Virulence of Anastomosis Groups of *Rhizoctonia solani* and *Rhizoctonia*-like Fungi on Selected Germ Plasm of Snap Bean, Lima Bean, and Cowpea

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ABSTRACT

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Rhizoctonia solani AG-4 and AG-2 type 2, indigenous to the Georgia coastal plain, were highly virulent on cultivars of snap bean, lima bean, pole bean, and cowpea. Snap bean breeding lines B4175, B4173-2X, 208-8R, 5181 R, and Venezuela 54 were more resistant to high inoculum densities (187 and 492 colony-forming units [cfu] per 100 g of soil) of AG-4 than Eagle snap bean, but at 16 cfu/100 g of soil, there were no significant differences. None of the snap bean breeding lines were resistant to *R. solani* AG-2 type 2. *R. solani* AG-2 type 1 was highly virulent on cowpea cultivars and slightly to moderately virulent on beans. *Rhizoctonia*-like CAG-5 was moderately virulent on PI 165426 and Jackson Wonder lima bean. CAG-3 was highly virulent on cowpea cultivars, and CAG-4 was avirulent.

Root diseases caused by *Rhizoctonia solani* Kühn are limiting factors in commercial production of snap and pole bean (*Phaseolus vulgaris* L.) and cowpea (southern pea, *Vigna unguiculata* (L.) Walp.) in Georgia (18) and other areas (6,9,10,13). Resistance to *R. solani* in snap bean usually is associated with dark seed coats (1,4,15), an undesirable characteristic in commercial cultivars. White-seeded breeding lines of snap bean have been developed with resistance to *R. solani* (5,7,16), but the anastomosis group (AG) of *R. solani* (11,14) is rarely reported in studies on resistance. Recently, *Rhizoctonia*-like binucleate fungi have been separated into anastomosis groups (2,12), and isolates in several of the groups were pathogenic on bean (3). This research was initiated to determine the reactions of snap bean breeding lines and cultivars of snap bean, cowpea, lima bean (*P. lunatus* L.), and pole bean to different AGs of *R. solani* and *Rhizoctonia*-like fungi indigenous to the Georgia coastal plain.

MATERIALS AND METHODS

Isolates were grown on 3% cornmeal-sand (w/w) in flasks and stored dry in the laboratory at 20–30 C until used (2–12 wk). Tifton loamy sand (about 85% sand, 10% silt, and 5% clay) was treated with aerated steam (85 C for 30 min) and blended separately with 27, 54, and 80 mg of fertilizer (NPK) per kilogram of soil (0.6 mg/kg ~ 1 kg/ha) and cornmeal-sand inoculum of each isolate for 5 min in a concrete mixer. Cultures were blended at 1:200, 1:500, 1:2,000, or 1:5,000 (inoculum:soil, v/v) to prepare inoculum concentrations. Infested soils were placed in sterile wooden flats (8 × 40 × 60 cm). Because inoculum concentration may influence disease severity ratings, soil from each inoculum level of each isolate was collected from the mixer and assayed with a soil sampler (8) on tannic acid-benomyl agar to determine the number of colony-forming units (cfu) per 100 g of oven-dry soil (17).

A split-plot experiment with a randomized complete block design was used in each test. Whole plots were two replicates of different isolates mixed into soil separately and placed in flats, and subplots were one row of each cultivar or breeding line within each flat of soil. Four to 10 seeds were spaced 3–5 cm apart and planted 3 cm deep. Data were analyzed with a least-squares analysis of variance statistical program.

Isolates of *R. solani* used were AG-2 type 1, 27A from sweet sorghum and 145 from soil; AG-2 type 2, 17A, 30A, and 100 from corn in Cook, Colquitt, and Pierce counties, respectively; and AG-4, 78-5A from soil, 60 from okra, and 164 from lima bean. Isolates of *Rhizoctonia*-like binucleate fungi used were CAG-2 and JH-2 from peanut; CAG-3, 66-36, and 73 from soil; CAG-4 and 71 from spinach and 156 from soil; and CAG-5, 66-39, and 132 from soil.

