Mechanism of Biological Control in Soil Suppressive to *Rhizoctonia solani*

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ABSTRACT

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Soil suppressive to *Rhizoctonia solani* was generated by monoculture planting of successive crops of radishes at weekly intervals in soil infested with the pathogen. A modification of Koch's postulates was utilized to determine the mechanism of biological control associated with suppressive soil. Numbers of *Trichoderma* spp. propagules in the soil increased as suppressiveness increased, whereas inoculum density of *R. solani* was inversely proportional to the density of these *Trichoderma* spp. following radish monoculture. Successive plantings of cucumber also generated suppressiveness which was associated with population of *Trichoderma* spp. propagules. Suppressiveness did not develop and *Trichoderma* was undetectable in sugarbeet, alfalfa, and wheat monoculture. Thus, increase in population and constant association of *Trichoderma* with suppressiveness was observed. *Trichoderma* was isolated with high frequency from mycelial mats of *R. solani* incubated in suppressive soil, but only occasionally from those incubated in conducive soil. Thus, the suspected causal agent (antagonist) was isolated in pure culture. Finally, conidia of *Trichoderma* added to conducive soil, at the same density found in suppressive soil, induced suppressiveness and the same species could be reisolated from mycelial mats of *R. solani* incubated in the soil. Among the antagonists tested, *T. harzianum*, isolated from Fort Collins clay loam, was most effective in inducing suppressiveness. Suppressiveness during radish monoculture developed more rapidly in acid soils than in alkaline soils. This corresponded to reports of *Trichoderma* being strongly favored by acid conditions. The suppressive effect persisted longer in soils with low negative matric potential. This also agreed with other research worker's observations of a high incidence of *Trichoderma* propagules in moist or wet soil environments.

The induction of suppressiveness to pathogenicity and growth of *Rhizoctonia solani* Kühn by planting successive crops of radish at weekly intervals in soil infested with the pathogen has been described (13,14,35). In a Colorado clay loam, incidence of damping-off increased during the first three replants and declined thereafter. Other examples of soils becoming inhospitable to certain plant pathogens with monoculture have been described (eg, 10,30,34); however, the exact mechanisms associated with suppressiveness were not clearly identified.

Often it has been assumed that the induction of suppressiveness is associated with some form of biological control (1) but experimental evidence is lacking. In the radish monoculture system, disease incidence increased more rapidly in the first three successive replantings when host tissue was reincorporated in soil than when this tissue was removed (35). This suggests that the suppression of disease that occurred in later successive replantings was not due to a buildup of a compound in radish that is active against *R. solani* (35). No change in the conduciveness of the

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original soil was observed when successive crops of radishes were planted without the pathogen, and no change was observed in soil to which only *R. solani* was added. Thus, both pathogen and host had to be present to induce suppressiveness. This indicated that the pathogen had to be active to induce the development of postulated biological antagonists which benefited from the association of host and pathogen (13). These are indirect evidences for the role of a biological control phenomenon operating in the radish monoculture system, but increased confidence in the validity of these interpretations would be possible if mechanisms (2) were identified.

Henis et al (14) detected no correlations between suppressiveness of soil and the antagonism of various soil microflora with one exception; increase in soil lytic properties was associated with increase in propagule population density of *Trichoderma* spp. In this report we attempted to elucidate the mechanism involved in generating soils inhospitable to *R. solani* through monoculture. In addition, a modificiation of Koch's postulates was used to verify the mechanism. Finally, for further verification, certain environmental parameters were manipulated which should have favored the activity of the biological entity(ies) postulated to be instrumental in control.

MATERIALS AND METHODS

In all experiments a Fort Collins clay loam was used (4,14). Soil was sifted before use through a 2-mm screen and stored in galvanized cans. The moisture characteristics were similar to those reported for the same soil by Rouse and Baker (26), as determined by the method of Fawcett and Collis-George (9). As each experiment was initiated, soil moisture was adjusted to and maintained at 15% by weight of oven-dry soil which resulted in −0.7 bar matric potential.

Two kinds of plant-growing containers were used. For most experiments, small round plastic pots (80 mm deep, 78 mm bottom diameter, 110 mm top diameter) were filled with 100 g of soil. Seeds (32 per pot) were planted 1 cm deep. When large amounts of suppressive soil were required, large plastic flats (45.5 × 25.5 × 5.5 cm) containing 1,500 g of soil were used. One hundred, forty-four radish seeds were planted 2 cm apart and 1 cm deep in each flat. After seeding, pots or flats were covered with transparent Mylar® (E. I. Dupont de Nemours Co., Wilmington, DE 19898), which was secured by rubber bands to reduce evaporation, and incubated on benches at 25 ± 1 C under continuous illumination (approximately 5,000 lx).

Inoculum of *R. solani*, isolate R-3 (AG 4) as used previously (4,13,14,35), was produced on chopped-potato-soil (CPS) substrate (4,17). After incubation on CPS medium for 3 wk at 25 C, the mixture was air-dried for 24 hr and, in some cases, screened to yield large (>589 μm) or small (<250 μm) propagules (15,35). This inoculum was stored in gauze-covered flasks at room temperature.

Uniform samples of soil for plating on *Rhizoctonia* selective medium (17) were obtained with the multiple pellet soil sampler (15). Fifteen pellets (100 mg each) from each of five aliquots in a given treatment or control were incubated on the selective medium for 18–20 hr at 25 C. The number of pellets containing *R. solani* was counted with a stereomicroscope at ×20 to ×40 magnification Inoculum densities were determined by applying the multiple colonization correction to the proportion of pellets yielding colonies of *R. solani* (15).

