Modeling of Phenomena Associated with Soil Suppressive to *Rhizoctonia solani*

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ABSTRACT

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Soils became suppressive to *Rhizoctonia solani* after being planted to successive crops of radishes at weekly intervals when small propagules (mycelial fragments) (<250 μm) but not large propagules (>589 μm) of the fungus were used as inoculum. Disease incidence increased more rapidly when host tissue was reincorporated into the soil during three replantings than when host tissue was removed, which suggested that no chemical entity present in radish was suppressing the activity of *R. solani*. Lack-of-fit tests applied to data describing increase of disease within a single crop suggested a better performance of data modified by the simple interest transformation than of either the plot of nontransformed points or the compound interest model. None of these approaches to modeling, however, demonstrated better degree of fit than did regression plots of nontransformed data on disease observed from crop to crop in successive plantings. The ED_{50} values indicated by the inoculum density-disease incidence (ID-DI) curve was changed from approximately 8.0 in conducive soil to 21.5 propagules per gram in the suppressive soil. Thus, change in ED_{50} value indicated the substantial impact of biological control achieved through the use of suppressive soil. As the concentration of conducive soil in mixtures was increased relative to suppressive soil, the conducive index (CI) increased. The curve obtained when concentrations of conducive soil were plotted against the values of CI resembled an ID-DI relationship. The curve, modeled according to the log-log transformation, had a slope value of 1.222. If the suppressive mechanism in the soil resulted from microparasitism, this could be interpreted as a "rhizosphere" effect adjacent to the thallus of *R. solani*.

In 1976, two research groups in our laboratories independently observed suppression of growth and pathogenicity of *Rhizoctonia solani* Kühn in a Fort Collins clay loam initially infested with the pathogen and planted to successive crops of radishes at weekly intervals. One group observed the phenomenon in inoculated controls of an experiment in which various treatments were attempted to obtain integrated control (6). Subsequently, this induction of suppressiveness during radish monoculture was described in more detail and an hypothesis for the mechanism of biological control was formulated (7). In the other case, the investigators were attempting to describe more fully

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and model the epidemiology of Rhizoctonia damping-off of radish (4,5). An experiment was designed and set up to determine the interactions of *R. solani* and radishes planted in monoculture at successive weekly intervals. Development of *Rhizoctonia*-suppressive soil also was observed in the latter case. Thus, we conducted other experiments designed to investigate these initial observations; in this article we describe the phenomenon further and apply models for epidemiological analyses.

MATERIALS AND METHODS

A Fort Collins clay loam described previously (12) was used in all experiments; it was air dried and sieved (5-mm pore size) prior to distribution in containers.

Isolate R-3 (4) of *R. solani* was grown in chopped potato-soil (CPS) substrate (5). This inoculum mixture was air dried, passed through a screen (1-mm pore size), and stored in a gauze-covered flask at room temperature. Different sizes of inoculum units were obtained by either wet or dry screening. In the wet screening process, running water was applied to CPS inoculum on a 589-μm (pore size) screen and the residue on the screen was collected. This sample contained units of inoculum identified hereafter as large propagules. CPS inoculum similarly wet screened through a 250-μm screen was collected and designated the sample containing small propagules. CPS inoculum also was dry sieved through a 500-μm screen. Material that passed through the screen was considered to contain small propagules; the residue remaining on the screen contained large propagules.

Inoculum density was determined by using the multiple pellet soil sampler described by Henis et al (8) who utilized appropriate transformations. Samples (five replications per treatment, each containing 15 soil pellets [each pellet 100 mg]) were incubated on a nutrient medium selective for *R. solani* (9) in an incubator at 26 C for 18–20 hr. Hyphae of *R. solani* from the disks were counted using a stereomicroscope. Those pellets from which 10 or more hyphae emerged were considered to contain large propagules; those having only a few hyphae emerging, were considered to have contained small propagules (8).

Two kinds of containers were used for soil. In some experiments, plastic pots (80 mm deep, 78 mm bottom diameter, and 110 mm top diameter) containing 100 g of oven dry soil, were planted with 32 radish (*Raphanus sativus* 'Early Scarlet Globe') seeds per pot spaced and placed at a depth of 1 cm by a vacuum seed planter (6,7). Five replications were used routinely for each treatment. To obtain a larger amount of suppressive soil or to have a greater host sample size in some experiments, we used large rectangular plastic flats, 45.5 × 25.5 × 5.5-cm deep, each containing 1,500 g of oven dry soil. Radish seeds (144) were planted 2 cm apart at a depth of 1 cm in each flat. In this case, three replications were used in each treatment.

Initially, water potential was adjusted to −0.7 bars and this was maintained throughout the experiment. Containers were covered with transparent Mylar® (E. I. duPont de Nemours Co., Wilmington, DE 19898) secured by rubber bands to reduce evaporation, and were incubated on benches at 25 ± 1 C under continuous illumination (approximately 5,000 lx).

