Effect of Inoculum Concentration on Resistance of Lima Bean to Rhizoctonia solani

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ABSTRACT

The effect of inoculum concentration of *Rhizoctonia solani* on resistant and susceptible lima bean cultivars was determined. A linear relationship between inoculum concentration, disease severity, and seedling infection was observed with Jackson Wonder, a resistant cultivar. The relationship between disease severity and seedling infection for Fordhook 242, a susceptible cultivar, was linear up to 30% and 50% inoculum concentration, respectively. Low concentrations of inoculum are sufficient to test selections of lima beans for resistance to *Rhizoctonia*. In addition, at low concentrations, seedlings intermediate in reaction can be separated.

Additional key words: Phaseolus lunatus, disease severity.

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Hypocotyl rot of lima bean *(Phaseolus lunatus* L.) caused by *Rhizoctonia solani* Kuehn is a severe soilborne disease in most lima-bean growing areas in eastern United States. Invasion of the hypocotyls results in pre- and postemergence damping-off. The fungus persists in soil particles or organic debris as mycelia and sclerotia (2). Previously, studies were conducted on the effect of inoculum concentration on infection of snapbeans and colonization of green and dried plant segments by *R. solani* (9, 10). Methods for evaluating inoculum densities of various soil-borne pathogens and techniques for analyzing such data have been developed (1, 13). Davey and Papavizas (4) showed that the frequency of isolation of *R. solani* from soil with buckwheat-stem segments increased with an increase in inoculum densities. Good correlation between colonization of buried plant segments and pathogenicity were obtained (9, 10). However, effects of various concentrations of *R. solani* on symptom expression in resistant and susceptible bean plants have not been studied.

Concentration of inoculum affects symptom expression in apparently resistant melons to *Fusarium oxysporum* f. *melonis* (5), and symptom expression of beans is influenced by inoculum concentration of *F. oxysporum* f. sp. *phaseoli* (7). The work reported herein was initiated to determine the effect of various inoculum densities of *R. solani* on disease severity in susceptible and resistant cultivars of lima bean and to determine the effects of inoculum density on saprophytic colonization of plant segments by *R. solani*.

MATERIALS AND METHODS.—Isolates LB-1 and LB-3, both lima bean pathogens (15), were used in these tests. The isolates belong to anastomosing group 4.

Rhizoctonia solani was grown on sterilized grain oats. After incubation for 14 days, inoculum was added to Elsinboro sandy loam (pH 6.0) at a concentration of 1.0 g infected oats/kg of soil. The infested soil was continuously cropped with susceptible Fordhook 242 (FH 242) lima beans until all seedlings were severely infected with *R. solani* whenever the soil was planted. A noninfested sandy loam that had a pH of 6.2 was used as a diluent. The two soils were passed through a 5-mm sieve and mixed thoroughly in various proportions to obtain several graded inoculum concentrations of propagules of *R. solani*. Large quantities of soil were mixed in order to complete all tests with the same mixture.

Cultivars used in this study included: FH 242, which is susceptible in the seedling stage in both greenhouse and field tests; Jackson Wonder (JW), a resistant cultivar in both greenhouse and field tests; and S-400, which is somewhat susceptible in the seedling stage in the greenhouse, but is relatively resistant in the field (15). All treatments in the greenhouse were replicated five times in a randomized block design.

Bean seeds were planted in *Rhizoctonia*-infested soil, which had been adjusted to contain various concentrations of inoculum. Three weeks after planting, the seedlings were carefully removed from the soil and rated for disease severity on a 0-5 scale (4), and the percentage of diseased seedlings also was determined.

