Analyses Involving Inoculum Density of Soil-Borne Plant Pathogens in Epidemiology

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Recently there has been considerable interest in the mathematical description of biological processes. One of the most intriguing areas is in the epidemiology of disease, and more specifically in the relationship of inoculum to development of disease. Certainly here is a subject appropriate for mathematical description; inoculum incites disease, and the host-pathogen interaction should be amenable to quantitative analysis and explanation. In its extreme form, an understanding will be expressed as a systems analysis integrating all important factors associated with epidemiology into comprehensible mathematical biology relations.

A simple formula has been suggested (1, p. 419) for situations involving soil-borne pathogens:

disease = inoculum potential × disease potential.

Concepts of inoculum potential are not uniform (1, 16, 20), but in the formula above it would be defined as the energy available for colonization of a host at the surface of the host to be colonized (15). Given inoculum of a certain genetic capacity, inoculum potential would be a function of inoculum density and the effects of environment on inoculum (1). Disease potential in this context is the ability of the host to contract disease.

This paper attempts to describe in simple terms the models and analyses suggested for treatment of inoculum potential, emphasizing the density component. With this as a basis, experimental data will be treated and discussed in relation to the relevance, usefulness, and suitability of the analyses in studies of epidemiology.

Ways of treating data for analysis.—The treatment and significance of data involving inoculum density of soil-borne pathogens have been dealt with by a number of authors (1, 2, 3, 4, 5, 6, 12, 16, 24, 34). This information has not been brought together, integrated, and interrelated, however, and technical mathematical derivations in some cases have discouraged all but the most intrepid plant pathologists from applying theory to their data. The purpose of this section is to review suggested methods of mathematical treatment, show how they relate to each other, and show what can be gleaned from them when data are subjected to analysis. Detailed derivations in most cases will not be treated; these can be found in the literature cited.

In practice, as inoculum density increases, disease usually increases (environment being constant). This is the inoculum density-disease curvilinear relationship. Transformations suggested so far attempt to produce a straight line from this curve.

Why strive to set the crooked straight? A rectilinear plot of points is mathematically simple and can be more readily interrelated with other factors. It permits data interpolation, like the ED_{50} point. Furthermore, extrapolations are easier. The most important scientific contribution, however, may be that it permits a better understanding of the biological phenomena involved, and thus provides possible theory expansion.

Transformations are based on assumptions. How well a transformation performs may reflect the validity of the assumptions.

Multiple infections.—The percentage of diseased plants observed in an experiment does not necessarily reflect the number of successful infections. An individual plant may be invaded by a pathogen many times, but would be recorded only once as being diseased. To change the proportion of diseased plants (x) per unit [see van der Plank (28) pages 8 and 22 for a discussion of the use of "per unit"] to the average number of infections per unit, Gregory (17) employed the first unit of the Poisson distribution

$$q = e^{-y} \qquad 1$$

where q, is the probability of a host not being exposed to a successful infection ($1 - x$, as treated by van der Plank), and y is the number of successful infections.

Figure 1 illustrates what happens when more and more inoculum comes into contact with infection courts, according to equation *1*. Consider each small square as the area of belowground infection court on each of 100 host plants. Each point is a successful infection, the position determined from a table of random numbers. As more and more inoculum is added, more infection courts are encountered. At lower densities, each additional unit of inoculum usually encounters previously healthy tissue. In Fig. 1, 10 infections are in 10 different plants. When higher amounts of inoculum are added, however, more than one propagule may encounter a single infection court if the distribution is truly random. Thus, in Fig. 1, when 20 infections have occurred, only 19 hosts are diseased. The discrepancy between total number of infections and diseased individuals becomes larger as inoculum is increased.

Is random distribution of inoculum in soil a valid assumption? It is for frequently cultivated soils (26). If inoculum is undisturbed for relatively long periods, the assumption may not be valid (36).

