Factors Affecting Suppressiveness to *Rhizoctonia solani* in Soil

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ABSTRACT

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Pathogenicity and growth of *Rhizoctonia solani* was suppressed in soils initially infested with this pathogen and planted to successive weekly crops of radishes. Suppressiveness developed in 5 wk in a Fort Collins clay loam. In untreated soil, or in soil uninfested with *R. solani* but planted with radishes weekly for 5 wk, almost 100% damping-off occurred when inoculum was added at a density of five propagules per gram of soil. In contrast, only 46% damping-off occurred in suppressive soil at 40 propagules per gram. When aliquots of soil were amended weekly with dead mycelium of *R. solani* or chitin at 1 g/kg, little suppressiveness was observed even when amendments continued for 5 wk. The soil became suppressive, however, when amended weekly with viable dried yeast at 1 g/kg soil. Samples of five cultivated soils collected in California were planted every 7 days with radishes over a 9-wk period. After this treatment, two of the soils infested with *R. solani,* became highly suppressive, one was as conducive as at the beginning of the experiment, and two were intermediate in suppressiveness. This indicated that soils may vary in capacity to develop suppressiveness in monoculture. No correlation was detected between suppressiveness of the soil and antagonism of the soil microflora as assayed in vitro. There was, however, a greater increase in soil lytic properties and populations of *Trichoderma* spp. in suppressive soils than in conducive soils. Three assays are suggested to develop quantitative measurements of the degree of suppressiveness in soils: a simple comparison of disease incidence (DI) in suppressive and conducive soils; generation of inoculum density/disease incidence curves for various soils (probably the most sensitive indicator of suppressiveness); development of a value for conducive index for soilborne pathogens (eg, *R. solani*) capable of growing through soil.

Additional key words: antagonism, biological control, soil fungi, soil microbiology.

Although monoculture of a crop plant species generally is thought to increase damage from soilborne plant pathogens, the reverse sometimes is true, as revealed by the take-all decline phenomenon. Gerlach (5) increased antagonism to *Gaeumannomyces graminis* in soil in a glasshouse by growing four successive wheat crops a year. Shipton et al (10) reported that survival of *G. graminis* was poor in soils of long-term wheat monoculture and good in soils having no history of recent cereal culture. A similar phenomenon, under laboratory conditions, was recently reported for *Rhizoctonia solani* Kühn following five successive weekly planting of radish (6). The intensity of the antagonism, as quantitatively evaluated by the conducive index (CI), was greater than that of soils amended with a wheat-bran culture of an antagonistic isolate of *Trichoderma harzianum* Rifai. In the present study, we examined the effect of time, number of replants, inoculum level, organic amendments, and soil location on suppressiveness of the soil to disease induced by *R. solani.*

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MATERIAL AND METHODS

Soils. A Fort Collins clay loam (Table 1) was used in most experiments. Five other soils from California also were used (Table 1). Soils were air dried and sieved (2-mm pore size) prior to distribution in pots and the water potentials were brought to about −0.7 bars.

Preparation and application of inoculum and rating of disease. Modified (4) chopped potato-soil (CPS) inoculum was used either as pellets (7) placed at the center of a pot or mixed into the soil at various concentration. Radish (*Raphanus sativus* 'Early Scarlet Globe') was used as the host. Plastic pots (78 mm diameter at the bottom and 110 mm diameter at the top) containing 100 g. (dry

weight) of soil were planted with radish seeds, covered with transparent Mylr® (E. I. du Pont de Nemours Co., Wilmington, DE 19898), and incubated for 7 days under illumination of ~5,000 lux.

To quantify the degree of suppressiveness of the soil, two assays were used in the analogue system described above. The first involved planting 20 seeds per pot in soil mixed with inoculum. Percentage of seed germination in the absence of inoculum was determined from appropriate uninoculated controls. Thus, disease incidence (DI) could be calculated as follows:

$$DI = \frac{A-X}{A} = 1 - \frac{X}{A} \qquad \text{Eq. 1}$$

in which A is the number of symptomless seedlings in the noninoculated control and X is the number of symptomless seed lings in the inoculated treatment (6). In this equation, DI is measured per unit; percent DI can be determined by multiplying by 100.