Percent disease incidence (DI) was caluclated, as reported previously (7). Conducive index (CI) was calculated as described previously (6), and it reflected the ability of *R. solani* to grow and induce damping-off when inoculum was introduced into the center of a pot containing eight rows of radish seeds, four seeds in each, radiating from the inoculum source. A completely suppressive soil would yield a CI value of 0 and a completely conducive soil would have a value of 1.

In some cases, data were plotted nontransformed or transformed to simple interest (ln 1/[1−y]) and compound interest (ln y/[1−y]) models (13) in which y stands for DI. The curves generated were analyzed to determine the best-fit model. The first order linear regression equation

$$y = \alpha + \beta t \quad (1)$$

and the second order linear regression equation

$$y = \alpha + \beta t + \gamma t^2 \quad (2)$$

were used in analysis. The second order linear regression equation was used to indicate curvature. The simple and compound interest models should give statistically significant coefficients when the second order equation is used (10). Coefficients were tested for significance, R^2 values were calculated, and lack-of-fit tests were performed.

Inoculum density-disease incidence (ID-DI) curves, generated by methods described previously (4,5,12), for conducive and suppressive soils were plotted nontransformed and with semi-log, log-probit, and log-log transformations (1). Slope values and ED_{50} values (inoculum density required for 50% infection) were calculated for quantitative analysis of data (2).

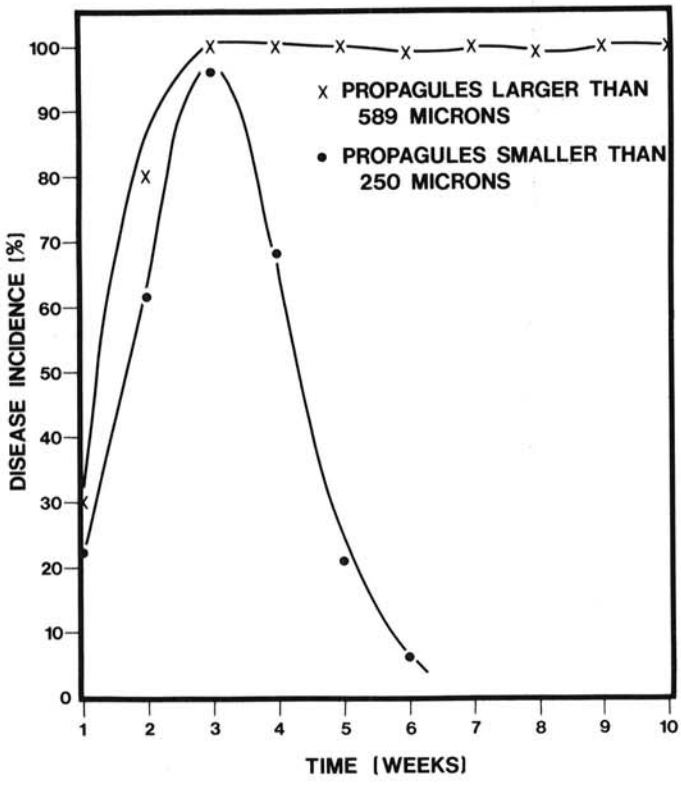

Fig. 1. Effect of propagule size of initial inoculum of *Rhizoctonia* on development of suppressive soil with successive crops of radishes replanted in the soil at 7-day intervals.

RESULTS

Effect of propagule size on development of suppressive soil. Large (>589 μm) and small (<250 μm) propagules of *R. solani* were each mixed in soil in small pots at initial inoculum densities of 4.5 and 3.8 propagules per gram, respectively. Radishes were seeded repeatedly at weekly intervals for 10 wk. A low incidence of damping-off was observed by the sixth replanting in soil initially infested with small propagules (Fig. 1). In soil originally infested with large propagules, disease incidence increased to 100% by the third replanting and remained at that level through the 10th wk.

Inoculum density in soil originally infested with large propagules increased to 20 propagules per gram after 3 wk and remained at that level for the duration of the experiment. *R. solani* colonies observed emerging from soil pellets from large inoculum treatments during this period predominantly had 10 or more hyphae per pellet and, thus, were classified as large (8). In soil from the treatments originally infested with small propagules, pellet

incubation tests revealed predominantly small propagules after repeated replants. The pathogen was not detectable in soil after the sixth replant.

