Influence of Exogenous Nutrition on Virulence of Rhizoctonia solani

A. R. Weinhold, R. L. Dodman, and Tully Bowman

First and third authors, Associate Professor and Staff Research Associate, respectively, Department of Plant Pathology, University of California, Berkeley 94720. Present address of second author: Queensland Wheat Research Institute, Toowoomba, Queensland, Australia.

Supported in part by Crops Research Division, ARS, USDA Grant No. 12-14-100-9163(14), and Cotton Incorporated Grant No. 68-115.

Accepted for publication 20 September 1971.

ABSTRACT

Virulence of *Rhizoctonia solani* is markedly influenced by the quantity of available nutrients. Sources of nutrients and the capacity of the pathogen to utilize these materials are important considerations in understanding disease development in nature. Mycelium growing from inoculum deficient in nitrogen rapidly absorbed nitrogen-containing compounds from the external environment and utilized them for pathogenic activities. The potential importance of soil as a source of nutrients for *R. solani* was shown by the fact that in the presence of soil, disease severity in cotton resulting from inoculation with nitrogen-deficient mycelium was increased. The addition of nitrogen to the soil resulted in a further increase in lesion development.

Additional key words: cotton, seedling disease.

At all levels of inoculum nutrition, seed inoculation resulted in greater disease severity than did hypocotyl inoculation. This supports the suggestion that seed exudates are an important source of nutrients available to soil-borne pathogens in nature. Seedling survival and lesion area on survivors were related to inoculum nutrition and initial distance between inoculum and seed. As this distance was increased, higher levels of inoculum nutrition were required for severe seedling damage. This study showed that the nutrients available to *R. solani* include those present in the propagule, the soil solutions, and the host exudates.

Phytopathology 62:278-281.

The importance of nutrition in promoting maximum virulence has been demonstrated for several plant-pathogenic fungi, including *Rhizoctonia solani* Kuehn (1, 4, 6, 7, 10, 11, 12, 13). The relative importance of exogenous versus endogenous nutrition as sources of external nutrients has important ecological implications. If, for example, the pathogen must rely to a large extent on exogenous nutrients, control through reduction of available energy sources is possible.

The possible role of nutrients in pathogenesis of *R. solani* from sources such as seed exudate has been considered (3, 5). In previous studies, the nutritional level of the pathogen was not regulated; therefore, it was difficult to obtain quantitative data on the relative contribution of exogenous nutrients. Recent investigation of the influence of *R. solani* nutrition on disease severity emphasized the importance of this factor (12), and provided a basis for investigating the role of various nutrient sources in nature.

The purpose of this study was to determine the ability of *R. solani* to increase in virulence through utilization of external nutrients. The extent to which the soil solution and host exudates may contribute to pathogen nutrition was also considered.

MATERIALS AND METHODS.—Five-day-old seedlings of *Gossypium hirsutum* L. 'Acala 4-42' (Family 77) were washed to remove planting mix adhering to the roots. The plants were placed on glass plates, inoculated, then incubated in a growth chamber at 28 C under continuous light, as previously described (12). The support medium, either washed sand or soil, was placed beneath the hypocotyls and was used to cover the roots. External nutrients were applied by moistening the sand or soil with solutions of the materials under investigation. The liquid was applied to the soil with an atomizer to avoid overwetting. The soil moisture content at the point of inoculation was maintained just below saturation.

Inoculum was prepared by growing *R. solani* on a basal medium containing 20 g glucose, 1.7 g KH_2PO_4, 0.75 g $MgSO_4 \cdot 7H_2O$, and essential micronutrients in 1 liter of distilled water (12). L-asparagine was added at various concentrations, depending upon the experiment. Glucose was autoclaved separately and added aseptically. The medium was dispensed into petri dishes (20 ml/plate) and seeded with mycelium grown on the basal medium containing 0.5 g/liter asparagine.

Inoculum was grown for 5 days at 28 C. Two-mm discs were cut from the mycelial mat and placed on the support medium, 1 mm from each hypocotyl. The roots and hypocotyls were covered with aluminum foil, and the plates were placed in plastic-covered pans containing liquid corresponding to that used to moisten the support medium. Small holes were punched in the foil envelope to maintain the support medium in a moist condition.

A sandy loam soil from the San Joaquin Valley of California, selected because of the low natural infestation of *R. solani*, was used in greenhouse tests. Twenty-cm plastic pots were used, and seed were planted at a depth of 5 cm. When hypocotyls were to be inoculated, seed were placed 0.5 cm below the soil surface, and the pots were covered with aluminum foil. After germination, inoculum was placed adjacent to the hypocotyls, and a 5-cm layer of soil added.