Figure 2-A shows how infections and disease are related in this scheme. From a table, as in the appendix, Table 3, of van der Plank's book (28), the pro-
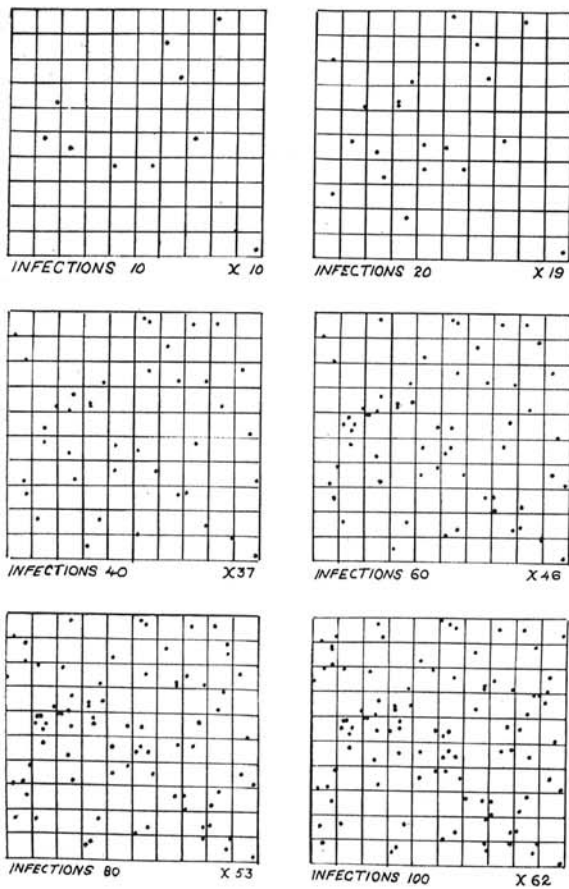
Fig. 1. Graphic presentation of the multiple infection or semilogarithmic transformation based on the Poisson distribution. There are 100 squares in each of the six plots. Each square represents a host infection court. Each dot is an infection, and its position is determined from a table of random numbers. Below each plot is recorded the number of infections and the number of plants that should be diseased (x).
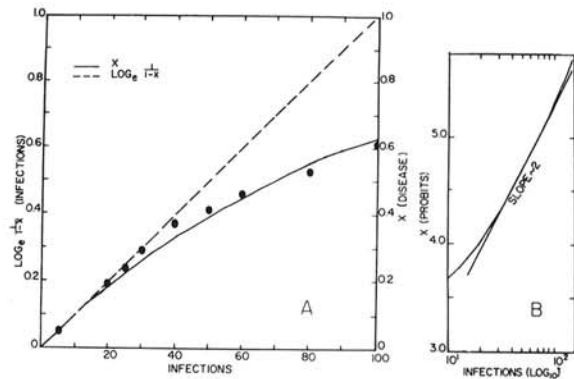

Fig. 2. The multiple infection correction and its relation to logarithmic probability. **A)** Relationship of disease (x) to theoretical number of infections as determined by $Log_e \dfrac{1}{1-x}$; ● represents points obtained from Fig. 1. **B)** Relationship of semilogarithmic transformation to logarithmic probability. An idealized situation in which infections increase with a slope of 1.0 and the resultant disease (x), taken from **A)**, is plotted in probit units. The slope near ED_{50} is ca. 2 probit units/log unit, and is represented by the straight line (27).

portion of disease (x) can be determined from the number of infections or $Log_e \dfrac{1}{1-x}$. The points of x in the example in Fig. 1 are also shown in Fig. 2-A, demonstrating the correlation with mathematical predictions.

Thus, one can note the proportion of diseased plants in populations, estimate the number of infections from the transformation $Log_e \dfrac{1}{1-x}$, plot this in relation to inoculum density on a semilog basis, and theoretically obtain a straight line.

Once this happy conclusion is drawn, most authors treating the subject hasten to describe the limitations. Especially at high inoculum densities, inoculum units may compete for single susceptible infection courts, the multiple infection transformation is not adequate, and curves are not straightened.

Logarithmic probability.—This system is endorsed by Dimond & Horsfall (12, 20) because, in their view,

the basic assumptions are more realistic than for other transformations. These assumptions and theory have been developed in some detail by Bliss (7) and Finney (13) for agents toxic to biological entities. The principles applicable to plant pathogens in soil are as follows:

1) Susceptibilities in a population are distributed normally, the change in per cent kill per unit dose being smallest near mortalities of 0 and 100%, and largest near 50%.

2) This variation in susceptibility ideally follows the normal curve of error shown in Fig. 3-A. The numbers along the base (abscissa) are equivalent to actual dosages or inoculum levels. Areas under the graph would give the proportions of individual organisms affected by each particular lethal dose. One inoculum dose, however, not only produces symptoms in hosts susceptible to it but to all the ones susceptible to lower doses. For example, the individuals responding to a dose inciting symptoms at a value 1.5 SD below the mean (3.5 probit) would be included in the area of the shaded portion of the curve. This could be stated as a proportion; that is, the ratio of the shaded to the total area under the curve.

3) For each observation of the proportion of mortality, then, the expected dosage can be inferred (if susceptibilities follow the normal curve). The unit used to designate inferred dosage is the probit. It is equal to 1 SD, and is used to eliminate minus and plus values about the mean (the mean is assigned a probit value of 5.0). Thus, the probit is merely a convenient "mathematical device for solving otherwise intractable equations" (13). Quoting values in probit levels eliminates the need for working explicitly with a mathematical formulation of the normal distribution.

4) A more convenient cumulative normal frequency distribution can be obtained by plotting the propor-
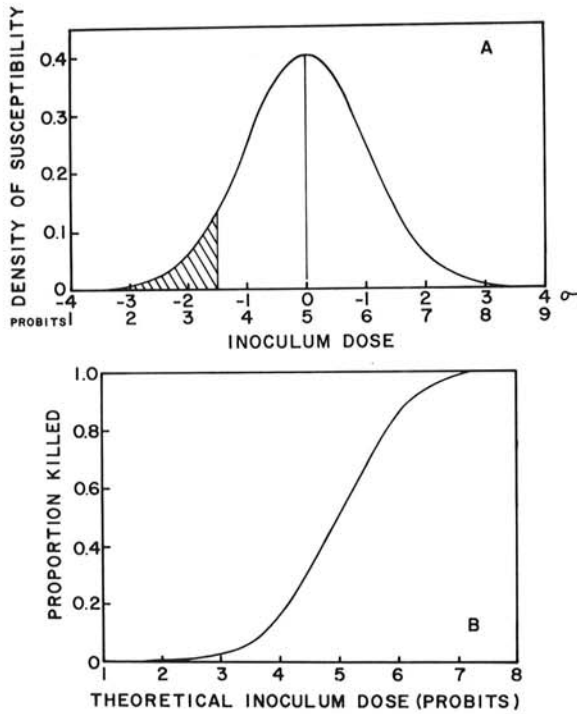
Fig. 3. The basis of the logarithmic-probability transformation. **A)** The normal distribution of susceptibilities (proportions of individuals affected by each particular dose) in a population showing the numbers of individuals per unit in a population susceptible to given inoculum levels in terms of probits. Each probit is equal to 1 SD; the mean (SD = 0) is equal to 5.0 probit units. All the individuals in the shaded portion should respond to a level of inoculum inciting symptoms at a standard deviation of −1.5 or 3.5 probit. **B)** S-shaped curve resulting from plotting the relationships in **A)** [adapted from Bliss (7)].

