Colonization of Host Tissues and Infectivity of *Rhizoctonia solani*

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ABSTRACT

Saprophytic colonization of various host tissues by *Rhizoctonia solani* and their influence on hypocotyl rot of lima beans was studied. Leaf tissue was the most suitable substrate for colonization by *R. solani*, whereas root tissue was least susceptible. Soil amended with lima bean leaf tissue gave the highest disease index on lima bean hypocotyls and permitted the highest percentage of beet seeds to be colonized. Soil amended with root segments gave a lower disease rating on lima bean hypocotyls than the control, but did not alter colonization of the beet seeds.

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It is common practice in the Eastern United States to grow two successive crops of beans per year. Plant debris from the first crop is incorporated into the soil either by plowing and disking or disking alone and the second crop is planted 4 to 14 days after mechanical harvest of the first crop. Field observations indicate that the second of the two successive crops of beans is usually more severely damaged by *Rhizoctonia* than the first crop.

Studies of pathogenicity and competitive saprophytic ability of *Rhizoctonia solani* Kuehn have been reported (2, 3, 6). Saprophytic activity of this pathogen has been estimated by its ability to survive as sclerotia (4), colonize dead substrate buried in soil (4, 5), colonize nonhost plants (5), and invade seeds buried in soil (10).

Organic amendments generally influence symptom development and the severity of root disease. Crop residues (7, 11) have been added to soils in an attempt to suppress diseases. The effect of specific amendments on *Rhizoctonia* infection varies. Infection may be reduced, or not affected, depending on the residue used (6, 7, 8).

The importance of prior addition to soil of host tissue in promoting maximum pathogenicity has been demonstrated for some plant-pathogenic fungi (9, 18), but few studies have evaluated the influence of specific parts of the host plant on *R. solani* hypocotyl rot of bean. The present study was conducted to determine the extent of colonization by *Rhizoctonia* of specific parts of the host plant (lima bean, *Phaseolus lunatus* L.) in soil in successive cropping regimes, and the influence of these host tissues on the incidence of hypocotyl rot of lima bean seedlings.

MATERIALS AND METHODS.—In this study, a sandy loam soil with a pH of 5.9 and a moisture-holding capacity (MHC) of 36% was artificially infested with *R. solani*. The soil was cropped repeatedly to 'Fordhook 242' lima beans until all seedlings were infected by *Rhizoctonia*. Infested soil was mixed with noninfested soil (1:4, w/w), and the MHC was adjusted to about 50%.

Tissue segments (1 to 2 cm long) of roots, hypocotyls, petioles, and leaves were excised from 6-week-old 'Jackson Wonder' (resistant to *R. solani*) and Fordhook 242 (susceptible to *R. solani*) lima beans grown in autoclaved soil. Two grams of each tissue were mixed with about 300 g of soil and incubated 6, 24, and 48 hr in wide-mouth glass jars. After the incubation period, the tissue segments were recovered by sieving, washed for 20 min in running tap water, transferred to paper towels, partly dried, and placed in petri plates containing 15 ml of water agar (2%), to which streptomycin sulfate and aureomycin hydrochloride (100 mg of each/liter) had been added. A similar method for isolation of *Rhizoctonia* from buckwheat segments was described by Papavizas & Davey (14). Five segments of each tissue were plated on each of five petri plates for the two cultivars of lima bean. The plates were incubated at 25 C for 24 hr, then examined microscopically for growth of *R. solani*.

The effect of host tissue on competitive saprophytic ability of *R. solani* was studied by burying the beet seeds in soil, a method similar to that described by Pan (12). Tissue segments (2 cm long) were mixed with lightly infested soil and incubated at 25 C for 3 weeks. During that time *Rhizoctonia* colonized a percentage of the tissue segments in the presence of other soil microorganisms. At the end of the incubation period, nondecomposed tissue segments were recovered from the soil and discarded. Nearly all of the leaf tissue had decomposed. One gram of beet seed was mixed with 300 g of sieved soil from each treatment and incubated for 3 days at 25 C. After incubation, the beet seeds were recovered and washed for 20 min in running tap water. The seeds were then prepared and plated on the water agar similar to the method described for tissue segments. The plates were examined 24 hr after incubation, and the percentage of beet seed colonized by *Rhizoctonia* determined.

The effect of various tissue segments on the pathogenicity of *Rhizoctonia* in soil was studied by growing lima beans in soil previously amended with segments (0.5%, w/w) and incubated for 3 weeks. Ten

seeds of Jackson Wonder or Fordhook 242 lima bean were planted. Each treatment consisted of four replications in a randomized block design. Infection was evaluated by washing the seedlings and indexing typical lesions of *R. solani* on the hypocotyls. Disease severity was estimated, using an infection rating scale from 0 (no visible infection) to 5 (plants completely girdled) as originally suggested by Davey & Papavizas (7).