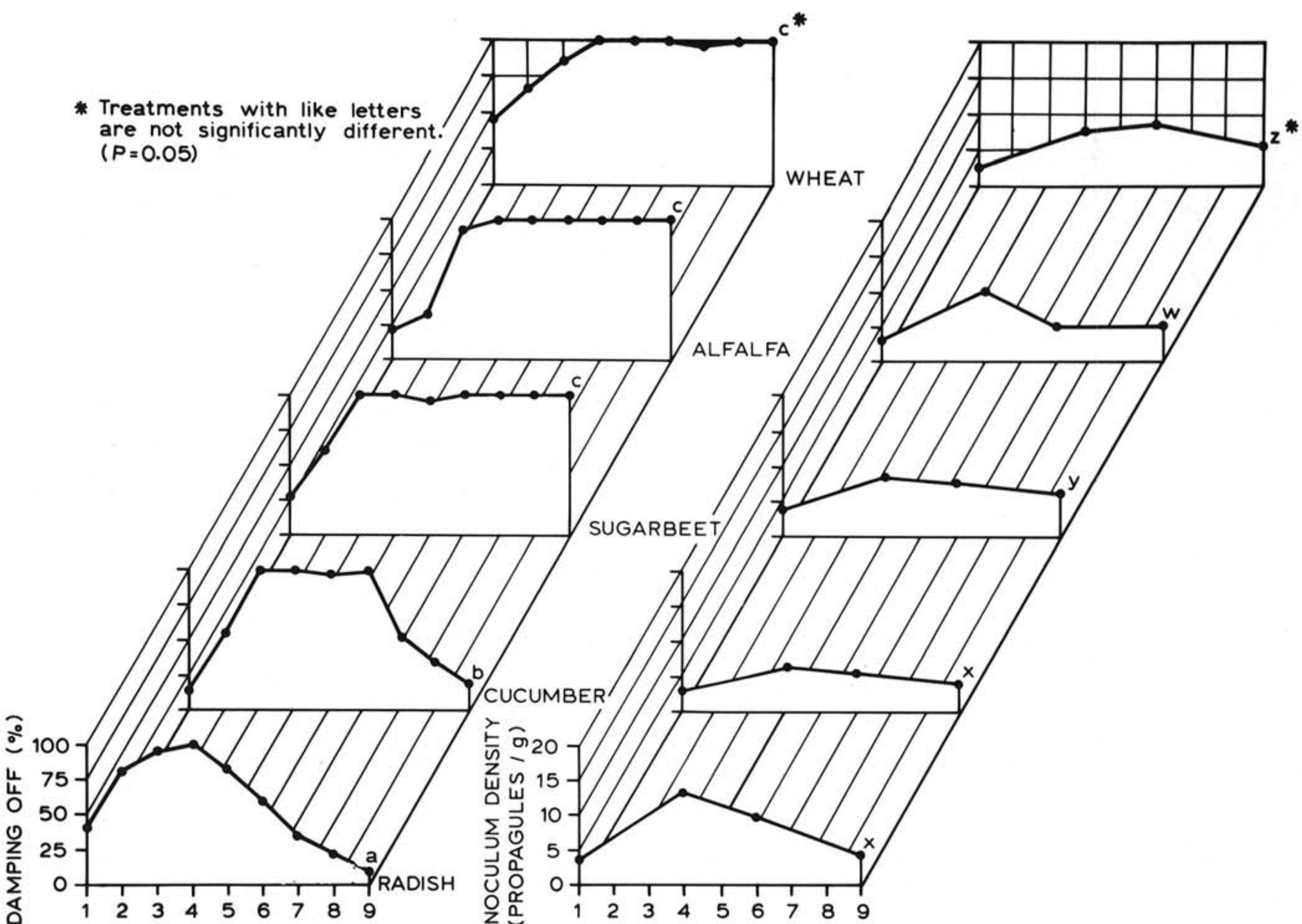

Fig. 1. A, Rhizoctonia damping-off occurring in various plants during monoculture replanted at weekly intervals. Disease incidence values in wheat reflects the presence of superficial brownish discoloration of host tissue but not death. **B,** Inoculum density of *Rhizoctonia solani* during the course of the experiment. Multiple comparison analysis of data was used to determine significance and there were five replications for each host.

Disease and relative suppressiveness of soils were assessed either by calculation (13) of disease incidence (DI) or conducive index (CI). The CI reflected the ability of *R. solani* to grow and induce damping-off when CPS inoculum was introduced into the center of a pot containing eight rows of radish seeds, four seeds in each, radiating from the inoculum source. The CI value of a completely suppressive soil is 0; a completely conducive soil has a value of 1.

Attempts were made to isolate soil microflora specifically antagonistic to *R. solani* from suppressive soil for comparison with those present in conducive soil by burying mycelial mats of the pathogen to act as "bait." Flame-proof nylon net (14) with 1 mm^2 holes was cut into 10 mm^2 pieces. The squares were placed in a petri dish with 10 ml of distilled water and autoclaved. Four of these pieces were placed around the margin of M-4 agar (31) in petri dishes. A mycelial disk from a culture of *R. solani* was introduced into the center of the medium. After incubation at 25 C for 6 days, the nylon nets, covered with hyphae of *R. solani* were incubated in soils for 24 hr and then removed and rinsed under tap water for 3 min to remove as many "casual" contaminants as possible. Mats were then incubated on rose bengal-streptomycin agar (21) supplemented with 100 μg/ml pentachloronitrobenzene which was semiselective for isolation of *Trichoderma* spp. Casein glycerol medium (32) also was used for isolation of *Streptomycetes* spp. and potato dextrose agar was employed as a nonselective medium.

Multiple comparisons analysis and, when appropriate, linear regressions were used for statistical analysis. The significance level of $P = 0.05$ was used throughout.

RESULTS

Development of Rhizoctonia suppressive soil in monoculture with different hosts. Radish (*Raphanus sativus* L., 'Early Scarlet Globe'), cucumber (*Cucumis sativus* L., 'Long Marketer'), sugarbeet (*Beta vulgaris* L., cultivar 'MonoHy A-1'), alfalfa (*Medicago sativa* L., 'Ranger'), and wheat (*Triticum aestivum* L., 'Twin') were replanted repeatedly at weekly intervals in monoculture in small pots. A mixture of large and small propagules of *R. solani* was used as inoculum (200 mg CPS/100 grams of soil). DI was observed 1 wk after each replanting (Fig. 1A). Pre- and postemergence damping-off were recorded for all hosts except wheat in which DI reflects superficial brownish discoloration of tissue, but not death of the host. After nine successive replantings, DI was significantly lower in soils planted with radish or cucumber than with sugarbeet, alfalfa, or wheat. The inoculum densities of *R. solani* found in soils at the end of the experiment were 3.9 (radish), 4.0 (cucumber), 6.1 (sugarbeet), 5.0 (alfalfa), 5.5 (wheat) propagules per gram of soil (Fig. 1B). Inoculum densities were significantly lower in soil planted with radish or cucumber than in soil planted with other host plants. The density of *Trichoderma* spp. was increased from levels not detectable at 10^{-4} soil dilutions to 9.4×10^5 propagules per gram of soil under radish, and 5.3×10^5 propagules per gram of soil under cucumbers after nine successive plantings. *Trichoderma* spp. could not be detected at the end of the experiment in soil dilution plates (10^{-4}) derived from monocultures of sugarbeet, alfalfa, and wheat.