tions of mortality on the ordinate against the expected dose (or inoculum level) in probits along the abscissa (Fig. 3-B). Tables for this conversion are available (7, 13). The typical S curve often observed under experimental conditions is still apparent, however, corresponding to a normal distribution of susceptibilities.

5) Variation in response of biological tissue to toxicity or inoculum level often has a geometrical rather than arithmetical distribution. Thus, it would be more appropriate to distribute inoculum level values on a logarithmic scale. In practice, there is usually a linear relationship between percentage or proportion killed (converted to the appropriate probit value) and the logarithm of the inoculum density.

The position of curves indicates how efficient propagules are in inciting symptoms; i.e., the farther to the right the curve is, the more propagules are required to produce an infection. With fungicides, some suggest that slope differences in dosage-response curves indicate a different mode of action, and this interpretation has also been advanced for the inoculum density-disease curve (12, 20). This hypothesis has yet to be adequately demonstrated, but slope differences may be due also to other factors discussed below.

How may the multiple infection and logarithmic-

probability transformations be related? If the values for percentage of disease (x) developed according to the principles of the multiple infection transformation from Fig. 2-A (representing a "perfect" situation) are transported to logarithmic-probability paper, the relationship is as shown in Fig. 2-B. The curve is slightly convex, but the slope at ED_{50} is very close to 2 probit units/log unit (27).

Models based on three-dimensional relationships: the log-log transformation.—Dimond & Horsfall (12) have called attention to the fact that the literature suggests a simple linear relationship between the logarithm of the proportion of diseased plants and the logarithm of the inoculum density. Indeed, they showed a straight line relation when values of percentage of diseased plants are plotted against the amount of inoculum from the multiple infection correction up to about 60% disease units on a log-log basis. This may be one reason why plotting inoculum density-disease data on a log-log basis straightens the curve. But models illustrating interactions between host and soilborne pathogens in three-dimensional space also predict straight line relationships on a log-log basis (4).

Inoculum in soil is usually dormant, and becomes active when suitable substrates become available, as for example, in the infection court of a host (1, 33). Using this principle, four general types of pathogen-host relationships may exist in soil: I = those involving nonmotile (fixed) inoculum distributed about a fixed infection court; II = nonmotile inoculum invaded by a motile infection court; III = motile inoculum about a fixed infection court; and IV = motile inoculum invaded by a moving infection court. Examples of each of these models may be found in the literature.

It is possible to compute values for inoculum density-successful infection slopes for each model (4). Solid geometry is employed using a lattice of tetrahedrons with each point of intersection of lines being the position of a propagule in soil. Thus, the sides of the tetrahedrons are the distances between propagules (1). If a rhizosphere influence exists in Model I, the slope of the inoculum density-successful infection curve on an arithmetic (or log-log) plot should be 1.0 if every propagule under the influence of the root produced an infection. This perfect situation obviously does not occur in nature, but the number of successful infections in any case would be a function of the number (N) of propagules in the rhizosphere volume and the probability (p) of these germinating and penetrating in the infection court or pN. Plotting various values of p and N on an arithmetic basis will produce straight lines with various slopes (2, p. 281); these transferred to log-log paper are parallel straight lines, and all have a slope of 1.0 (2, p. 283). For a rhizoplane influence in this model, the slope would be 0.67 in a log-log plot of the data. For Model II, the slope is also 0.67 log-log. Models III and IV were originally calculated for successful infections as a function of distance between motile propagules. The latter value is difficult to determine experimentally. Thus, a propagule moving from a zoosporangium could be considered, for mathematical purposes, as a germination hypha, and Models

III and IV should have slopes similar to I and II, respectively.

The models then suggest that an inoculum density-successful infection slope can be predicted for a given host-pathogen relationship. This also implies that the extent of previously unknown host influence may be deduced in certain instances (e.g., whether there is a rhizosphere or rhizoplane influence in Model I) from experimentally derived slopes. Similar models with the same slopes are used in physical chemistry to describe absorption of atoms and molecules on surfaces (18). More specific applications in theory of inoculum, biological control, and fungitoxins have been reviewed (2, 3).

Use of the models requires a knowledge of the number of successful infections. The multiple infection correction can supply this information when the proportions of disease are known.

Predicted success in single spore inoculum.—S. D. Garrett (*personal communication*) has derived an equation based on the hypothesis of independent action (16). This hypothesis states that only one of a number of infective propagules will be capable of causing infection and will have the opportunity to do so.

