

Association of Seed Coat Factors with Resistance to *Rhizoctonia solani* in *Phaseolus vulgaris*

Krishna Prasad and Jack L. Weigle

Research Assistant and Professor, respectively, Department of Horticulture, Iowa State University, Ames 50010. Present address of senior author: Department of Horticulture, University of Maryland, College Park 20742.

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ABSTRACT

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Black-seeded cultivars of *Phaseolus vulgaris* were resistant to *Rhizoctonia solani* (*Thanatephorus cucumeris*) infection, while most white-seeded cultivars were susceptible to both seed infection and preemergence damping-off. The seed coats of most white-seeded beans cracked readily before emergence. Extracts of white seed coats stimulated growth of

R. solani. The seed coats of resistant black-seeded cultivars adhered tightly to the cotyledons. Extracts of black seed coats contained phenolic compounds that inhibited growth of *R. solani*.

Additional key words: growth inhibition.

Rhizoctonia solani Kuhn [*Thanatephorus cucumeris* (Frank) Donk] causes seed infection and damping-off of *Phaseolus vulgaris* L. (bean). Growth of *R. solani* is increased by nutrients from external sources (6, 12, 15, 22), and seed exudates provide such a source (7,16). Chemical components of the seed coat also may determine the extent of seed infection in a manner similar to the control of onion smudge (8); high phenolic content of bean seed coats has been related to host resistance to pathogenic infection (3, 19). Resistance to *R. solani* exists in beans with colored seed coats (9, 11, 12, 13), and the genetic system that controls pigment formation in the seed coat also controls production of other phenols (4). The purpose of this study was to determine the relative importance of various seed-coat factors in protecting the germinating seed from *R. solani* infection.

MATERIALS AND METHODS

The *P. vulgaris* cultivars used as host plants included Harvester, Tendergreen, Red Kidney, Venezuela 54, and plant introduction (P.I.) 165426. Harvester and Tendergreen have been classified as susceptible to *R. solani*, while Venezuela 54 and P.I. 165426 were classified as resistant (9, 12). Red Kidney has been used as the host plant for *R. solani* experiments (1). Harvester and Tendergreen have white seed coats, Red Kidney has a red seed coat, and the other two cultivars have black seed coats and a smaller seed size.

The isolate of *R. solani* used in these experiments was obtained from bean hypocotyls grown in a naturally

infested soil from Fruitland, Iowa. Identification of the pathogen was based on the presence of septal pores, multinucleate cells, typical cell morphology, and the branching characteristic (10, 11).

The bean seeds were surface sterilized with 0.525% sodium hypochlorite for 5 minutes, and rinsed five times with sterile distilled water. The pathogen was grown in a cornmeal-sand (1:39, w/w) medium for 10 days, and then mixed (1:30, v/v) with a pasteurized soil-sand (3:1, v/v) growing media in a greenhouse bench. Air temperatures fluctuated between 21 C and 27 C. Plant age was determined from time of seeding in the bench. Seed coats were artificially cracked with a scalpel. The design of the intact vs. cracked seed coat experiment was a randomized complete block with five replications and 100 seeds per treatment per replication. Noninoculated controls were grown under similar conditions except that the pathogen was not added to the soil mix.

Extracts were prepared by boiling 25 g of removed seed coats in 100 ml ethanol (95%) for 5 minutes, then homogenizing in a blender for 3 minutes at 21 C. The homogenate was passed through four layers of cheese cloth and filtered through a Büchner funnel under partial vacuum. The filtrate was centrifuged at 2,000 g for 20 minutes at -6 C. The clear supernatant was evaporated to dryness and resuspended in 5 ml of distilled water. The suspended supernatants from the seed coat extracts were spotted on Whatman No. 1 filter paper (50 μ liters/spot), and partitioned with *n*-butanol: acetic acid: water (4:1:5, v/v) epiphase at 20 C for 24 hours during which time the solvent front moved approximately 30 cm. The paper chromatograms were dried overnight in the dark at 80 C.

