Effect of Propagule Size of Rhizoctonia solani on Saprophytic Growth, Infectivity, and Virulence on Bean Seedlings

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ABSTRACT

The effect of inoculum concn and propagule size of *Rhizoctonia solani* Kuehn on rate of infection of bean and on damping-off disease in artificially infested soil was tested. A linear relationship between inoculum concn, rate of infection, and disease intensity was observed at concn up to 0.25 g/kg soil. Virulence of mycelial homogenates prepared from a culture grown on yeast dextrose broth was higher than that grown on potato-dextrose broth. Mycelial homogenate was fractionated by precipitation and sieving procedures, and fractions composed of propagules of similar size were tested for saprophytic growth and virulence towards bean seedlings. In the absence of external nutrients, saprophytic growth was positively correlated with propagule size and volume. No symptoms were observed in bean seedlings grown in soil infested with propagules smaller than 250 μ in size at a rate of four/g, whereas with propagules of 250-1,000 μ in diam, 100% of the host plants showed symptoms 7 days after inoculation. When bean seeds were directly inoculated with the 400 to 500-μ fraction, 67% and 93% of the host plants showed symptoms 21 days after inoculation with two and four propagules/seed, respectively. When placed in groups of eight or more at a distance of 2 cm from each seed, a significant proportion (30% or more) of the host plants developed symptoms. The possible fitness of these results to some mathematical models is discussed. Phytopathology 60:1351-1356.

Additional key words: Phaseolus vulgaris, disease severity, inoculum placement, propagule fractionation, effect of media on virulence.

Studies on the relationship between population level of *Rhizoctonia solani* Kuehn and disease severity of susceptible crops have been carried out either with naturally (13, 17) or with artificially (11, 15) infested soil. However valuable these studies could have been, the data obtained did not allow for a full evaluation of the factors involved in the quantitative relationship between inoculum and disease caused by *R. solani,* because of the ill-defined conditions of the inoculum. In both naturally and artificially infested soil, *R. solani* populations are very heterogeneous, being composed of mycelial fragments and sclerotia of various sizes and shapes. In addition, *R. solani*-free soil usually has been artificially infested by adding the fungal culture along with its growth medium to the soil, thus upsetting the biological equilibrium of the natural soil and changing the nutritional status of the host plants. In our work, a procedure for the preparation of propagules of *R. solani* of a uniform size is described. Employing this method, the relationship between propagule size and saprophytic growth and the effects of propagule origin, size, number, and distance from host on disease severity of bean seedlings are examined in detail.

MATERIALS AND METHODS.—An isolate of *R. solani* from infected beans was used as a test organism. The following media were used: (i) yeast dextrose broth (YDB) or agar (YDA), composed of (%, w/v) Bacto peptone (Difco), 0.5; Bacto yeast extract (Difco), 0.5; glucose, 2.0 and Bacto agar (Difco), 2.0, in distilled water; (ii) potato-dextrose broth (PDB) or agar (PDA), prepared from fresh potatoes; (iii) Martin's rose bengal medium; and (iv) tap water agar (TWA) supplemented with chloramphenicol (Chloromycetin Lederle) at a final concn of 250 ppm. All media except YDB and YDA were prepared according to Johnson et al. (10).

The inoculum was prepared by growing *R. solani* for 5 days on PDA at 28 C and homogenizing the whole culture (mycelium and agar medium) in a sterile Waring Blendor for 30 sec. Five-ml samples of the homogenate were added to Roux bottles containing 85 ml YDB, which were then incubated for 7 days at 28 C. The mycelium was thoroughly washed with sterile distilled water and homogenized in a Waring Blendor for 30 sec. Fractionation of the homogenized mycelium was carried out as follows: Ten g wet, homogenized mycelium were suspended in 1 liter distilled water and poured into a 3 × 40-cm glass column. Within 3 min, the heavier propagules (pieces of compact mycelial mat plus sclerotia of various sizes) settled on the bottom, whereas the lighter mycelial fragments floated. The two fractions were separated by decantation. In order to eliminate hyphae protruding from the compact propagules, the inoculum was treated in a MSE ultrasonic vibrator for 5 min, and the compact propagules were separated again from the mycelial fragments. The suspension containing the heavier propagules was then passed through a series of sieves, heavier propagules thus being separated according to their diam into the following fractions: < 150 μ, 150-250 μ, 250-400 μ, 400-500 μ, and 500-1000 μ.