Competitive saprophytic activity of *R. solani* at different inoculum densities was evaluated by burying lima-bean hypocotyl segments (5 mm long) and beet seeds

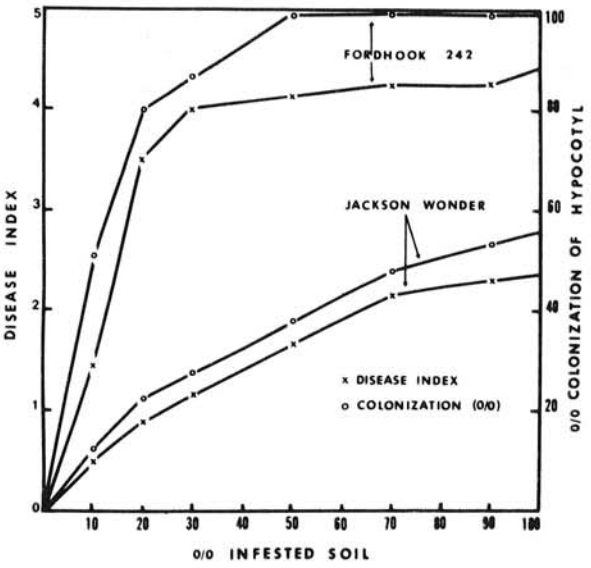

Fig. 1. Disease severity index for hypocotyl rot (caused by *Rhizoctonia solani)* in lima bean relative to inoculum concentration (percentage of infested soil in the soil mixture) and percentage colonization of 5-mm-long lima bean hypocotyl segments or beet seeds after three days of incubation in the soil mixtures.

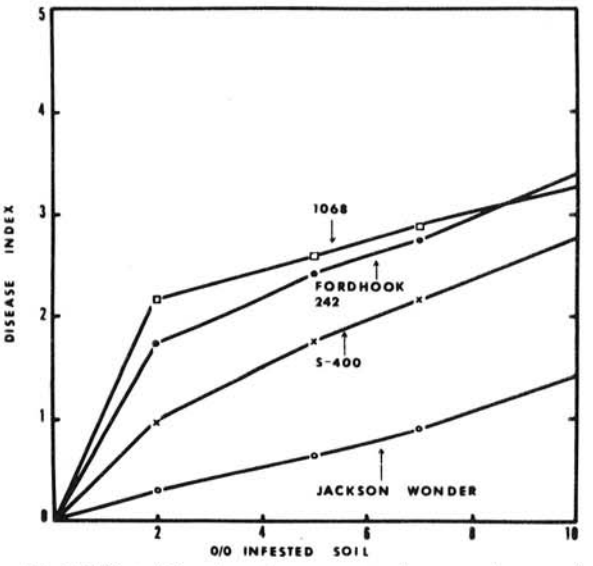

Fig. 2. Effect of four inoculum concentrations on resistance of lima-bean selections and cultivars to infection by *Rhizoctonia solani.*

in soil in which susceptible and resistant lima-bean cultivars had been growing. Hypocotyl segments or beet seeds buried in fallowed soil, served as the control. The beet seeds and hypocotyl segments were incubated in the soil for 3 days, recovered by sieving, washed 15 minutes in tap water, and placed on water agar amended with streptomycin sulfate and aureomycin hydrochloride, each at 100 mg/liter (8, 9). Results were recorded as

percentage of beet seeds or hypocotyl segments yielding *R. solani*.

RESULTS.—*Disease severity and host-tissue colonization as influenced by inoculum density.*—The relationship between average disease index and inoculum concentration is shown in Fig. 1. A near-linear relationship between disease and inoculum concentration was observed with concentrations up to 30% infested soil for FH 242 and up to 100% for JW. Disease severity for FH 242 was not affected by inoculum concentrations higher than 50%. However, disease severity of JW was markedly increased as inoculum concentrations increased.

Colonization of lima-bean hypocotyl sections and disease-severity index at the various inoculum levels were positively correlated, which agrees with an earlier report (14). Hypocotyl sections of FH 242 were colonized more readily than those of JW at various concentrations. All of FH 242 segments were colonized in soil mixtures of 50% or higher; whereas the highest percentage of colonization for JW was 58% in the undiluted infested soil.

Effect of inoculum concentration on disease expression of lima-bean cultivars.—An attempt was made to determine the minimum concentration at which differences could be measured on four different cultivars. Infested and noninfested soil mixtures of 0 to 10% were made. All other procedures were the same as in the earlier test.