The second rating (6) used in some experiments was the conducive index (CI). To determine this value, 32 radish seeds were planted in each pot in eight rows, four seeds per row, radiating from the center of each pot. A single-pellet soil sampler was used to place a pellet (200 mg dry weight of CPS inoculum containing about 900 propagules of *R. solani* as determined by methods described previously [7]) in the center. CI was determined after 7 days of incubation by applying the formula (6) in Eq. 1. The CI estimates inoculum potential (or the ability of inoculum in the infection court to induce damping-off). In addition, it measures the ability of *R. solani* to grow in a given soil and thus gives a better idea of the overall suppressivesness of the soil.

Soil amendments. The following organic amendments were used: a lyophilized culture of Fleischmann's® viable dry yeast (Standard Brands Inc., New York, NY 10022); chitin (unbleached, Nutritional Biochemical Co., Cleveland, OH 44128); dead thallus of *R. solani* prepared by growing the fungus for 10 days on yeast-dextrose broth (YDB) in a standing culture at 25 C, washing the mycelial mat first in water, then in acetone, drying the mat for 24 hr at room temperature, grinding it with a mortar and pestle, and sieving it through a 0.5-mm sieve; cellulose powder (Solka-Floc, grade BW-200, Brown Co., Chicago, IL 60606), and glucose. All amendments were applied to soil at a rate of 1 g/kg each week. In experiments lasting more than 1 wk, soil from replicate pots were bulked every week, amendments and water (usually 50 ml/kg soil) were added, and the soil was redistributed in the same pots.

Microbial analyses. M-4 glucose medium (11) was used for isolations and enumerations of soil bacteria and actinomycetes and for testing their antagonism toward *R. solani*. Soil fungi were counted on rose bengal/streptomycin agar (8) supplemented with chloramphenicol at 100 μg/g. In vitro antibiotic activity of soil bacteria and actinomycetes toward *R. solani* was examined as follows: plates with M-4 medium were inoculated with potential antagonists, six in each petri dish, by depositing a loopful of the antagonist on an area approximately 1 cm^2 spaced equidistant from each other and 1 cm from the edge of the plate. After incubation for 2 days at 25 C, *R. solani* was introduced by placing a 2-mm-diameter plug from a yeast-dextrose agar (YDA) culture in the center of the plate. Measurements of areas around each potential antagonist in which *R. solani* was unable to grow were recorded after an additional 3 days of incubation at 25 C. Lysis of cells of *R. solani*, if it occurred, also could be observed microscopically when the fungus penetrated the colony of the test organism.

In vitro antagonism (2) of soil microfloral isolates against *R. solani* was examined as follows: 10-mm-diameter disks were cut from flame-proof nylon net (with square openings ~1 mm^2) were cut with a cork borer. The disks were placed in petri dishes (about 100 per 95-mm-diameter petri plate) with 10 ml of distilled water, and autoclaved. If water was not added, the disks curled irreversibly. Several disks were picked up with flamed forceps, vigorously shaken to remove water held in the net pores, and placed on an M-4 agar base (with no glucose or yeast extract) in two rows along the edge of the plate. A 2-mm-diameter disk of a culture of *R. solani* growing on YDA was introduced into the center and then incubated for 3 days at 25 C. During incubation, the fungus reached the edge of the plate and colonized the disks. Five sterile 22 × 22-mm cover glasses (No. 1) were placed at equal distances from each other on M-4 agar (without glucose or yeast extract). One nylon disk overgrown by a culture of *R. solani* was placed on each cover slip and one drop (drop size, 16 drops per milliliter) of a suspension of a test organism was added. In addition, one drop of complete M-4 medium was added to each disk. The plates were incubated for 3–7 days and observed daily for inhibition of growth (macroscopically) and lysis (microscopically). The agar on which the cover slips were placed prevented drying of the cultures and enabled growth and detection of lytic activity of a test organism against *R. solani*. The cover slip prevented escape of the fungus from the candidate antagonist into the agar and the drop of M-4 solution supplied dilute nutrients to both the pathogen and test organism.