The probability (P) that a host will be infected by a density of spores (d) would be:

$$P = 1 - (1 - p)^d \qquad 2$$

where p is the probability that any one spore will succeed in infecting.

Writing the formula in terms of p, the equation becomes:

$$p = 1 - (1 - P)^{1/d} \qquad 3$$

and for the ED_{50} value of P the equation is:

$$p = 1 - 0.5^{1/d} \qquad 4$$

This would allow a prediction of the per cent success of individual propagules in an infection court when the inoculum density-infection curve is known.

Garrett has shown that a testable example may be found in the literature. Wastie (37) placed spores of *Botrytis fabae* and *B. cinerea* on broad bean leaves at various densities, and observed lesions. The value of d at ED_{50} for *B. fabae* was 4, and for *B. cinerea* was 500. Substituting in equation *4,* the calculated per cent success in single spore inoculum is 16 and 0.14, respectively. Wastie actually observed 13 and 0.9% in his experiments.

This could also be used to determine the number of successes predicted for propagules in soil. Chocolate spot of broad bean presents a fortunate situation, however, in that one spore inciting a successful infection produces one lesion. In many cases, symptom expression in soil-borne diseases is not so readily related to a unit of inoculum. Thus, the units would have to be in terms of the increments of inoculum density and disease used for any particular situation.

Synergism.—Synergism in the context of this paper means the concurrent ingress by two or more individual propagules in which symptoms produced are of greater magnitude than the sum of the effects of each

propagule acting alone. Van der Plank (28) prefers to call this mutual interference.

The sum of the evidence, as reviewed by Garrett (16), is that propagules do act independently. As the inoculum level increases, however, there is more opportunity for inoculum to pool energy, and synergism may result.

How can synergism be detected in an inoculum density-disease experiment? In Fig. 4-A, the percentage of diseased individuals has been computed from the multiple infection transformation, with infections increasing with a slope of one as a result of increasing inoculum density in a rhizosphere, as postulated for Model I (4). A regression line drawn from points on the exponential slope would pass near the origin. If synergism occurred at higher densities, however, there would be more successful infections than the model predicts, the slope would be more than 1.0, and a regression line drawn from this area would pass to the right of the origin (28).

In Fig. 2-B, the same reasoning would imply that a slope greater than 2.0 (16, 27) based on logarithmic-probability would indicate synergism for a rhizosphere effect, Model I. But for a rhizoplane effect, Model I predicts a slope of 0.67 (log density-log infections), as does Model II (4). Thus, slopes should be steeper than 0.67 log-log for these situations if propagules infect synergically; on logarithmic probability paper, the slope should be greater than 1.31 near ED_{50} (Fig. 4-B).

Analyses of examples in the literature.—It is appropriate that the analyses just reviewed should be applied to data contained in the literature. Several ground rules regarding selection of examples will be observed, however. Since models employing solid geometry assume a situation in accord with current concepts of soil ecology (e.g., inoculum essentially dormant until propagules germinate in response to the presence of an adjacent infection court), experiments performed using soil steamed or fumigated prior to an experiment (31, 38), or artificial wounding of plants to allow ingress of a pathogen (8), will not be treated. Similarly, invasions of atypical infection courts or use of excessive amounts of organic material with the inoculum in experiments (29) would violate the assumptions of the models.

For each set of data, figures will show (A) the raw data plotted arithmetically; (B) multiple infections transformed from disease (x) as given by $\text{Log}_e \dfrac{1}{1-x}$ (28, 34) related to inoculum density (the semilogarithmic transformation) (12); (C) logarithmic-probability with computed slopes (20); and (D) multiple infections plotted as a function of inoculum density on a log-log basis with computed slopes (4). Standard linear regression analysis was used to determine slopes and lines drawn on the graphs. In the latter two transformations, the regression coefficient was tested for significance by the t-test.