No attempt was made to precisely regulate soil moisture. At the beginning of each test the moisture content at seed depth was ca. 17%. This was obtained

by bringing soil to maximum water-holding capacity (26.5% moisture) by subirrigation, then allowing the soil to dry to the desired moisture content. During the course of the test, pots were subirrigated whenever the top 2.5 cm of soil was dry. The moisture content of soil added after hypocotyl inoculation was 17.0-17.5%.

In growth chamber tests, disease severity was determined 48 hr after inoculation by measuring the area of macerated hypocotyl tissue. In greenhouse tests, seedling emergence and survival were recorded. Sixteen days after inoculation, the lesion area on surviving plants was determined.

RESULTS.—*Effect of external nutrients available in sand.*—In these tests, inoculum was grown on a medium containing 0.5 g/liter asparagine. When grown on this medium, the fungus is weakly virulent. When the sand was moistened with a solution containing supplemental nitrogen, virulence was markedly increased (Table 1). The increased virulence occurred when either asparagine, $(NH_4)_2HPO_4$, or KNO_3 was supplied at a concentration as low as 30 mg N/liter (Table 1).

When the inoculum was grown on a medium with a limiting concentration of carbon source, an external supply of glucose increased virulence. Inoculation with mycelium grown on the basal medium containing 2.0 g/liter asparagine and 1.2 g/liter glucose instead of the usual 20 g/liter resulted in lesions with a mean area of 7.3 mm^2. Moistening the sand with a solution containing 5 g/liter glucose increased the lesion size to 23.5 mm^2 as compared to a mean lesion area of 79 mm^2 when plants were inoculated with mycelium grown on a high nutrient medium (basal plus 2.0 g/liter asparagine and 20 g/liter glucose).

Influence of field soil on pathogen virulence.—In growth chamber tests, field soil was used in place of sand to support cotton hypocotyls and to cover the seedling roots. The inoculum discs were placed on the surface of the soil ca. 2 mm from the hypocotyl.

The presence of soil resulted in an increase in disease severity. Inoculum grown on basal medium containing 0.5 g/liter asparagine produced lesions with a mean area of 8.0 mm^2 on seedlings supported by sand. When seven soils from various areas of the San Joaquin Valley of California were used, the mean lesion areas ranged from 12.3 to 32.5 mm^2. These values are based on three experiments with three 10-plant replications/experiment. Inoculation of the hypocotyls of seedlings growing in these soils in the greenhouse produced similar results.

To determine whether or not soil microorganisms were playing a role in increasing disease severity, the soil was sterilized by autoclaving. When this soil was used as a seedling support, there was no effect on mean lesion area. This indicates that the difference in lesion severity between sand and soil was not due to activities of the soil microflora.

If the influence of soil in increasing lesion size is due, at least in part, to the presence of nutrients, the addition of nitrogen should result in a further increase in disease severity. The purpose of supplying additional nitrogen to soil was also to compare the ability of *R. solani* to obtain nitrogen from both soil and sand. A soil was selected that had a relatively small influence on disease severity. Air-dry soil was placed on the plates and moistened to just below saturation with solutions containing either ammonium phosphate or potassium nitrate at concentrations of 0.5, 0.25, and 0.12 g/liter.

When the soil was moistened with water and the seedlings inoculated with mycelium grown on the basic medium plus 0.5 g/liter asparagine, the resulting mean lesion area was 13.7 ± 2 mm^2. Moistening the soil with solutions containing 0.5, 0.25, and 0.12 g/liter $(NH_4)_2HPO_4$ resulted in mean lesion areas of 16 ± 2, 22 ± 4, and 21 ± 6 mm^2, respectively. The use of solutions containing the above concentration of KNO_3 resulted in mean lesion areas of 31 ± 8, 24 ± 7, and 26 ± 5 mm^2. Based upon the dry weight of soil used/plate (100 g) and the volume of liquid applied (30 ml), the use of solutions containing 0.5 g/liter $(NH_4)_2HPO_4$ and KNO_3 added 32 and 21 ppm N, respectively. These data are based on three experiments, each with three 10-plant replications. The standard error values indicate there was considerable variation. This, however, was among

TABLE 1. Effect of external nitrogen on virulence of *Rhizoctonia solani*

Nitrogen source	Mean lesion area (mm^2)[a]									
	Concentration of chemical applied to sand (mg/liter)									
	2,000	1,000	500	250	125	60	30	15	8	0
Asparagine	6[b]	27	75	59	70	53	33	15	8	
$(NH_4)_2HPO_4$	44	45	57	44	64	54	43	15	19	
KNO_3	43	44	48	33	47	28	25	3	6	
Control (N-deficient inoculum)										7
Control (N-adequate inoculum)										80

[a] Solutions of chemicals were used to saturate the sand, and cotton plants were inoculated with mycelium grown on basal medium plus 0.5 g/liter asparagine (N-deficient inoculum). Nitrogen-adequate inoculum grown on basal medium plus 2.0 g/liter asparagine.