This method of employing cover slips on agar also was used to determine quantitative lytic activity of soils following treatments. Drops of tenfold soil dilutions were placed on the cover slips as described previously. Five replications were used and relative lytic ability was determined as a function of the dilution end point of activity by the most probable number technique (1).

RESULTS

Effect of successive plantings on disease incidence. Three kilograms of Fort Collins clay loam were infested with CPS inoculum at five propagules per gram and equally distributed into

TABLE 1. Properties of soils used in investigations of the induction of soil suppression of *Rhizoctonia solani*

Location	Soil series name	Soil pH	Organic matter (%)	Lime[a]	Conductivity (mmhos/cm)	NO^-_3-N (μg/g)	P_2O_5 (μg/g)	K_2O (μg/g)	DPTA[b]-extractable micronutrients Zn (μg/g)	Fe (μg/g)
Fort Collins, CO	Fort Collins clay loam	8.2	1.9	High	0.4	4	10	139	16.5	2.5
Shafter, CA	Hesperia fine sandy loam	7.0	1.0	Low	2.1	11	16	218	1.3	8.2
Bidart, CA	Delano loamy sand	7.2	0.8	Low	1.5	6	6	133	1.0	9.0
Kamn, CA	Panoche sandy loam	7.9	1.0	High	1.2	6	5	356	0.7	2.0
Westside, CA[c]	Panoche clay loam	7.8	0.7	Low	2.1	61	3	269	0.7	4.1
Boston, CA	Panoche clay loam	7.7	1.3	Low	5.5	15	1	407	0.6	4.4

[a] Lime: low = <1%, high >2%.
[b] Acronym DPTA stands for diethylenetriamine penta-acetic acid extraction.
[c] The Westside Field Station of the University of California.

30 pots. Another 3-kg sample of soil was similarly distributed but without inoculum. The 60 pots were planted with radish seeds (20 per pot) and incubated for 7 days. Radish seedlings were uprooted and removed, soils of replicates of the same treatments were combined in, adjusted for moisture, redistributed in the pots, and replanted. This process was repeated five times.

Complete preemergence damping-off developed after three plantings (Fig. 1). After the fifth successive planting, however, emergence of radish seedlings from the soil originally infested with *R. solani* was as good (98%) as that initially observed in the uninfested control treatment (95.5%) or that obtained in that control after five plantings (88%).

Suppressiveness of the soils in the treatments described above after five replantings was compared with that of the original untreated soil by comparing CI values (6), or by challenging with fresh CPS inoculum to give different inoculum densities of *R. solani* up to 40 propagules per gram (Fig. 2). The maximum amount of damping-off in the infested replanted soil was 46% even when seeds were subjected to fresh inoculum at a density of 40 propagules per gram of soil. In contrast, disease incidence in either uninfested soil replanted with radishes originally without inoculum or in nontreated soil (not planted to radish) was 100% when *R. solani* was added at an inoculum density of 10 propagules per gram of soil. This situation was reflected in the CI values: 0.98 for untreated soil, 0.70 for uninfested soil repeatedly planted with radishes but without *R. solani,* and 0.09 for soil repeatedly replanted with inoculum of *R. solani* added with each successive planting as well as for the CI test.

At the end of this experiment, the soil of replicates belonging to the same treatment were bulked, moisture was adjusted, the soil was redistributed in the pots, planted (20 radish seeds per pot), and incubated for 7 days. Less than 5% damping-off was observed in the monoculture treatments previously infested and replanted, whereas 89–100% of the seedlings damped-off in the controls (replanted previously only with radishes or not planted at all) (Fig. 3).