Counts of viable propagules were made on Martin's rose bengal agar supplemented with chloramphenicol instead of streptomycin.

A sandy-loam, slightly alkaline soil was used. The

sieved, air-dried soil was thoroughly mixed with the inoculum. One-kg samples were distributed in 12.5 × 12.5 × 5-cm plastic containers. They were irrigated to field capacity and planted with 20 bean seeds each (*Phaseolus vulgaris* L. 'Brittle Wax') at equal distances from each other, in five replicates. All experiments involving plant growth were carried out in a greenhouse at 18-26 C.

For evaluating infection, seedlings were thoroughly washed under running tap water and their hypocotyls were cut into 5-mm segments, placed on TWA, incubated for 36 hr at 28 C, and observed for typical growth of *Rhizoctonia*.

Disease severity was estimated according to Sneh et al. (17), using an arbitrary scale originally suggested by Davey & Papavizas (5).

RESULTS.—*Effect of growth medium on virulence of R. solani.*—Potato-dextrose broth (PDB) and yeast dextrose broth (YDB) were compared for their influence on the virulence of the fungus. The effect of the composition of the growth medium on the virulence of *R. solani* has been already pointed out by Shephard & Wood (16).

R. solani was grown on a shallow layer of YDB and of PDB for 7 days at 30 C. Soil was infested with fungal homogenate, sown with bean seeds, and incubated in the greenhouse. Seedlings were examined for symptoms after 21 days.

Inoculum prepared from *R. solani* grown on PDB is clearly less virulent than that grown on YDB at a concn of 0.25 and 0.5 g/kg soil. Thus, at 0.25 g/kg, most seedlings were free of the fungus after 21 days, whereas with the same amount of inoculum from YDB, most seedlings were infected with the fungus, and showed typical symptoms of damping-off disease. However, in soil inoculated with 1 and 2 g/kg, differences between the two inocula were less prominent (Table 1).

Microscopic examination showed that YDB-grown propagules were much more compact than those grown on PDB. Colonies were visible after 24 hr when YDB-grown propagules were placed on Martin's rose bengal agar medium, but growth of PDB-grown propagules was not observed for 48 hr. All further experiments were carried out with inoculum grown on YDB.

Effect of inoculum concn of R. solani on infection and disease severity of bean seedlings.—The relationship between inoculum concn, using unfractionated mycelial homogenate, and the proportion of infected and diseased bean seedlings is practically linear up to 0.25 g/kg soil. Higher inoculum concn do not affect infection rate and disease severity after 14 and 21 days, as these already had reached a max at 0.25 g/kg on the 14th day. There is an increase in infection rate but not in disease severity with increasing inoculum concn up to 1 g/kg, 3 and 5 days after planting. When observed 7 days after planting, disease severity is almost linearly correlated with inoculum concn up to 1 g/kg soil (Fig. 1-A, B).

The relationship between average disease index of the host population and inoculum concn and time is shown in Fig. 1-C. A linear relationship is observed at concn up to 0.25 g/kg soil.

Effect of propagule size on disease severity.—Various fractions of homogenized mycelium of *R. solani* were mixed with soil at a final concn of four propagules/g. Groups of 100 seedlings were examined at intervals for symptoms, and the healthy ones were examined for possible colonization by *R. solani*. The results (Fig. 2) show that addition of propagules smaller than 250 μ to natural soil did not cause disease in bean seedlings. Moreover, although a small proportion of the host population (less than 10%) became infected after 5 days, no disease symptoms developed during the observation period (21 days). On the other hand, in soil infested with propagules larger than 250 μ, all seedlings were infected by the pathogen within 5 days, and symptoms developed within 7 days.