The effect of removal of host tissue during successive replants. The inoculum density of *R. solani* was adjusted to 2.9 small propagules per gram (dry-screened CPS inoculum passing through a 500 μm screen) in soil and radishes were planted repeatedly at 4-day intervals. In one treatment, plants were reincorporated into the soil before the next replanting. In the other treatment, plant material of the preceding crop was removed by hand. Inoculum densities were higher when plant tissue was reincorporated into the soil (Fig. 2). The curves which represented plots of inoculum densities over time resembled those generated from plotting disease incidence over time (compare Fig. 2A and 2B). Disease incidence was higher if plant tissue was returned to the soil than if the host tissue was removed at the second and third plantings.

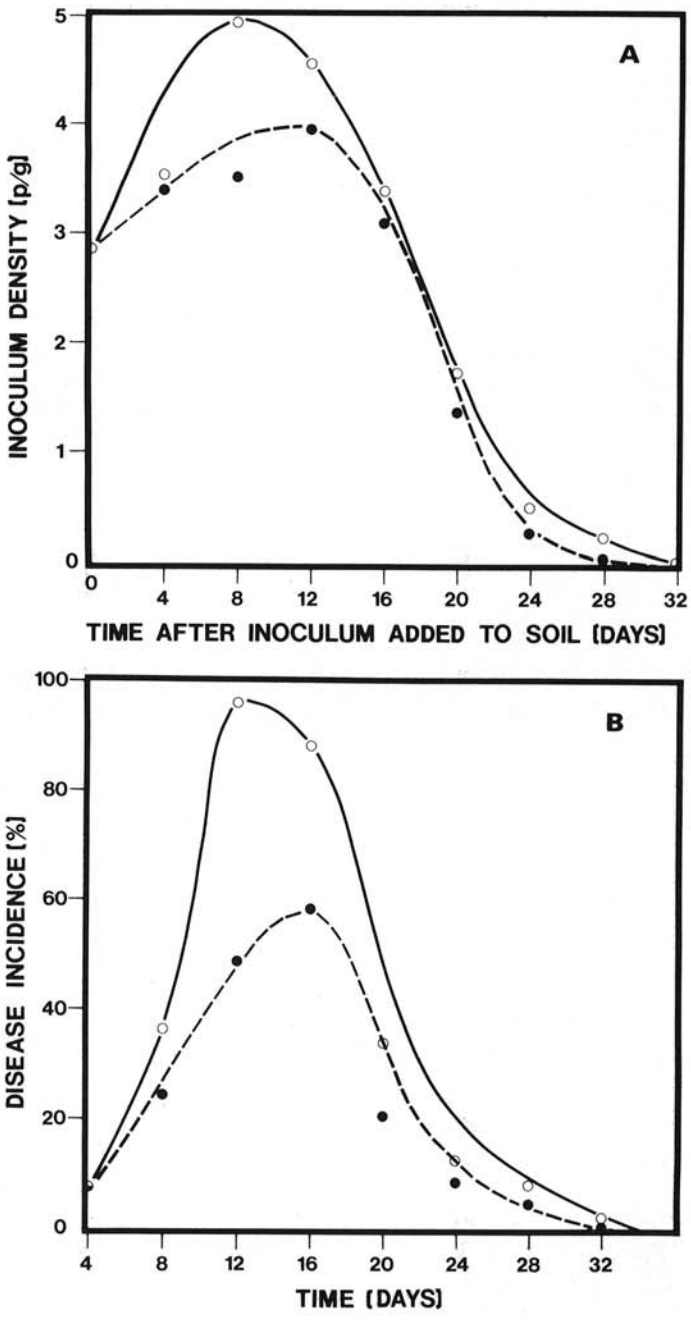

Fig. 2. The effect of removal of host tissue from successive crops of radishes replanted at 4-day intervals: **A,** inoculum density of *Rhizoctonia solani* against time; **B,** preemergence damping-off of radish against time (o—o plants were reincorporated into soil; •---• plants were not reincorporated into soil).

The increase of disease in a single crop. Radishes were planted in flats containing 3.0 small propagules (<500 μm)/g of soil. Small propagules induced predominantly postemergence damping-off. Damping-off incidence was recorded every 12 hr for 7 days beginning 3.5 days after planting. Transformed and nontransformed data were plotted (Fig. 3A). In each case the coefficients were not significantly different. Data transformed according to the simple interest equation $\ln 1/(1-y)$ had a higher R^2 value than did those that were either nontransformed or transformed according to the compound interest model using Eq. 1 but not Eq. 2 (Table 1). With Eq. 1, the lack-of-fit was significant for nontransformed data and highly significant for the compound interest model (Table 1). However, there was no significant lack-of-fit for any model with Eq. 2.

The increase of disease between crops. Nontransformed, simple, and compound interest transformed data representing the increase of disease when each crop of radishes was planted during the first four replants in the treatment in which the plant tissue was removed from the soil (Fig. 2B) were analyzed (Fig. 3B). Table 2 gives the values for coefficients, R^2, and the lack-of-fit tests in which the first degree (Eq. 1) and second degree (Eq. 2) linear regression equations were used. Coefficients and R^2 values did not differ significantly and there was no significant lack-of-fit in any model.