In the last experiment, CI values were determined for soils infested initially with *R. solani* and repeatedly replanted with radishes. CI values of 0.4 ± 0.05, 0.3 ± 0.04, 0.02 ± 0.002, and $0.01 \pm$

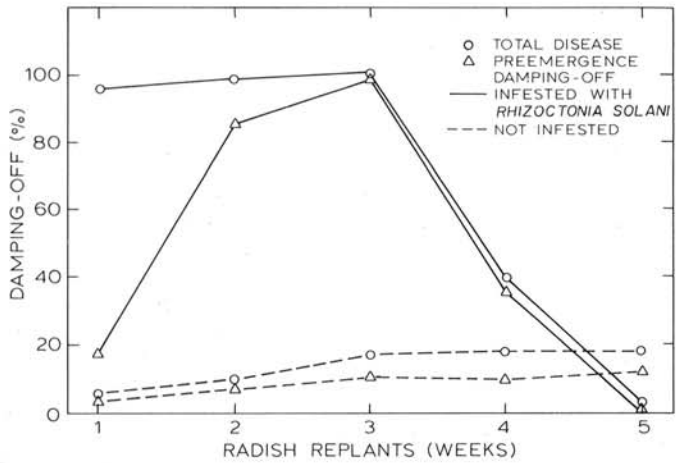

Fig. 1 Damping-off of radishes induced by *Rhizoctonia solani* during successive replantings.

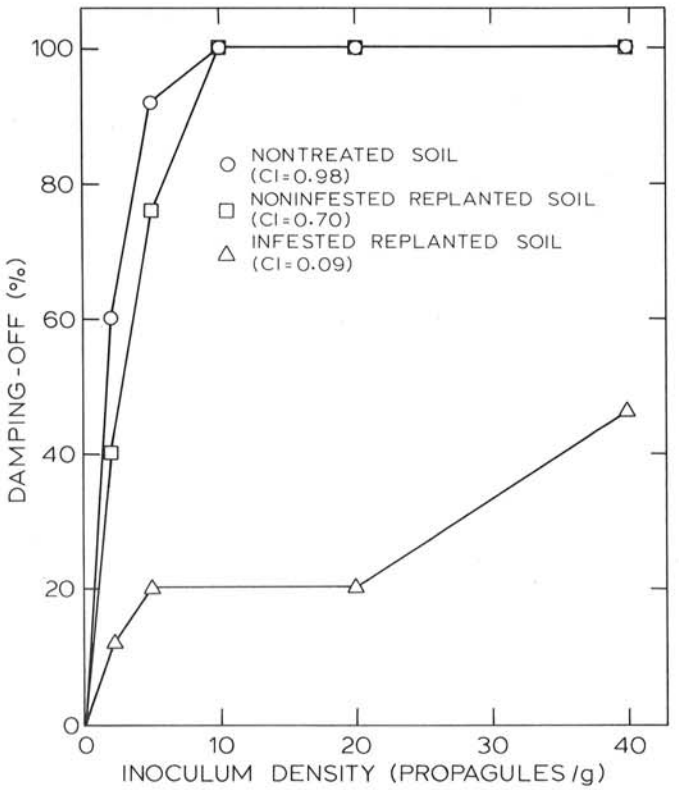

Fig. 2. Inoculum density/disease incidence relationships in soils conducive or suppressive to *Rhizoctonia solani.* Soil was induced to be suppressive to *R. solani* by planting five crops of radish at 1-wk intervals in soil infested with the pathogen. Conducive indices (CI) are given in parentheses after each soil treatment.