The dried paper chromatograms were then cut into 10 equal R_f ranges: 0.0–0.10, 0.10–0.20 ... 0.90–1.00. The substances contained in these ranges were eluted with 5 ml of sterile distilled water. Then 0.75 ml of the solution from each range was evaporated on a sterile petri dish. Fifteen ml of potato-dextrose agar (PDA) was added, and the dishes were agitated gently to mix the contents. The plates were seeded with agar disks (~2 mm in diameter) from the margin of *R. solani* cultures grown for 3 days on PDA at 21 C. Colony diameter was recorded after 72 hours of incubation.

Amino acids from the seed coat extract were separated on a Dowex 50-X4 (200- to 400-mesh) resin column at 10 C into acidic, basic, and neutral fractions (11, 20, 21). These fractions were concentrated in vacuo to 10 ml, and 100 μ -liters of the fraction was spotted on unidimensional ascending paper chromatograms using a butanol: acetic acid: water (4:1:5) solvent system. When the chromatograms were dry, they were sprayed with 0.3% ninhydrin solution in 95% ethanol, and developed at 80 C overnight in the dark. Identifications of amino acids were based on their R_f values compared with those of known standards. Quantitative determinations were not attempted.

The flavonoid compounds were extracted from 25 g of excised seed coats in 100 ml solvent as reported for anthocyanins (5), flavonoid glucosides (17), and leucoanthocyanins (2). Separation and partial purification of these compounds were done on Whatman No. 1 paper chromatograms. Identification was based on their R_f values and color reaction to NH_3 , AlCl_3 and Na_2CO_3 under both visible light and ultraviolet (UV) radiation (17). The number of these flavonoid compounds located on paper chromatograms were recorded. Phenolic substances were isolated from seed coat extracts by a series of extraction and precipitation methods (18). The number of spots detected on paper chromatograms under UV radiation and treated with diazotized sulfanilic acid (DSA) were estimated visually and recorded.

RESULTS

In a comparison of the effect of inoculation with *R. solani* on the germination of the resistant cultivar Venezuela 54 and the susceptible cultivar Tendergreen, *R. solani* retarded germination percentage and emergence of Tendergreen, but had little effect on Venezuela 54 (Fig. 1).

Cracking the seed coats increased the percentage of damping-off considerably for all cultivars (Table 1). Approximately one-third of the seedlings from the black-seeded cultivars with cracked seed coats did not damp off, while the loss for the other cultivars with comparable treatment was nearly total. It was noted that a large percentage of the seed coats of the white- or red-seeded cultivars normally cracked upon hydration during germination, and were very easily removed. In contrast, the seed coats of the black-seeded cultivars adhered tightly to the cotyledons and generally remained intact until the radical emerged. Many times, the seed coats of these cultivars remained over the cotyledons until after emergence, but the seed coats of the other cultivars separated from the cotyledons before emergence in most instances.

TABLE 1. Percentages of damping-off in *Phaseolus vulgaris* cultivars with either intact or cracked seed coat at 30 days after planting in *Rhizoctonia solani*-infested growing media^a

Cultivar	Seed coat color	Condition of seed coat	
		Cracked (%)	Intact (%)
Venezuela 54	black	62.4 x ^b	3.5 x ^b
P.I. 165426	black	63.1 x	15.8 x
Red Kidney	red	94.3 y	69.9 y
Tendergreen	white	97.6 y	74.7 y
Harvester	white	98.5 y	76.3 y

^aEach value is based on 500 seeds.

^bPercentages in each column followed by the same letter are not statistically different ($P = 0.05$) according to Duncan's multiple range test.

