The Effect of Iron and Boron Amendments on Infection of Bean by *Fusarium solani*

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ABSTRACT

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Mineral nutrition may affect the interaction of plants with microorganisms because of perturbations in phenol metabolism. On bean (*Phaseolus vulgaris*), *Fusarium solani* f. sp. *phaseoli* produces hypocotyl lesions in which phenolic components accumulate. Lesion size was increased 59–91% by growing bean seedlings in hydroponic solutions containing lower (5 μM $FeCl_3$) rather than higher (50 μM $FeCl_3$) iron content. Absence of boron in the nutrient solutions caused a 47–75% increase in lesion size compared to those in seedlings grown with 25 μM borate. In nutrient solutions lacking boron but containing 5 μM $FeCl_3$, the lesion size was 183% larger than for plants grown with 50 μM $FeCl_3$ and 25 μM borate. Although iron and boron deficiencies both contributed to increased lesion size, low availability of these nutrients differentially affected the accumulation of lignin. Absence of boron increased the accumulation of polymerized phenolics in the lesion area by about 10%; whereas, low iron availability reduced lignin formation by about 30%.

Fusarium solani (Mart.) Sacc. f. sp. *phaseoli* (Burk.) Snyd. and Hans. is the causal agent of root rot of bean (*Phaseolus vulgaris* L.). The disease is prevalent in alkaline loam soils (1,2) and is characterized by reddish-brown lesions that develop on the hypocotyl and lateral roots. Oxidized polyphenolics and phenolic phytoalexins accumulate in the lesions (10). The dramatic effects of infection by *Fusarium* on phenolic metabolism suggest that disease severity could be affected by plant nutritional deficiencies that perturb phenolic pathways. Lewis (5) suggested that borate deficiency enhances production of cinnamoyl residues and phenolic phytoalexins while repressing the polymerization of phenoxy radicals to lignin. The deposition of lignin in plant tissues challenged by microorganisms has been associated with a defense response (3,9). Effective lignification requires the function of an iron-containing enzyme, peroxidase. Consequently, one effect of iron deficient nutrition could be an impaired ability to produce lignin because of reduced peroxidase activity.

The purpose of this research was to investigate the effects of iron and boron nutrition on disease severity and lignin formation in beans infected by *F. solani*. Hydroponic culture was used to obtain precise control of iron and boron supplied to the plants.

MATERIALS AND METHODS

Culture of *F. s.* f. sp. *phaseoli*. Cultures of *F. s.* f. sp. *phaseoli* were isolated from infected Pinto beans from Southern Utah (2). The cultures were maintained by bimonthly transfers onto potato-dextrose agar. Conidial suspensions were obtained by flooding 5-day-old plates with sterile water, rubbing the agar surface, and filtering this wash through cheesecloth. Suspensions were centrifuged at 10,000 *g* for 10 min and the pelleted spores were resuspended in sterile distilled water. After repeating the centrifugation procedure twice, spores were resuspended and their concentration was determined with a hemacytometer. Suspensions were used as inoculum within 2 hr.

Growth of bean seedlings. Seeds of *P. vulgaris* 'Dark Red Kidney' were surface sterilized twice by a 10-min immersion in 1% sodium hypochlorite (NaOCl). Culls were discarded and the cleaned seeds were transferred to vermiculite in plastic flats. The vermiculite had been immersed previously in 2 M HCl for15 min and the acid was removed by extensive water washing. Immediately prior to use, the vermiculite was sterilized with 1% NaOCl for 15 min and thoroughly washed with double-deionized water.