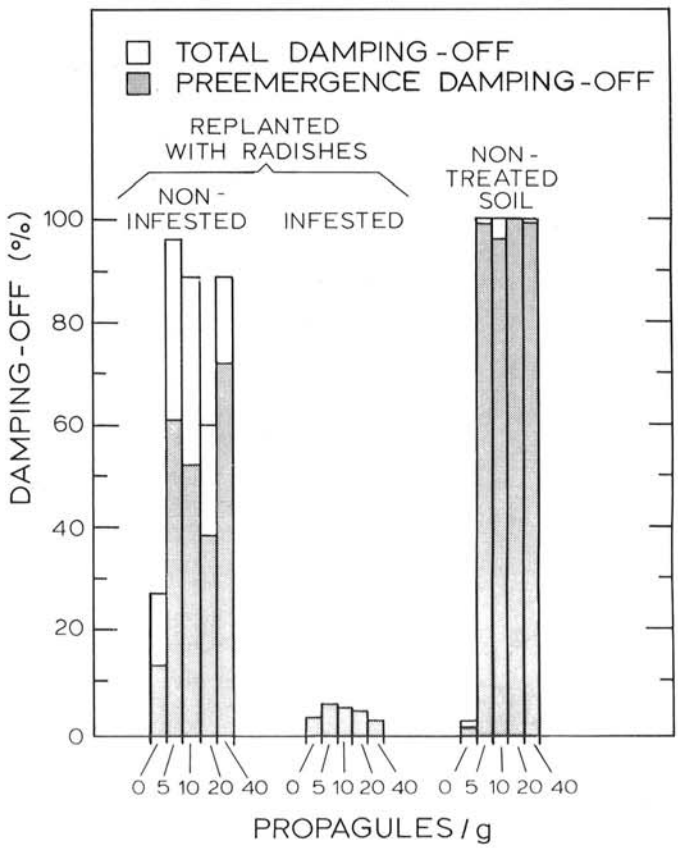

Fig. 3. Damping-off of radishes in soils conducive or suppressive to *Rhizoctonia solani.* Soils were originally induced to be suppressive to *R. solani* by planting five crops of radish at 1-wk intervals. Immediately afterwards, the suppressive and conducive soils were exposed to various inoculum densities (Fig. 2). These densities are given on the abscissa in propagules per gram. Soil from the replicates of each treatment at each inoculum density was bulked, its moisture was adjusted, and the soil was redistributed into the pots. Each pot contained 100 g soil and was planted with 20 radish seeds. There were five replications.

0.006 were obtained with the soils infested with 5, 10, 20, and 40 propagules per gram of soil, respectively.

Effect of organic amendments on suppressiveness of soil to *R. solani*. Aliquots of Fort Collins clay loam were amended each week with 1 g of dead mycelium of *R. solani* per kilogram of soil, viable dry yeast, or chitin, each at 1 g/kg of soil. The control was unamended soil. Every 7 days for 5 wk, soil moisture was adjusted to −0.7 bars), 1-kg subsamples were taken, and amendments were added again at the same rate to the remaining original sample. Subsamples were split into 500-g pairs, one infested with CPS inoculum (five propagules per gram of soil), distributed in pots (100 g/pot), planted with radish seeds (20 seeds per pot), and incubated for 7 days to determine DI values. After 5 wk, disease incidence per unit was: unamended control, 0.9; yeast-amended, 0.1; mycelium-amended, 0.66; and chitin-amended, 0.68 (Fig. 4). In a parallel experiment, soil samples amended in the same way with glucose (1 g/kg soil each week) or cellulose (1 g/kg or 4 g/kg soil each week) had DI values of 0.98, 0.83, and 0.94 respectively, after 5 wk of incubation.

Development of suppressiveness in various soils. Samples of five different soils from California each were divided into ten 100-g lots and planted with radish seeds (32 per pot). Five pots of each soil were infested with *R. solani* by placing one CPS pellet in the center of each pot. Five pots of each soil were uninfested, but were planted with the same number of radish seeds (controls). After incubation for 7 days, these soils had CI values of 0.80 to 0.89 (Fig. 5). The soil of replicates of each treatment was bulked (the original pellet containing *R. solani* furnishing the inoculum in infested treatments) and the process of bulking of replicates and replanting was repeated eight times during 8 wk. Seed germination was recorded each week to determine the percentage nonemergence of radish seedlings (Fig. 5).

