

Integrated Control of *Rhizoctonia solani* Damping-Off of Radish: Effect of Successive Plantings, PCNB, and *Trichoderma harzianum* on Pathogen and Disease

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Published with the approval of the Director of the Colorado State University Experiment Station, as Scientific Journal Series Paper 2304.

The senior author is supported by funds from National Science Foundation Grant KEB76-02223. This research was also supported in part by funds from Western Regional Project W-147.

Accepted for publication 28 November 1977.

ABSTRACT

HENIS, Y., A. GHAFFAR, and R. BAKER. 1978. Integrated control of *Rhizoctonia solani* damping-off of radish: Effect of successive plantings, PCNB, and *Trichoderma harzianum* on pathogen and disease. *Phytopathology* 68:900-907.

When applied to soil at rates of 0.04-0.15 g/kg (dry weight basis), wheat-bran cultures of *Trichoderma harzianum* protected radish seedlings from damping-off induced by *Rhizoctonia solani* and also increased radish germination in noninfested soils. Protection lasted for five successive weekly plantings. Pentachloronitrobenzene (PCNB) at 4 µg/g soil (active) added with *T. harzianum* inoculum had an additive effect on disease control and a synergistic effect on the decrease in inoculum density of *R. solani* propagules. In the absence of *T. harzianum* PCNB alone delayed the decrease of viable *R. solani* propagules. At a relatively low initial inoculum density (five propagules/g soil) when radishes were replanted every week, inoculum concentration rose during the first 3 wk. Cultures of *T. harzianum* added to this soil

permitted no increase in inoculum density. With high inoculum levels (80 propagules/g soil) *T. harzianum* accelerated reduction in population of *R. solani* in comparison with nontreated controls. After four or five successive plantings of radish in infested, nonamended soil, however, incidence of *Rhizoctonia* damping-off decreased substantially. A conduciveness test was developed and used for quantitative evaluation of the ease with which disease increased in a given soil. Soil conduciveness declined to a minimum in the nonamended, infested treatment after five successive plantings. The concept of incorporating soil conduciveness (along with inoculum quality and inoculum concentration) into the capacity portion of the inoculum potential is suggested.

Additional key words: biological control.

The literature on biological (3, 5) and chemical (16, 20, 21) control of *Rhizoctonia solani* Kühn is voluminous. It covers seed treatments, soil fungicides, modification of cultural practices, amending soil with plant residues and specific substances to induce changes in soil microflora, and direct introduction of biological antagonists into soil. However, no single treatment provides a satisfactory control of *R. solani*. Fungicides are practical and widely used, but can favor other pathogens (10). Moreover, the use (often extensive and excessive) of chemicals for pest control is a growing concern to public health authorities and environmentalists. With the exclusion of crop sequence and fertilization practices (7, 16), however, practical use of biological control of root diseases has been doubted on both theoretical and practical grounds (1). The successful introduction of integrated, chemical-biological control in entomology has encouraged plant pathologists to renew their efforts towards this goal (3).

The few reports so far on integrated control of *R. solani* deal with the combined effect of chemical or physical factors and the indigenous microflora (8, 18, 19, 21). Attempts to control *R. solani* by simultaneous use of pentachloronitrobenzene (PCNB) and *Trichoderma* sp. were reported recently (6). With development of the soil-

pellet sampler (12) for the study of population dynamics of *R. solani* in soil, it became possible to study the combined chemical-biological effect on both the pathogen population and the incidence of root diseases in the soil. In this study, the effect of single treatments of soil with *Trichoderma harzianum* Rifai, PCNB, or both on damping-off of radish and on the inoculum density of *R. solani* was examined. Observations were made following successive plantings of radish seeds in a soil with an initially low-to-medium population density of *R. solani*, as compared with those in a soil infested with a high population of that fungus.