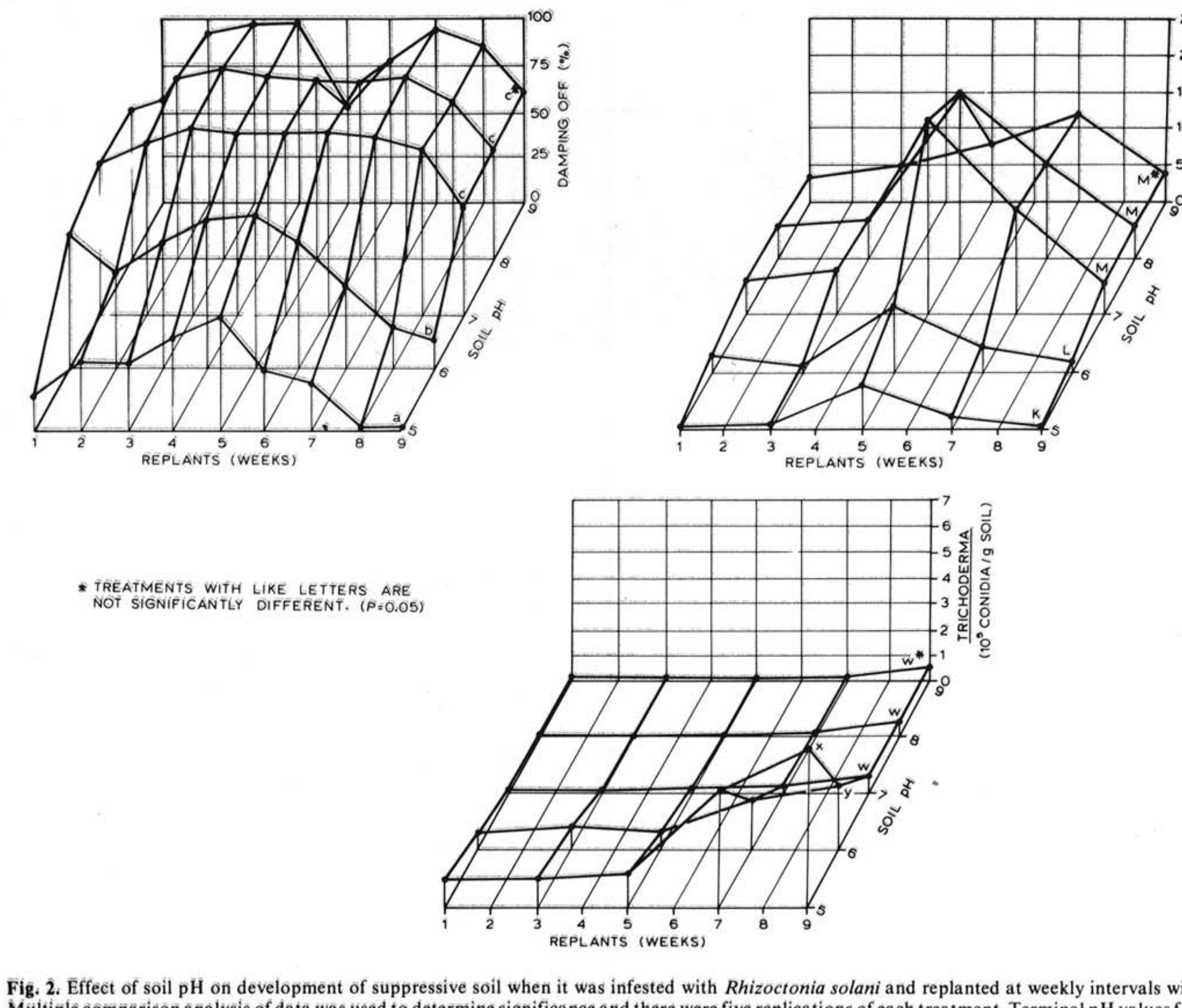

Fig. 2. Effect of soil pH on development of suppressive soil when it was infested with *Rhizoctonia solani* and replanted at weekly intervals with radish. Multiple comparison analysis of data was used to determine significance and there were five replications of each treatment. Terminal pH values for the unit increments of (original) pH were 6.0, 7.0, 7.3, 7.4, and 7.5, respectively, at the end of the experiment.

Effect of soil pH on development of suppressiveness. The pH of Fort Collins clay loam (initial pH 8.2 [14]) was adjusted at unit increments from 5 to 9 with 0.1 N H_2SO_4 or 0.1 N NaOH. Soil reaction was measured by the method of Schofield and Taylor (28). A mixture of large and small propagules was used in a pellet placed initially in the center of small pots for determination of CI with radish as the host. For subsequent replantings soil from replications at each pH value of soil was bulked and mixed, redistributed into the pots in 100-g portions, and radishes replanted. Seven replantings were made at weekly intervals. After the eighth replanting, CI values were obtained with fresh CPS inoculum placed at the center of each pot.

DI values generally decreased as soil pH decreased (Fig. 2A). Final CI values increased from 0.1 to 0.21, 0.43, 0.47, and 0.43 as initial pH of the soils was adjusted in unit increments from 5 to 9, respectively.

Inoculum densities of *R. solani* before CI values were determined after final replanting ranged from 0 to 4.2 propagules per gram of soil in direct proportion ($r = 0.877$) to the initial pH value of the soils (Fig. 2B). In soils with initial pH values of 5.0 and 6.0 inoculum density of *R. solani* was significantly lower than that found at initial pH values of 7.0, 8.0, and 9.0.

The density of *Trichoderma* spp. isolated in dilution plates ranged from 5×10^4 to 6.8×10^5 propagules per gram of soil in the various treatments at the end of the experiment and was inversely related to inoculum density of *R. solani* (Fig. 2). Regression analysis reflected a high value for the correlation coefficient ($r = -0.84$) for this relationship (Fig. 3).