Germination increased after 5 wk in the infested Delano loamy sand (Bidart) and Hesperia fine sandy loam (Shafter) soils, but not in the Panoche clay loams (Westside Field Station and Boston) or the Panoche sandy loam (Kamn). After 8 wk, some decrease in disease incidence was observed in the latter three soils. At the conclusion of the experiment, the soil from five pots of each treatment were combined, adjusted for moisture, redistributed in six pots (80 g/pot) and planted with 32 seeds per pot. To determine CI, three pots of each series were infested with one CPS inoculum pellet in the center and the other three uninfested pots served as controls. All soils originally infested with *R. solani,* except the Panoche sandy loam (Kamn), developed suppressiveness as measured by the CI (Fig. 5). In the replanted, uninfested Panoche clay loam (Bidart, CA) sample, however, CI values could not be determined because of an increase in damping-off induced by a *R. solani* native to the soil. In the Hesperia fine sandy loam (Shafter, CA), reduction in CI value was observed also in the replanted uninfested treatment.

Microflora present following radish monoculture. Total counts of soil bacteria and fungi following 5 wk of radish monoculture were higher than in nontreated soil (Table 2). Counts in soil infested with *R. solani* and planted repeatedly with radish were only slightly higher than in comparable uninfested controls. Proportions of microorganisms antibiotic, lytic, or antagonistic to *R. solani* did not differ among treatments. A pronounced increase in frequency of isolation of colonies of *Trichoderma* spp. was observed, however, in the suppressive soil infested with *R. solani* and repeatedly planted with radish. Lytic activity of this same treatment, as determined by the most probable number technique, also was higher. *Trichoderma* spp. isolated from soil in this treatment were parasitic to *R. solani* in two-membered culture.

DISCUSSION

Development of antifungal suppressiveness soils during monoculture was reported for *G. graminis* (5,9,10) and *R. solani* (6). For these organisms, the evidence suggests that the mechanism of suppressiveness is related to the biological characteristics of certain soils.

Methods for precise measurement of suppressiveness in soils are needed in epidemiology to determine the amount of biological control generated in contrast to other methods, and in studies on the mechanisms involved. In this study, we provide three methods for analysis:

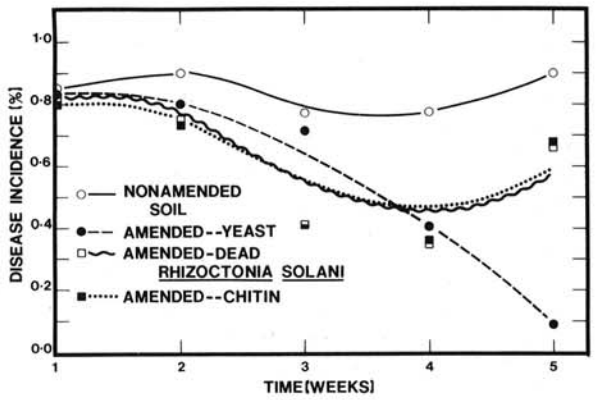

Fig. 4. The effect of organic amendments added in small increments (1 g/kg soil) each week on preemergence damping-off of radish induced by *Rhizoctonia solani.* Every 7 days for 5 wk, soil moisture was adjusted, 1-kg subsamples were taken, and amendments were added each time to this and the remaining original sample at the rate of 1 g/kg of soil. Subsamples were split into 500-g pairs, one infested with *R. solani* (five propagules per gram of soil), distributed in pots (100 g/pot), planted with radish (20 seeds per pot), and incubated for 7 days to determine disease incidence (DI) values.