RESULTS.–*Isolation of Rhizoctonia spp. from host tissue buried in infested soil.*–The amount of colonization of plant segments after 6, 24, and 48 hr of incubation in *Rhizoctonia*-infested soil, as measured by the percentage of plant segments colonized, was influenced by the kind of host tissue used and the length of the incubation period (Table 1). Leaf tissue of both resistant and susceptible cultivars was the most suitable for isolating *R. solani* and was 96 to 100% colonized, respectively. Petiole segments of both resistant and susceptible cultivars (51 and 60%, respectively), and the hypocotyl segments of Fordhook 242 (53%) were extensively colonized after 48 hr. Root and hypocotyl segments of Jackson Wonder yielded the lowest percentage of *Rhizoctonia* isolates (14 and 12%, respectively).

None of the tissue was colonized when incubated for less than 6 hr. Hypocotyls and petioles were colonized more intensively 48 hr after incubation, which is consistent with similar results obtained by other workers (5, 7).

Effect of age of host tissue on colonization by Rhizoctonia.–Specific host tissues of different maturities, buried in soil (Fig. 1), were compared for their influence on colonization of beet seeds. Colonization of beet seeds was highest from soil previously amended with leaf tissue at all stages of maturity for both cultivars. On the other hand, colonization of beet seeds was lowest in soil amended with root segments. The source of tissue, and stage of hypocotyl or petiole maturity, significantly influenced beet-seed colonization. The percentage of beet seeds colonized by *Rhizoctonia* in soil amended with immature hypocotyl tissue, was greater than in soil amended with mature tissue. For example, the percentage of beet seeds colonized in soil amended

TABLE 1. Isolation of *Rhizoctonia* from parts of host tissue recovered after various intervals of burial in infested soil

Tissue	Isolation from indicated tissue[a]					
	Cultivar					
	Jackson Wonder (Resistant)			Fordhook 242 (Susceptible)		
	Incubation time (hr)			Incubation time (hr)		
	6	24	48	6	24	48
	(%)	(%)	(%)	(%)	(%)	(%)
Leaf	0	80 a[b]	96 a	6	96 a	100 a
Petiole	0	3 a	51 b	3	27 b	60 b
Hypocotyl	0	2 b	14 c	0	12 c	53 b
Root	0	2 b	12 c	0	8 c	27 c

[a] Percentage of buried segments of tissue colonized from 14-day-old plants.

[b] Numbers followed by the same letter are not significantly different at the 5% level.

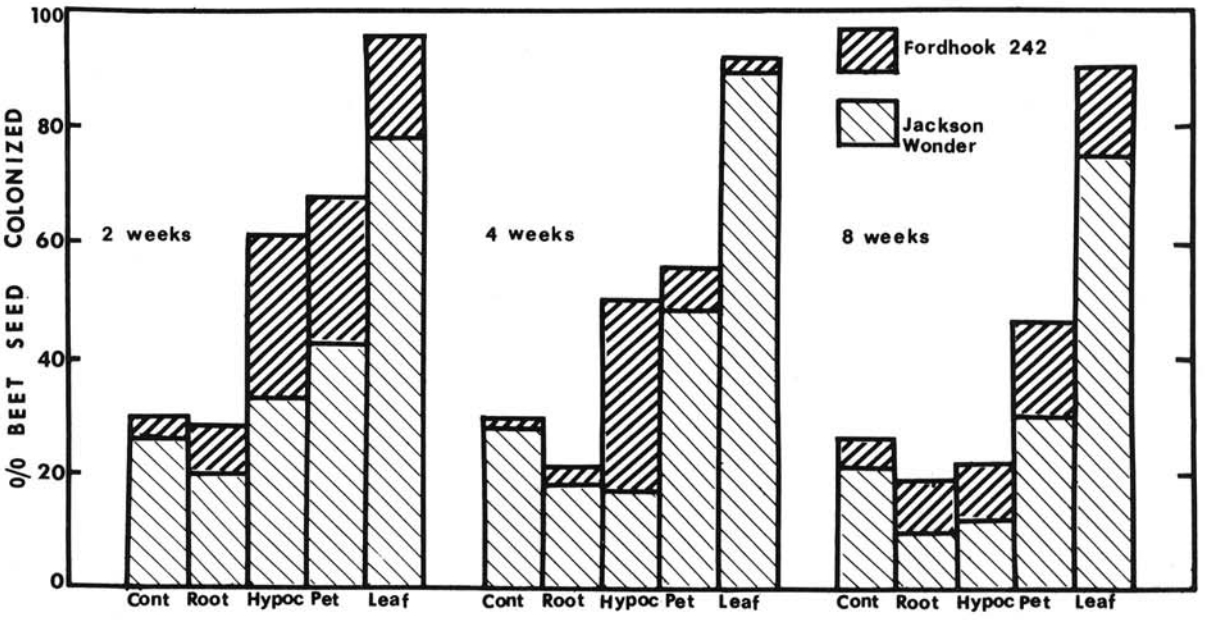

Fig. 1. Saprophytic (3-day) colonization of beet (*Beta vulgaris* L.) seed incubated in soil amended with specific plant parts of two lima bean cultivars (*Phaseolus lunatus* L.) for 2, 4, or 8 weeks. Seedlings of cultivar 'Jackson Wonder' are resistant to invasion by *Rhizoctonia solani*, and those of 'Fordhook 242' are susceptible.

with hypocotyl segments 2, 4, and 8 weeks old were 33, 18, and 12 for Jackson Wonder and 61, 50, and 22 for Fordhook 242, respectively. Colonization of beet seed from soil amended with leaf tissue at different stages of maturity remained relatively stable, regardless of the cultivar.