Snap bean breeding lines tested were Venezuela 54, B4173-2X, PI 165426, and B4175 (J. E. Wyatt, USDA Vegetable Laboratory, Charleston, SC); Wisconsin RRR45 and Wisconsin RRR46 (D. J. Hagedorn, University of Wisconsin); and 924R, 204-8R, and 5181 R (M. H. Dickson, New York State Agricultural Experiment Station, Geneva). The first two lines have black, the third has brown, and the rest have white seed coats, respectively. Cultivars tested were Eagle snap bean; Dade pole bean; Jackson Wonder and Bridgeton lima bean; and Iron, Clay, Brabham, Coronet, Dixie Cream, Worthmore, White Acre, Pinkeye Purple Hull, Big Boy, and Mississippi Silver southern pea.

Plants were grown in a greenhouse for 2 wk at night-day soil temperature ranges of 10–16 to 20–36 C and watered as needed. Roots and hypocotyls were rated for disease severity on a scale where 1 = <2, 2 = 2–10, 3 = 11–50, and 4 = >50% discoloration and decay of root and hypocotyl tissues; 5 = dead plant. Previous experiments had shown that maximum damping-off usually occurred 10–14 days after planting in greenhouse tests.

RESULTS

Pathogenicity. *R. solani* AG-4 and AG-2 type 2 were highly virulent on cultivars of snap bean, lima bean, pole bean, and cowpea (Tables 1–3) and many of the snap bean breeding lines. At extremely high inoculum densities (187 or 492 cfu/100 g of soil), B4175, B4173-2X, 208-8R, 5181 R, and Venezuela 54 were more resistant to AG-4 isolates than Eagle snap bean. In contrast, at the moderate inoculum density of 16 cfu/100 g of soil, there were no significant differences among entries. The two Wisconsin breeding lines did not show resistance to AG-4, and Wisconsin RRR46 was more susceptible than Eagle to AG-2 type 2 at 95 cfu/100 g of soil (Table 1). None of the snap bean breeding lines were resistant to AG-2 type 2.

The binucleate *Rhizoctonia*-like CAG-5 caused little injury to Eagle and most breeding lines, but it caused slight and moderate root and hypocotyl disease severity on Jackson Wonder lima bean and snap bean breeding line PI 165426, respectively (Table 2). Isolates of AG-2 type 1, CAG-2, CAG-3, and CAG-4 caused little or no injury on the beans (Tables 1 and 2).

All cowpea cultivars tested were highly susceptible to isolates of AG-4, AG-2 type 1, AG-2 type 2, and one isolate of CAG-3 but were resistant to isolates of CAG-2, CAG-4, and CAG-5 (Table 3). Reducing the inoculum density of an AG-

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4 isolate from okra from a high inoculum density of 42 to a low inoculum inoculum density of 6 cfu/100 g of soil increased emergence and height of 2-wk-old cowpea seedlings and reduced root and hypocotyl disease severity significantly (Table 3).

After cowpea cultivars were harvested, soils infested with *R. solani* AG-2 type 1 and CAG-3 were remixed separately, and Pinkeye Purple Hull and Eagle were replanted. Cowpea seedlings grown in soil infested previously with the AG-2 type 1 isolate were killed, and there was moderate root rot on Eagle. In soil infested previously with the CAG-3 isolate, there was slight to moderate disease severity on both cultivars.

Symptoms. Isolates of AG-4 caused typical cortical decay and reddish brown sunken cankers or girdling of the hypocotyl and roots, commonly referred to as Rhizoctonia root rot in edible legumes (9). Isolates of AG-2 type 2 also caused reddish brown sunken cankers and occasionally light red to gray to black water-soaking at the base of the stem and upper hypocotyl. Isolates of AG-2 type 1 usually caused tan to light brown lesions that were restricted to the cortex, and rarely, reddish brown sunken cankers. Isolates of CAG-3 and CAG-4 caused typical reddish brown lesions but these were usually small (0.5–1.5 cm) and shallow (<2 mm). Isolates of CAG-5 caused superficial reddish brown hypocotyl lesions and numerous similar lesions on the taproots and fibrous roots.

Only AG-4 caused severe preemergence damping-off consistently. With AG-2 type 2, plants usually emerged, but frequently, there was postemergence damping-off 10–14 days after planting. Other isolates usually did not cause damping-off.

In five other pathogenicity experiments in 1979 and 1980, 28 isolates of AG-4, 13 isolates of AG-2 (not identified to type), and two isolates of CAG-4 from corn, snap bean, lima bean, cowpea, soybean, cucumber (fruits), peanut (seed), cotton, and soil were tested separately for pathogencity to numerous crops including Bridgeton lima bean, Dade pole bean or Astro snap bean, and Pinkeye Purple Hull cowpea. All AG-2 isolates and 25 of the AG-4 isolates were moderately to highly virulent on the legumes. One CAG-4 isolate was avirulent and one isolate was slightly virulent.