At five of these low-inoculum levels (Fig. 2) there were significant differences in disease severity between susceptible and resistant lima-bean cultivars. Jackson Wonder showed few symptoms, FH 242 and 1068 were severely rotted, while S-400 showed less disease than FH 242. Differences in disease severity increased as the inoculum concentration increased. Coefficient of correlation determinations of *Rhizoctonia* hypocotyl rot plotted against inoculum concentration were high (0.98 to 0.99). An analysis of variance of the data in Fig. 2 gave significant differences between JW and the other cultivars at all concentrations used. Slope values of the disease severity rating curves of the four cultivars ranged from 0.138 for JW to 0.219 for S-400.

Effect of inoculum concentration on plant emergence and infection of susceptible and resistant cultivars.—The relationship between inoculum concentration and the percentage of infected lima-bean seedlings is practically linear up to 50% and 100% (undiluted soil) infested soil mixture for FH 242 and JW, respectively.

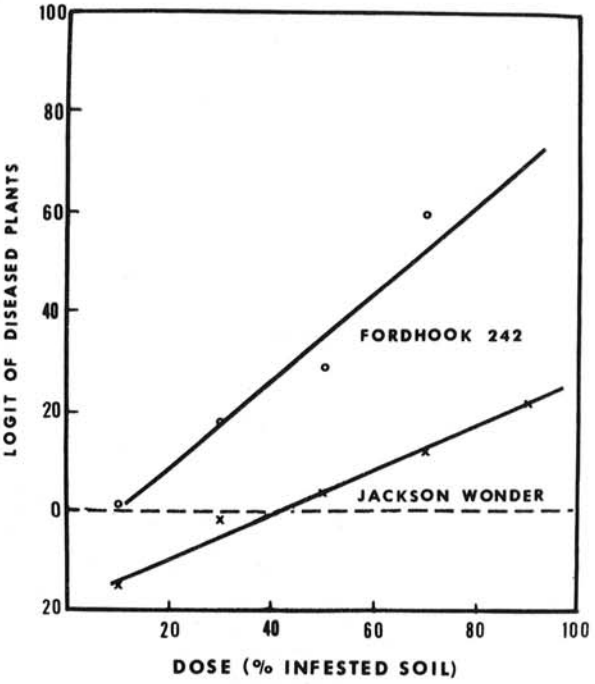

Fig. 3. Effect of inoculum concentration (percentage of infested soil in the soil mixture) on disease (hypocotyl rot of lima bean) incidence and seedling emergence.

The logistic curves for diseased plants plotted against dose (% infested soil) was not parellel for JW and FH 242 when logit analysis was used (Fig. 3). Thus the inoculum concentration for equal response is not the same for percentage of diseased plants. For example, a soil mixture containing 50% infested soil resulted in 95% infection of FH 242 seedlings, but 100% infested soil was required to infect all of JW seedlings. Although all JW seedlings were infected at the greatest inoculum level, lesions were small, and the disease index was low.

High inoculum concentration of *R. solani* reduced the emergence of FH 242 seedlings, but had little or no effect on emergence of JW. Plant emergence in 100% infested soil was 60 and 90% for FH 242 and JW, respectively.

Effect of inoculum concentration on colonization of beet seeds.—The effect of inoculum concentration on saprophytic colonization of substrate by *R. solani* has been extensively studied (4, 11, 13). In the present work,

TABLE 1. Colonization of beet seeds by *Rhizoctonia solani* at various times in infested soil in which resistant Jackson Wonder and susceptible Fordhook 242 lima beans were growing and control infested soil in which no plants had been grown

Infested soil in soil mixture (%)	Colonization[a] of beet seeds after specified weeks								
	Jackson Wonder			Fordhook 242			Control		
	2	4	8	2	4	8	2	4	8
10	8	5	4	8	11	6	9	7	4
30	10	7	7	18	15	10	19	9	6
50	60	54	51	63	58	53	66	49	31
70	65	60	58	66	62	60	69	52	46
90	67	62	59	65	62	62	69	54	50
100	71	67	66	75	71	63	75	69	59

[a]Percentage of buried beet seeds colonized.

emphasis was placed on the influence of the host on the ability of *R. solani* to colonize substrate.