Figure 5 gives a diagrammatic and highly idealized picture of the complexities which might be found in a typical density-disease relationship plotted arithmeti-
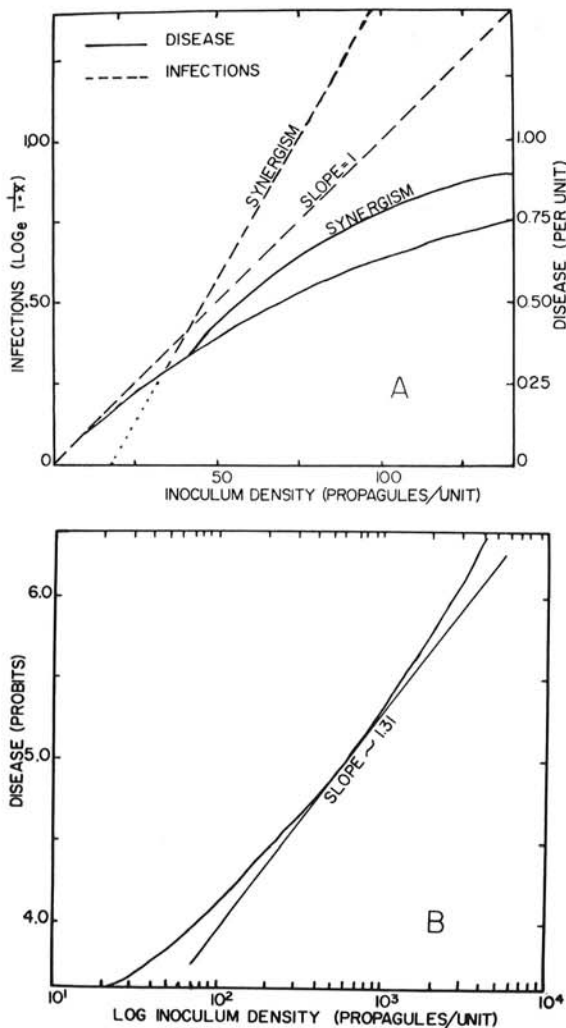
Fig. 4. Influences of synergism on slopes; A) example showing how disease and infections increase above predicted values during synergism; B) conversion of log-log slope of 0.67 to log-probit scale. Synergism in such a situation should give a slope greater than 1.31 near ED_{50}.
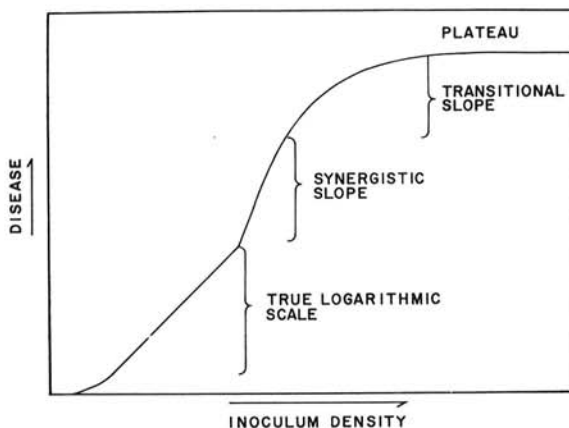

Fig. 5. Idealized picture of complexities involved in the inoculum density-disease curve.

cally. At very low densities, the increase in disease is small in response to increasing units of inoculum or infection conforming to normal distribution. As more inoculum is added, propagules may act independently, and multiple infection corrections are adequate theoretically, resulting in a straight line plotted on log-log paper. More inoculum may result in synergistic action in some cases. As multiple infections increase and propagules begin to compete for limited sites on the infection court, a transitional zone is reached, eventually ending in a plateau when all the hosts are dead or there is no increase in host response.

Bearing these complexities in mind, the points for use in analyses were selected as objectively as possible from the logarithmic part of the curve. First there must be at least three points for regression analysis. Obviously, due to limitations of logarithmic and probit transformations, values near 0 and 100 have to be omitted. The points can be selected from raw data as being logarithmic, and sometimes the semilogarithmic transformation is helpful in detecting where the multiple infection transformation does or does not correct adequately. Also, the log-log analysis reveals when the plot of points is linear, thereby conforming to the model. Synergistic action is suspected, if the slopes are over the values predicted by logarithmic-probability or log-log transformations. In some cases, examples are treated below in which points on the transitional slope or plateau occur to show how the analyses are affected.

Examples of models with fixed infection courts.— Martinson (25) exposed radishes to various inoculum densities of *Rhizoctonia solani* at different temperatures and concentrations of a fungistatic compound, pentachloronitrobenzene (PCNB). Analyses of the appropriate data, according to the criteria immediately above, are presented in Fig. 6. The curves (Fig. 6-A) suggest that some points may have reached the transitional slope, but the semilogarithmic transformation (Fig. 6-B) suggests adequate corrections for multiple infections. Synergism is suggested by all slopes on log-probability paper (Fig. 6-C), except when PCNB was present. A fungistat, such as PCNB, would be expected to reduce the slope, as only those propagules near or touching the infection court would be capable of germinating and penetrating (2). Slopes on log-log paper appear to be near 1.0.