In one test with an AG-1 web blight isolate from lima bean in southern Georgia, root disease severity on lima bean was slight and the fungus was not considered a serious root pathogen.

DISCUSSION

Snap bean breeding lines from New York and South Carolina were highly resistant to *R. solani* AG-4 isolates from Georgia, whereas the breeding lines from Wisconsin were not. In contrast, none of the breeding lines were more resistant to AG-2 type 2 than commercial cultivars of snap bean and pole bean tested. This

Table 1. Root and hypocotyl disease severity ratings in 2-wk-old beans and cowpea grown in soil infested with *Rhizoctonia solani* AG-4, AG-2 type-2, or binucleate *Rhizoctonia*-like CAG-3 or CAG-5[x]

	Colony-forming units per 100 g of oven-dried soil at planting								
	AG-4 (soil)[y]		AG-2 type-2 (corn)		CAG-3 (soil)		CAG-5 (soil)		
Breeding lines and cultivars	492	103	95	16	32	8	523	63	Control
Wisconsin RRR36	5.0 a[z]	4.0	4.0 abc	3.0	1.5	1.6	1.6 c	1.7 bc	1.5
Wisconsin RRR46	5.0 a	4.5	4.8 a	3.9	2.0	1.8	1.5 c	2.0 abc	1.8
Venezuela 54	4.5 ab	4.4	3.3 c	2.9	2.0	1.5	1.6 c	1.8 abc	1.6
PI 165426	5.0 a	4.2	4.5 ab	3.3	1.7	1.8	3.0 a	1.9 abc	1.8
B4175	4.2 b	4.3	4.1 abc	3.7	1.8	1.8	1.6 c	1.5 c	1.2
B4173-2X	3.8 b	4.0	3.6 bc	3.1	2.0	1.8	1.6 c	1.6 bc	1.4
Eagle	5.0 a	4.6	3.8 bc	3.3	1.4	1.3	1.4 c	1.5 c	1.1
Dade pole bean	5.0 a	4.2	3.4 c	3.5	1.5	1.1	1.6 c	1.4 c	1.3
Jackson Wonder lima bean	5.0 a	4.0	3.5 c	3.4	1.6	1.8	2.0 b	2.4 a	1.3
Pinkeye Purple Hull cowpea	5.0 a	4.2	3.6 c	2.5	1.9	1.3	1.7 c	2.1 ab	1.6

[x]Root and hypocotyl disease severity: 1 = <2, 2 = 2–10, 3 = 11–50, 4 = >50% discoloration or decay; 5 = dead or dying plant.
[y]Source of isolate.
[z]Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. No letters indicates no significant differences.

Table 2. Root and hypocotyl disease severity ratings in 2-wk-old beans and cowpea grown in soil infested with *Rhizoctonia solani* AG-4, AG-2 type 1, AG-2 type 2, or binucleate *Rhizoctonia*-like CAG-4[x]

	Colony-forming units per 100 g of oven-dried soil at planting							
	AG-4 (soil)[y]		AG-4 (okra)	Ag-2 type 2 (soil)	AG-2 type 2 (corn)	Ag-2 type 1 (sorghum)	CAG-4 (spinach)	
Breeding lines and cultivars	187	16	140	<8	<8	<8	<8	Control
Venezuela	3.7 b[z]	3.7	3.3 b	1.1	3.1	1.0	1.0	1.2
PI 165426	5.0 a	3.3	5.0 a	1.6	2.8	1.2	1.0	1.1
B4175	3.8 ab	3.7	3.5 b	1.3	2.9	1.2	1.0	1.0
B4173-2X	4.2 ab	3.8	3.1 b	1.5	3.5	1.1	1.1	1.0
924 R	4.0 ab	3.7	4.0 b	1.7	3.3	1.2	1.1	1.0
208-8R	3.4 b	3.4	3.5 b	1.2	3.8	1.4	1.2	1.1
5181 R	3.1 b	4.2	3.7 b	1.6	3.0	1.0	1.0	1.0
Eagle snap bean	5.0 a	4.3	5.0 a	1.3	3.5	1.6	1.0	1.0
Jackson Wonder lima bean	3.8 ab	3.4	3.8 b	1.8	2.3	1.3	1.0	1.8
Pinkeye Purple Hull cowpea	5.0 a	4.4	5.0 a	1.9	3.1	1.5	1.5	1.0

[x]Root and hypocotyl disease severity: 1 = <2, 2 = 2–10, 3 = 11–50, 4 = >50% discoloration or decay; 5 = dead or dying plant.
[y]Source of isolate. AG-4 from soil and AG-2 type 2 from corn were the same isolates shown in Table 1.
[z]Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. No letters indicates no significant differences.