Infection and disease of bean seedlings caused by propagules of various sizes placed in direct contact

TABLE 1. Virulence of *Rhizoctonia solani* grown on potato-dextrose broth (PDB) and on yeast dextrose broth (YDB)

Growth medium	Amount of inoculum (wet mycelium) g/kg soil	% Noninfected seedlings[a]	Seedlings infected with *R. solani*[b]		Avg disease index[c]
			% without symptoms	% Showing symptoms	
PDB	0	94	0	6[d]	0.15 A*
	0.25	97	3	0	0.00 A
	0.5	67	7	26	0.54 B
	1	4	16	80	2.96 C
	2	3	15	82	2.83 C
YDB	0	95	3[d]	2[d]	0.02 A*
	0.25	23	10	67	2.26 B
	0.5	1	0	99	3.39 C
	1	1	4	95	3.42 D
	2	0	0	100	3.91 E

[a] Healthy and uninfected seedlings.
[b] Incubation time 21 days.
[c] The sum of all diseased plants multiplied by their corresponding indices divided by the total number of the plants. Treatment means are statistically different at the 5% level of probability (Newman-Keuls range test) if they do not have a common letter. Parallel treatment means of PDB and YDB are statistically different if they are not followed by an asterisk.
[d] Probably caused by indigenous *R. solani* propagules present in some samples of the natural soil used.