Inoculum density-disease incidence relationships in conducive and suppresive soils. Radishes were planted in pots containing small propagules (<500 μm) at various inoculum densities. ID-DI curves were generated for nontransformed and multiple infection, log-log, and log-probit transformed data (Fig. 4). An inoculum density of 50 propagules per gram was necessary to induce 90% pre- and postemergence damping-off in the suppressive soil, whereas 17.6 propagules per gram induced 92.7% disease in conducive soil.

Calculated slope values for the log-log transformation of ID-DI curves were 1.572 for conducive soil and 1.299 for suppressive soil. ($P = 0.0127$ and $P = 0.0298$, respectively, when statistical analysis was applied to test for the predicted slope of 1.0 [1,2].) Calculated ED_{50} values in the log-log transformation were 8.0 propagules per gram for conducive soil and 21.5 propagules per gram for suppressive soil. ED_{50} values for the log-probit transformation were 7.4 and 19.4 propagules per gram for conducive and suppressive soils, respectively.

For data subjected to both the log-log and log-probit transformations, ID-DI curves for conducive soil nearly paralleled that of the suppressive soil. Although the curves differed in position, their slopes did not differ significantly.

Effect of concentration of suppressive soil on disease. Suppressive soil was blended (v/v) with conducive soil at various concentrations, and CI were generated for all dilutions. Data resulting from observations of pre- and postemergence damping-off were plotted as a function of the concentration of conducive soil (Fig. 5A). We also plotted the log-log transformation (Fig. 5B). The slope value in this case is 1.222 ($P = 0.0404$ when statistical analysis was applied to test for the predicted slope of 1.0 [1,2].) The concentration of conducive soil required to induce a CI of 0.5 (ED_{50}) was 0.375 per unit.

DISCUSSION

If small propagules were used for inoculum, suppressive soil was generated after six plantings (Fig. 1). When large propagules were incorporated, soil remained nonsuppressive. Previous reports (7,8) suggested that the factor inducing suppression of *R. solani* in soils under radish monoculture is biological in nature. Thus, the biological entity (or entities) responsible for the suppression in soil was not measurably antagonistic to large propagules. This was reflected in assays for inoculum density; inoculum density in soils originally infested with either small or large propagules increased during three radish monoculture replantings. After that period, the inoculum density of small propagules decreased to extinction although the density of large propagules remained at approximately the same level.

Soil that became suppressive to *R. solani* during radish monoculture was generated in the Fort Collins clay loam only in

TABLE 1. Comparison of linear regression equations for data from the increase over time of postemergence damping-off of radish in a single crop caused by *Rhizoctonia solani* to determine best fit of curves using various models in which y represents disease incidence

Linear regression equation and model	Regression coefficients				Lack-of-fit test			
	α	β	γ	R^2	Mean square lack-of-fit	Mean square pure error	F^c	P
$y = \alpha + \beta t$								
Nontransformed	− 0.519[b]	0.197[b]		0.957	0.006	0.002	2.793	0.0751
ln 1/(1−y)	01.023[b]	0.339[b]		0.984	0.006	0.008	0.767	0.5668
ln y/(1−y)	− 4.761[b]	0.917[b]		0.930	0.270	0.040	6.740	0.0044
$y = \alpha + \beta t + \gamma t^2$								
Nontransformed	− 1.701[b]	0.712[b]	−0.054[a]	0.996	0.001	0.002	0.371	0.7754
ln 1/(1−y)	− 2.007[a]	0.767[a]	−0.045	0.993	0.003	0.008	0.375	0.7727
ln y/(1−y)	−12.261[b]	4.180[b]	−0.344[b]	0.999	0.022	0.040	0.550	0.6577

[a] Different from 0 at $P < 0.05$.
[b] Different from 0 at $P < 0.01$.
[c] Degrees of freedom for the first order equation are four for the numerator and 12 for the denominator. Degrees of freedom for the second order equation are three for the numerator and 12 for the denominator; t is in units of 12 hr.