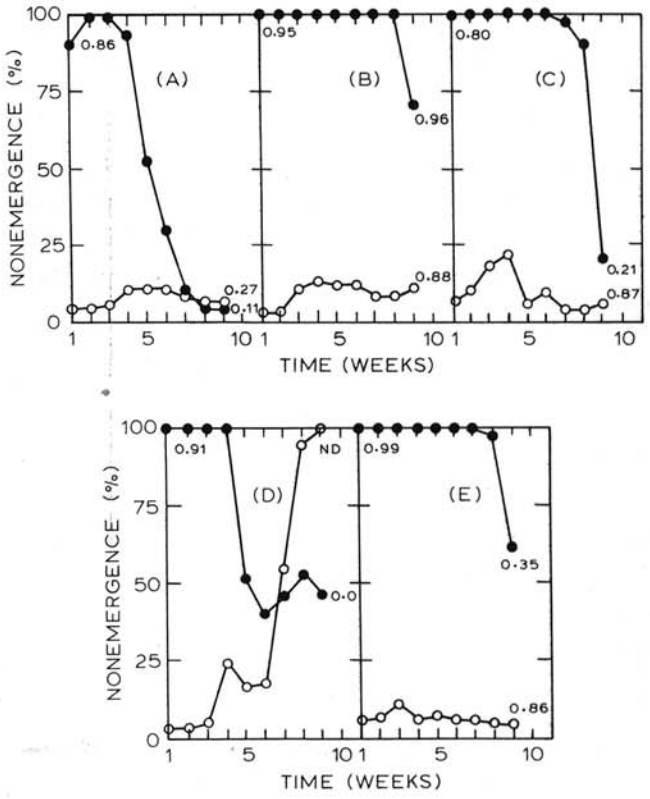

Fig. 5. Development of suppressiveness to *Rhizoctonia solani* in five soils collected in California when radishes were planted weekly for 9 wk. Numbers adjacent to curves designate conducive index (CI) values at the beginning and end of the various treatments. **A**, Hesperia fine sandy loam (Shafter, CA); **B**, Panoche sandy loam (Kamn, CA); **C**, Panoche clay loam (Westside Field Station of the University of California); **D**, Delano loamy sand (Bidart, CA); and **E**, Panoche clay loam (Boston, CA). Legend: •—• *R. solani* added to soil at the beginning of the experiment (five propagules per gram); •---• *R. solani* not added to soil.

TABLE 2. Antibiotic, lytic, and antagonistic activity against *Rhizoctonia solani* in suppressive and nonsuppressive Fort Collins clay loam soil

Treatments	Colonies[a] exerting inhibitory effects (%)	Colonies[b] having lytic activity (%)	Colonies[a] having antogonism (%)	Total[c] bacteria (colonies/g)	Total[d] fungi (colonies/g)	*Trichoderma*[e] sp. (colonies/g)	Lytic activity[f]		
							Average (units/g)	95% confidence limit	
								Upper	Lower
Untreated soil (nonsuppressive)	15	56	26	9.0×10^6	1.8×10^4	1.5×10^2	68	224	20
Radish monoculture									
Not infested with *Rhizoctonia solani* (nonsuppressive)	17	57	30	2.4×10^8	5.3×10^5	2.0×10^2	330	1,000	100
Infested with *Rhizoctonia solani* (suppressive)[g]	6	63	27	2.7×10^8	1.2×10^6	4×10^6	110,000	360,000	33,000

[a] Proportion of isolates out of 100 random colonies with inhibitory or lytic activity against *R. solani* in vitro.
[b] Proportions of antagonistic isolates out of 100 random colonies isolated from the soils using cover slips placed on M-4 agar (without glucose or yeast extract). Nylon disks overgrown with *R. solani* placed on each cover glass and one drop of suspension of a test organism added together with a drop of M-4 medium. Plates were incubated for 3–7 days and observed daily for inhibition of growth and onset of lysis.
[c] Soil dilutions cultured on M-4 medium (11).
[d] Soil dilutions cultured on rose bengal-streptomycin-chloramphenicol medium (RBC).
[e] Soil dilutions cultured on RBC supplemented with 10 ppm pentachloronitrobenzene.
[f] As observed on agar-cover slip method (see [b] above) using tenfold soil dilutions, five replicates in each, and calculating lytic activity by the most probable number method (1).
[g] Radish monoculture consisted of placing radish seeds (20 per pot containing 100 g soil) in pots, incubating at 25 C, and replanting at weekly intervals for 5 wk.