Persistence of suppressiveness in soil held at different matric potentials. Relatively large amounts of soil suppressive to *R. solani* was obtained with soil in large plastic flats replanted nine times with radishes (35). A mixture of large and small propagules was used as inoculum. During the last replanting, the CI value was 0.21. This suppressive soil was mixed and divided into four equal parts. These samples were adjusted to moisture matric potentials of −1.35, −13.5, −87, and −7,000 bars. During a 140-day period, the soils at these matric potentials were maintained at 25 ± 1 C. At intervals, CI values, inoculum densities of *R. solani*, and colony counts of *Trichoderma* spp. in dilution plates were determined. Conducive soil not subjected to monoculture was maintained under the same conditions at −13.5 bars as a control.

The persistence of suppressiveness, as reflected by CI values, at these matric potentials were plotted according to the log-probit transformation (5) in Fig. 4. CI values for the conducive soil (control) remained relatively constant compared with the initial value of 0.81 over the 140-day period. F values indicated significant differences in slope values at the four matric potentials compared with the control when CI were plotted over time; also, the slope value at −7,000 bars was significantly greater than at −87 bars and both were significantly greater than at −13.5 or −1.35 bars. Generally, negative slope values increased as matric potential values decreased.

Inoculum density of *R. solani* was at low levels and remained relatively constant during the 140-day period at all water potentials (Table 1). Generally, as CI values increased (soils became more conducive), the density of *Trichoderma* spp. in the soils decreased (compare Table 1 with Fig. 4).

Isolation of potential antagonists in suppressive soil. Mycelial mats of *R. solani* supported by nylon nets were used to bait potential antagonistic microorganisms from suppressive soil. Thirty mycelial mats were buried in each of three soils: nontreated soil (conducive), soil planted with radish weekly for 9 wk but not infested with *R. solani* (conducive), and soil planted with a radish monoculture for a similar period but infested with *R. solani* (suppressive).

Trichoderma was isolated (21) from 25 of the 30 mycelial mats buried in suppressive soil, from only 10% of the mats buried in the nontreated control, and from 13% of the mats in soil not infested with *R. solani* but repeatedly planted with radishes (Table 2). The recovery of *Trichoderma* from the latter two soils was significantly lower than from the former.

Frequency of isolation of *Streptomyces* spp. on casein glycerol medium from mats was not significantly different among the three soils (Table 2). Various fungi and bacteria were isolated on PDA from the mycelial mats but no clear relationship between frequency of these isolates and suppressiveness or conduciveness was observed. The frequency of bacteria associated with the mats in the various soils is given in Table 2; there were no significant differences among treatments.

Conidia of *T. harzianum* Rifai, the species most commonly found in Fort Collins clay loam when suppressiveness was induced, were added to conducive soil at 10^6 conidia per gram of soil. Controls were also prepared of conducive soil without addition of *T. harzianum* and also conducive soil which was infested with *R. solani* (200 mg CPS/gram of soil). Mycelial mats of *R. solani* were incubated in soil for 24 hr, washed, and plated on the semiselective

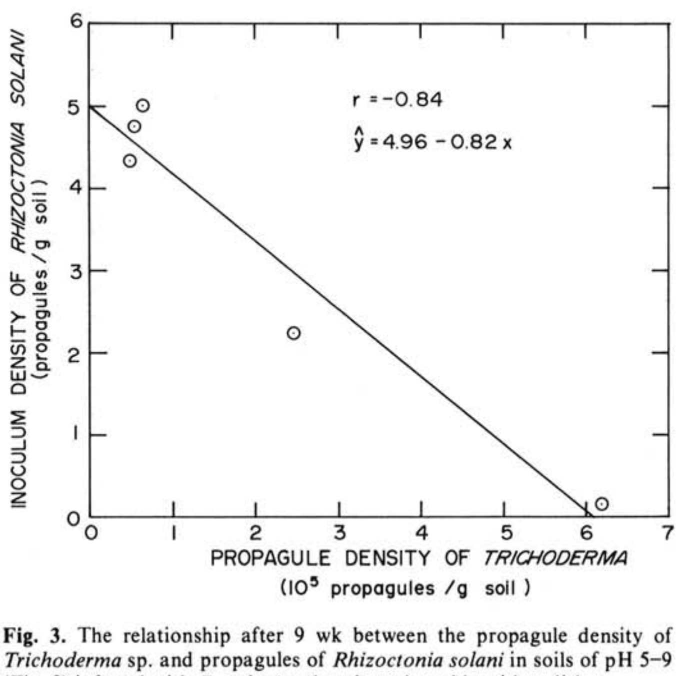

Fig. 3. The relationship after 9 wk between the propagule density of *Trichoderma* sp. and propagules of *Rhizoctonia solani* in soils of pH 5–9 (Fig. 2) infested with *R. solani* and replanted weekly with radishes.

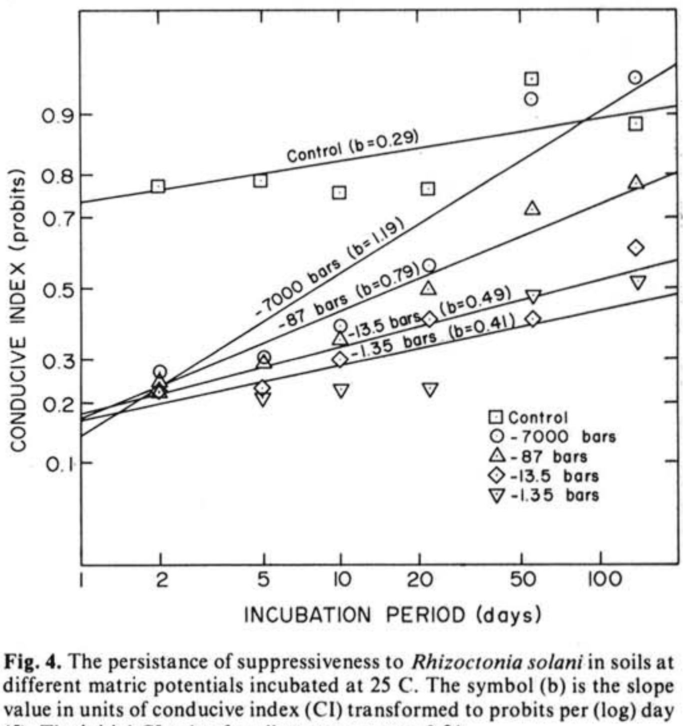

Fig. 4. The persistance of suppressiveness to *Rhizoctonia solani* in soils at different matric potentials incubated at 25 C. The symbol (b) is the slope value in units of conducive index (CI) transformed to probits per (log) day (5). The initial CI value for all treatments was 0.21.