TABLE 2. Relative amounts of amino acids and phenolic substances in *Phaseolus vulgaris* seed coat extract as determined by paper chromatography

Cultivar	Seed coat color	Water extract	Compounds detected	
			Amino acids ^a (no.)	Phenols ^b (no.)
Venezuela 54	black	purple	8–10	3
P.I. 165426	black	purple	8–10	2
Red Kidney	red	red	12–14	1
Tendergreen	white	...	14–16	0
Harvester	white	...	14–16	0

^aNumber of amino acids detected by ninhydrin spray on paper chromatograms.

^bNumber of spots detected on paper chromatograms by ultraviolet radiation and reaction to diazotized sulfanilic acid.

TABLE 3. Numbers of flavonoid compounds found in seed coat extracts of *Phaseolus vulgaris* cultivars having different seed coat colors

Cultivar	Seed coat color	Flavonoid compounds ^a		
		Anthocyanin	Flavonol glucoside	Leucoanthocyanidin
Venezuela 54	black	2	4	1
Red Kidney	red	1	2	0
Harvester	white	0	0	0

^aNumber of flavonoid compounds detected on paper chromatogram were based on their R_f values and color reaction to NH_3 , AlCl_3 , and Na_2CO_3 under visible light and ultraviolet radiation.

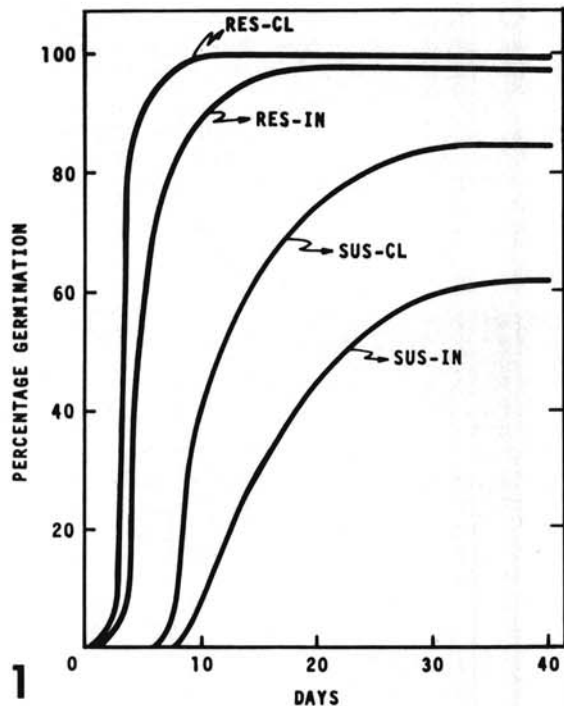


Fig. 1. Effect of *Rhizoctonia solani* on seed germination of *Phaseolus vulgaris* cultivars Venezuela 54 (RES) and Tendergreen (SUS). Legend: CL = control; IN = inoculated.

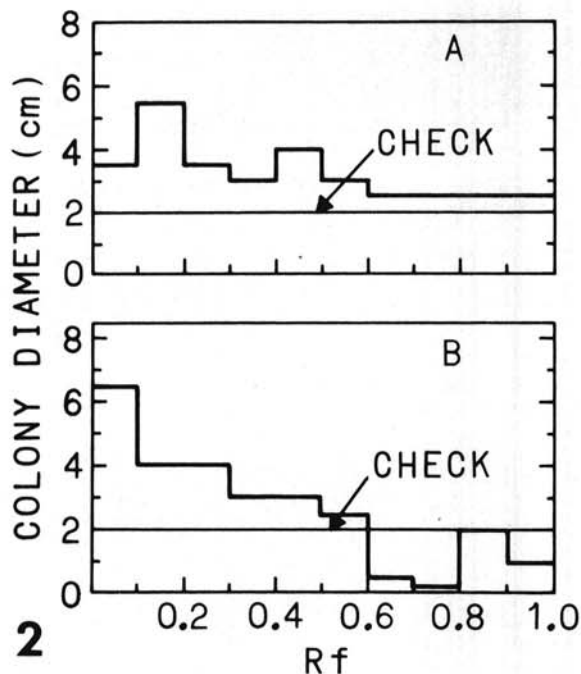


Fig. 2-(A, B). Colony growth of *Rhizoctonia solani* on potato-dextrose agar in response to the addition of different R_f ranges obtained by paper chromatography from alcohol extracts of *Phaseolus vulgaris* seed coats of A) susceptible cultivar Tendergreen, B) resistant cultivar Venezuela 54.