The plants were grown under greenhouse conditions at 16–22 C and were watered with double-deionized water. After growth to the first trifoliate leaf stage, each seedling was washed and transferred separately to a 1-L polystyrene container for growth in hydroponic culture. Opaque, brown containers were used to exclude light from the nutrient solutions. The presterilized containers were filled with sterile nutrient solution prepared as described by Hoagland (4) but modified in iron and borate amendments. Either 5 or 50 μM of FeEDDHA or $FeCl_3$ were used as the iron source. Boron, as H_3BO_3, was either omitted or added at 25 μM. In addition, the nutrient solution contained 5 mM $Ca(NO_3)_2$, 1 mM K_2PO_4, 5 mM KNO_3, 1 mM NH_4NO_3, 2 mM $MgSO_4$, 0.4 μM $ZnSO_4$, 0.15 μM $CaSO_4$, 4 μM $MnCl_2$, and 0.025 μM Na_2MO_4. The initial pH of the nutrient solution was adjusted to 6.7 with 1 M KOH. The electrical conductivity was between 2,300 and 2,400 μmhos. Each plant was held in position in its container by a sterilized styrofoam plug. The solutions were aerated from the bottom of the containers by air entering from tubing attached to a pump. The volume of the solution was adjusted during the growth period either with half-strength Hoagland's solution, deficient in iron or borate, or with deionized water to keep within the designated electrical conductance range.

The treatments were arranged in a randomized block design with five replicates. Each experiment was conducted three to five times. Designated containers were supplemented at the time of transplant with 10^3 conidia of *F. s.* f. sp. *phaseoli*. Containers with control plants did not receive fungal inoculum. The four nutrient combinations were; 50 μM Fe^{3+} plus 25 μM borate; 5 μM Fe^{3+} plus 25 μM borate; 50 μM Fe^{3+} plus zero borate; and 5 μM Fe^{3+} plus zero borate.

Samples of nutrient solution were removed at selected times for examination of pH, chemical composition, and microbial populations. Bean seedlings were harvested 18 days after transplanting and were examined for the presence of lesions at the root-stem interface. The weight of the whole plant and root were recorded. Samples of plant tissue were oven dried at 105 C for 1.5 hr prior to measurement of iron and lignin content. The iron content in dried leaf tissue was determined by the Soils Laboratory at Utah

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State University. Leaf tissue was selected for iron determination because chlorosis of developing leaf tissue was an early symptom of iron deficiency.

Determination of lignin. For each seedling, the lower 5-cm of the root and the root-shoot interface section were dried and ground to pass through a 40-mesh screen. Lignin was extracted from these tissues and quantified (6).

Determination of *o*-dihydroxyphenols. The nutrient solutions were assayed for the first 8 days after transplant for the presence of *o*-dihydroxyphenols (7,8). Caffeic acid (Sigma Chemical Company, St. Louis, MO) was used as the standard.

RESULTS

Growth of uninoculated bean plants under hydroponic conditions was impaired 16–20% by reducing iron in the nutrient solution from 50 to 5 μM $FeCl_3$ (Table 1). Leaves of plants grown in 5 μM $FeCl_3$ developed chlorosis after 16 days. No chlorosis was observed in plants with 50 μM $FeCl_3$ or 50 μM Fe EDDHA nutrition. Absence of added boron decreased growth 15–30%, except for plants grown in 5 μM iron in which no additional effect of boron deficiency was observed (Table 1). Leaves of plants grown in the absence of added boron had a rough appearance and showed leaf-edge cupping at 18 days.

Inclusion of inoculum of *F. solani* in the nutrient solutions dramatically reduced plant weight gain under all conditions (Table 1). With 5 μM $FeCl_3$ nutrition, marginal interveinal chlorosis was observed at 18 days in leaves from plants inoculated with *F. solani*. Foliage remaining on inoculated plants growing in 50 μM $FeCl_3$ was deep green throughout the experimental period. Lesions at the root-shoot interface of inoculated plants were first visible after 5 days and enlarged with time. Borate amendments did not affect the timing of chlorosis or lesion formation. *F. s.* f. sp. *phaseoli* was reisolated from the root-shoot interface of inoculated plants but not from the control seedlings. Root apices of plants inoculated with *Fusarium* frequently were dark brown compared with the white color of those of uninoculated seedlings. Segments of the brown root apices plated on potato-dextrose agar yielded colonies of *F. solani*.