MATERIALS AND METHODS

Inoculation and assessment of disease.—An axenic culture of chopped potato-soil (CPS) inoculum of *R. solani* isolate R-3 was prepared (12). A Fort Collins loamy sand (sieved through a 2-mm sieve) was infested by mixing it with the inoculum in a twin-shell blender. After adjusting moisture to 15% (about -0.7 bars water potential), 100-g portions were distributed in 80-mm-deep, conical, plastic pots, (78 mm diameter at the bottom and 110 mm diameter at the top). Soil in each pot was planted with 10 radish (*Raphanus sativus* 'Early Scarlet Globe') seeds having 99% germinability. Seeds were planted at a depth of 1 cm. The pots were covered with

transparent Mylar® (E. I. DuPont de Nemours Co., Wilmington, DE) plastic sheets, secured by rubber bands to reduce evaporation. All treatments were randomly arranged on benches and incubated at 25 ± 1 C for 7 days under continuous illumination, using ten white 40-W, 120-cm-long neon lamps (approximately 5,000 lux) per bench. The light source was 1 m above the pots. Seed germination and pre- and postemergence damping-off were recorded on the 7th day of incubation. In experiments involving replanting in soil containing an initially low inoculum density (five propagules/g soil), disease for each planting was recorded the 7th day after planting, the seedlings were removed, and the soil in all the replicates of each treatment was bulked together and weighed. Water content of the soil was readjusted to 15% moisture-holding capacity (MHC) (usually by adding 50 ml water/kg of soil), the soil was redistributed in the pots, replanted with 10 radish seeds each, and incubated for an additional 7 days. To study survival under various treatments in heavily infested soil (80 propagules/g soil), 7 kg of soil were incubated at 25 ± 1 C for 7 wk. Subsamples of 1 kg soil were taken after 1, 2, 3, 5, and 7 wk of incubation, distributed in 10 plastic pots and planted with 10 radish seeds each. At each sampling, soils of the main treatments were weighed for adjustment to 15% MHC and thoroughly mixed.

Typically, five replications of each treatment were used and experiments were repeated at least once.

Counting *Rhizoctonia solani* propagules in the soil.—Inoculum density of *R. solani* in soil was determined using the multiple pellet soil-sampler (12) and the selective medium of Ko and Hora (15). Large propagules were identified by their production of profuse hyphal growth from the interior of soil pellets.

Evaluation of soil conduciveness to damping-off and to activity of *Rhizoctonia solani*.—Soil was distributed in plastic pots (8.5 cm in diameter) so that each contained 100 g. Thirty-two radish seeds were planted in each pot with a vacuum planter, in eight rows, four seeds in each, radiating from the center of each pot. Seeds were planted at a depth of 1 cm. A pellet (200 mg dry weight) of CPS inoculum containing about 900 propagules of *R. solani* was obtained using a single pellet soil-sampler (12), placed in a groove at the center of the pots at a depth of 1 cm and covered with soil. Then the pots were covered with Mylar film and incubated for 7 days at 25 ± 1 C under illumination as described above.

Soil conduciveness was defined as the incidence of disease in a given plant population (32 radish seeds arranged around the inoculum source in this case), as expressed by the proportions of healthy seedlings developing in the infested and in the noninfested treatments. Thus, conduciveness can be described by the linear function

$$CI = \frac{A - X}{A} = 1 - \frac{X}{A} \quad \text{Eq. 1}$$

where CI is the conduciveness index, A is the number of symptomless seedlings in the noninoculated control, and X is the number of symptomless seedlings in the inoculated treatment. The number of plants affected by *R. solani*, therefore, is $A - X$. By definition, the limits of

X are 0 (minimum) and A (maximum), whereas those of CI are 0 and 1. The more suppressive the soil is, the less the value of the CI. Conduciveness is an inversely linear function. Soil suppressiveness can be described similarly by a suppressive index (SI) as

$$SI = 1 - \frac{X}{A} \quad \text{Eq. 2}$$

In this case, however, X denotes the number of damped-off, rather than healthy plants, in the infested treatment. Both indices measure the ability of *R. solani* to grow in a given soil and to induce damping-off. When the proportions of CI and SI are defined mathematically, their sum should be one; they are complementary (rather than reciprocal) to each other.

In this study, changes in activity of *R. solani*, as influenced by various soil properties, are defined in terms of conduciveness as described in Equation 1.

Culture of *Trichoderma harzianum* inoculum mix.—An isolate of *T. harzianum* was used that grows in culture in the presence of 100 ppm PCNB and is antagonistic to *R. solani*. Erlenmeyer flasks (250-ml) containing 10 g wheat bran and 20 ml tap water autoclaved for 1 hr on 2 successive days and the wheat bran then was seeded with a suspension of conidia obtained from a yeast-dextrose agar slant. Cultures were incubated in the dark at 25 C for 3 days. Flasks were exposed to light on tables (described previously) for 2-3 days, shaken vigorously, and incubated for 3-4 more days. One gram of this culture contained 4.1×10^9 conidia.

