Root Rot Resistance in Common Bean Germ Plasm of Latin American Origin

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ABSTRACT

Beebe, S. E., Bliss, F. A., and Schwartz, H. F. 1981. Root rot resistance in common bean germ plasm of Latin American origin. Plant Disease 65:485-489.

Resistances of common bean (*Phaseolus vulgaris* L.) to *Fusarium solani, Rhizoctonia solani, Sclerotium rolfsii*, and *Pythium* spp. were studied. N203 (PI 203958) displayed only intermediate field resistance to *F. solani*, whereas Cornell 2114-12 and several tropical cultivars were highly resistant. Resistance to *Pythium* spp. was common among colored-seeded tropical materials. Resistances to *R. solani* and *S. rolfsii* were measured by plant survival, because hypocotyl lesions and disease severity had little effect on plant yield. Resistance to *R. solani* was comparable to that of other Latin American bean cultivars. Many tropically adapted cultivars demonstrated resistance to the pathogens superior to that of previously reported sources.

Little research has been conducted on the effect of pathogens inciting root rot in common beans (*Phaseolus vulgaris* L.) grown in Latin America. Published reports about specific soilborne pathogens suggest their wide distribution throughout bean production regions (2). Barros (1) reported that several *Fusarium* spp. were pathogenic to beans in Colombia. *F. oxysporum* is a serious problem in Ecuador (8) and coastal Peru, where it occurs within other *Fusarium* spp. (6). *F. solani* is an important pathogen in São Paulo, Brazil, particularly in combination with *Rhizoctonia solani* (2). *R. solani* and *Sclerotium rolfsii* cause occasional problems in Ecuador and Peru. *S. rolfsii* is a serious problem in the Narino highlands and frequently at lower elevations in Valle, Colombia. At least four *Pythium* spp., including *P. debaryanum* and *P. ultimum* (6), infect beans in Latin America. Bean production in Venezuela is seriously affected by these root rot pathogens (5).

MATERIALS AND METHODS

Inoculum preparation. We obtained isolates of *F. solani* (Mart.) Appel & Wr. f. sp. *phaseoli* (Burk.) Snyd. & Hans., *Pythium* spp., *S. rolfsii* Sacc., and *R. solani* Kühn from infected bean plants grown at CIAT near Palmira, Colombia. They were used as inocula in field tests of bean accession reactions to each of the four pathogens.

Fifteen-liter metal cans were washed and partially filled with rice hulls. Two liters of a nutrient solution (20 g of refined sugar and 2.3 g of KNO_3/L of nonchlorinated water) were added to

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Research supported in part by CIAT; College of Agricultural and Life Sciences, The Graduate School, University of Wisconsin, Madison; and by a National Science Foundation Graduate Fellowship awarded to the senior author.

0191-2917/81/06048505/$03.00/0

Table 1. Disease index used to evaluate bean plants for resistance to *Rhizoctonia solani*, *Fusarium solani*, and *Sclerotium rolfsii*

Extent of infection[a]	Depth of infection[b]		
	Shallow	Intermediate	Deep
Slight	1	4	7
Intermediate	2	5	8
Extensive	3	6	9

[a] For *R. solani*, slight = 1–2, intermediate = 3–4, extensive = ≥5 lesions/hypocotyl. For *F. solani* and *S. rolfsii*, slight = 1 cm, intermediate = 2 cm, and extensive = ≥3 cm of infected tissue measured from base of hypocotyl.

[b] Scale also includes 0 (no symptom development) and 10 (dead or missing plants).

each can, which was then sealed, sterilized for 1 hr at 120 C, cooled, and resterilized 2 days later.

Each can was seeded with starter inoculum, which was obtained by macerating two agar colonies of a specific pathogen isolate in 60–70 ml of sterile, deionized water. The agar slurry was evenly distributed over the surface of the medium with a large, sterile pipette or glass tube. The can was resealed and agitated to mix the agar slurry with the sterilized rice hulls and nutrient solution. *Pythium* spp. grew best on a whole corn kernel medium prepared by filling a 15-L can one-third full of dry corn, which was then covered with nonchlorinated water. Cans were sealed, sterilized, and inoculated.

