The Role of the Cuticle in Resistance of Beans to *Rhizoctonia solani*

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ABSTRACT

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The resistance of 3-wk-old Red Kidney bean plants to *Rhizoctonia solani* is associated with the inability of the fungus to form infection cushions and penetrate the hypocotyl. Two factors suggested to be important in this resistance are increased calcification of cell walls and increased cuticle thickness. Cuticle permeability of hypocotyls of 1- and 3-wk-old seedlings was determined by immersing them in dyes. Dyes penetrated the cuticle of the younger, but not the older, plants. When hypocotyls of 3-wk-old plants were rubbed with cotton wetted with water or chloroform, gently abraded with Carborundum, rinsed with chloroform, or grown in a mist chamber, they became permeable to dyes. After inoculation, the fungus formed infection cushions and lesions on these treated older plants similar to those formed on young plants. Simple infection cushions were more common on treated older plants, whereas complex infection cushions predominated on young seedlings. The ability of the fungus to form infection cushions on older, more calcified plants after the cuticle has been altered suggests that cuticle thickness plays a more important role than calcification of cell walls in the resistance of older plants to *R. solani*.

Additional key word: *Phaseolus vulgaris*.

The susceptibility of bean seedling hypocotyls to *Rhizoctonia solani* Kühn decreases until they are about 3 wk old. Two explanations have been proposed for this phenomenon. Bateman and Lumsden (2) found a higher calcium content in older hypocotyls. These data, together with the observation that walls from older plants are less readily macerated by fungal endopolygalacturonase, led to the hypothesis that the pathogen was unable to macerate calcified walls of older hypocotyls and cause lesions. Others suggested that resistance of radish, groundnut, and cotton to *Rhizoctonia* is determined prior to penetration and is associated with the inability of the pathogen to form infection cushions (6,12,16). Infection cushion formation apparently requires exudates (8) which are reduced by the thicker cuticle of older plants (12). Thus, older bean plants may be resistant because the quantities of the exudates are insufficient to stimulate infection cushion formation.

Stockwell and Hanchey (14) confirmed by chemical analysis and ultrastructural histochemistry that the calcium content of hypocotyl walls increases with age. Additionally, we found that cuticle thickness was greater on older hypocotyls.

The purpose of the present research was to determine the role of the cuticle in resistance of older bean hypocotyls to *R. solani*.

MATERIALS AND METHODS

Bean (*Phaseolus vulgaris* L., 'Red Kidney') seeds were planted in 10-cm-diameter plastic pots containing steamed soil and placed in a greenhouse. *R. solani* isolate MAB, AG 4, kindly provided by D. F. Bateman, North Carolina State University, was grown in PDA slant tubes. Plants were inoculated with *R. solani* by placing approximately five sclerotia from PDA slant tubes adjacent to the hypocotyl at the soil line. Inoculated hypocotyls were moistened with distilled water and placed in a humidity chamber for 48 hr at 25 C. The disease incidence (DI) was determined by dividing the number of infected plants 72 hr after inoculation by the total number of plants inoculated. The DI was expressed as a percentage. In all cases, when lesions developed, the fungus was reisolated.

Alteration of the cuticle. To determine the effect of cuticle removal on resistance, hypocotyls of 1- or 3-wk-old plants were treated by one of the following methods, then rinsed with distilled water and inoculated as described above. Control plants were rinsed with distilled water. Hypocotyls of some plants were rubbed three times with cotton (on an orangewood stick) moistened with either distilled water or chloroform. The hypocotyl surface of other plants was gently abraded with Carborundum. To avoid physical effects (such as surface topography changes) associated with rubbing, stems were rinsed with 1 ml of chloroform or the plants were grown in a mist chamber (90% RH, 25 C) for 3 wk, to retard cuticle development. After treatment, hypocotyls were examined under fluorescence microscopy for changes in cuticular autofluorescence. Fresh cross sections of hypocotyls were mounted in distilled water on a glass slide and examined with an Olympus BMF fluorescent microscope with two OG-12 excitation filters and a 515-nm barrier filter.

Staining with methylene blue or neutral red. Staining with methylene blue or neutral red was used to determine the relative permeability of the cuticle to dyes. Bean plants, 1 or 3 wk old, untreated or treated as described in the previous section, were gently removed from soil, rinsed, and immersed in either 0.1% (w/v) neutral red or 0.1% (w/v) methylene blue for 10 min. Upon removal from the dye, plants were rinsed with distilled water, and the dye-staining pattern on the hypocotyl surface was observed.