RESULTS

Effect of pentachloronitrobenzene (PCNB) and *Trichoderma harzianum* on the inoculum-density versus disease incidence relationship.—Soil was inoculated with various amounts of CPS inoculum of *R. solani* and with *T. harzianum* inoculum mix at a rate of 2 g (fresh weight)/kg dry soil. A relatively low concentration (4 μ g/g soil) of PCNB was added in some treatments with or without the *T. harzianum* mix and the soils were incubated for 1 wk at 25 C. Soils were then distributed in pots, 100 g/pot, five replicates per treatment, planted with radish seeds (10/pot), and incubated under illumination for 7 days. The initial pathogen level required for 100% disease in the control, *T. harzianum* mix, PCNB, and combined treatments, was 20, 40, 40, and 80 propagules/g, respectively [Fig. 1-(A to D)]. Both *T. harzianum* and PCNB improved seed emergence in the *Rhizoctonia*-free controls and reduced *R. solani*-caused postemergence damping-off.

Effect of *Trichoderma* and pentachloronitrobenzene (PCNB) on *Rhizoctonia solani* added initially at low inoculum densities.—Soil was amended with CPS inoculum of *R. solani* at five propagules/g soil, amended with *T. harzianum* mix (0.1 - 4 g/kg soil), PCNB (4 μ g/soil), or both, incubated for 7 days, and planted five successive times with radish seeds at 1-wk intervals.

Disease control was obtained with all concentrations of *T. harzianum* mix used; i.e., 0.1-4.0 g/kg soil, as well as with PCNB during the first, second, and third

successive plantings (Fig. 2-A). Seed germination in noninoculated, nonamended controls was reduced by about 40% throughout the experiment. *Trichoderma harzianum* mix added to soil gave progressively better control until the third planting; thereafter control by *T. harzianum* became less impressive than disease decline in the nonamended, infested control. Yet, most *T.*

harzianum levels of 0.5 g/kg soil and above were effective in control throughout the five successive plantings.

Pentachloronitrobenzene ($\mu\text{g/g}$ soil) effectively controlled *Rhizoctonia* damping-off in the first planting only. Thereafter it became less effective than most of the *T. harzianum* infestation levels of 0.5 g/kg soil and above.