Cans containing the inoculated media were incubated in the greenhouse at 20–28 C. *F. solani* and *R. solani* required 3–5 days and *S. rolfsii* and *Pythium* spp. required 5–7 days for maximum mycelial growth or sclerotia production. The cans were then opened for slow evaporation of the excess moisture and nutrient solution.

The mycelial-infested media were ground in a Wiley mill to produce the fine-textured mixture used as inoculum in field and greenhouse trials. Several isolates of each pathogen were grown individually but mixed before inoculation. The inoculum of *F. solani* contained

Table 2. Reaction of *Phaseolus vulgaris* accessions to inoculations by four soilborne pathogens

CIAT number	Identification	*Fusarium solani*	*Pythium* spp.	*Sclerotium rolfsii*		*Rhizoctonia solani*	
		Disease index	% emergence	Disease index	% survival	Disease index	% survival
G02959	Pecho Amarillo	3.20	54	7.03	46	7.55	60
G01164	Chimbolo	1.75	61	6.83	74		
G03807	Bico de Ouro	1.74	59	7.33	55	7.70	55
G03153	Frijol de Parra	3.08	78	3.50	91	6.70	80
G04454	ICA-Tui	1.75	75	2.78	85	7.75	60
G03974	Jin 10B	2.29	61	4.45	87		
G03353	Puebla 152 (black)	2.77	46	5.75	74	8.70	30
G04421	C63-S-630B	2.62	81	7.92	61	7.05	65
G04495	Porrillo Sintético	1.68	76	4.72	78		
G04459	Nep 2	2.21	49	8.94	17	9.75	5
G00881	N203	2.70	70	7.95	49	7.50	85
G05694	Cornell 49-242	2.54	65	7.22	63	8.75	40
G05704	Canario Divex	2.89	18	8.80	30	8.30	45
G04494	Diacol Calima	3.73	76	4.08	87	7.00	75
G04791	Honduras 46	1.51	76	6.53	77		
G04470	Pompadour	3.13	23	8.53	30		
G04789	Cubagua	1.84	78	3.64	80	8.85	25
G04456	Jamapa	2.19	69	4.44	81	7.00	75
G04830	Rio Tibagi, Lote 10	1.94	78	4.69	79		
G04461	Porrillo 1	1.88	52	6.86	58		
G04446	Puebla 152 (brown)	2.44	72	6.78	67	8.00	45
G05719	Aurora	2.74	30	7.26	42	9.80	5
G05749	Venezuela 54	2.52	76	6.70	80	8.90	35
G04197	Black Turtle Soup	2.44	61	5.19	83		
G01540	PI 284703	4.62	71	6.39	87	6.50	80
	FF12-13-1	2.42	63	7.78	24		
	PI 224730	1.61	74	7.39	79		
G00982	PI 224737	3.81	41	9.20	17		
G01770	PI 309726	4.90	70	7.86	57		
G01817	PI 309801	4.70	71	7.36	39		
G02270	PI 311917	3.14	83	7.11	60		
G02316	PI 311975 (brown)	4.72	68	6.97	68		
G02316	PI 311975 (black)	3.00	61	6.06	59		
G02323	PI 311987	2.04	85	6.80	76		
G02324	PI 311989	1.92	46	5.28	66		
G02326	PI 311991	2.17	78	6.39	72		
G02350	PI 312028	2.60	72	6.44	58		
G02353	PI 312033	1.40	76	4.83	81		
G02360	PI 312041	2.75	78	6.47	59		
G02362	PI 312043	2.23	55	5.89	65		
G02381	PI 312062	2.58	83	5.89	80		
G02390	PI 312077	2.70	67	7.92	48		
G02808	PI 319606	2.55	59	5.64	78		
G05697	Cornell 2114-12	1.46	76	5.67	85	6.45	95
G06651	15R-55	1.49	70	4.17	81	8.55	50
G04498	Sanilac	2.36	32	8.48	23	9.70	8
	Resistant check[a]	2.70	74	4.14	88	6.40	83
	Mean	2.73	62	6.46	63	9.20	53
	LSD (0.05)	1.22	20.8	2.08	22.4	1.75	32.7

[a] PI 203958 for *F. solani* and *Pythium* spp.; Porrillo Sintético for *S. rolfsii* and *R. solani*.

seven isolates, that of *R. solani* four, that of *S. rolfsii* eight, and that of *Pythium* spp. five.