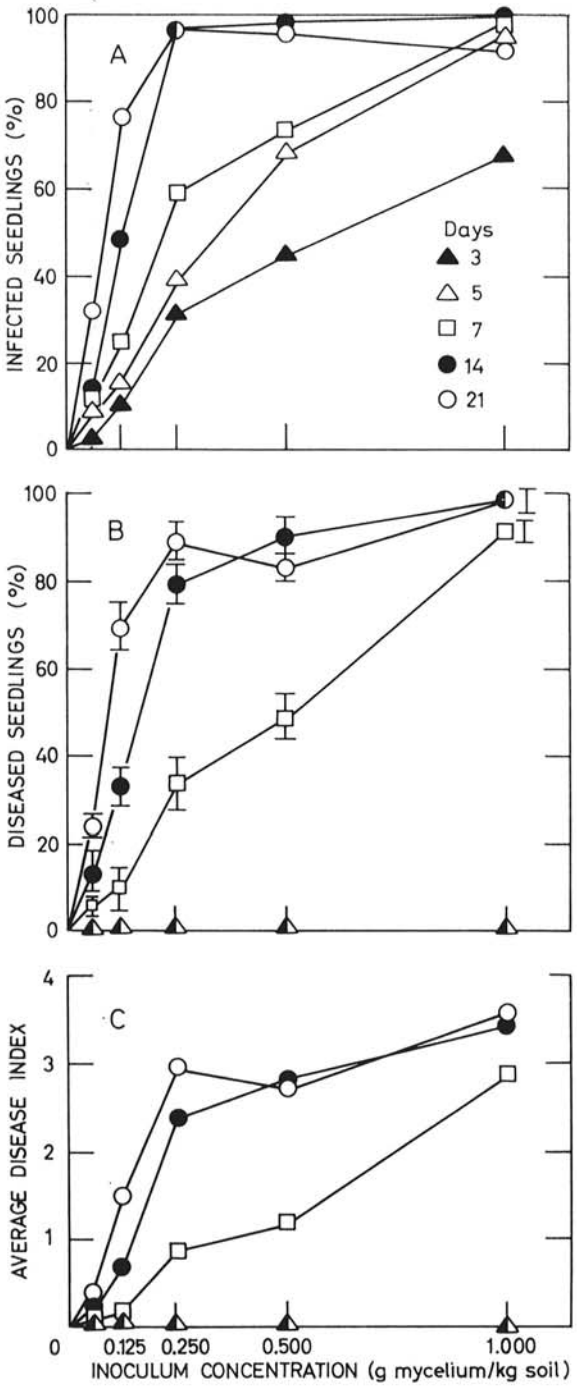

Fig. 1. Infection (A), disease (B), and average disease index (C) in bean seedlings as a function of inoculum concn of *Rhizoctonia solani* and time. Standard errors (standard deviations of the means) are enclosed by brackets. Average disease index is defined as the sum of all diseased plants multiplied by their corresponding indices divided by the total number of the plants.

with the seeds.—It was considered that propagules smaller than 250 μ may not provide sufficient energy for emerging hyphae to reach the infection court.

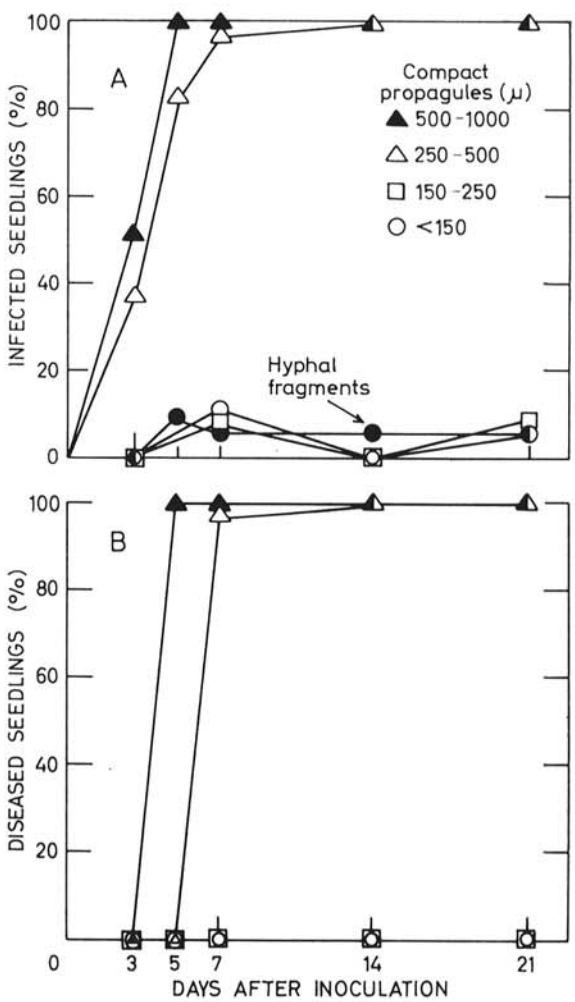

Fig. 2. Infection (A) and disease (B) of bean seedlings as a function of size of *Rhizoctonia solani* propagules and time. Concentration of hyphal fragments and compact propagules in soil: 4/g.

Therefore, individual propagules were applied directly to the bean seeds at sowing time by means of a fine needle, and the developing bean seedlings were tested for infection and for visible lesions after 21 days.

All symptomless seedlings were found to be free of *Rhizoctonia*. Application of propagules smaller than 150 μ did not result in disease development at a rate as high as 36/seed. On the other hand, application of propagules of the 500-μ fraction resulted in disease development in all the host population when applied directly at a rate of 32/seed; however, when applied at a rate of 8/seed, 15% of the seedlings still showed no symptoms after 21 days of incubation (Table 2).

In another experiment, bean seeds were directly inoculated with various numbers of propagules of the 400 to 500-μ fraction, and the seedlings examined for symptoms after 21 days.