TABLE 1. Survival of propagules of *Rhizoctonia solani*[a] and *Trichoderma* spp.[b] in Fort Collins clay loam (suppressive after radish monoculture to *R. solani*) held at various matric potentials[c]

Incubation period and microorganisms used in survival tests	Survival at matric potentials			
	−7,000 bars (propagules/g)	−87 bars (propagules/g)	−13.5 bars (propagules/g)	−1.35 bars (propagules/g)
1 day				
R. solani	0.3	0.3	0.3	0.3
T. harzianum	7.3×10^5	9.6×10^5	7.9×10^5	9.7×10^5
2 days				
R. solani	0.3	0.3	ND[d]	0.4
T. harzianum	7.5×10^5	13×10^5	4.8×10^5	9.5×10^5
5 days				
R. solani	0.4	0.6	0.3	0.6
T. harzianum	7.3×10^5	13.5×10^5	9.3×10^5	12.3×10^5
10 days				
R. solani	0.6	0.6	0.7	0.7
T. harzianum	7.4×10^5	13.2×10^5	11.8×10^5	20.2×10^5
22 days				
R. solani	0.7	0.6	0.9	1.2
T. harzianum	4.6×10^5	9.6×10^5	8.6×10^5	15.6×10^5
55 days				
R. solani	0.3	ND[d]	0.9	0.3
T. harzianum	2.0×10^5	1.0×10^5	4.2×10^5	3.7×10^5
140 days				
R. solani	0.3	ND[d]	0.4	0.3
T. harzianum	0.95×10^4	1.5×10^4	4.0×10^5	0.3×10^5

[a] Inoculum densities of *R. solani* determined according to the method of Henis et al (15).
[b] Densities of *T. harzianum* determined by counting colonies developing on dilution plates containing nutrient medium composed of rose bengal streptomycin agar (21) supplemented with 100 μg/g pentachloronitrobenzene.
[c] Matric potentials determined by method of Fawcett and Collis-George (9) and densities averaged from five replications in each treatment. Neither *R. solani* nor *T. harzianum* were detected in a conducive soil control held at −13.5 bars. Treatments were incubated at 25 ± 1 C.
[d] *R. solani* not detected (ND) in 45 soil pellets each weighing 100 μg.

TABLE 2. Analysis of antagonistic microbial activity against *Rhizoctonia solani* in suppressive and nonsuppressive Fort Collins clay loam soil

Treatment	Microorganisms isolated (%)[a]		
	Trichoderma sp.[b]	Streptomyces sp.[c]	Bacteria[d]
Nontreated soil (nonsuppressive)	10 x	23 w	40 z
Radish monoculture			
without *R. solani* (nonsuppressive)	13 x	17 w	43 z
with *R. solani* (suppressive)	83 y	23 w	50 z

[a] Means followed by the same letter are not significantly different ($P = 0.05$).
[b] *R. solani* was grown in M-4 medium (31) so that hyphae formed on a nylon net on the surface of the agar. After 24 hr of incubation in the various soil, 30 mats from each were washed to remove casual contaminates and incubated on rose bengal-streptomycin-chloramphenicol (21) supplemented with 100 ppm PCNB.
[c] Mats of *R. solani* placed on casein glycerol medium (32).
[d] Mats of *R. solani* placed on potato dextrose agar.

medium for isolation of *Trichoderma* spp. Fifteen percent of the mycelial mats from the conducive Fort Collins clay loam, 5% of the mats in the same soil infested with *R. solani*, and 75% of the mats in the soil to which conidia of *T. harzianum* had been added (of the 20 mats introduced into each soil) yielded cultures of the candidate antagonist.

Effect of Trichoderma into conducive soil. Conidia of *T. harzianum* (previously used in our investigations [13] and designated as the "Israel isolate") were harvested from 3-wk-old cultures growing on PDA. Two concentrations, 5.7×10^3 and 5.7×10^5 conidia per gram were added to conducive soil. One lot of soil (control) was not infested. These were divided into five replications in small pots and CI's were determined by introducing a mixture of large and small propagules of *R. solani* into the center of inoculated treatments. After seven weekly plantings of radish, CI's were determined again.

In the conducive soil not infested with *Trichoderma*, the initial CI of 0.75 was reduced to 0.47 after seven replantings. In contrast, only a 0.5% DI was observed and CI's were only 0.05–0.10 after the first planting when *T. harzianum* was introduced at a density of 5.7×10^5 conidia per gram of soil. This approximated the density of *Trichoderma* spp. usually recovered in suppressive soil (14). At a density of 5.7×10^3 conidia per gram of soil, the final CI was 0.38. The CI's for soils in which the two concentrations of *T. harzianum* were introduced were both significantly lower than in the noninfested control.

The ability of various isolates from soils to induce suppressiveness in the conducive Fort Collins clay loam was further tested by determining CI values at various densities of potential antagonists. Four isolates of *Trichoderma* spp. were used: *T. harzianum*, isolated from suppressive Fort Collins clay loam soil from mycelial mats of *R. solani* by the methods described above; *T. harzianum* (Israel isolate); *T. koningii* Rifai, isolated from Panoche sandy loam soil in Kamn, CA (14); *T. aureoviride* Rifai, isolated from Panoche clay loam soil at the Westside Field Station, CA. The cultural characteristics and taxonomy of these isolates matched descriptions of Rifai (25).

These four isolates and an isolate of *Aspergillus* sp. commonly recovered from Fort Collins clay loam (used as an infested control) were cultured on PDA. Conidial suspensions were introduced into conducive soil at concentrations from 10^1 to 10^6 conidia per gram of soil. CI's, with small propagules as inoculum, were determined for these treatments (Fig. 6). At a density of 10^6 conidia per gram of soil, the CI (0.04) for *T. harzianum*, (Fort Collins isolate) was significantly lower than the CI in soil infested with *T. harzianum* (Israel isolate) (CI = 0.10). Soils infested with either isolate of *T. harzianum* also had significantly lower CI values than soils with either *T. koningii* or *T. aureoviride*. Soils infested with any of the four *Trichoderma* spp. isolates had significantly lower CI values than did noninfested soil or soil infested with *Aspergillus* sp.