TABLE 2. Comparison of linear regression equations for increase of postemergence damping-off of radish by *Rhizoctonia solani* in successive crops to determine best fit of curves with various models in which y is disease incidence

Linear regression equation and model	Regression coefficients				Lack-of-fit test			
	α	β	γ	R^2	Mean square lack-of-fit	Mean square pure error	F^c	P
$y = \alpha + \beta t$								
Nontransformed	−0.098[b]	0.045[a]		0.978	0.005	0.006	0.844	0.4
ln 1/(1−y)	−0.227	0.071[a]		0.983	0.010	0.019	0.519	0.6139
ln y/(1−y)	−3.249[a]	0.242[b]		0.952	0.377	0.160	2.356	0.1569
$y = \alpha + \beta t + \gamma t^2$								
Nontransformed	−0.179	0.065	−0.001	0.984	0.008	0.064	1.333	0.2816
ln 1/(1−y)	−0.2000	0.064	0.000	0.983	0.019	0.019	1.000	0.3466
ln y/(1−y)	−4.434[a]	0.537[a]	0.015	0.998	0.160	0.123	0.768	0.4061

[a] Different from 0 at $P < 0.05$.
[b] Different from 0 at $P < 0.01$.
[c] Degrees of freedom for the first order equation are two for the numerator and eight for the demoninator. Degrees of freedom for the second order equation are one for the numerator and eight for the demoninator; t is in units of 4 days.

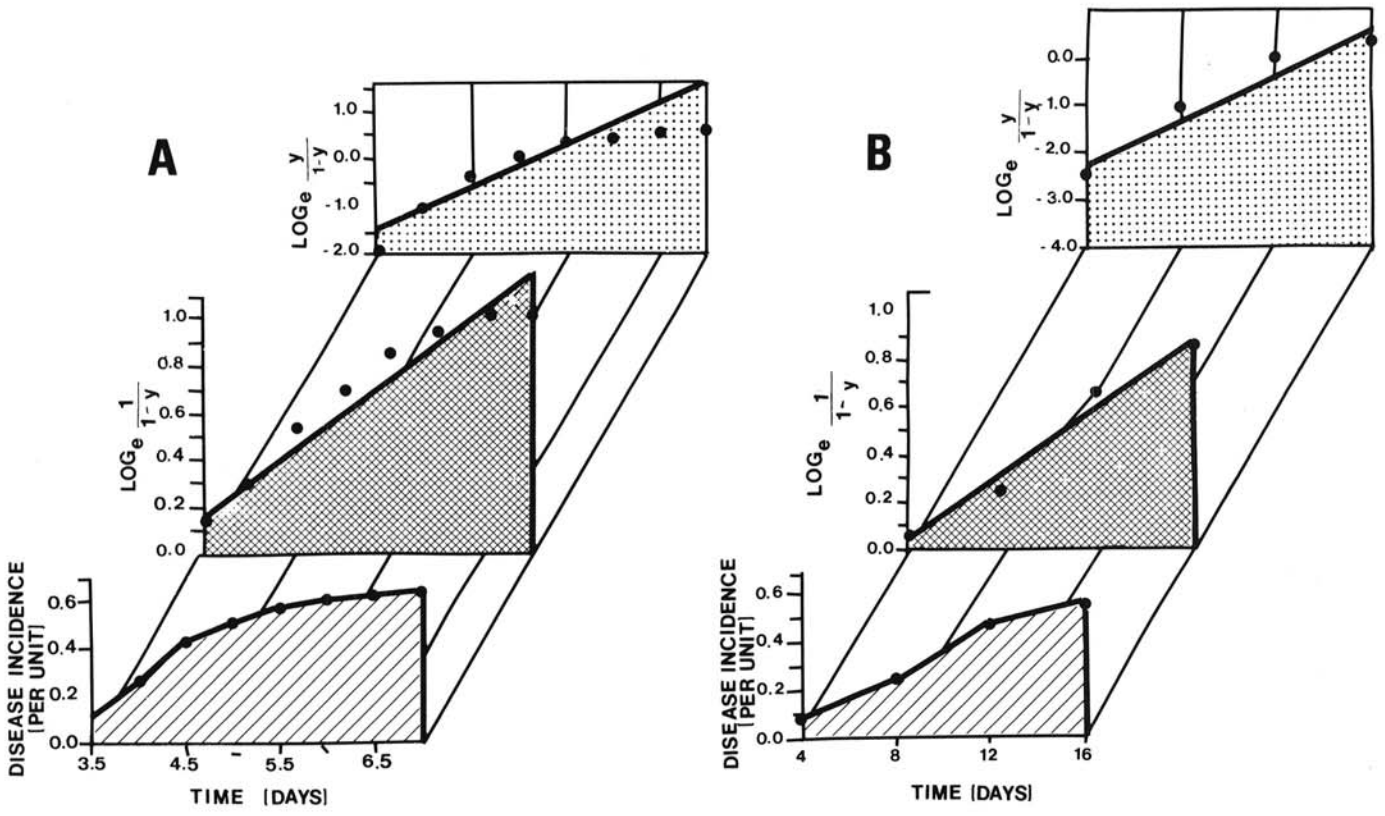

Fig. 3. The increase of damping-off induced by *Rhizoctonia solani* over time: **A,** within a single crop; **B,** over successive crops replanted in the same soil at 4-day intervals.

the presence of both pathogen and host (6). No change in suppressiveness was observed when only the pathogen was added. Similarly, no change was observed when successive crops of radish were planted without the pathogen. This suggests that the pathogen had to be active in the presence of the host to induce the development of postulated antagonists which gained benefit from the association of host and pathogen. Further evidence that the host alone did not induce suppressiveness was provided by comparing disease incidence in experiments in which the previous crop was removed after each planting or incorporated in soil. If host tissue provided a chemical entity toxic to the pathogen, disease incidence should have been lower when host tissue was added to soil and higher when it was not. Just the opposite effect was observed (Fig. 3B) during the second and third replantings. When host tissue was removed, inoculum levels in soil were lower than in plots in which radish plants were reincorporated into the soil before each replanting (Fig. 3A).