Infection cushion morphology. The method of El-Samra et al (5) was used to study the morphology of infection cushions. Bean hypocotyls of 1-, 2-, and 3-wk-old plants were wrapped with cellophane, rinsed, and inoculated as previously described. Three-week-old plants were rinsed with water or chloroform, or rubbed with Carborundum before wrapping. After 48 hr, the cellophane was removed, stained with 0.1% trypan blue in lactophenol, rinsed, mounted in lactophenol on glass slides, and observed with a light microscope. Infection cushions were counted and categorized, according to complexity of branching and size as defined by El-Samra et al (5). Infection cushions in category I are simple with few branches, while those in category V are large and highly branched. Complex infection cushion types III, IV, and V were grouped since they are morphologically similar and differ primarily in size.

RESULTS

Effect of cuticle alteration on lesion formation. *R. solani* rarely formed infection cushions or lesions on untreated older bean plants. However, lesions formed if the cuticle of older plants had

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been abraded or the plants had been grown in a mist chamber (Table 1). Susceptible, 1-wk-old plants had a DI of 70%, whereas the DI on 3-wk-old plants was 3%. Treatment of the hypocotyl of 3-wk-old plants by rubbing, rinsing with chloroform, or by prior growth in a mist chamber increased the DI to 62–78%. Lesions on older plants were similar to those found on young plants. They extended into the cortical tissue, were elliptical, and became brown and sunken as they matured. Lesions formed only in treated areas. The application of chloroform seemed in itself damaging because the hypocotyl appeared to be water-soaked and developed a brown coloration. After inoculation, however, lesions developed and the fungus was reisolated from the tissue.

Staining. Placing plants in solutions of either neutral red or methylene blue was a quick, reliable method to illustrate irregularities in the cuticle of bean hypocotyls. The two dyes stained areas of the hypocotyl equally well where the cuticle was thin.

Two distinct staining patterns were found on the hypocotyl surfaces of 1-wk-old seedlings grown in soil: streaks and patches. Thin vertical streaks generally occurred on the aboveground portion of the hypocotyl. Streaks were less common on the sides of the hypocotyl beneath the cotyledons or on seedlings grown on moistened filter paper. Areas of the hypocotyl that stained as small, diffuse patches were commonly found at or beneath the soil line or associated with the point of emergence of secondary roots.

The hypocotyls of 3-wk-old untreated bean plants did not stain with either dye. However, hypocotyls of plants that were rubbed with chloroform- or water-soaked cotton, rubbed with Carborundum, or rinsed with chloroform stained readily in the treated areas. Additionally, there was a decrease in cuticular autofluorescence in the treated areas when compared to nontreated plants. The hypocotyl of plants grown in a mist chamber stained in patches along its entire length. In all cases, regardless of age, the primary and secondary roots stained heavily with the dyes. These results suggest that dye penetration occurred only through thin or abraded areas of the cuticle.

Infection cushion morphology. Infection cushions that formed on cellophane wrapped around bean hypocotyls prior to inoculation were easily observed. Hyphae of *R. solani* branched frequently and formed numerous infection cushions, the majority of which were complex, on 1-wk-old plants (Table 2). The proportion and pattern of complexity of infection cushions on 2-wk-old plants was similar to that on 1-wk-old bean plants.

On 3-wk-old nontreated hypocotyls, fungal hyphae were sparsely branched, and no infection cushions were observed. However, when 3-wk-old plants were rinsed with chloroform or rubbed with Carborundum, infection cushions formed. Lesions also developed on the hypocotyls of older plants that had been subjected to these treatments. The infection cushions that formed on these older, treated plants were primarily simple (types I and II). More complex forms of infection cushions (types III, IV, and V) were less common on older treated bean plants than on young plants.

DISCUSSION

The cuticle plays an important role in the resistance of older bean hypocotyls to *R. solani*. Altering the cuticle of older plants stimulated the formation of infection cushions, subsequent penetration, and lesion development. As seedlings emerge from the soil, they are subjected to physical forces, such as abrasion of the hypocotyl by soil particles as the seedling elongates upward. This is evidenced by the presence of areas on the hypocotyl that stained as streaks where it was exposed to the soil; such streaks rarely occurred on seedlings grown on moistened filter paper. Areas that were permeable to methylene blue and neutral red in these experiments, such as primary and secondary roots, have been reported to exude ninhydrin- and silver nitrate-reactive compounds (11,13). It may be inferred that areas on the hypocotyl that are permeable to dyes are also areas of increased exudation. After the hypocotyl emerges from the soil and elongates, it is no longer permeable to dyes. Stockwell and Hanchey (14) showed that the cuticle of older plants is thicker and (as judged by the lack of staining in the present study) intact. The quantity of exudates from hypocotyls decreases over time (6,12,13), which may be related to increasing cuticle thickness.