Field inoculations. Three nurseries were established at CIAT in April 1977 to evaluate resistance to *F. solani, Pythium* spp., and *S. rolfsii* individually. Each nursery consisted of 56 cultivars planted in three replications in a randomized complete block design. These cultivars included 17 entries reportedly resistant to *F. solani* (3), the New York breeding line 2114-12, and several CIAT breeding parents that represented various seed color classes. Each block also included 10 plots of a susceptible control (Sanilac) and 10 plots of a resistant control (PI 203958 in the *F. solani* and *Pythium* spp. plots, and Porrillo Sintético in the *S. rolfsii* plots).

The field was prepared in beds that measured 60 cm from center to center and 15 cm high. Eight days before planting, the *F. solani* and *Pythium* spp. nurseries received a pentachloronitrobenzene (PCNB) treatment (9 kg/ha) to control native propagules of *R. solani* and *S. rolfsii.* Entries were planted (18 seeds) in a 1.5-m-long furrow made in the center of each bed with a hoe. Inocula of *F. solani* and *Pythium* spp. were applied in each row, 100 ml/m. Sclerotia of *S. rolfsii* were applied 400–500/m. Seed and inocula were covered with soil immediately after inoculation. Subsequent studies indicated that adequate levels of native *F. solani* propagules were present in CIAT soils and that artificial inoculation was not necessary.

Greenhouse inoculation with *R. solani.* Eight accessions reported to be resistant to *R. solani* and 203 CIAT breeding parents were planted in a CIAT greenhouse seedling test (two replicates). Inoculum was prepared and mixed into screened, unsterilized sand at 800 ml per greenhouse flat (46 × 34 × 6 cm).

Fourteen entries and a resistant (Porrillo Sintético) and susceptible (Sanilac) check were sown in each flat. Flats were watered initially with 600 ml of tap water and moistened as needed for plant growth. Seedlings were evaluated for resistance 2 weeks after planting.

Symptom evaluation. Near flowering times, a disease index rating based on extent and depth of hypocotyl infection was used to evaluate severity of *F. solani* infection. The extent and depth of infection were each divided into three gradations (Table 1). Extent was measured as 1, 2, or 3 cm or more of infected tissue above the base of the hypocotyl. Depth was classified as shallow, when confined to the outer 50% of the cortical tissue; intermediate, when the pathogen had penetrated to the midpoint of the cortex or beyond but had not entered the vascular system; or deep, when the pathogen had entered the vascular system or pith.

We measured repeatability by selecting 35 *Fusarium*-infected hypocotyls at random and making two successive evaluations of them. The correlation between the two sets of values was 0.88, suggesting that the rating method was repeatable.

Table 3. Effect of *Rhizoctonia solani* inoculation and chemical control treatments on seed yield and number of surviving bean plants

Cultivar	Inoculated			Uninoculated		Benomyl/PCNB[y]	
	Seed yield (kg/ha)	Surviving plants (per 50)	Disease index[x]	Yield (kg/ha)	Surviving plants (per 50)	Yield (kg/ha)	Surviving plants (per 50)
Porrillo Sintético	1726 a[z]	27.3 bc[z]	6.46	1963 a	43.0 a	1611 a	42.0 a
Puebla 152 (brown)	622 c	5.7 d	9.16	1435 ab	36.0 ab	1895 a	30.0 b
Sanilac	125 d	1.7 d	9.53	1083 bc	29.7 b	726 c	18.0 c

[x] Disease index based on measurement of extent and depth of infection.

[y] Pentachloronitrobenzene applied 8 days before planting at 9 kg/ha.

[z] Average of three replications. Values followed by the same letter do not differ significantly at $P = 0.05$ using Duncan's new multiple range test.

Table 4. Greenhouse test of bean lines reported to be resistant to *Rhizoctonia solani*

Bean line	Surviving plants (per 10)[a]	Disease index[b]
PI 109859	9.0	6.20
PI 165426 (black)	6.5	7.30
PI 165426 (white)	0.5	9.65
PI 165435	3.0	8.75
PI 163583	4.5	8.65
PI 226895	7.0	7.55
Cornell 2114-12	9.5	6.45
Venezuela 54	3.5	8.90

[a] Mean of two replicates of 10 seeds each.