Lesions were larger on plants grown in 5 μM $FeCl_3$ than in 50 μM $FeCl_3$, by 91% in the presence of borate and 62% in the absence of added borate (Table 1). Boron deprivation increased lesion size by 75% with 50 μM $FeCl_3$ and 48% with 5 μM $FeCl_3$ nutrition (Table 1). Inoculated plants supplemented with 50 μM FeEDDHA developed lesions similar in size to those obtained with 5 μM $FeCl_3$ nutrition. The lesion size was enhanced 87% in plants growing in boron deficient-FeEDDHA media.

Fusarium infection increased the amount of material extractable from the root-shoot interface by a procedure designed for lignin estimation (6). In treatments with 50 μM FeEDDHA or 50 μM $FeCl_3$, lignin at the interface area increased 43–54% with infection by *Fusarium* (Table 1). When 5 μM $FeCl_3$ was supplied, infection by *Fusarium* again increased lignin content relative to uninoculated plants but only by 10% with added borate and by 20% without it. Plants infected by *Fusarium* and grown with 5 or 50 μM $FeCl_3$ produced about 10% more lignin when the nutrient media lacked borate supplementation.

The intense green color of leaves of seedlings infected by *Fusarium* and the effect of low-iron nutrition in promoting disease severity encouraged additional studies of plant properties related to iron metabolism. In infected plants supplied with 50 μM $FeCl_3$, terminal leaves contained more iron than those of control plants by 108% in the presence and 67% in the absence of added borate (Table 2). Leaves from infected plants grown with 5 μM $FeCl_3$ supplement did not show elevated iron content (Table 2). The pH of solutions containing 5 μM $FeCl_3$ and uninoculated plants was stable at 6.7 for the first 5 days after transplant. The pH decreased daily to 5.2 in the presence and 3.8 in the absence of added borate by day 8. The pH of the media for both uninoculated plants in 50 μM $FeCl_3$ and of inoculated plants remained between 6.0 and 7.0 during the 8-day period.

The accumulation of *o*-dihydroxyphenols in the nutrient medium was examined because of a proposed role in iron utilization of caffeic acid secreted by plant roots (8). With uninoculated plants grown in nutrient solutions containing either 5 μM or 50 μM $FeCl_3$ no *o*-dihydroxyphenols were detected in the medium for 2 days after transplanting. Subsequently, there was a linear increase up to 4 μM by day 8. In contrast, with Fusarium-infected plants, *o*-dihydroxyphenols increased by day 1 to 7 μM with 5 μM $FeCl_3$ and 9.5 μM with 50 μM $FeCl_3$. These concentrations declined during the next 6 days: at day 8 the concentration, 4 M, did not differ from that of uninoculated plants.

DISCUSSION

Hydroponic culture permitted study of the effects of limited iron and borate availability and of their interaction with the fungal pathogen, *F. solani* f. sp. *phaseoli*, on the growth of bean seedlings. Both boron and iron deficiencies reduced growth of uninfected plants which confirms the role of these elements as essential growth factors. Growth of the plants was dramatically reduced by inoculation of the seedlings with *F. s.* f. sp. *phaseoli*. Hydroponic culture permitted rapid development of the same type of lesion that is observed following natural infection by *F. solani* of beans in soil. The speed of lesion formation suggested that hydroponic culture may predispose the plant to infection by this pathogen. Hydroponic culture clearly differs from field growth conditions in physical, microbial, and nutrient parameters that may influence disease-related parameters.

TABLE 1. The effects of boron and iron deficiency on fresh weight gain, lesion length, and lignin accumulation for bean seedlings uninfected or infected with *Fusarium solani* f. sp. *phaseoli*

Nutrient conditions[a]	Fresh weight gain[b] (g)		Lesion length (cm)[c]	Lignin content[d] (mg/g dry wt)	
	Uninfected	Infected by *F. solani*		Uninfected	Infected by *F. solani*
50 μM FeEDDHA plus:					
25 μM borate	14.5 a	1.5 a	2.3 a	177 a	259 a
Zero borate	12.4 a	3.2 b	4.3 b	NA	NA
50 μM $FeCl_3$ plus:					
25 μM borate	14.4 a	4.1 b	1.2 c	160 a	230 b
Zero borate	10.2 b	3.4 b	2.1 a	160 a	246 a
5 μM $FeCl_3$ plus:					
25 μM borate	12.2 a	4.2 b	2.3 a	168 a	185 c
Zero borate	12.7 a	2.2 a	3.4 b	157 a	189 c

[a] Plants were grown for 18 days under aerated hydroponic culture in media containing the iron and borate concentrations as described in Materials and Methods.