The direct application of two propagules to each bean seed resulted in disease development in 67% of

TABLE 2. Disease severity in bean seedlings as affected by the size and number of the propagules of *Rhizoctonia solani* applied directly to the seeds

Propagule size, μ	Propagules/seed	% Noninfected seedlings[a]	% Seedlings infected with *R. solani*[b]	Avg disease index[c]
<150	0	100	0	0 A
	9	100	0	0 A
	36	100	0	0 A
150-250	13	78	22	0.55 A
	39	27	73	2.4 B
500-1,000	8	11	89	3.1 C
	32	0	100	3.3 C

[a] Healthy and uninfected seedlings.
[b] All infected seedlings showed symptoms; incubation time, 21 days.
[c] The sum of all diseased plants multiplied by their corresponding indices divided by the total number of plants. Treat-Treatment mears are statistically different at the 5% levelcf probability (Newman-Keuls range test) if they do not common letter. Incubation time, 21 days.

the host population. But in order to obtain 100% diseased plants, it was necessary to apply as many as 64 propagules/seed. With 32 propagules/seed, 7% of the seedlings still did not show any symptoms. A pronounced difference in disease index was observed with two propagules as compared with four propagules or more/seed. Seedlings infected with 4 to 32 propagules showed an average disease index between 2.8 and 3.3, whereas with those inoculated with 64 propagules, the disease index was 4.3 (Table 3).

Effect of propagule size on saprophytic growth of R. solani in the absence of external nutrients.—Propagules of various sizes were placed on glass slides and incubated in a water saturated atmosphere at 30 C, and the diam of the growing colonies, as well as the number of hyphal extensions emerging from each propagule, was recorded. The results, summarized in Table 4, show a positive relationship between saprophytic activity (as measured by multiplying colony diam by the number of hyphal extensions per colony) (B. Sneh, *personal communication*) and propagule size. When this experiment was carried out on TWA, growth of all propagules from 150 to 1,000 μ was similar, possibly indicating the ability of *R. solani* to utilize the small amount of nutrients present as contaminants in the TWA.

Effect of propagule distance from the seed on disease severity in bean seedlings.—Groups of 32 propagules 400-500 μ in size were placed in the soil at 2-3 cm depth at various distances from the bean seeds, and the developing seedlings were examined for symptoms after 21 days. A difference in infection index was observed with propagules placed at 0-2.5 cm from the seed as compared to 4-7.5 cm (Table 5).

Effect of number of propagules, placed 2 cm from seed, on disease development.—In this experiment, propagules were placed in the soil at a fixed distance from the seed (2 cm) in groups containing from 1 to 32 units of the 400 to 500-μ fraction. When 1 or 2 propagules were placed at 2-cm distance from each bean seed, no symptoms were observed in any of the developing seedlings after 21 days of incubation. On the other hand, when placed in groups of 8 or more, symptoms appeared in a significant proportion, though not in all of the developing seedlings (Table 6). As none of the hypocotyls came in direct contact with the propagules after germination, the seedlings were infected by *Rhizoctonia* as a result of the saprophytic growth of the fungus through natural soil.

DISCUSSION.—Severity of damping-off of bean seedlings disease by *R. solani* was found to be linearly correlated with inoculum concn, up to 0.25 g/kg soil.

TABLE 3. Disease severity in bean seedlings as affected by the number of propagules of *Rhizoctonia solani* applied directly to the seeds

Propagules/seed	% Seedlings free of *R. solani*[a]	% Infected seedlings[b]	Avg disease index[c]
0	100	0	0 A
2	33	67	1.9 B
4	3	97	3.3 C
8	8	92	2.8 BC
16	14	86	3.1 C
32	7	93	2.9 BC
64	0	100	4.3 D

[a] Healthy and uninfected seedlings.
[b] All infected seedlings showed symptoms.
[c] The sum of all diseased plants multiplied by their corresponding indices divided by the total number of the plants. Treatment means are statistically different at the 5% level of probability (Newman-Keuls range test) if they do not have a common letter. Incubation time, 21 days; propagules size, 400-500 μ.