The data of Richardson & Munnecke (30), involving similar experiments with the damping-off pathogen, *Pythium irregulare*, with various concentrations of methylmercuric dicyandiamide, are not as straightforward. Many of the points are on the transitional slope. Percentage damping-off is relatively high at the lowest density used except at the higher concentrations of fungicide. Curves characteristic of the transitional slope are apparent (Fig. 7-A). Semilogarithmic transformations (Fig. 3-B) do not appear to correct adequately for multiple infections. All transformations, however, appear to correct best at 2.5 and 5.0 ppm of fungicide. Poor correlation between inoculum density and disease is apparent at lower inoculum densities in the latter instance, however. Figure 7-C and -D indicate that
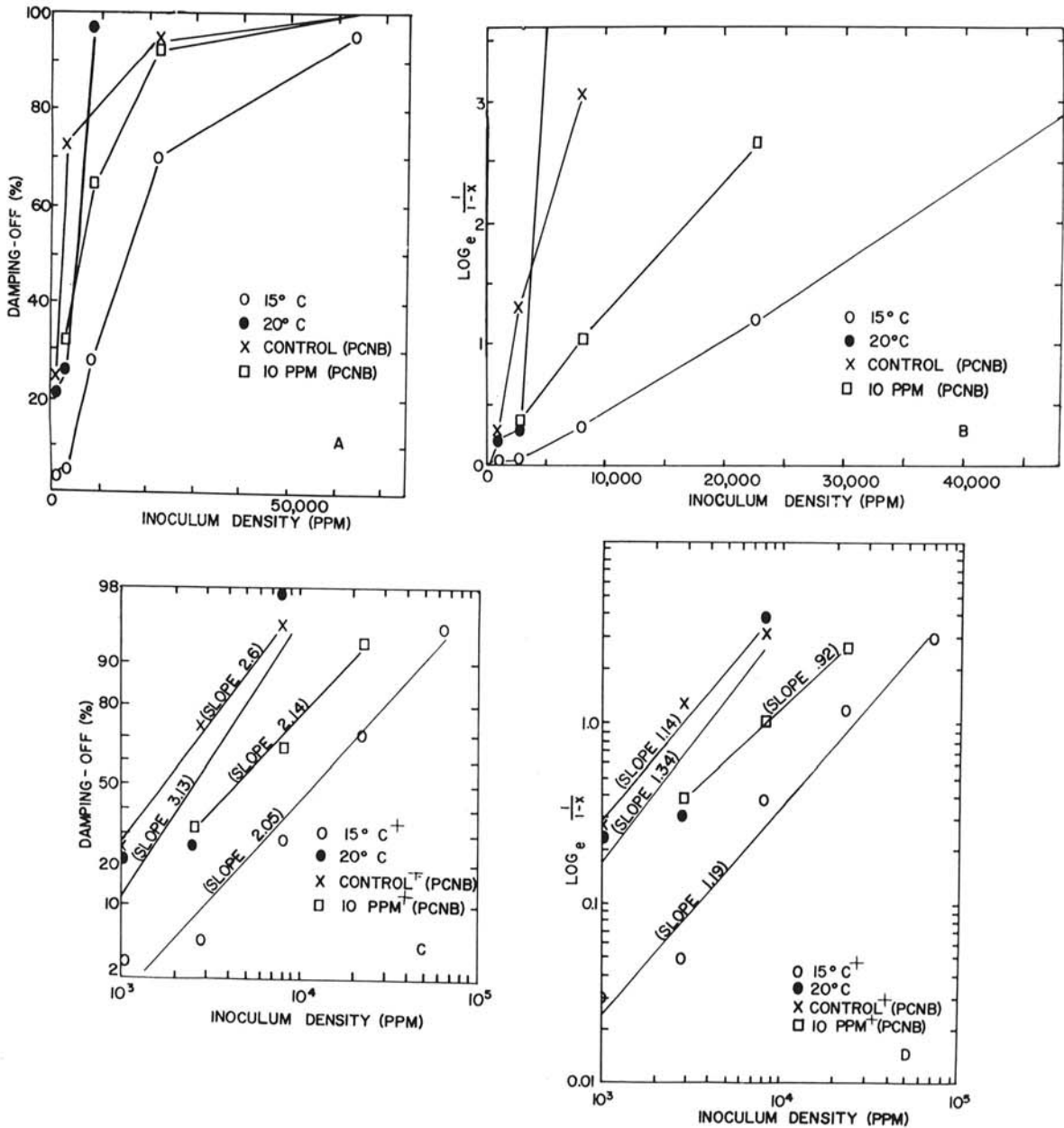
Fig. 6. Analyses of data of Martinson (25) for Rhizoctonia damping-off. **A)** Arithmetic plots; **B)** semilogarithmic transformation; **C)** log-probit transformation. The correlation coefficients are significantly positive at the 5% level of significance for those marked + in the last two transformations.

points lie in a reasonably straight line for the 2.5-ppm concentration of fungicide and especially for the four points at the lower inoculum densities. The slope for these on a log-log basis is 1.06. Synergism is not readily apparent, but again, transitional slopes would not reflect this phenomenon.

Garrett's transformation should give some idea of the relative success of propagules acting as independent units in the presence of various concentrations of fungicides. Table 1 shows how the data of Richardson & Munnecke (30) can be used in this manner. From the inoculum density required to kill 50% of the hosts (n) at any particular concentration of fungi-

cide (Fig. 7-C), the value of p (success of inoculum units at ED_{50}, equation 4) can be calculated. From this, the effectiveness of the fungicide at a given concentration can be determined relative to the nontreated control.

Sneh et al. (34) inoculated bean with various densities of *Rhizoctonia solani*. Comparison of Fig. 8-A and B indicate good correction for multiple infections as reported in their paper. Points conform to a straight line in either logarithmic-probability (Fig. 8-C) and in log-log plots (Fig. 8-D). The latter analysis would suggest a slope of about 1.0.