For increase of disease without multiplication, which occurs during a single season for most soilborne pathogens, van der Plank (13) suggested the correction factor $\ln 1/(1-y)$ for transformation of observed disease incidence over time. With Eq. 1, coefficients, R^2 values, and lack-of-fit tests (Table 1), confirmed that data modified by this transformation best describes the development of the Rhizoctonia damping-off disease in radish. Equation 2, however, did not indicate significant differences among analyses when data were plotted nontransformed, or transformed to simple or compound interest. The first-degree equation (Eq. 1) describes a straight line. The second-degree equation (Eq. 2), which was used by Last et al (10), describes a curved line. The simple and compound interest transformations straighten curves describing the progress of an epidemic over time when the first-degree equation (Eq. 1) is used. Thus, the improved performance of $\ln y/(1-y)$ with Eq. 1 suggests that this transformation provided the best description of development of disease for Rhizoctonia damping-off in a single crop.

For soilborne pathogens, a significant portion of the inoculum is generated on the host substrate and is returned to the soil after harvest. Therefore, as successive crops of radish were grown in monoculture, the pathogen (*R. solani*) may have multiplied through successive generations from crop to crop in a form similar

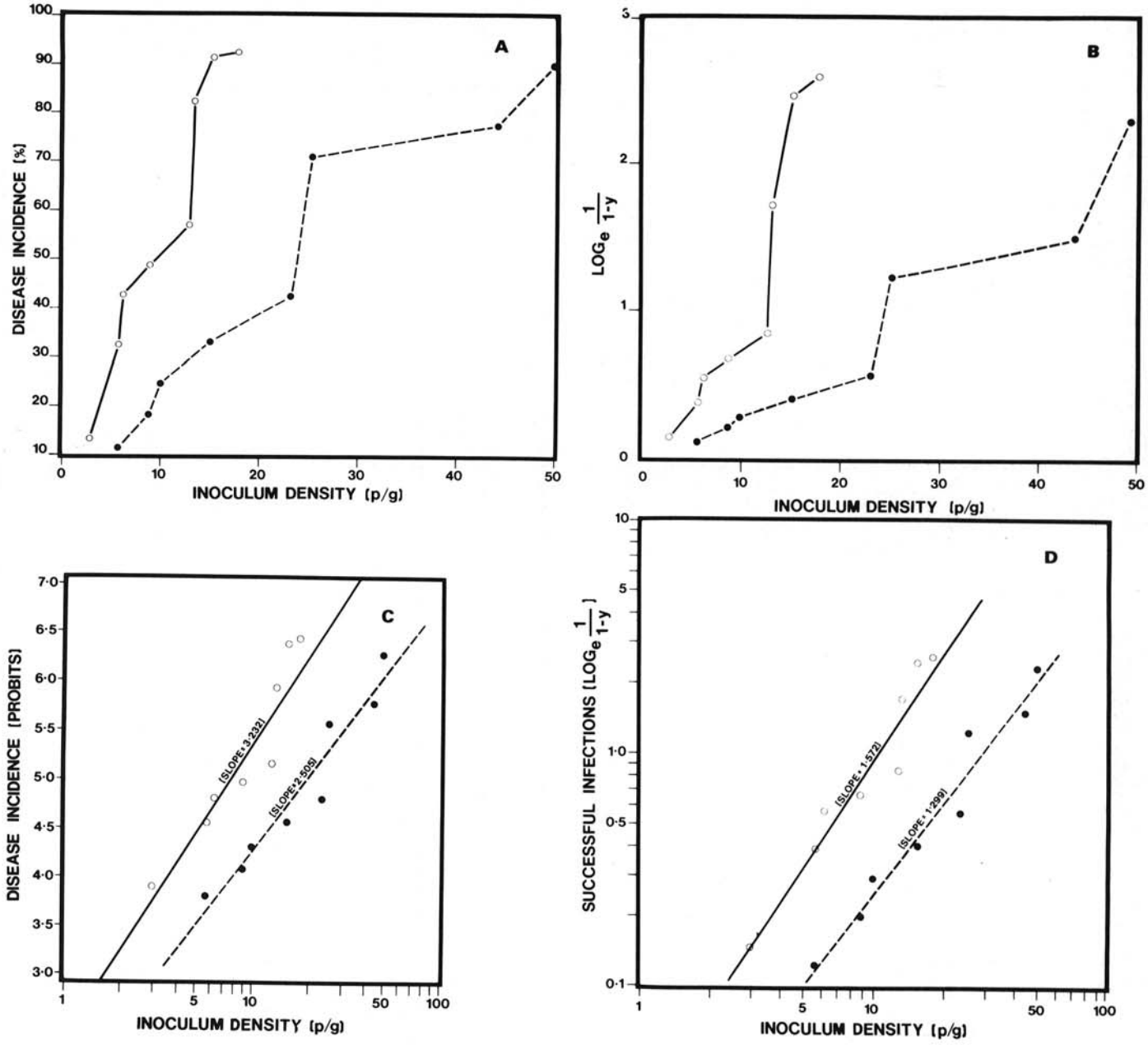

Fig. 4. Inoculum density-disease incidence relationships of Rhizoctonia damping-off of radish in conducive (o—o) and suppressive (•—•) soils; **A,** nontransformed; **B,** semi-log; **C,** log-probit; **D,** log-log transformations.

to compound interest (2). Use of Eq. 1 and Eq. 2, however, did not establish that ln $y/(1-y)$ was more appropriate for transformation of data represented in Fig. 3B than either ln $y/(1-y)$ or plotting the nontransformed data. This is a case in which the data fit any model. In such cases, Waggoner (14) suggests that it is logical to choose a model that is simple it it fits the growth curve of an epidemic reasonably well.

The Rhizoctonia damping-off system with radish as a host is an example of the fixed infection court (seed or below-ground stem in this case) and fixed pathogen (inoculum distributed at random in soil germinating in response to the presence of the host [3]). There is evidence (1,4,5,12) that the host in this case exerts a rhizosphere effect so that the slope value of the log-log (transformed) ID-DI curve should be approximately 1.0 (3). In our experiments, slope values of ID-DI curves were not significantly different (>2 [log-probit, Fig. 4C] or >1 [log-log, Fig. 4D]) at $P=0.01$, but they were different at $P = 0.05$. If the slope values were >1 (log-log) or >2 (log-probit [11]), the increased values can be ascribed to synergism which has been noted in plant disease systems involving *Rhizoctonia* spp. (1,2,4,5,12). Rouse and Baker (12) developed a mathematical analysis using the log-log transformation of the ID-DI curve to determine the difference in areas under the curve as influenced by various treatments (Table 3). According to that type of quantitative analysis of biological control, the disease reduction induced by suppressive soil was less than that obtained with a cellulose amendment, but greater than that induced when chitin was added to soil.