(i) The simplest assay consists of growing the indicator host (radish) in soil infested with the pathogen and determining subsequent disease incidence (DI, Eq. 1). For some host-pathogen systems in which inoculum density has a relatively small effect on disease development in comparison with other components of inoculum potential (3), inoculum concentration in soil need not be adjusted within critical limits. For *R. solani,* however, inoculum density profoundly influences disease incidence (4); thus, an initial inoculum level of five propagules per gram of soil was chosen because that is the minium level capable of inducing almost 100% damping-off (Fig. 2) in untreated Fort Collins soil. Any increase in suppressiveness (reflected by decreased disease incidence) in the soil should be detectable at this inoculum density (examples in Fig. 1 and 4).

(ii) Data from previous research (6) indicated that disease incidence in the infested Fort Collins soil repeatedly replanted with radishes correlated directly with inoculum density, accounting in part for reduction in inoculum potential in suppressive soils. The magnitude of suppression becomes more impressive, however, when inoculum density disease incidence curves are generated to compare responses in conducive and suppressive soils (Fig. 2). In conducive soils, five propagules per gram of soil was adequate to produce almost 100% disease incidence. In contrast, 40 propagules per gram in suppressive soil only induced 46% damping-off. This type of assay is clearly a more sensitive indicator of the impact of suppressiveness on disease incidence.

(iii) The preceding assays measure inoculum potential sensu Garrett (3); ie, energy available for infection at the surface of the hosts' infection court. An additional component that can be measured is the ability of a fungus (like *R. solani*) to grow through soil. The conducive index (6, Eq. 1) reflects both the capability for growth through soil to reach a susceptible host infection court and the subsequent inoculum potential. Examples of experiments in which CI was calculated are in Fig. 2 and 5.

Soils amended weekly in small increments with viable yeast cells, killed hyphae of *R. solani*, or chitin became slightly suppressive (compared with nontreated controls) after 3 wk (Fig. 4). After 5 wk, soil amended with viable yeast was highly suppressive. Thus far, highly suppressive soils have been generated only from soils containing a high population of viable fungal thalli; specifically, in the radish monoculture system in which development and activity of *R. solani* was stimulated by the presence of the host (6, and Fig. 1) and by weekly supplements of viable yeast. This could be interpreted to indicate that an antagonistic microflora develops in suppressive soils specifically in response to activity by living entities and that these entities may provide substrates for microparasites. The inability of dead mycelium of *G. graminis* to induce significant influences on take-all decline (5) also seemed to hold for *R. solani* in the radish monoculture system over a 5-wk period.

There was no correlation between suppressiveness of the soil to *R. solani* and antibiosis, antagonism, or lytic ability to the pathogen by isolates of the soil microflora. On the other hand, there was a substantial increase in both the lytic activity of the soil and *Trichoderma* populations in infested soil repeatedly replanted with radishes (Table 2). Members of *Trichoderma* sp. are well know for parasitic (lytic) attack on other fungi and isolates from suppressive soil were parasitic to *R. solani* in two-membered cultures. Even though involvement of soil microflora other than *Trichoderma* in soil suppressiveness to *R. solani* was not demonstrated (Table 2), participation of other microorganisms cannot be excluded.

Soils apparently vary in capacity to become suppressive after monoculture. As measured by DI, Hesperia fine sandy loam (Shafter) and Delano loamy sand (Bidart) soils infested with *R. solani* became highly suppressive after repeated plantings of radish and Panoche clay loam from the Westside Field Station and Boston sites was intermediate in response (Fig. 5). Panoche sandy loam (Kamn) showed no change in CI after nine replantings; the uninfested Hesperia fine sandy loam (Shafter) that was repeatedly replanted exhibited a reduction in CI: it was the first soil of those analyzed in our laboratory in which this property was identified. These results suggest that soils contain quantitatively and qualitatively different associations of microflora with different potentials for induction of antagonism under monoculture.

The prospects for "immunizing" soils against soilborne pathogens are fascinating and deserve further study, both in the laboratory and in the field. No information is available on the persistence of the suppression described in this paper, whether it can be transferred to another soil, and how widespread the capacity is among soils. Information of that kind should improve prospects for biological control of plant pathogens in soil.

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