Table 3. Emergence, root and hypocotyl disease severity ratings, and heights of 2-wk-old cowpea cultivars grown in soil infested with *Rhizoctonia solani* and binucleate *Rhizoctonia*-like fungi[w]

Isolate	Source	cfu[x]	Emergence	RHDI[y]	Height (cm)
AG-2 type 1	Soil	54	0.0 d[z]	5.0 a	0.0 b
AG-2 type 2	Corn	6	1.0 c	5.0 a	1.4 b
AG-4	Okra	42	0.6 cd	4.9 a	1.4 b
AG-4	Okra	6	5.1 b	3.9 b	8.0 a
AG-4	Lima bean	48	0.8 cd	4.8 a	1.7 b
CAG-2	Peanut	60	8.6 a	1.5 c	9.6 a
CAG-3	Soil	48	0.1 d	5.0 a	0.2 b
CAG-4	Soil	6	7.6 a	2.0 c	9.2 a
CAG-5	Soil	24	8.6 a	1.9 c	9.4 a
Control	···	0	7.8 a	1.6 c	9.6 a

[w] Average of 10 cultivars; there were no significant cultivar × isolate interactions.
[x] cfu = Colony forming units per 100 g of oven-dried soil at planting.
[y] RHDI = root and hypocotyl disease index: 1 = <2, 2 = 2–10, 3 = 11–50, and 4 = >50% discoloration and decay; 5 = dead or dying plants.
[z] Numbers followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test. Emergence is the mean from 10 seeds planted.

might be expected, because *R. solani* AG-2 type 2 is a pathogen primarily associated with multiple-cropping systems including corn and peanut in Georgia (17), and AG-2 (type not given) was reported as a weakly virulent pathogen of snap bean in New York (6). The breeding line PI 165426 was more susceptible to CAG-5 than Eagle or Dade, although disease severity was only slight to moderate. The fungus is very common in soils in the Georgia coastal plain and could be a potentially serious pathogen on beans with PI 165426 germ plasm in their background.

Jackson Wonder was the lima bean cultivar most resistant to *R. solani* in other research (20), but it was very susceptible to some isolates of *R. solani* AG-4, moderately susceptible to *R. solani* AG-2 type 2, and slightly susceptible to CAG-5 in these experiments. Reduced inoculum concentrations were beneficial in separating resistant from susceptible cultivars in Indiana (19), but in this research, higher inoculum concentrations appeared to give better separation of cultivars and breeding lines than lower concentrations. A more thorough investigation is needed with a range of inoculum concentrations to determine the optimum inoculum density for separations of breeding lines in the greenhouse.

Cowpea is the most widely grown edible legume vegetable in Georgia, and none of the major cultivars planted in the state were resistant to *R. solani* AG-4, AG-2 type 1, or AG-2 type 2 or to CAG-3. Thus, there is a need for screening cowpea germ plasm for sources of resistance to *R. solani* and binucleate *Rhizoctonia*-like fungi.

Even with the large variety of *R. solani* and *Rhizoctonia*-like AGs indigenous to soils in the Georgia coastal plain, *R. solani* AG-4 is isolated most frequently from roots and hypocotyls of edible legumes and is found more commonly in soils (5–50 cfu/100 g) than any other AG. Therefore, breeding lines tolerant to AG-4 in this study (Venezuela 54, B4175, B4173-2X, 924 R, 208-8 R, and 5181 R) would probably be very useful in developing cultivars resistant to AG-4 in field tests. One snap bean breeding line, B-4175, is also resistant to root-knot nematode (21); however, only field studies will determine the cultivars that will be best suited to coastal plain soils. *Pythium irregulare* in cool weather and *P. aphanidermatum, P. myriotylum,* and *Sclerotium rolfsii* in warm weather are serious pathogens of bean and cowpea in Georgia (13) and resistance to other pathogens must be considered in programs to select improved cultivars.

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