The pH of the Fort Collins clay loam soil was adjusted from 5.0 to 9.0 in unit increments. A suspension of conidia of *T. harzianum* (Fort Collins isolate) was added to the soil at a concentration of 9.2×10^5 conidia per gram of soil. The CI at each soil pH was determined by using small propagules of *R. solani* (< 280 μm) as inoculum. Soil initially adjusted to pH 5.0 or 6.0 and supplemented with a *Trichoderma* sp. was more suppressive than soil at pH 7.0, 8.0, or 9.0 (Table 3).

The ability of *T. harzianum* (Fort Collins isolate) to induce suppressiveness was compared with four other isolates of the genus obtained from Abbott Laboratories (Long Grove, IL 60047). Quantitative analyses of CI's with a mixture of large and small propagules of *R. solani* as inoculum were generated for these isolates at various infestation densities in Fort Collins clay loam (originally conducive before addition of the *Trichoderma* sp. isolates). The data presented in Fig. 7 indicate a statistically superior performance by the Fort Collins isolate in comparison with the other isolates except for the ATCC isolate at an infestation density of 10^5 condia per gram of soil.

Antagonism of *T. harzianum* against *R. solani* in vitro. A thin layer of PDA was poured on sterilized glass microscope slides. *T. harzianum* (Israel and Fort Collins isolates) and *R. solani* each were introduced on opposite ends of slides. The slides were placed on V-shaped glass rods and incubated at 25 C in sterilized petri dishes with 5 ml of sterile water to maintain moisture.

When the two organisms made contact on the slide, *Trichoderma* branched, attached itself to (Fig. 8A), and coiled around the hyphae of *Rhizoctonia* (Fig. 8B). Later, lysis of hyphae of *Rhizoctonia* was observed, and cells became separated at the septae (Fig. 8C-D). No hyphae of *Trichoderma* were observed to penetrate hyphae of *R. solani.*

DISCUSSION

The prime objective of this study was to determine the mechanisms associated with the induction in soil of suppressiveness to *R. solani* following monoculture (13,14,35). Proof of the pathogenicity of a potential causal agent to a higher plant host is conventionally accomplished through the application of Koch's postulates which include: (i) demonstration of a constant association of the candidate organism with the disease, (ii) the isolation of the suspected causal agent in pure culture from a host with typical symptoms or responses, (iii) the inoculation of the pathogen on healthy plants of the same species on which the pathogen appears and production of the same host response (symptom), and (iv) the reisolation of the pathogen from the host and its identification showing that same organism introduced at inoculation was reisolated. These postulates were followed to provide evidence for the participation of antagonistic *Trichoderma* spp. in the induction of suppressiveness by *Trichoderma* spp. to *R. solani* in soil following plant monoculture and our observations and results that constitute fulfillment of the postulates are presented under (i)–(iv) below. The "pathogen" was *Trichoderma*; the "host" was *R. solani*. "Host response" was measured by effects of treatments on inoculum density of *R. solani* and/or DI and CI.

(i) Suppressiveness induced in soil infested with *R. solani* in monoculture was accompanied by a corresponding increase in the population density of *Trichoderma* propagules (Fig. 1 and 2). Increase in density of this antagonist also was accompanied by a decrease in inoculum density of *R. solani* (Fig. 3). Further, in soil containing *R. solani* radish and cucumber monoculture eventually resulted in an increase in suppressiveness with a corresponding increase in the frequency of isolation of *Trichoderma.* This antagonist was not detected after similar monoculture (in soil also infested with *R. solani*) with sugarbeet, alfalfa, or wheat and the soil remainded conducive (Fig. 1). In all cases, therefore, there was a constant asssociation of high densities of *Trichoderma* with low values of DI, CI, and inoculum density of *R. solani.* Low densities of the postulated antagonist were associated in all cases with conduciveness.

(ii) *T. harzianum* was isolated much more often from mycelial mats of *R. solani* incubated in suppressive soil generated during radish monoculture. *Trichoderma* rarely was isolated from mats exposed to conducive soil (Table 2). Thus, the isolation in pure culture of the suspected suppressive agent from mycelium of *R. solani* was accomplished, fulfilling Koch's second postulate.

TABLE 3. The effect of soil pH on the conducive index of Fort Collins clay loam soil infested with *Trichoderma harzianum*[a] (Fort Collins isolate)

Soil pH[b]	Conducive index
5	0.066 x[c]
6	0.068 x
7	0.106 y
8	0.110 y
9	0.114 y

[a]Concentration of 9.2×10^5 conidia per gram of soil.
[b]Original pH of Fort Collins clay loam was 8.2 and was adjusted with 0.1 N H_2SO_4 or 0.1 N NaOH. Soil pH was measured by the method of Schofield and Taylor (28).
[c]Means followed by the same letter are not significantly different ($P = 0.05$).

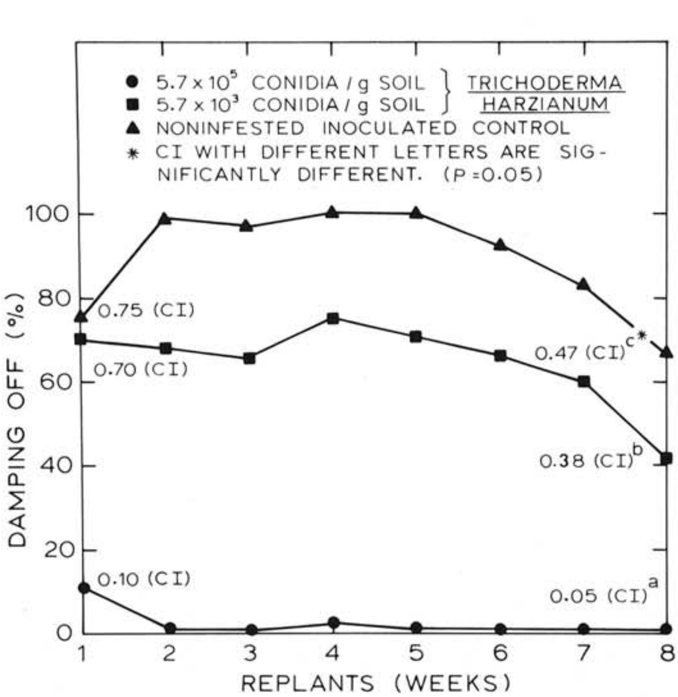

Fig. 5. Disease incidence (DI) and conducive indices (CI) of damping-off of radishes when soil (Fort Collins clay loam) conducive to *Rhizoctonia solani* was infested with conidia of *Trichoderma harzianum* (Israel isolate) and replanted weekly for 8 wk.