A decrease in exudation from older plants could have a significant effect on disease development. Numerous researchers have shown that the formation of infection cushions is an important stage of pathogenesis (4,6,7,9,10,15,16). Infection cushion formation is stimulated by chemical, not topographical, factors (6,8,12). On young bean seedlings numerous complex infection cushions are formed, whereas on older plants resistance to *R. solani* is characterized by a lack of infection cushions. Marshall and Rush (10) found that *R. solani* AG 1 did not form infection cushions on resistant rice cultivars. They suggested that wax or associated compounds inhibited infection cushion formation or increased wax deposition decreased exudation of a stimulator of infection cushion formation. The removal of wax deposits by chloroform increased the susceptibility of these cultivars. El-Samra et al (5) found simple infection cushions were more commonly formed on cotton cultivars susceptible to *R. solani* AG 4, whereas more complex forms predominated on resistant cultivars. Their work suggested that one factor of genetically based resistance of cotton to *R. solani* AG 4 was related to the type of infection cushion formed. Our results suggest that the type of infection cushion is not the determining factor for successful penetration of bean hypocotyls. As bean plants age, even with treatments that alter the cuticle, the total number of infection cushions decreases and the morphology of infection cushions is predominantly of the simple types. These results suggest that not only do the total amounts of exudates decrease, but that exudate quality is changed.

While it is clear that chemical factors influence the ability of *R. solani* to form infection cushions and to penetrate hypocotyl tissues, the question of how *R. solani* degrades the calcified cell walls of older bean plants remains unanswered. Bateman (1) and

TABLE 1. Incidence of Rhizoctonia hypocotyl canker on young and older bean plants

Treatment of hypocotyl prior to inoculation	1-wk-old plants		3-wk-old plants	
	Infected/ inoculated	DI[a] (%)	Infected/ inoculated	DI (%)
None	33/47	70	1/36	3
Rubbed with Carborundum	10/12	83	15/24	62
Rubbed with water-wetted cotton	...[b]	...	15/23	65
Rubbed with chloroform-wetted cotton	...	...	11/14	78
Rinsed with chloroform	11/12	92	12/18	67
Grown in a mist chamber	...	...	11/17	65

[a] Disease incidence (DI) = (total number of plants infected/total number of plants inoculated) × 100. Infection was defined as the presence of a lesion on the hypocotyl from which *R. solani* was reisolated.
[b] Treatment was not performed.

TABLE 2. Infection cushion formation on cellophane-wrapped bean hypocotyls inoculated with *Rhizoctonia solani*

Plant age (wk)	Hypocotyl treatment	Plants (no.)	Infection cushions (no.)	Type of infection cushion (%)[a]		
				I	II	(III, IV & V)
1	None	16	467	10.5	25.3	64.3
2	None	18	129	17.8	27.9	54.2
3	None	12	0	0	0	0
	Chloroform-rinsed	12	226	38.0	59.7	2.2
	Carborundum-rubbed	12	117	31.6	63.2	5.1

[a] Infection cushion types I and II (see ref. 5) represent simple infection cushions. Complex infection cushion types III, IV, and V were grouped. Each type of infection cushion present on the cellophane is expressed as a percentage of the total number of infection cushions.

Bateman and Lumsden (2) showed that pectolytic enzymes produced by *Rhizoctonia* are unable to macerate calcium pectate, and Stockwell and Hanchey (14) confirmed that calcium is present in higher concentrations in older bean hypocotyl cell walls. Additional work is needed to isolate the groups of enzymes active in vivo in lesions formed on older treated bean plants. In vitro studies by Bateman et al (3) showed that hemicellulytic enzymes with substantial activity are produced by *R. solani.* Hemicellulytic enzymes may play a greater role in cell wall degradation on these older bean tissues than pectolytic enzymes. The results suggest that resistance of older bean plants is related to increased cuticle thickness and consequent reduced exudation and infection cushion formation. Thus, cell wall calcification and the inability of endopolygalacturonase to macerate walls of older plants (3), although of interest, are less important determinants of resistance of older bean plants to *R. solani.*

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