Saprophytic activity of *R. solani* decreased as incubation of infected substrate in soil increased; however the rate of decrease was greater in uncropped infested control soil than in soil cropped to beans (Table 1). For example, with JW in 100% infested soil, the percent colonization after 2 and 8 weeks was 71 and 66% respectively, whereas in control soil, colonization was 75 and 59% respectively. Colonization was about equal at the various inoculum concentrations in both soils where plants were grown.

DISCUSSION.—Little is known about the response of susceptible and resistant hosts to inoculum concentrations of *Rhizoctonia,* since few resistant hosts are available for study. The studies reported here showed that disease severity of hypocotyl rot of susceptible lima bean seedlings by *R. solani* was linearly correlated with inoculum concentrations up to 30% infested soil, with further increases in inoculum concentrations resulting in relatively small increase in disease severity (Fig. 1). However, disease severity and inoculum concentration was linear up to 100% infested soil (undiluted soil) for the resistant cultivar. Thus, increasing inoculum concentration markedly increased disease severity for the resistant cultivar. The work reported here for the susceptible cultivar agrees with that of Sneh et al. (13) and Richards (12). They showed that there was a linear relationship up to a given level of inoculum, and further increases in inoculum concentrations caused only small increases in disease severity. However, Das and Western (3) reported a decrease in disease severity with heavy inoculum levels. Papavizas (9) reported similar results using autoclaved soil. Differences in results obtained here and elsewhere with similar and different research methods may be ascribed to specific conditions of the experiment or to modifications introduced into the method used. Techniques of infestation of soil may account for the apparent difference in disease severity. For example, in the present study, and in the experiments by Sneh et al. (13), successive crops of a susceptible bean cultivar were grown in lightly infested soil to increase inoculum potential.

The curvilinear relationship between inoculum densities and disease severity for a susceptible cultivar indicates the availability and early saturation of readily infectable sites or limited host response to infection. The linear relationship obtained with the resistant cultivar indicates the operation of a generalized host reponse to infection. It should be noted from this study that the inherent resistance of the host should be considered in studies involved in analysis of inoculum potential and disease severity of soilborne fungi.

These studies show that the percentage of seedlings infected, as measured by the number of plants showing visible lesions of *R. solani,* increased with an increase in inoculum concentration. Again, the relationship depends on the inherent resistance of the host plant tested. This was demonstrated when all FH 242 seedlings were infected at a soil inoculum concentration of 50%, whereas 100% infested (undiluted) soil was required to infect all of the JW seedlings. Although JW seedlings became infected, lesions were superficial and rarely girdled the hypocotyl, even at the highest inoculum levels. The hypocotyls of FH 242 were severely infected at 50% and all higher concentrations. Kendrick and Allard (6) reported similar observations for a tolerant lima-bean line.

The need for low inoculum levels in setting up a breeding program for *Rhizoctonia* resistance is indicated (Fig. 2), since high levels may overcome resistance that would provide adequate protection under field conditions. These results demonstrate that differences in disease reaction as well as incidence occurs among the cultivars tested at low levels of inoculum. Thus, lines with intermediate resistance can be selected that otherwise might be discarded as susceptible when tested at high inoculum levels.

On the basis of substrate colonization when an actively growing host was present, it can be concluded that heritable characters of the host did not appear to influence saprophytic activity of the pathogen (Table 1). Saprophytic activity of *Rhizoctonia* was about equal in soil from both the resistant and susceptible cultivars. However, the percentage of hypocotyls infected by *Rhizoctonia* was greater in the susceptible cultivar than the resistant one. This is particularly significant in view of the fact that *Rhizoctonia* mycelia and sclerotia were observed microscopically by Boosalis and Scharen (2) in decomposed organic debris in soil; and because the pathogen can assume a parasitic status from its saprophytic condition in the colonized substrate (14).

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