TABLE 3. Amount of control achieved by each biological control treatment calculated as change in efficiency of inoculum (12)[a] including damping-off by *Rhizoctonia solani*

Treatment	Average amount of control[a]
Cellulose	0.61117[b]
Chitin	0.34453[b]
Suppressive soil	0.45303

[a] Amount of control calculated as $\Delta A = 0.5[(b_1-b_1')\ (I^2-I^2_o)]+(b_o-b_o')\ (I-I_o)$ in which ΔA equals the difference in the area under the ID-DI (inoculum density-disease incidence) curve (log-log transformed data) because of disease control, b_o and b_1 are regression coefficients for the curve generated in the nonamended control or conducive soil, b_o' and b_1' are the regression coefficients for the amended treatment or suppressive soil, and I_o and I are the inoculum density limits of integration. The ID_{10} and ID_{90} values of the nonamended control or conducive soil were arbitrarily chosen for the limits of integration. These are the values of inoculum density required to give 10 and 90% disease incidence, respectively.

[b] Calculations from Rouse and Baker (12).

The ED_{50} value in the ID-DI curve was changed from approximately 8.0 in conducive soil to 21.5 propagules per gram in the suppressive soil with the log-log transformation. These values were similar whether the log-probit (Fig. 4C) or log-log (Fig. 4D) transformations were used. This change in ED_{50} value indicates the substantial impact on biological control achieved through the use of the suppressive soil.

As the concentration of conducive soil was progressively increased in relation to suppressive soil, the CI increased. The curve obtained when concentrations of conducive soil were plotted against the values of CI (Fig. 5A) resembled an ID-DI relationship (2). If a biological entity (or entities) capable of microparasitism in the suppressive soil is postulated (7), addition of suppressive soil could be viewed as an "inoculation" of *R. solani* by a pathogenic agent. A measure of the "micropathogen's" ability to influence activity of *R. solani* can be obtained by the CI. Thus, relative inoculum densities of the postulated micropathogen and "disease" of *R. solani* can be determined from data presented in Fig. 5A, and the log-log transformation of the ID-DI curve can be applied. Such analysis yields a slope of 1.222. This indicates a "rhizosphere" effect adjacent to the thallus of *R. solani* (3). Necessarily, measurements of micropathogenic activity have to be made indirectly (ie, by measuring values in terms of CI) so that the rationale and conclusions reached immediately above could be only chimerical.

