass, and wheat was not affected.

Table 1. Pathogenicity of *Rhizoctonia solani* AG 5 isolate R230 on seedlings and mature plants of nontarget species.

Host	Seedling survival[a]		Foliar or stem necrosis[b]
	R230	Control	
Alfalfa	56	64	18 ± 8
Corn	92	95	2 ± 3
Kentucky bluegrass	50	68*[c]	2 ± 4
Smooth bromegrass	33	42	2 ± 3
Soybean	27	79**	0
Spotted spurge	—[d]	—	58 ± 8
Tall fescue	63	87	2 ± 4
Wheat	84	82	2 ± 3

[a]Measured 20 days after planting.

[b]Percent of total number of stems or leaves with necrosis 4 days after inoculation. Values are means ± s.d.

[c]* and ** indicate significant difference at p = 0. 10 and 0.05, respectively, between R230 and control according to F-test.

[d]Not tested.

R230 applied to foliage caused necrosis on all plant species to varying degrees (Table 1). Disease on spotted spurge was the most severe, with 58% of the shoots exhibiting necrosis following a 4-day period under high humidity. In one experiment, spotted spurge shoots continued to rot after transfer to lower humidity, and the plants died after 1 month. However, necrosis ceased when the plants were moved from high to low humidity in a second experiment. In all other species, necrosis developed only under high humidity, but stopped once humidity was reduced. During the high moisture periods, stem lesions girdled 18% of the shoots in alfalfa, soybean exhibited only cotyledon rot, and grass species sustained less than 5% necrosis of sheaths and lower blades.

Pathogenicity of R230 to crop plants shown in this study supports the findings that AG 5 has a wide host range including legumes (13), turfgrass (11), and sugarbeet (*Beta vulgaris* L.) (17). There is a precedent for using a nonspecific pathogen as a mycoherbicide (14), namely *Phytophthora palmivora* (8), the pathogen of milkweed vine (*Morrenia odorata* Lindl.). This was justified, however, because the fungus was only mildly virulent on nontarget plants at high inoculum levels (14). The potential for R230 to cause severe symptoms on seedlings and mature crop plants precludes its use as a biocontrol agent in areas under cultivation. The fungus may still be safely used if field applications were restricted to sites where the pathogen is endemic and where no susceptible crops will be grown. Before this strategy can be employed, the impact of R230 on native grasses and forbs must be assessed.

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