TABLE 4. Effect of propagule size of *Rhizoctonia solani* on hyphal growth from propagules on glass slides in absence of external nutrients[a]

Propagule size, μ	Colony diam, cm	Avg no. of extensions/propagule	Colony diam (cm) × avg no. of hyphal extensions[b]
500-1,000	1.28	19	24.3 A
250- 500	0.68	9	6.12 B
150- 250	0.44	5	2.20 B
<150	0.52	3	1.56 B

[a] Incubated at 28 C in a water-saturated chamber for 120 hr.
[b] Treatments means are statistically different at the 5% level of probability (Newman-Keuls range test) if they do not have a common letter.

TABLE 5. Effect of distance of propagules of *Rhizoctonia solani* from infection site on the severity of damping-off of bean seedlings

Distance of propagules from host, mm[a]	% Diseased seedlings	Avg disease index[b]
0	100	2.9 B
10	100	3.6 A
25	75	2.2 B
40	9	0.26 C
50	6	0.23 C
75	0	0.17 C

[a] Placed in groups of 32/seed.

[b] The sum of all diseased plants multiplied by their corresponding indices divided by the total number of the plants. Treatment means are statistically different at the 5% level of probability (Newman-Keuls range test) if they do not have a common letter.

Further increase in inoculum concn resulted in a relatively small increase in disease severity, as estimated by the disease index method. These results, obtained with artificially infested soil, are similar to those reported earlier for naturally infested soil by Richards (14) and by Sneh et al. (17). On the other hand, the decrease in disease severity in soil heavily infested with *R. solani,* reported by Sanford (15) and by Papavizas & Davey (13) for naturally infested soil, and by Das & Western (4) for artificially infested soil, was not observed in our experiments. It should be noted that the technique used for testing the effect of fungus concn on disease severity in naturally infested soil involved dilutions with *Rhizoctonia*-free soil.

In the case of artificially infested soil, this difference in results could be explained by the different methods of inoculum preparation. Most authors use the whole culture (fungus plus growth medium) as inoculum (9, 11, 16). Consequently, changes which undoubtedly affect disease severity may take place in the composition and activity of the soil microflora, as well as in the nutritional status of the host plant. On the other hand, in naturally infested soil conditions may become more favorable for the fungus upon dilution with *Rhizoctonia*-free soil, enabling it to multiply and spread faster, and to cause more damage to the host plant.

TABLE 6. Effect of number of propagules placed 20 mm from the seed on damping-off of bean seedlings caused by *Rhizoctonia solani*

Propagules/seed	% Diseased seedlings	Avg disease index[a]
1	0	0 A
2	0	0 A
4	11	0.1 A
8	40	1.7 B
16	30	0.8 AB
32	63	2.0 C

[a] The sum of all diseased plants multiplied by their corresponding indices divided by the total number of the plants. Treatment means are statistically different at the 5% level of probability (Newman-Keuls range test) if they do not have a common letter. Incubation time, 21 days; propagules size, 400-500 μ.

Under a given set of conditions, development and severity of damping-off of bean seedlings by *R. solani* depends on the number of hyphae reaching infection courts and on the susceptibility of plants to infection, which in turn depend upon their age. The higher the inoculum level and the younger the seedlings, the more severe the disease. The onset of disease as well as its development are very short at high inoculum level. At lower inoculum concn, however, average disease severity increases at a constant rate during 21 days, which is within the period of host sensitivity to *R. solani* (6). At higher inoculum levels, a higher proportion of infected, as compared to diseased seedlings, was observed during the first 2 weeks of the experiments. After 21 days, however, proportions of infected and diseased plants were similar, indicating that most of the infected plants eventually developed disease symptoms.