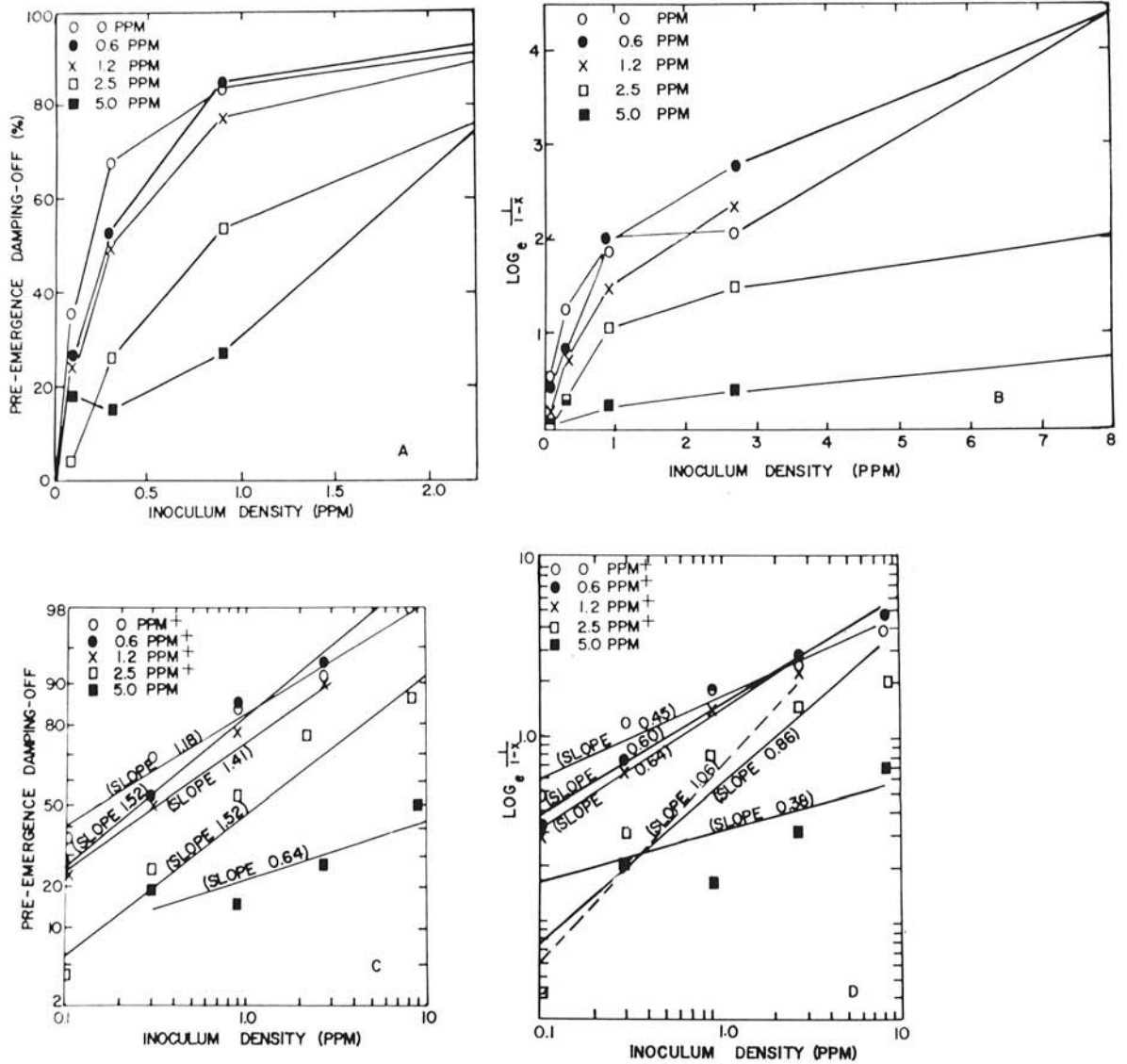The method used by Sneh et al. (34) to expose a

Fig. 7. Analyses of data of Richardson & Munnecke (30) for Pythium damping-off. **A)** Arithmetic plot; **B)** semilogarithmic transformation; **C)** log-probit transformation; **D)** log-log transformation. The correlation coefficients are significantly positive at the 5% level of significance for those marked + in the last two transformations.

host to various densities of a soil-borne pathogen is highly recommended. Populations of a pathogen are increased by growing a succession of susceptible hosts. Then the soil is appropriately diluted with raw soil not containing the pathogen. This insures "naturally cultured" inoculum in an ecological environment characteristic for both host and pathogen.

Other data using the same host and conforming to Model I have been recently reported, although the experiments were done in 1959 (3). Lesions incited by *Fusarium solani* f. *phaseoli* at various inoculum densities were counted on bean hypocotyls. This was reported as a function of temperature and C/N ratio in the soil. As data were in terms of numbers of lesions, it was necessary to estimate the maximum disease units or lesions in order to convert to a per unit basis.

The maximum number chosen was 15, as this is where lesions converged and the host infection court was obscured.

The data from experiments at 18 C are presented in Fig. 9. No synergism is apparent, and slopes on log-log paper are from 0.59 to 0.65. Although not shown, plotted data collected at 20 C yielded log-log slopes of 0.52, 0.68, and 0.68 at C/N ratios of 10, 20, and 28, respectively.

Cook (11) inoculated wheat with various inoculum densities of *Fusarium roseum* f. *cerealis*. This host-pathogen relationship probably conforms to Model I. Only three inoculum levels were used, however, and the highest level was probably on the transitional slope. Values for slopes are low (0.46-0.43 log-log) as a result, and interpretations are not possible.