[b] Based on extent and depth of infection.

We further validated the *F. solani* scale by determining the correlations between visual ratings of hypocotyl symptoms, damage to fibrous feeder roots observed through a stereoscope, and macroscopic symptoms on the primary and large lateral roots. Fragments of feeder roots washed from the soil were evaluated on a scale of 0 (no discoloration) to 4 (very severe discoloration or death). Large roots were evaluated in terms of visible lesions and degree of pruning.

Similar disease index ratings were devised for *S. rolfsii* and *R. solani* (Table 1). Shallow, intermediate, and deep lesions were defined as being limited to the cortex, having penetrated the vascular system, and having developed within the pith, respectively. Extent of infection by *S. rolfsii* was graduated as for *F. solani.* Extent of infection by *R. solani* was quantified by number of lesions, because they were easily counted. Repeatability tests for *R. solani* and *S. rolfsii* ratings yielded correlations of 0.94 and 0.97, respectively, for successive evaluations of sets of hypocotyls infected with each pathogen. The *R. solani* scale was applied to both seedling and adult plants and the *S. rolfsii* scale to plants 3 wk or older.

We evaluated resistance to *Pythium* spp. by using emergence percentage, or the number of viable seedlings divided by the number of seeds planted. Several lines were later selected for inclusion in root rot tests at Hancock, WI, where *Pythium* spp. have been reported in a root rot complex (7).

Evaluation of yield losses. We conducted a factorial experiment of three cultivars × three soil treatments to measure yield loss caused by *R. solani.* Treatments were arranged in a randomized complete block design with three replications. Preliminary screening had determined that Porrillo Sintético, Puebla 152 (brown-seeded), and Sanilac were resistant, intermediate in susceptibility, and highly susceptible, respectively.

One soil treatment was an application of benomyl and pentachloronitrobenzene to control populations of native soil fungi and to estimate yield reduction caused by direct inoculation with soil pathogens. No fungicide was used to control *Pythium* spp. The second treatment was inoculation with 100 ml of *R. solani* inoculum over the seed in 1 m of furrow, which in turn was covered with 300 ml of untreated rice hulls per meter and topped with soil. Third, we maintained an untreated control.

Seeds were hand-planted every 8 cm in 60-cm-wide beds. Each plot was four rows wide and 3 m long, and the center 2 m of the inner rows was harvested at maturity. Inoculated plants were rated on a 10-point scale as having no lesions (0), shallow lesions (1–3), intermediate lesions (4–6), and deep lesions (7–9) (Table 1). Average plant yields were calculated for uninoculated and fungicide-treated plots, and plants were grouped by disease severity in the inoculated plots. An analysis of variance was performed on plot yield and number of plants harvested.

In a similar test of yield loss caused by *S. rolfsii,* we planted one resistant cultivar (Sanilac), one having intermediate resistance (C63 S-630-B), and seven resistant cultivars (ICA-Tui; Jin 10B; Porrillo Sintético; Cornell 2114-12; Diacol Calima; Cubagua; and Rio Tibagi, Lote 10). Each cultivar received three soil treatments. *S. rolfsii* was applied at the rate of 360 sclerotia per meter. The fungicide treatment included captan to control *Pythium* spp.

Table 5. Effect of *Sclerotium rolfsii* inoculation on seed yield of bean plants

	Uninoculated	Inoculated			
		Yield (g/plant) of plants with			
Bean line	Yield[a] (g/plant)	No lesions	Shallow lesions	Intermediate lesions	Deep lesions
Sanilac	7.4	17.0	15.0	13.6	8.7
Frijol de Parra	5.0	7.8	9.7	5.0	5.2
ICA-Tui	6.2	7.9	8.9	5.9	5.4
C63-S-630-B	5.2	7.2	5.0	6.2	6.3
Porrillo Sintético	7.0	8.9	8.4	7.9	5.0
Cornell 2114-12	7.4	9.3	8.0	8.7	4.2
Diacol Calima	8.6	12.3	10.0	10.0	12.0
Cubagua	5.3	7.0	6.3	3.9	2.8
Rio Tibagi, Lote 10	4.7	6.3	7.7	6.2	7.6
Mean	6.3	9.3	8.7	7.5	6.4

[a] Average yield per plant.