Three mechanisms may be involved in increasing disease development with time: (i) increase in inoculum concn of the pathogen through both saprophytic and parasitic growth; (ii) occurrence of new infections originating either from previously infected courts during the sensitivity period, or from saprophytically growing hyphae, which reach the infection courts during the observation period; and (iii) great variability in germination time of the *R. solani* propagules.

The use of fractions composed of propagules of similar size has enabled us for the first time to examine quantitatively the effect of size, number, and distance from the host of *R. solani* propagules on disease severity of bean seedlings. Our results indicate clearly that hyphae emerging from propagules smaller than 150 μ cannot incite disease symptoms in bean seedlings, regardless of their concn in the soil. On the other hand, disease developed in the total plant population when propagules 500-1000 μ in size were mixed with soil to give an average concn of only 4 propagules/g.

The dependence of virulence on propagule size in *R. solani* is probably correlated with its nutrient reserves (2, 16), which determine the ability of the propagule to produce hyphae which, in the absence of external nutrients, reach the infection court or the rhizosphere, where specific root exudates or toxins influence the infection process and the development of symptoms (6). In general, the problems involved in the *R. solani*-bean system are strikingly similar to those concerning the inoculum potential of spores of *Botrytis cinerea* and *B. fabae sardiña* placed on leaves of *Vicia faba* (7); a minimal number of spores is required to incite disease, depending on species, spore size, and spore nutrition.

It seems that the propagules' energy is used by *Rhizoctonia* for (i) saprophytic growth through the soil; (ii) infection of the host; and (iii) inciting disease. Only propagules larger than 150 μ may provide enough energy to the emerging hyphae for all three purposes. Moreover, when placed together at a distance from the host, propagules can provide energy

to the hyphae of each other. Thus, infection of the host does not occur when two propagules are placed 2 cm from the host, but infection does occur when 32 propagules are placed together at the same distance. These findings emphasize the importance of location and distribution of the pathogen in the soil.

The use of fractions composed of propagules of similar size allows for a better understanding of the relationship between inoculum concn of *R. solani* and severity of damping-off disease of bean seedlings. Van der Plank (18) and Baker et al. (1) developed mathematical models which express the relationship between disease and inoculum level. The possible use of these models in the prediction of disease severity in crops infected by *R. solani* is discussed elsewhere (8). Here we demonstrated that hyphae emerging from propagules of *R. solani* present in the soil volume around the bean seed at 5-cm diam are capable of inciting disease. According to Baker et al. (1), if the propagules germinate in the rhizosphere and are under a directional stimulus toward the infection court, the volume of influence would be in the form of hollow cylinder, and additional inoculum in this volume should result in a proportional increase in infections. Indeed, this type of relationship was observed at relatively low inoculum concn 7 days after inoculation. It differs from that observed by Martinson (11), who obtained a direct relationship on a semi-logarithmic scale between inoculum concn of *R. solani* and damping-off disease of radish seedlings.

It should be noted that in another set of experiments (17), results obtained were successfully fitted into Van der Plank's equation (18), which assumes a random distribution and homogeneity of the pathogen and a homogeneous population of the host. Indeed, it can be shown that plotting $\log_e \frac{1}{1-y}$ (when y is the proportion of diseased plants after 7 days; Fig. 1-B) instead of y, against inoculum concn, would also yield a straight line. Thus, our results seem to fit into both mathematical models, in spite of their different approaches.

According to Papavizas (12), the density of viable propagules of *R. solani* in naturally and artificially infested soil is greater in the very coarse and coarse fractions than in the very fine sand and in the silt-clay fractions. This pattern of distribution was verified by the segment colonization method as well as by pathogenicity tests with beans and radishes. This also agrees with our results, which demonstrate the importance of propagule size. But in our studies inoculum consisted of propagules only, with no accompanying external food reserves, whereas in naturally infested soil germinating propagules use the particulate soil organic matter, in which they are embedded, as a food base. It would be of interest to compare the effectiveness of internal and external food base in inciting disease in susceptible hosts. Such studies may lead to the improvement of the direct examination method developed by Boosalis & Scharen (3) for the quantitative estimation of *R. solani* in the soil. A better prediction of disease potential of a given soil may be achieved by recording, in addition to the relative abundance of organic matter particles harboring *R. solani*, the size of these particles.