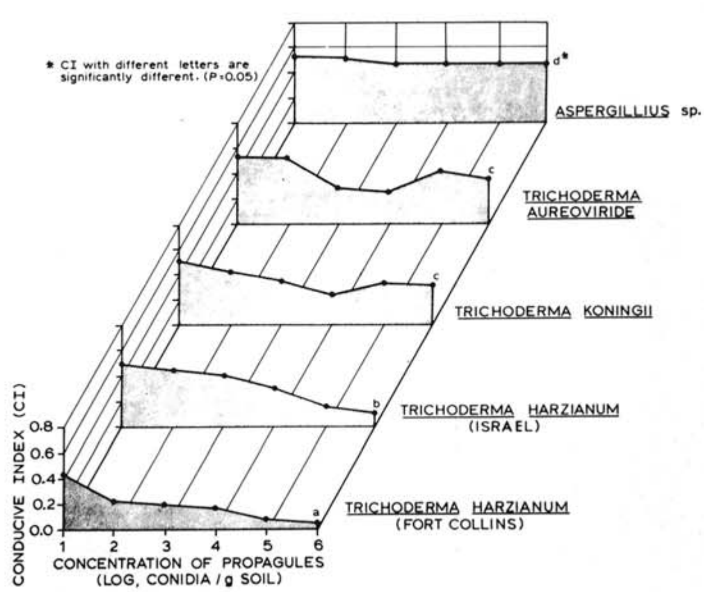

Fig. 6. Average conducive indices (CI) of soil originally conducive to *Rhizoctonia solani* after infestation with conidia of various isolates of *Trichoderma harzianum, T. koningii,* and *T. aureoviride* each at six propagule densities. CI with different letters are significantly different.

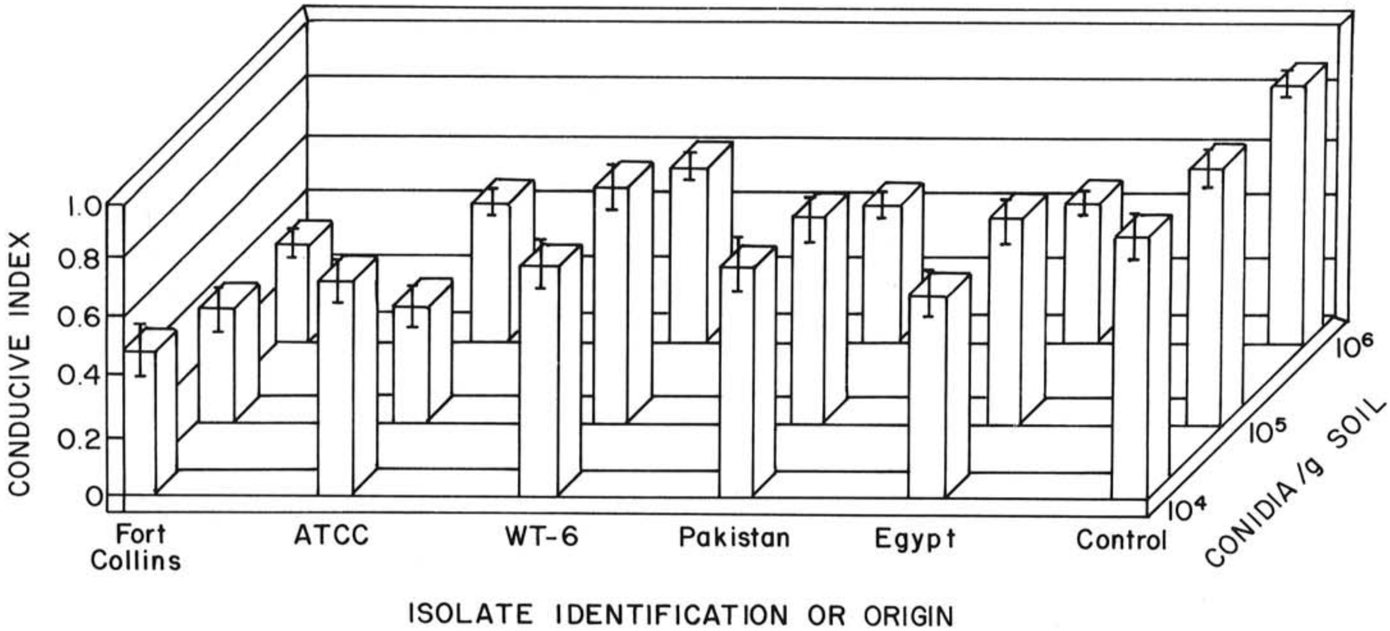

Fig. 7. Conducive indices (CI) of soil originally conducive to *Rhizoctonia solani* after infestation with conidia of isolates of *Trichoderma* spp. at three densities. Brachets indicate LSD values which were 0.17 for 10^4 conidia pet gram of soil, 0.15 for 10^5 conidia per gram of soil, and 0.09 for 10^6 conidia per gram of soil at $P = 0.05$.