[b] Each value based on at least three experiments with three 10-plant replications/experiment.

TABLE 2. Cotton seedling damage resulting from inoculating seed and hypocotyls with inoculum grown on various concentrations of nitrogen

Asparagine concentration used to grow inoculum (g/liter)	Seed inoculated[a]			Hypocotyls inoculated[b]		
	Survival 16 days after inoculation		Mean lesion area on survivors (mm^2)	Survival 16 days after inoculation		Mean lesion area on survivors (mm^2)
	Mean plants/pot	%		Mean plants/pot	%	
2.00	0	0		5	36	82
0.50	0	0		7	50	80
0.25	6	40	23	14	100	60
0.12	8	53	20	12	86	10
0.06	7	47	16	12	86	1
0.00	9	60	19	13	90	2
Control (noninoculated)	15	100	16	14	100	9

[a] Based on three experiments: three one-pot replications/experiment; 20 seeds planted/pot.
[b] Based on three experiments: three one-pot replications/experiment; 14 hypocotyls inoculated/pot 5 days after seeding.

experiments, and not among replications within an experiment. In each test, the lesion area was approximately doubled by additional nitrogen.

Influence of inoculum nutrition on disease resulting from seed and hypocotyl inoculation.—The pathogen was grown on the basic medium containing 2.0, 0.5, 0.25, 0.12, 0.06, and 0.0 g asparagine/liter. Mycelium was used to inoculate seed at time of planting and hypocotyls of 5-day-old seedlings in field soil in the greenhouse. Temperature was constant at 27 C. The soil used was from the San Joaquin Valley of California, and had a low level of natural infestation of *R. solani*.

Inoculation of seed resulted in reduced seedling survival compared with hypocotyl inoculation at all levels of inoculum nutrition (Table 2). When seeds were inoculated with mycelium grown on media containing 2.0 or 0.5 g/liter asparagine, emergence was low, and none of the seedlings survived. Hypocotyl inoculation with the same inoculum resulted in 36 and 50% survival, respectively. The surviving plants, however, had extensive lesion development.

Inoculation of seed with mycelium grown on a medium containing 0.25 g/liter asparagine resulted in 40% survival as compared with 100% for hypocotyl inoculation. Using inoculum grown on media containing lower amounts had little effect on seedling survival as compared with 0.25 g/liter asparagine inoculum. There was, however, a decrease in lesion size on surviving plants, particularly when hypocotyls were inoculated (Table 2).

Influence of inoculum nutrition on growth of R. solani through soil and subsequent pathogenic activity.—The relationship of inoculum nutrition and distance of inoculum from the host was investigated by placing inoculum of various nutritional levels at different distances from cottonseed at the time of planting in field soil. The tests were conducted in the greenhouse at 27 C, using soil from the San Joaquin Valley, California. Inoculum consisted of mycelium grown on the basic medium plus 2.0, 0.5, 0.25, 0.12, 0.06, and 0.0 g/liter asparagine. Two-mm discs were placed either 3 or 6 cm from the seed.

Seedling survival and the lesion area on survivors were related to both inoculum nutrition and initial distance between inoculum and seed (Table 3). When inoculum was placed 6 cm from the seed, a higher level of inoculum nutrition was required for severe seedling damage than when the inoculum was at a distance of 3 cm.

DISCUSSION.—An understanding of the factors that influence the disease-producing capacity of plant pathogens, such as *R. solani*, is necessary for the development of more effective control measures. The quantity of nutrients available to the pathogen is one factor that can markedly affect disease severity.

In an earlier report, the effect of the utilizable nutrient contained within the fungus was emphasized (12). It was shown that when the mycelium of *Rhizoctonia* was deficient in either carbon or nitrogen, it could grow vegetatively, but its ability to attack the stems of cotton seedlings was markedly reduced.

The present study has shown that nutritionally deficient mycelium can rapidly utilize nutrients present in the external environment to increase virulence and utilize them for pathogenic activities. We believe that the reduced disease severity at high levels of asparagine is due to catabolite repression (*unpublished data*).

The increased disease resulting from inoculation with nutritionally deficient mycelium in the presence of field soil suggests that soil contains sufficient available nutrients to appreciably affect pathogenesis by *R. solani*. The fact that this response is not negated by autoclaving the soil shows that it is not due to activities of the soil microflora. The suggestion that soil may contain nutrients utilizable by *R. solani* is supported by the demonstration that disease severity is increased by the addition of nitrogen to the soil.