Table 6. Disease reactions of Latin American cultivars and two susceptible checks to the root rot complex endemic at Hancock, WI

CIAT number	Identification	Disease index[a]
G04446	Puebla 152 (brown)	16.9
G04142	Porrillo 70	17.8
G04525	ICA-Pijao	18.0
G02006	PI 310740	22.7
C04495	Porrillo Sintético	24.9
G04830	Rio Tibagi, Lote 10	26.4
G03065	Blanco 137	30.2
G04789	Cubagua	31.8
G04791	Honduras 46	42.2
G05749	Venezuela 54	56.4
G06651	15R-55	61.3
G05704	Canario Divex	91.8
G04498	Sanilac[b]	81.1
	Tenderette[b]	94.2
	LSD (0.05)	12.7

[a] Based on symptoms of 0 = none, 20 = slight, 50 = moderate, 80 = severe, 100 = very severe, plant dead or missing. Average of three replications.

[b] Susceptible check.

RESULTS

Resistance to *F. solani*. In *F. solani* tests, ratings of hypocotyls and feeder roots had a correlation of 0.47, which is less than the 0.51 required for significance at the 0.05 level. Considering these results and the role of *F. solani* as a cortical pathogen, we used the hypocotyl rating in all subsequent tests. The positive relationship of hypocotyl to feeder root damage suggested a common genetic capacity to limit infection by the pathogen.

Plant density affected the ability to distinguish differences in resistance to *F. solani* on the basis of hypocotyl symptoms. Known resistant and susceptible lines could be distinguished in the field at spacings of 5.6 and 8 cm within the row but not at 16 cm (S. E. Beebe, *unpublished*).

Differences in resistance to *F. solani* were observed among the 47 lines field-tested (Table 2). Among the CIAT breeding parents, black-seeded lines were generally more resistant than red-seeded lines; however, no strong relationship between color and resistance was apparent. Black-seeded lines showing good resistance included Chimbolo; Porrillo Sintético; Cubagua; Rio Tibagi, Lote 10; 15R-55; and Porrillo 1. Honduras 46 (a dark red-seeded line) and Cornell 2114-12 also showed good resistance.

Results varied for the U.S. introductions from Mexico that are reported to be resistant (3).PI 224730, PI 311987, PI 311989, and PI 312033 (all black-seeded) showed the highest resistance. Other accessions were intermediate or susceptible, including PI 312077 and PI 319606, which had performed well in seedling and field tests (3) and had been confirmed in other seedling tests. N203 (PI 203958), long used for its resistance in the United States and which has shown resistance in seedling and field tests (3,9), also was intermediate in the CIAT field test.

Yield losses caused by *R. solani*. In *R. solani* tests, inoculated plots of susceptible Sanilac yielded only 11% as much seed as uninoculated plots. Fewer plants survived than of resistant Porrillo Sintético (Table 3). Plots of Porrillo Sintético with and without inoculation had no significant yield differences, although fewer plants survived in the inoculated plots. Yield per plant was greater in inoculated than in uninoculated plots because of lower density, leading to reduced competition and yield compensation.

The surviving plants of Porrillo Sintético allowed us to relate yield to symptom severity. Plants with no lesions yielded 20.2 g of seed per plant. Those with shallow, intermediate, and deep lesions yielded 19.7, 17.6, and 11.0 g, respectively. Uninoculated plots sown at recommended rates produced about 12.0 g per plant, little more than infected plants with deep lesions.

R. solani infection caused severely affected plants to develop more slowly, to suffer greater competition, and to yield less than uninfected plants; however, plot yield was affected only when stands were severely reduced. Plant survival under severe attack was probably a better criterion for assessing resistance than lesion development.

Resistance to *R. solani*. Data from a disease index based on hypocotyl ratings and a score based on number of surviving plants were analyzed statistically to compare their efficiency in distinguishing resistance to *R. solani* (Table 2). Ratings based on hypocotyl lesions were more time consuming to compile and provided no better means of distinguishing resistance than did ratings based on the number of plants surviving inoculation. Several CIAT bean selections were as resistant as lines previously reported to be so (Tables 2 and 4).