LITERATURE CITED

1. Baker, R., C. L. Maurer, & Ruth A. Maurer. 1967. Ecology of plant pathogens in soil. VII. Mathematical models and inoculum density. Phytopathology 57:662-666.
2. Blair, I. D. 1943. Behavior of the fungus *Rhizoctonia solani* Kühn in the soil. Ann. Appl. Biol. 30:118-127.
3. Boosalis, M. G., & A. L. Scharen. 1959. Methods for microscopic detection of *Aphanomyces euteiches* and *Rhizoctonia solani* and for isolation of *Rhizoctonia solani* associated with plant debris. Phytopathology 49:192-198.
4. Das, A. C., & J. H. Western. 1959. The effect of inorganic manures, moisture and inoculum on the incidence of root disease caused by *Rhizoctonia solani* Kühn in cultivated soil. Ann. Appl. Biol. 47:37-48.
5. Davey, C. B., & G. C. Papavizas. 1959. Effect of organic soil amendments on the *Rhizoctonia* disease of snap beans. Agron. J. 51:493-496.
6. Flentje, N. T. 1959. The physiology of penetration and infection, p. 76-87. *In* C. S. Holton, G. W. Fischer, R. W. Fulton, Helen Hart, & S. E. A. McCallan [ed.] Plant Pathology Problems and Progress, 1908-1958. The University of Wisconsin Press, Madison.
7. Garrett, S. D. 1966. Spores as propagules of disease. Vol. XVIII Colston papers (18th Symposium of the Colston Research Soc., University of Bristol) 18: 309-319. Butterworth Scientific Pub., London.
8. Henis, Y. 1970. Significance of population level of *Rhizoctonia solani* in soil. *In* T. A. Toussoun [ed.] Root disease and soil borne pathogens. Calif. Univ. Press (in press).
9. Houston, B. R. 1945. Culture types and pathogenicity of isolates of *Corticium solani*. Phytopathology 35:371-390.
10. Johnson, L. F., E. A. Curl, J. H. Bond, & H. A. Fribourg. 1959. Methods for studying soil microflora-plant disease relationships. Burgess Pub. Co., Minneapolis, Minn.
11. Martinson, C. A. 1963. Inoculum potential relationships of *Rhizoctonia solani* measured with soil microbiological sampling tubes. Phytopathology 53: 634-638.
12. Papavizas, G. C. 1968. Survival of root infecting fungi in soil. VIII. Distribution of *Rhizoctonia solani* in various physical fractions of naturally and artificially infested soils. Phytopathology 58:746-751.
13. Papavizas, G. C., & C. B. Davey. 1961. Saprophytic behavior of *Rhizoctonia* in soil. Phytopathology 51:693-699.
14. Richards, B. L. 1923. Soil temperature as factor affecting the pathogenicity of *Corticium vagum* on the pea and the bean. J. Agr. Res. 25:431-450.
15. Sanford, G. B. 1941. Pathogenicity tests on sugar beets or random isolates of *Rhizoctonia solani* Kühn from potato. Can. J. Agr. Sci. 21:746-749.
16. Shephard, M. C., & R. K. S. Wood. 1963. The effect of environment and nutrition of pathogen and host, in the damping-off of seedlings by *Rhizoctonia solani*. Ann. Appl. Biol. 51:389-402.
17. Sneh, B., J. Katan, Y. Henis, & I. Wahl. 1966. Methods for evaluating inoculum density of *Rhizoctonia* in naturally infested soil. Phytopathology 56:74-78.
18. Van der Plank, J. E. 1963. Plant diseases epidemics and control. Academic Press, N.Y. 349 p.