When the seed coat extract supernatants were separated by paper chromatography, all R_f ranges from Tendergreen increased the diameters of *R. solani* colonies by 5-35% compared with the control; the greatest increases occurred at R_f 's 0.10-0.20 and 0.40-0.50 (Fig. 2). Ranges 0.0 to 0.10 through 0.50 to 0.60 from cultivar Venezuela 54 also increased colony diameters, but ranges 0.60 to 0.70, 0.70 to 0.80, and 0.90 to 1.0 reduced colony size. *Rhizoctonia solani* growth on range 0.80 to 0.90 from the Venezuela 54 extract was the same as the control.

Other chromatographic analyses of the seed coat extract supernatants demonstrated that seed coats of Tendergreen and Harvester contained the highest amounts of amino acids, while those of Venezuela 54 and P.I. 165426 had the lowest content, and Red Kidney was intermediate (Table 2). A reverse trend was indicated for phenolic content, with Venezuela 54 seed coats showing a higher content than P.I. 165426. No phenols were detected in Tendergreen or Harvester seed coat extracts. Chromatographic determination of the number of flavonoids present in the seed coat extracts indicated a trend among cultivars similar to that found for all phenols (Table 3).

DISCUSSION

This experiment confirmed reports (11,12) that a considerable variation in susceptibility to preemergence damping-off caused by *R. solani* exists among *P. vulgaris* cultivars. The results also agree with those of other workers (14) which showed that a difference in percentage and rate of emergence occurs among cultivars even in the absence of this pathogen.

A comparison of seedling emergence from seed with intact seed coats with that from seed with artificially cracked seed coats during germination in the presence of *R. solani*, indicated the importance of an intact seed coat in the resistance of *P. vulgaris* to seed infection by this pathogen. Since many of the seed coats of Red Kidney, Tendergreen, and Harvester cracked during the initial hydration of the seed, they only protected the germinating seed from infection for a very short time. When all seed coats were artificially cracked before planting, there was almost no emergence for these three cultivars.

The seed coats of Venezuela 54 and P.I. 165426 usually remained intact during hydration, and the percentage of seedlings which damped-off was low. Cracking the seed coats of these two cultivars before planting increased the percentage of seedlings which damped-off. These data indicate that, under the conditions of this experiment, approximately two-thirds of the resistance to *R. solani* damping-off in these cultivars is related to the seed coats remaining intact until they are burst by the hypocotyl during germination.

The resistance level of Venezuela 54 with cracked seed coats was considerably greater than that of Tendergreen with similar treatment, so factors other than the soundness of the seed coat must be involved. Since the seed coat extract from Tendergreen increased the size and density of *R. solani* colonies as compared with the control, while a similar extract from Venezuela 54 reduced these measurements, the chemical content of the seed coat must be important in determining the level of

resistance of a particular cultivar. Other tests indicated the presence of phenols in the black-seeded cultivars, but not in the white-seeded ones. These phenols may be a factor in the superior resistance of the black-seeded cultivars, similar to the phenols responsible for onion smudge resistance in dry, colored onion scales (8).

In the opinion of the authors, the differences in levels of resistance to *R. solani* found among the cultivars used in this experiment were related primarily to the integrity of the seed coat, and secondarily to the phenol content of the seed coat. Since colored seed are not preferred by the processing industry, the relation between seed coat color and phenolic content is unfortunate (4). The importance of an intact seed coat in determining the level of resistance indicates the possibility, however, of the development of new white-seeded cultivars with a moderately high level of resistance to this pathogen.

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