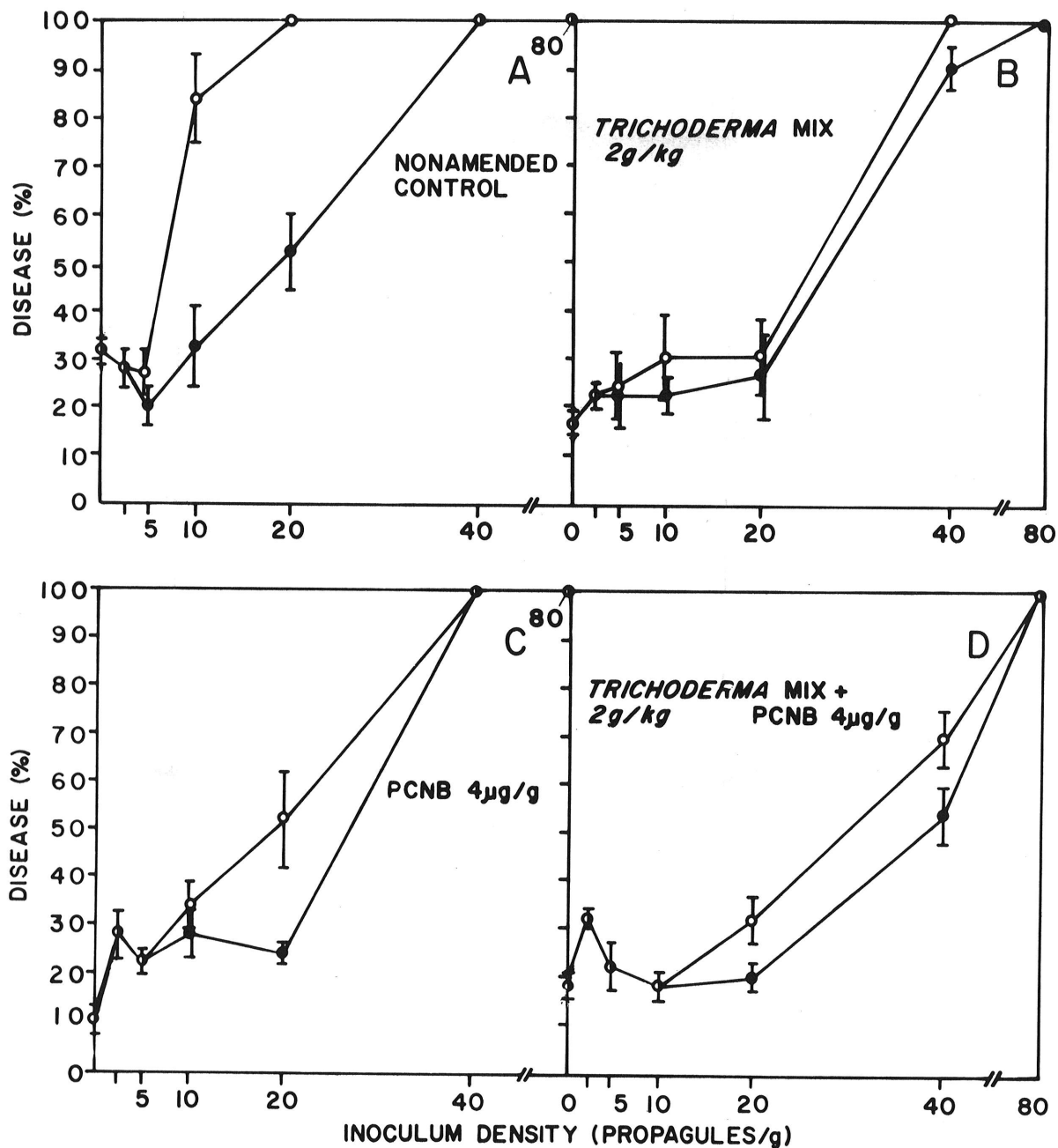


Fig. 1-(A to D). Effect of *Trichoderma harzianum* and pentachloronitrobenzene (PCNB) on the inoculum-density-disease incidence relationship in damping-off of radish induced by *Rhizoctonia solani*. Treatments were incubated for 7 days before planting. Disease was recorded after a further incubation for 7 days in A) nonamended soil, B) soil amended with *T. harzianum* mix at 2 g/kg soil, C) soil amended with PCNB at 4 $\mu\text{g/g}$ soil, or D) *T. harzianum* + PCNB. Bars indicate standard error of the mean (S_x) for two similar experiments.

Moreover, the disease decline after three replantings observed in the inoculated, nonamended soil, was not observed in the PCNB treatments. Combined treatment with PCNB at 4 $\mu\text{g/g}$ and *T. harzianum* inoculum at 2 g/kg gave an additive effect during the first and second plantings.

In general, disease severity was directly proportional to the inoculum density of *R. solani* [Fig. 2-(A, B)]. *Trichoderma harzianum* inoculum mix at rates suppressive to disease prevented a build-up of propagules of *R. solani* in the soil. However, even in the nonamended, inoculated controls, the inoculum density of *R. solani* declined sharply during the fourth and fifth plantings and reached a level similar to that of the *Trichoderma*-amended soil. The highest inoculum density occurred after five successive plantings in the PCNB-treated soil. Combined treatment with PCNB and *T. harzianum*, however, was the most efficient in reducing the inoculum density of *R. solani*.

Effect of *Trichoderma harzianum* and pentachloronitrobenzene (PCNB) on *Rhizoctonia solani* added initially at high inoculum densities.—Soil initially infested with a CPS inoculum to provide a final concentration of 80 propagules/g soil, was divided for the following treatments: (i) nonamended control, (ii) *T. harzianum* mix (2 g/kg soil), (iii) PCNB (4 $\mu\text{g/g}$ soil), and (iv) *T. harzianum* plus PCNB. Soil samples were incubated at 25 C in 5-kg plastic containers covered with Mylar. Separate 1-kg samples of soil were taken from each treatment after 1, 2, 3, 5, and 7 wk of incubation, readjusted for moisture content, remixed, distributed in 10 plastic pots (100 g soil/pot), and planted with 10 radish seeds per pot. Every experiment was repeated twice with

similar results. Parallel samples were examined for inoculum density of *R. solani* propagules.

Disease incidence remained high after the first and second plantings in spite of a marked decline in *R. solani* propagule count, especially in the *T. harzianum* (T) and T-PCNB combined treatments [Fig. 3-(A, B)]. By the third planting, however, decline in disease incidence occurred especially in the combined treatment. Slowest decline in either disease or *R. solani* propagule counts were observed in the PCNB-treated soil.