TABLE 2. *Rhizoctonia* disease severity on lima bean seedlings as affected by host tissues from resistant and susceptible cultivars of two different maturities

Tissue[b]	Infection index[a]			
	Fordhook	Jackson Wonder	Fordhook	Jackson Wonder
	2 weeks		6 weeks	
Root	0.32 a[c]	0.35 a	0.31 a	0.12 a
Hypocotyl	3.89 c	0.68 a	0.62 b	0.31 b
Petiole	2.84 b	1.63 ab	1.28 c	0.97 c
Leaf	4.09 d	1.94 b	3.50 d	1.49 d
Control	3.22 c	1.26 ab	3.18 d	1.32 d

[a] Severity rating on individual plants on a scale of 0 (no disease symptoms) to 5 (hypocotyls completely girdled).

[b] Tissue incubated in soil for 3 weeks before planting lima bean seeds. Lima beans were grown in soil before adding tissue.

[c] Numbers followed by the same letter are not significantly different at the 5% level.

Incidence of Rhizoctonia hypocotyl rot in soil amended with host tissue.—When Jackson Wonder and Fordhook 242 lima beans were planted in infested soil in which their respective host tissue had been incubated for 3 weeks and then removed, there was a significant decrease in infection of hypocotyls of both cultivars in soil amended with root tissue when compared to the control (Table 2). Amending soil with leaf tissue, resulted in a slight increase in hypocotyl rot of both cultivars, but resulted in a significant increase only when 2-week-old Fordhook 242 leaf tissue was used. The disease rating in soil amended with immature hypocotyl and petiole sections was not different from that of the control.

When mature tissue (6-week) was used, the pattern was similar to that for immature tissues, except that there was a significant decrease in hypocotyl rot in soil amended with mature Fordhook 242 hypocotyl tissue.

DISCUSSION.—*Rhizoctonia* may persist in soil as a saprophyte in tissues colonized during parasitism, or by colonizing dead plant tissues, within which it can remain for long periods of time (4, 13). Therefore, investigation of the soil factors which mediate saprophytic activity of *R. solani*, and the role of the host tissue involved, are prerequisite to an understanding of persistence of this pathogen under various cropping systems. *Rhizoctonia* is of great interest because it is a pioneer colonizer of fresh organic matter lying on or in soil. It is also parasitic on many plants. Therefore, conditions that affect its saprophytic existence in soil in the absence of a susceptible host may influence its subsequent pathogenic capabilities.

Rhizoctonia invades lima bean leaf and petiole tissues readily. Under warm, humid conditions it is capable of infecting all aerial plant parts of snap beans (19, 20) and soybeans (1), regardless of age. In the present investigation, lima bean leaves at all stages of maturity were suitable substrate for colonization by *R. solani*. In addition, the incidence of hypocotyl rot increased significantly compared to the control (Table 2), when a susceptible cultivar was grown in soil amended with immature-leaf tissue and was greater than, or equal to, that of the control in all other treatments. Thus, colonized leaves and, to a lesser extent, petioles are probably important sources of inoculum in fields where two successive crops are grown per year. Since leaf tissues decompose rapidly, infested leaves are probably not a source of inoculum when only one crop is grown per year. Coons & Kotila (6) showed that when one crop of beans was grown each season, the incidence of hypocotyl rot was not increased. These findings may account for part of the differences in the incidence of hypocotyl rot between one planting and two successive plantings.

From the experiments on the influence of host tissue from resistant and susceptible cultivars, it was learned that the resistant cultivar provided a substrate less suitable for colonization of *R. solani* than susceptible cultivar tissue, except where leaf tissue was used. However, the percentage of *R. solani* colonies isolated, and the incidence of hypocotyl rot was always lower from Jackson Wonder segments than from those of Fordhook 242. The leaves and petioles of both cultivars were suitable substrates for colonization. Thus, resistance to *R. solani* did not extend to petiole and leaves in Jackson Wonder, nor was leaf maturity a factor. Other lima bean tissues showed clear differences in the percentage of infection and percent of colonization between the two cultivars.

Root segments were always less vigorously colonized by *Rhizoctonia* spp. than other segments in both susceptible and resistant cultivars. This suggests that roots and mature hypocotyls are not readily available substrates for *R. solani*. Bateman & Lumsden (3) showed that calcium content of hypocotyl tissue increased with age, and that susceptibility was inversely related to calcium content. Highly lignified tissues, such as roots and mature hypocotyls, are also less readily macerated by enzyme preparations.

It would be of particular interest to determine whether removal of petioles and leaves of bean would affect disease incidence under field conditions.

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