Great interest is being expressed in biological control of plant pathogens by means of suppressive soils. These studies provide a mathematical basis for the phenomena associated with development of suppressiveness and these descriptions can be the

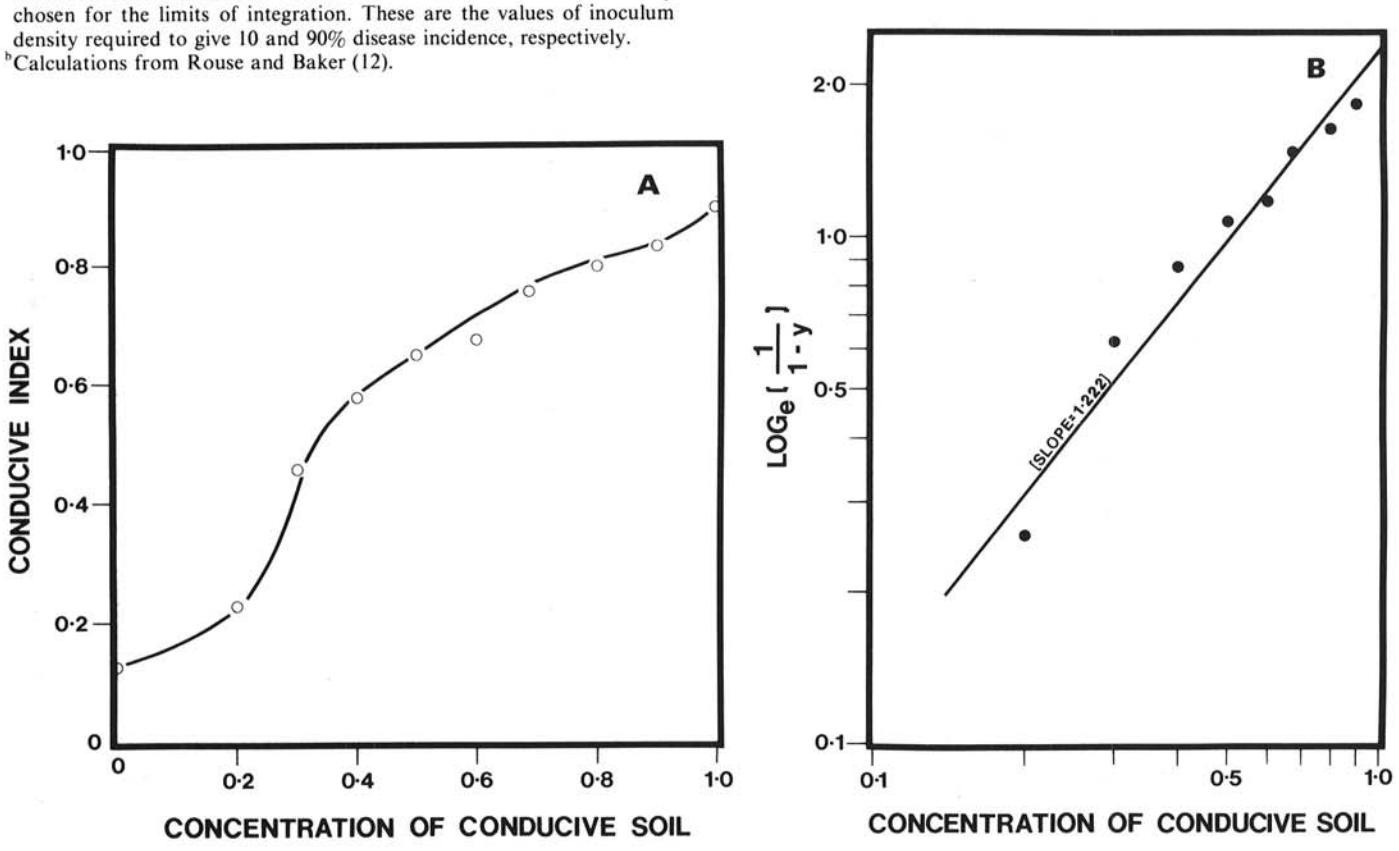

Fig. 5. Curves representing data that show the effect of concentrations of suppressive and nonsuppressive soil on damping-off induced by *Rhizoctonia solani* in mixtures of suppressive soil: **A,** nontransformed; **B,** log-log transformation, in which y = conducive index (6).

basis for interpretations and extrapolations of the mechanisms associated with the potential value of this kind of biological control.

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