Yield losses caused by *S. rolfsii*. In *S. rolfsii* tests, hypocotyl lesions were evident on plants of all lines, and the susceptible check Sanilac exhibited considerable stand reduction due to damping-off and seedling wilt. Although cultivars differed in yield, no significant differences were found between inoculated plots and those receiving either no inoculation or a fungicide treatment. For most cultivars, fewer plants survived in the inoculated plots, but the remaining plants were able to compensate.

As with *R. solani*, yield was related to symptom severity. Plants with deep lesions usually yielded less than those with shallow lesions, but some lines showed little difference among disease classes (Table 5). Plants with deep lesions sometimes produced yields similar to those of lesion-free plants grown in uninoculated plots. Percentage of germination and plant survival were better criteria for assessing resistance than lesion development. Most losses occurred during the seedling stage, but *S. rolfsii* also caused death of older plants.

Resistance to *S. rolfsii*. We observed preemergence and postemergence damping-off of plants up to 3 wk after germination. No bean line was immune to infection, and differences in resistance were inferred from percentage of surviving plants and disease index (Table 2). As with *R. solani*, percentage of survival was as useful in assessing plant resistance as lesion severity on hypocotyls.

Two Venezuelan lines (Cubagua, Black Turtle Soup) showed good resistance. Other resistant lines came from a wide geographic range including Honduras, Brazil, and Colombia, but with no concentration of resistant germ plasm from a specific area.

Resistance to *Pythium* spp. Lines grown in the Pythium plot had different percentages of germination but showed little postemergence damping-off (Table 2). N203 and Cornell 2114-12, which were expected to be resistant, emerged well; the susceptible check Sanilac showed an average emergence of only 32%. Many CIAT materials performed as well as the resistant checks. The few white-seeded lines tested were susceptible, with other seed color classes generally more resistant.

In the Hancock tests, most Latin American cultivars had good emergence, excellent vigor, and low disease indexes, while susceptible checks consistently had high disease indexes (Table 6). Resistance to damping-off and feeder root loss caused by *Pythium* spp. was common among the colored-seeded materials of Latin American origin.

DISCUSSION

Resistance of Latin American germ plasm to the soilborne pathogens studied was more common than expected, perhaps because less stringent criteria of resistance were used than by previous investigators. For example, we evaluated resistance to *R. solani* principally on the basis of seedling survival rather than lesion development. Data from yield experiments suggested that plant survival was a more realistic criterion of resistance; further, it was more practical for evaluating a large number of materials (4).

Similarly, although few reports have been made of resistance to *S. rolfsii*, several lines evaluated in this study were considered resistant on the basis of plant survival. However, we do not know whether this resistance is effective during all stages of growth. Further research is needed to determine the correlation between seedling and adult plant resistance and to study the effect of time of infection on yield loss.

Many lines were as resistant to *Pythium* spp. as previously reported sources (N203 and Cornell 2114-12), which suggests that resistance is widespread within Latin American germ plasm. These findings agree with the observation that Pythium root rot is seldom reported to be damaging in Latin America (6).

Some lines were nearly as resistant to *F. solani* as was Cornell 2114-12, and many were intermediate in their resistance. Most bean lines reported to be sources of resistance would not be of much value in Latin America because they are susceptible to other prevalent diseases, adapt poorly, and have undesirable agronomic traits.

Not only was resistance to the various pathogens widespread in Latin American germ plasm, but several lines were resistant to more than one pathogen. Porrillo Sintético was resistant to all four pathogens, and Rio Tibagi, Lote 10; Honduras 46; Frijol de Parra; and ICA-Tui were resistant to two or three.

The intermediate to high levels of root rot resistance in Latin American germ plasm may be caused by natural selection during repeated exposure to soilborne pathogens, as well as by directed selection of resistant lines in national programs. Relatively few materials were screened in this study, and many more Latin American cultivars may possess resistance to one or more root rot pathogens. Before these or other reported sources of resistance are used in a Latin American breeding program, locally adapted cultivars should be carefully screened for their potential resistance and value as parents in improving dry bean production.

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