[b,c,d] Fresh weight gain, lesion length, and lignin content were determined as described in Materials and Methods. Means with a common letter within columns were not significantly different, $P = 0.05$, according to Duncan's new multiple range test. NA = not available.

TABLE 2. The effect of iron and borate deficiency in iron content of leaves of bean uninfected and infected with *Fusarium* f. sp. *phaseoli*

Nutrient treatment[a]	Iron content[b] (μg/g dry weight)	
	Uninfected	Infected by *F. solani*
50 μM $FeCl_3$ plus		
25 μM borate	370 a	625 a
Zero borate	425 b	710 a
5 μM $FeCl_3$ plus		
25 μM borate	375 a	320 b
Zero borate	310 c	300 b

[a] Plants were grown for 18 days under aerated hydroponic culture in media containing the iron and borate concentrations described in Materials and Methods.

[b] Iron contents of leaves from plants uninoculated or inoculated with *F. solani* were determined 18 days after growth under described nutrient conditions. Means with a common letter within columns were not significantly different according to Duncan's multiple range test, $P = 0.05$.

Disease severity was increased by seedling growth in nutrient solutions with restricted iron and borate availability. These nutrient stresses cumulatively enhanced disease, as measured by reduction in plant growth and the increased lesion size. Lesion size varied also with the form of iron supplied to the plants. Lesions on plants supplemented with 50 μM FeEDDHA were larger than those observed on plants grown in 50 μM $FeCl_3$. This variability in lesion size may relate to differences in the ability of the infected plant and pathogen to utilize iron in the chelated and nonchelated form.

Borate effects were studied because of the suggested connection between boron nutrition and phenol metabolism. Lewis (5) reported that boron deficiency in plants limited polymerization of phenolics. Consequently, borate-deficient tissues are predicted to have reduced lignin levels but higher amounts of low-molecular-weight phenolics. In uninfected beans, however, the amount of lignin extracted from the root-shoot interface was not influenced by borate nutrition. The occurrence of reddish-brown pigmented lesions, characteristic of infection by *F. solani*, in both borate-supplemented and borate-deficient plants suggested that oxidized polyphenols were produced even with limited boron nutrition. The amount of lignin extracted from the lesion area was not decreased but slightly increased in plants grown in the absence of added borate. The lack of the predicted borate effect in uninoculated plants may reflect early secondary wall development in the root-shoot zone when borate from the seed was available. Results from the tissues infected by *F. solani* may suggest differential regulation of phenolic metabolism in healthy and pathogen-challenged tissues.

The effects of limited iron nutrition on lignification of the root-shoot tissue, like boron nutrition, were only apparent for the plants infected by *F. solani* and not for uninoculated plants. Reduced lignification in the lesioned tissue that accompanied low-iron (5 μM $FeCl_3$) nutrition may reflect impaired activity of the plant peroxidase, a heme enzyme. Peroxidase is an essential enzyme for lignin formation. The relationship between root peroxidase activity and growth under iron-limited conditions is currently being studied in our laboratory.

The infection of bean seedlings by *F. solani* affected other phenomena associated with iron metabolism. Lowered pH of the nutrient media was not observed with the inoculated plants under low iron availability although acidification did occur with the uninoculated plants. Secretion of *o*-dihydroxyphenols was accelerated with infections by *Fusarium* and was independent of iron availability. The leaves of plants grown under adequate iron nutrition accumulated more iron when infected by *F. solani*. These effects re-emphasize the complexity of interactions between nutritional and pathogen-induced stresses on plant metabolism. However, enhanced susceptibility of bean to infection by *F. solani* under limited iron and borate nutrition was demonstrated successfully in hydroponic culture. Consequently, the reproducible growth conditions possible with hydroponics may permit the gathering of physiological and biochemical data that would be more difficult to obtain with plants grown in pot culture or in the field.

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