Decline in propagule counts was accompanied first by a decrease in preemergence damping-off correlating with an increase in postemergence damping-off followed by reduction in disease incidence. This pattern was observed within 2 wk in the combined treatment, 3 to 4 wk in the *T. harzianum* treatment, and 4 to 5 wk in the PCNB and control treatments. Different relationships, however, seemed to exist between propagule counts and disease in the different treatments. Thus, in the fifth planting, differences between treatments were more pronounced in disease severity and incidence than in propagule counts of *R. solani*. Disease incidence was particularly low in the *T. harzianum* treatment.

Development and persistence of antagonism toward *Rhizoctonia solani* in soil.—Evaluations were made of the conducive index (Equation 1) after the fifth successive planting in the experiment with an initial low inoculum (five propagules/g soil) level and after 7 wk of incubation in the experiment in which there was a high initial inoculum density (80 propagules/g of soil). Highest values of the conduciveness coefficients were observed with raw soil (collected from the storage bin and originally used in the experiment) and with the combined

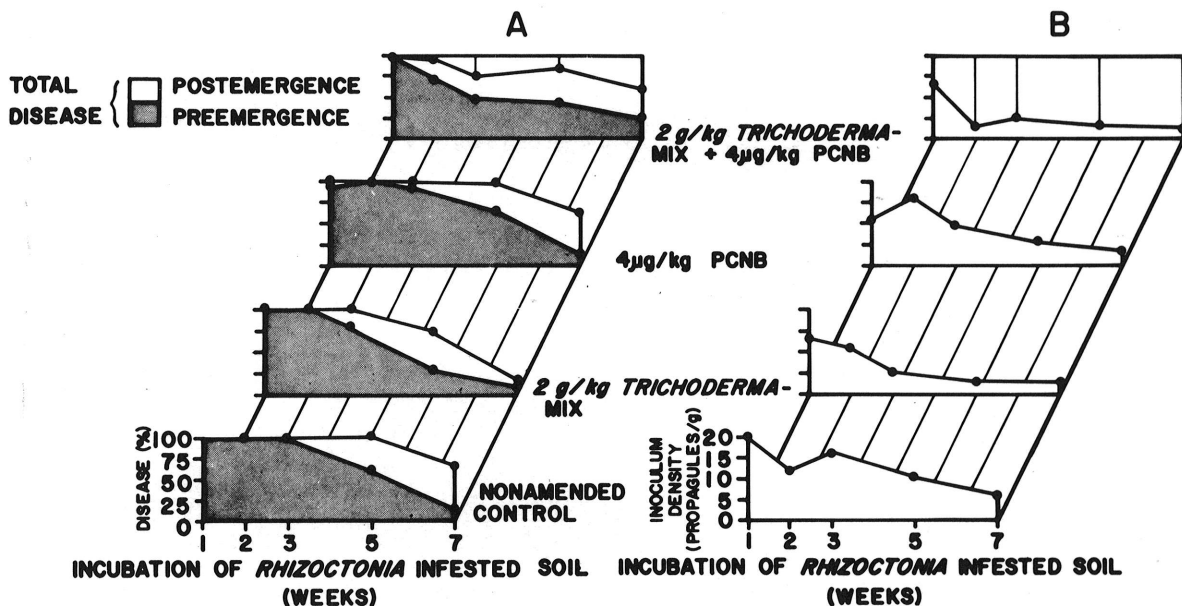


Fig. 2-(A, B). Effect of *Trichoderma harzianum* and pentachloronitrobenzene (PCNB) on the increase of A) radish damping-off and B) *Rhizoctonia solani* population during five successive plantings. Soil was initially infested with five propagules per gram, incubated for 7 days before the first planting and replanted every week for 5 wk. Standard errors of the mean did not exceed a total of 40%.

T-PCNB treatment in the heavily infested soil incubated for 7 wk at 25 C (Table 1). Single applications of *T. harzianum* inoculum, though initially effective in accelerating the decrease in inoculum density of initially introduced *R. solani*, and in preventing an increase of the pathogen in the soil, were not effective in protecting the plants against newly introduced pathogen in the assay used to collect data for Equation 1. Suppression of disease was highest in the nonamended inoculated (five propagules/g soil) control planted with radish seeds for 5 successive wk. In this treatment the suppression of *R. solani* surpassed all other treatments. Soil containing *T. harzianum* at levels of 0.5-4 g/kg soil followed by successive plantings was more suppressive than similarly treated soil incubated without the presence of the host for 7 wk. Soil suppressiveness increased 7 wk after the application of PCNB, but its combination with *T. harzianum* provided no significant decrease in the CI value.