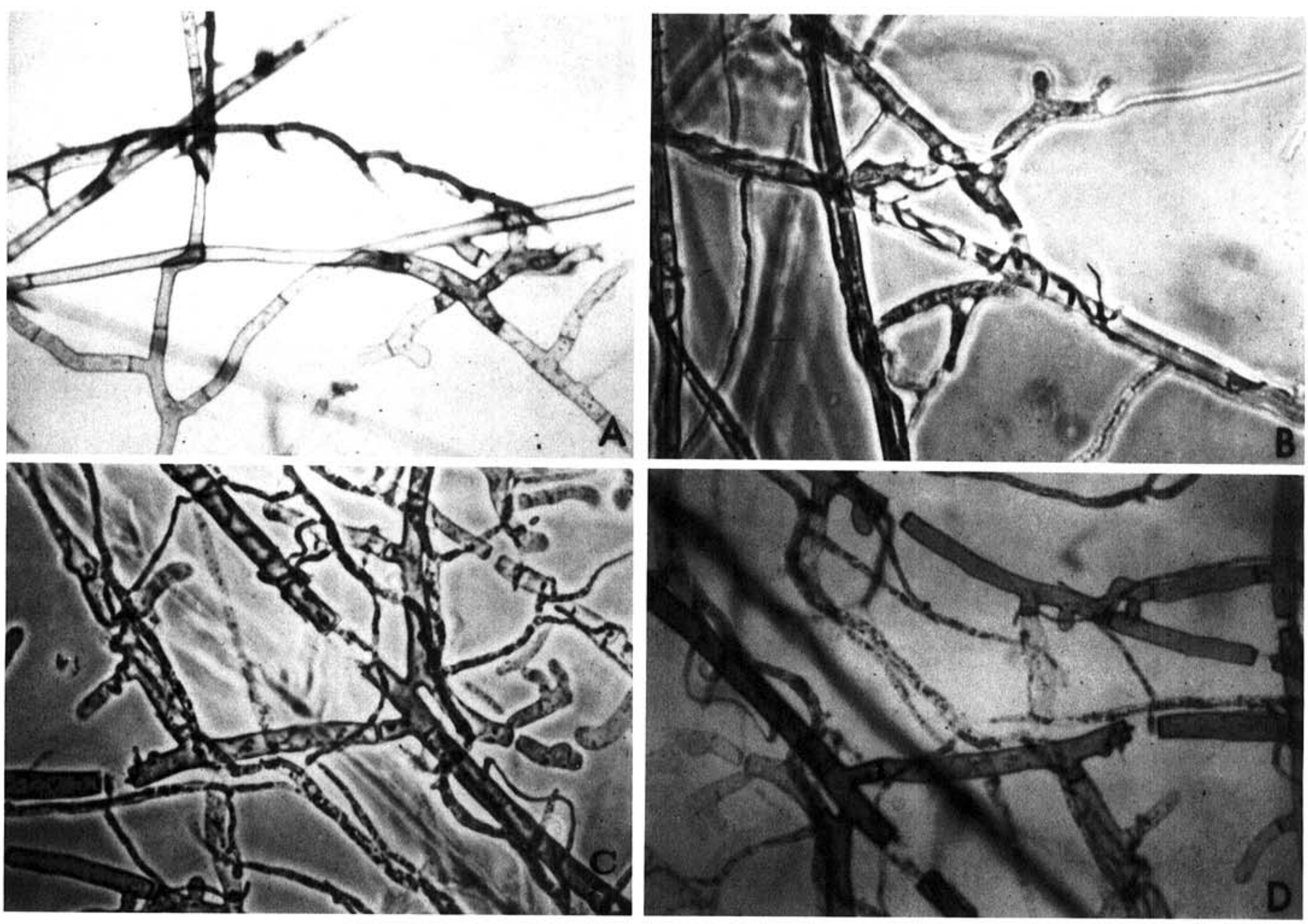

Fig. 8. Interaction between *Rhizoctonia solani* (larger hyphae) and *Trichoderma harzianum* (smaller hyphae) in vitro. **A, B,** contract and coiling of hyphae of *T. harzianum* around thallus of *R. solani* **C, D,** Lysis and separation of hyphae of *R. solani* at later state of interaction.

(iii) Infestation of conducive soils with species of *Trichoderma*, some of which were isolated from suppressive soils, induced large decreases in DI and CI (Fig. 5–7). Suppressiveness was induced in these treatments at density levels of *Trichoderma* found in suppressive soils ([14] and Fig. 1–3, and Table 1). *T. harzianum* isolated from the Fort Collins clay loam, which consistently has become suppressive to *R. solani* in monoculture (3,14,35), was the most effective of all the isolates in reducing the CI (Fig. 6 and 7). Therefore, introduction of the candidate antagonists into conducive soil at the same densities found in suppressive soil induced suppressiveness.

(iv) When mycelial mats of *R. solani* were placed in soil previously infested with *T. harzianum*, the candidate antagonist was very frequently reisolated in from the mats. In contrast, *T. harzianum* rarely was associated with the mats introduced into noninfested (conducive) soil. Thus, the antagonist was reisolated from *R. solani,* which fulfills the final postulate.

This is the first application of Koch's postulates to the identification of an antagonist responsible for induction of suppressiveness to a plant pathogen in soil.

Indirect evidence for participation of *Trichoderma* spp. in induction of suppressiveness also was obtained. Suppressiveness with radish monoculture could be induced more rapidly in acidified than in alkaline soil (Fig. 2). *Trichoderma* spp. have a reputation for being strongly favored by acid conditions (29,33). Again, survival of *Trichoderma* spp. generally was enhanced and suppressive effects were more persistent in moist soil than in drier soils (Fig. 4). *Trichoderma* spp. commonly inhabit soils having high moisture content (6,7,16,18,19,22–24,33). In contrast, *R. solani* persists for the longest periods in soils with low matric potential (3,5). These observations suggest that induction of suppressiveness to *R. solani* during monoculture of a crop in the field may be enhanced in acid habitats and/or by manipulation of the frequency of soil irrigation. The same principles may be applied in conjunction with applications of *Trichoderma* spp. to soils for biological control.

Wijetunga and Baker (35) reported that soils became suppressive to *R. solani* when planted to successive crops of radishes at weekly intervals when small propagules ($<250\ \mu m$) but not large propagules ($>589\ \mu m$) were used as inoculum. In many of our experiments, mixtures of large and small propagules were used to determine whether it was possible to suppress these large units of inoculum. It was possible when the antagonists were introduced into soil at the densities found in suppressive soil (Fig. 5). Further, adjusting soil pH from alkaline to acid permitted an increase in the population density of *Trichoderma* during radish monoculture to a level which induced suppressiveness even when large propagules were in the inoculum (Fig. 2). Since large propagules probably are the long-term survival units in field soils (12), these observations also are of importance in the elaboration of practical biological control strategies in this type of system.

Penetration of *R. solani* hyphae by *T. harzianum* was not observed in two membered culture as reported by others (8,20); however, an intimate association of paired hyphae, lysis of cell contents, and disjunction of cell walls occurred in *R. solani* similar to that observed by Hadar et al (11). This in vitro antagonism has not been investigated in a natural habitat although Sanford (27) observed hyphae of *T. lignorum* adhering to the surface of hyphae of *R. solani* in soil.

Interest in the use of mechanisms associated with suppressive soils for practical plant disease control has expanded recently. Through the use of Koch's postulates and elaboration of environmental parameters influencing the system described in this report, the basic mechanisms involved and the entities responsible for inducing suppressiveness through monoculture have been identified for the first time.

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