The comparison of seed and hypocotyl inoculation using mycelium of varying nutritional levels provides

TABLE 3. Influence of inoculum nutrition of growth of *Rhizoctonia solani* through soil and subsequent pathogenic activity

Asparagine concentration used to grow inoculum (g/liter)	Inoculum distance from cottonseed (cm)			
	3		6	
	Surviving (% control)	Mean lesion area on survivors	Surviving (% control)	Mean lesion area on survivors
2.00	17[a]	183	49	71
0.50	20	203	70	43
0.25	64	30	78	13
0.12	70	25	110	2
0.06	105	0	100	3
0.00	100	0	112	0
Control (noninoculated)	100	0	100	0

[a] Values based on three pots; 12 seed planted/pot; mean survival in control, 8.6 seedlings. Data collected 16 days after inoculation.

an example of the type of interactions that may occur between inoculum nutrition and host exudates. The results of this comparison, coupled with the demonstration that *R. solani* can effectively utilize exogenous nutrients to increase virulence, provide strong support for the suggestion that seed exudates may play an important role in attack by seedling pathogens (2, 3, 5, 8, 9).

Distance of inoculum from host seed must be considered in evaluating the influence of pathogen nutrition on disease severity. Growth of the pathogen through soil requires the expenditure of energy. Therefore, the greater the distance between seed and inoculum, the larger the nutrient reserve in the inoculum required for seedling damage. This relationship was shown by placing inoculum of various nutritional levels at different distances from the seed (Table 3).

The extent to which *R. solani* causes seedling damage is dependent upon the interactions of numerous variable factors. One of the basic variables underlying these interactions is the supply of nutrients available to the pathogen. The killing of a seedling by *R. solani* requires nutrients for growth of the pathogen from the propagule to the host, formation of infection cushions, and production of sufficient metabolites (toxins, enzymes) to destroy the plant. The nutrients available for these activities are a combination of those present in the propagule, the soil solution, and the host exudates. Our results show that the reduction of nutrients from any of these sources would reduce the virulence of *R. solani*, and thus result in a significant reduction in disease severity. The modification of cultural practices, such as application of fertilizer after seedling emergence, might be a means of escaping damping-off by *R. solani*.

LITERATURE CITED

1. BANTTARI, E. E., & R. D. WILCOXSON. 1964. Relation of nutrients in inoculum and inoculum concentration to severity of spring black stem of alfalfa. Phytopathology 54:1048-1052.
2. FLENTJE, N. T., & H. K. SAKSENA. 1964. Pre-emergence rotting of peas in South Australia. III. Host-pathogen interaction. Australian J. Biol. Sci. 17:665-675.
3. HAYMAN, D. S. 1969. The influence of temperature on the exudation of nutrients from cotton seeds and on pre-emergence damping-off by Rhizoctonia solani. Can. J. Bot. 47:1663-1669.
4. HUNTER, R. E., & G. GUINN. 1968. Effect of root temperature on hypocotyls of cotton seedlings as a source of nutrition for Rhizoctonia solani. Phytopathology 58:981-984.
5. KAMAL, M., & A. R. WEINHOLD. 1967. Virulence of Rhizoctonia solani as influenced by age of inoculum in soil. Can. J. Bot. 45:1761-1765.
6. KRAFT, J. M., & D. C. ERWIN. 1968. Effects of inoculum substrate and density on the virulence of Pythium aphanidermatum to mung bean seedlings. Phytopathology 58:1427-1428.
7. PHILLIPS, D. J. 1965. Ecology of plant pathogens in soil. IV. Pathogenicity of macroconidia of Fusarium roseum f. sp. cerealis produced on media of high or low nutrient content. Phytopathology 55:328-329.
8. RAJAGOPALAN, K., & K. BHUVANESWARI. 1964. Effect of germination of seeds and host exudations during germination on root-rot disease of rice. Phytopathol. Z. 50:221-226.
9. SCHROTH, M. N., & R. J. COOK. 1964. Seed exudation and its influence on pre-emergence damping-off of bean. Phytopathology 54:670-673.
10. SIMS, A. C., JR. 1960. Effect of culture substrate on the virulence of single basidiospore isolates of Pellicularia filamentosa. Phytopathology 50:282-286.
11. TOUSSOUN, T. A., SHIRLEY M. NASH, & W. C. SNYDER. 1960. The effect of nitrogen sources and glucose on the pathogenesis of Fusarium solani f. phaseoli. Phytopathology 50:137-140.
12. WEINHOLD, A. R., T. BOWMAN, & R. L. DODMAN. 1969. Virulence of Rhizoctonia solani as affected by nutrition of the pathogen. Phytopathology 59:1601-1605.
13. WILLIAMS, F. J. 1965. Antecedent nitrogen sources affecting virulence of Colletotrichum phomoides. Phytopathology 55:333-335.