Effect of successive plantings on survival of *Rhizoctonia solani*.—Soil samples were taken from the noninoculated (CI = 0.838 ± 0.118) and inoculated radish monoculture treatments (CI = 0.163 ± 0.042). Plants in

these soils were reinoculated with CPS inoculum at a rate of 14 propagules/g soil, adjusted to 15% moisture, incubated at 25 C, and examined at 24-hr intervals for propagule concentration using the multiple pellet soil-sampler (12). The average daily death rates of *R. solani* propagules were 50 and 70% in the noninoculated and inoculated treatments, respectively (Fig. 4).

DISCUSSION

The possible use of *T. harzianum* in biological control of phytopathogenic fungi has been examined frequently. Wells et al. (24) used it against *Sclerotium rolfsii*, by mixing it with soil along with its growth medium. Backman and Rodriguez-Kabana (2) used it against *S. rolfsii* in peanuts in the form of granules composed of diatomaceous earth and nutrients. Kelley (14) employed a similar method against *Phytophthora cinnamomi* in pine trees but encountered complications when nutrient leakage from the granules resulted in an increase in disease severity. An indication of the feasibility of integrated control of *R. solani* was first reported by Georgopoulos and Wilhelm (8) who reported an

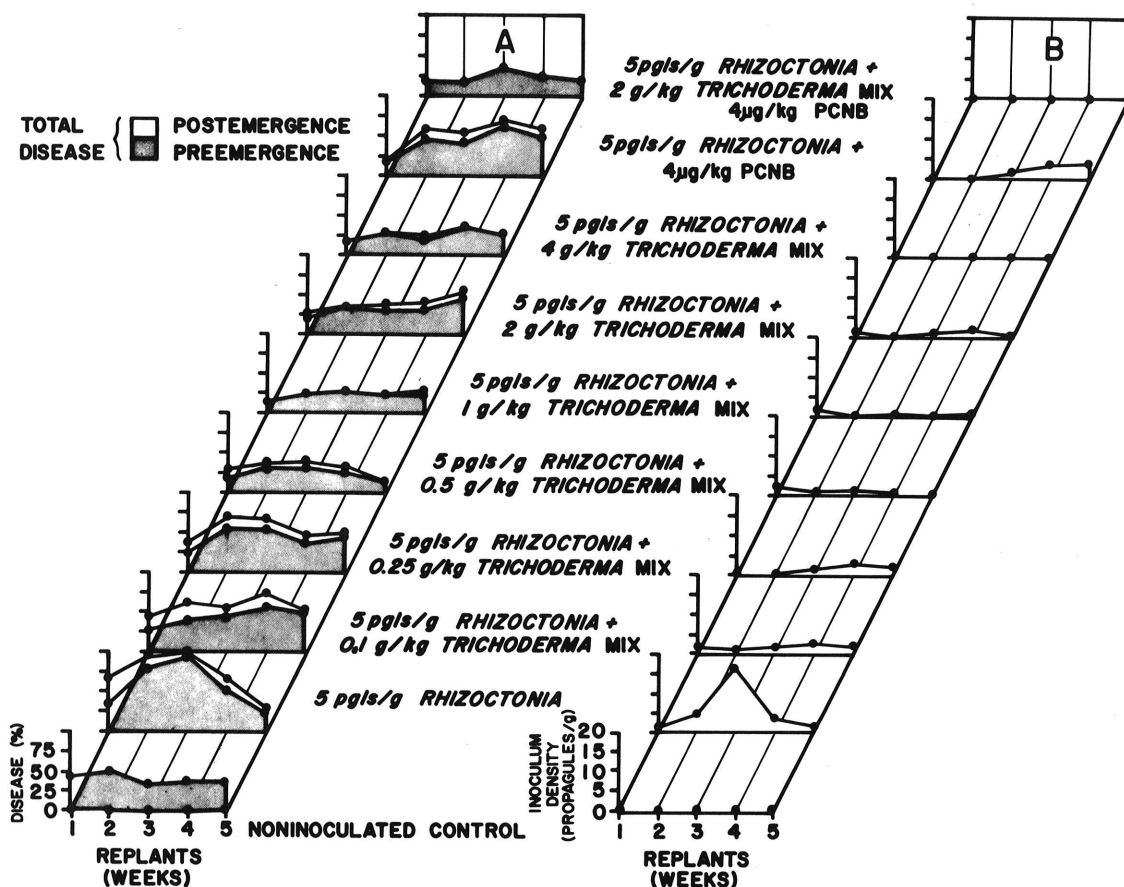


Fig. 3-(A, B). Effect of *Trichoderma harzianum* and pentachloronitrobenzene (PCNB) on the decline of A) radish damping-off and B) population of *Rhizoctonia solani* during 7 wk of incubation. Soil was initially infested with 80 propagules/g soil, incubated for 7 days before first planting and subsamples replanted after 1, 2, 3, 5, and 7 wk. Standard errors of the mean did not exceed a total of 40%.

enhanced death rate of the pathogen in PCNB-treated, presterilized soil following flooding with water effluent from a natural soil. Reports on integrated control of *R. solani* using *Trichoderma* sp. and fungicides include that of Curl et al. (6) who obtained control of damping-off in sterilized sand with PCNB and *T. harzianum*, and of Hadar et al. (*unpublished*) who demonstrated a synergistic effect of *T. harzianum* and PCNB on *R. solani* damping-off of eggplant seedlings in natural soil.

Wheat bran cultures of *T. harzianum* added to soil at rates ranging from 0.1-4 g/kg soil protected radish seedlings from damping-off induced by *R. solani*. Addition of PCNB at 4 µg/g soil along with *T. harzianum* resulted in an additive effect of both reduction of inoculum density and disease. In successive plantings of

radish in *R. solani*-infested soil, *T. harzianum* alone checked the increase of both pathogen and disease. It also accelerated the decline in population of the pathogen. In contrast, PCNB induced some disease control, but also slowed the rate of decline of both the population of *R. solani* and disease, probably because it inhibited organisms antagonistic to *R. solani* (e.g., actinomycetes) (13). Part of this reduction in control could result from the effect of PCNB on enhancement of other damping-off pathogens such as *Pythium* spp. (10). Our findings corroborate the report of Curl et al. (6) that, in sterilized sandy loam infested with *R. solani*, the biological control provided by *Trichoderma* sp. exceeded that of the chemical control provided by PCNB.

Since many clones of *R. solani* are capable of growth in

TABLE 1. Effect of successive plantings, addition of *Trichoderma harzianum*, and/or pentachloronitrobenzene (PCNB) on soil conduciveness to damping-off of radish seedlings caused by *Rhizoctonia solani*^a

Treatment	Symptomless plants in noninoculated control (A)	Symptomless plants in inoculated treatments (X)	Soil conduciveness index (CI) ^c	S _x ^d
Original soil (not incubated, treated, or exposed to host)	26	2.6	0.900	±0.046
Soil infested with 80 propagules/g soil of <i>R. solani</i> and incubated 7 wk before assay ^a				
Nontreated control	14	1.4	0.900	±0.087
T ^b (2 g/kg soil)	25.6	3.2	0.880	±0.043
PCNB ^b	1.8	0.2	0.888	± ND
T (2 g/kg soil) + PCNB	15.4	1.8	0.882	±0.055
Five successive plantings before assay at weekly intervals (noninoculated control)	29.5	4.8	0.838	±0.118
Soil infested with five propagules/g soil of <i>R. solani</i> with five successive plantings of radish at 1-wk intervals before assay				
Inoculated control	27.5	23.0	0.163	±0.042
T (0.5 g/kg soil)	27.5	11.4	0.586	±0.118
T (1 g/kg soil)	26.0	8.4	0.677	±0.089
T (2 g/kg soil)	27.5	7.2	0.738	±0.081
T (4 g/kg soil)	26.0	14.0	0.461	±0.120
PCNB	25.4	14.2	0.441	±0.107
T (2 g/kg soil) + PCNB	25.4	11.8	0.537	±0.116

^a Assay consisted of inoculum of *R. solani* introduced centrally into soil in a pot containing 32 radish seeds at various distances from the inoculum source. There were five replications.

^b Abbreviations: T = *T. harzianum* grown on wheat bran and mixed into soil at the time of infestation with *R. solani*, PCNB = pentachloronitrobenzene mixed into soil at 4 µg (active ingredient)/g of soil at time of infestation with *R. solani*.

^c Soil conduciveness index (CI) = (A-X)/X where A = number of symptomless seedlings in noninoculated control and X = number of symptomless seedlings in inoculated treatments. Complete conduciveness would have a value of 1; complete suppressiveness a value of 0.

^d Standard errors of the mean were calculated from the standard deviations between X average, X minimum, and X maximum, taking into consideration the larger values.

soil (5), the conducive index (Equation 1) was developed with this growth factor in mind as well as the ability of the pathogen to induce disease upon its arrival at the infection court. Thus, a standard percent disease rating based only on disease incidence in soil uniformly infested with the pathogen would not provide as complete a picture of potential suppressiveness of that soil.

The sharp decline in disease after 3 wk in the nonamended, inoculated treatments planted five successive times with radish (Fig. 2) was accompanied by a significant increase in suppressiveness (a reduced value of the conducive index, Table 1). This phenomenon superficially resembles the observation of reduced severity of take-all disease associated with repeated cropping of wheat following the opening of new polders in The Netherlands (9).

What is the mechanism for the induction of suppressiveness to soil when radishes were exposed to *R. solani* and repeatedly planted at 1-wk intervals in monoculture for 5 wk? As shown in Table 1, there was no change in the conducive index after 5 wk in the soils to which only *R. solani* had been added. Similarly, no change was observed when successive crops of radish were planted without the pathogen. Thus, increase in suppressiveness following repeated crops of inoculated plants cannot be due to a disease potential factor (4) or to fungitoxic chemicals produced by the host itself. Rather, this may be an example of increase in antagonistic populations postulated by Baker and Cook (3) in areas where both the pathogen and disease occur; that is, the pathogen must be active to induce the development of antagonists gaining some benefit from the association of

host and active pathogen. Such a mechanism suggests that profound influences must operate related to changes in inoculum potential (4). Capacity factors, especially the microbial environment, presumably influence the suppressiveness of the soil in the present case. Influences on inoculum quality involving genetic factors, propagule size (11, 17), pathogen nutrition (22, 23), and age also provide candidate hypotheses for mechanisms; however, the prime factor may be the influence of the suppressive factor on inoculum density. Figures 2 and 3 illustrate the correlation between inoculum density and incidence of disease over the 5-wk replanting period in soil infested with *R. solani*. Inoculum density declined during the period when suppressiveness was increased, eventually reaching a relatively low level at the end of the experiment.

No information is available regarding the effect of successive plantings on populations of and the diseases induced by *R. solani*. *Trichoderma harzianum* added to conducive soil, was effective both in preventing buildup and reducing the population of *R. solani*; PCNB on the other hand, primarily was fungistatic and reduced disease incidence. It retarded decline in pathogen population, however, in radish monoculture when compared with nontreated soil as shown in Fig. 3.

The increase in suppressiveness observed in the inoculated, monoculture treatment suggests development of a microflora more antagonistic than *T. harzianum* to *R. solani*. The suppressiveness generated by this monoculture system also increased the death rate of *R. solani* over time, indicating profound influences of the factor on survival of the pathogen (Fig. 4). On the other hand, enhanced survival of propagules of *R. solani* in soil treated only with PCNB calls for a screening of other fungicides more suitable for integrated control.

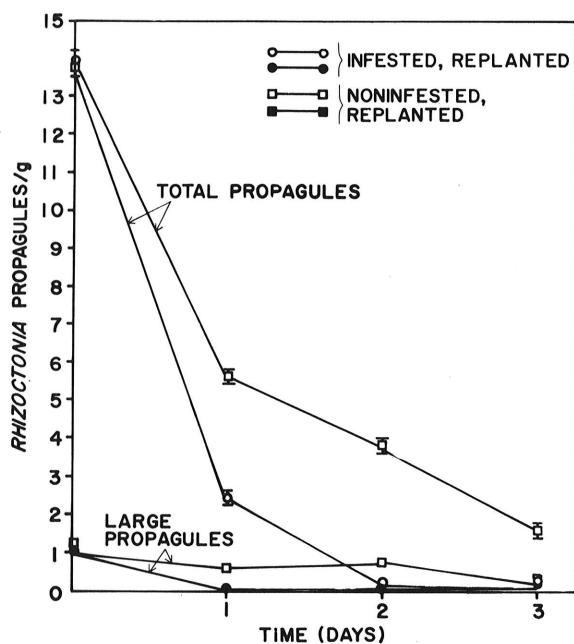


Fig. 4. Death rate of *Rhizoctonia solani* in replanted, infested, and noninfested soil. Soil was replanted four times. Bars indicate standard error of the mean.

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