TABLE 1. Relative toxicity of a fungicide in terms of the ability of the material to reduce the success of inoculum acting as independent units using Garrett's transformation from data of Richardson & Munnecke (30)

Fungicide conc	Inoculum density at ED_{50}	Success of inoculum units at ED_{50}	Effectiveness of fungicide[a]
ppm	*ppm*	*per unit*	*%*
0	0.16	.987	
0.6	0.26	.934	5.4
1.2	0.33	.878	11.1
2.4	1.02	.493	50.0

[a] Effectiveness =

$$\frac{\text{success of inoculum units at given fungicide concentration}}{\text{success of inoculum units of control}} \times 100.$$

The sclerotial population of *Sclerotium rolfsii* was determined by Leach & Davey (23) in various fields with corresponding records of losses in sugar beets. While the pathogen in this case attacks from the soil surface or slightly beneath with stem tissues providing most of the primary infection courts, the models should be valid as long as the effective inoculum occupies three dimensional space. Analysis of their data is presented in Fig. 10. The number of points in this case is considerably larger than in others reviewed in this paper, but they are also scattered. Multiple infection and log-probit transformations (Fig. 10-D) yield a slope of 0.90 which could be interpreted as conforming to a fixed infection court influencing a volume of soil near the stem. On log-probit paper, however, the slope is more characteristic of a plane effect (compare Figs. 4-B and 10-C).
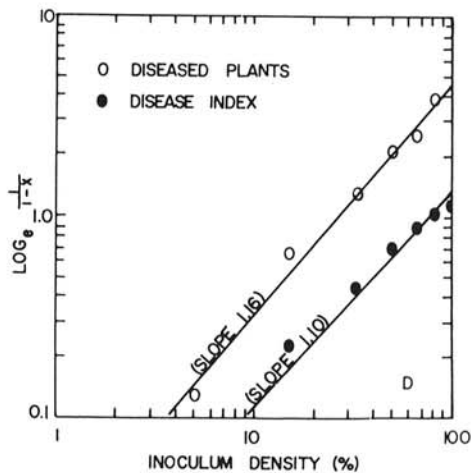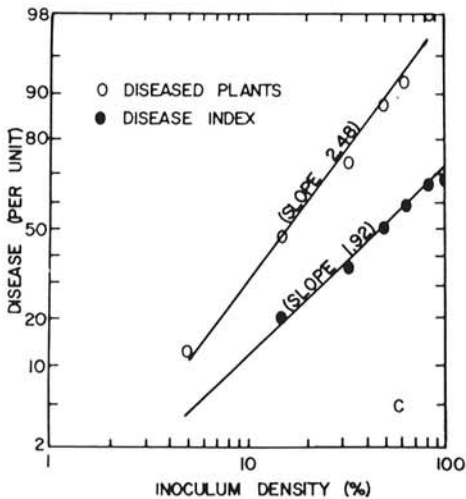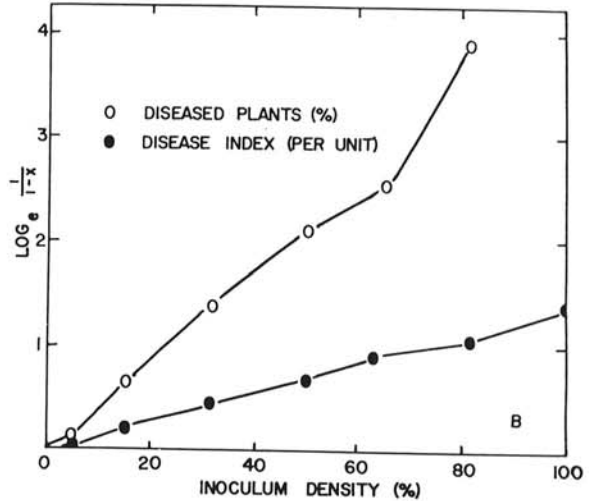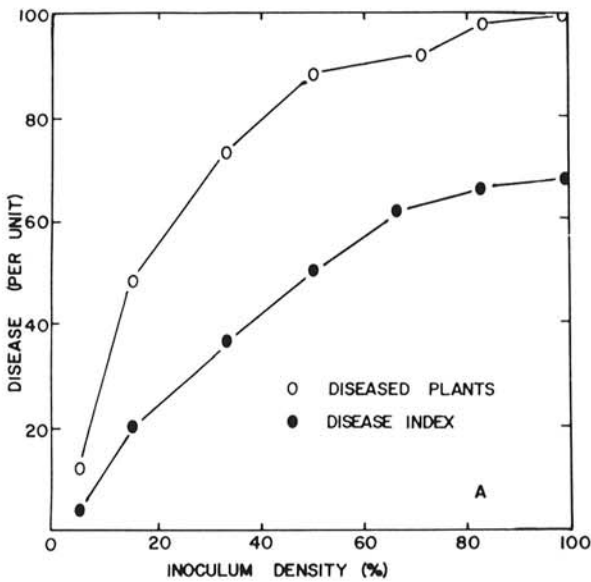

Fig. 8. Analyses of data of Sneh et al. (34) for Rhizoctonia stem rot of beans. A) Arithmetic plot; B) semilogarithmic transformation; C) log-probit transformation; D) log-log transformation. The correlation coefficients are significantly positive at the 5% level of significance in the last two transformations.

Fig. 9. Analyses of data of Baker (3) for Fusarium root rot of beans at 18 C. **A)** Arithmetic plot; **B)** semilogarithmic transformation; **C)** log-probit transformation; **D)** log-log transformation. The correlation coefficients are significantly positive at the 5% level of significance in the last two transformations.
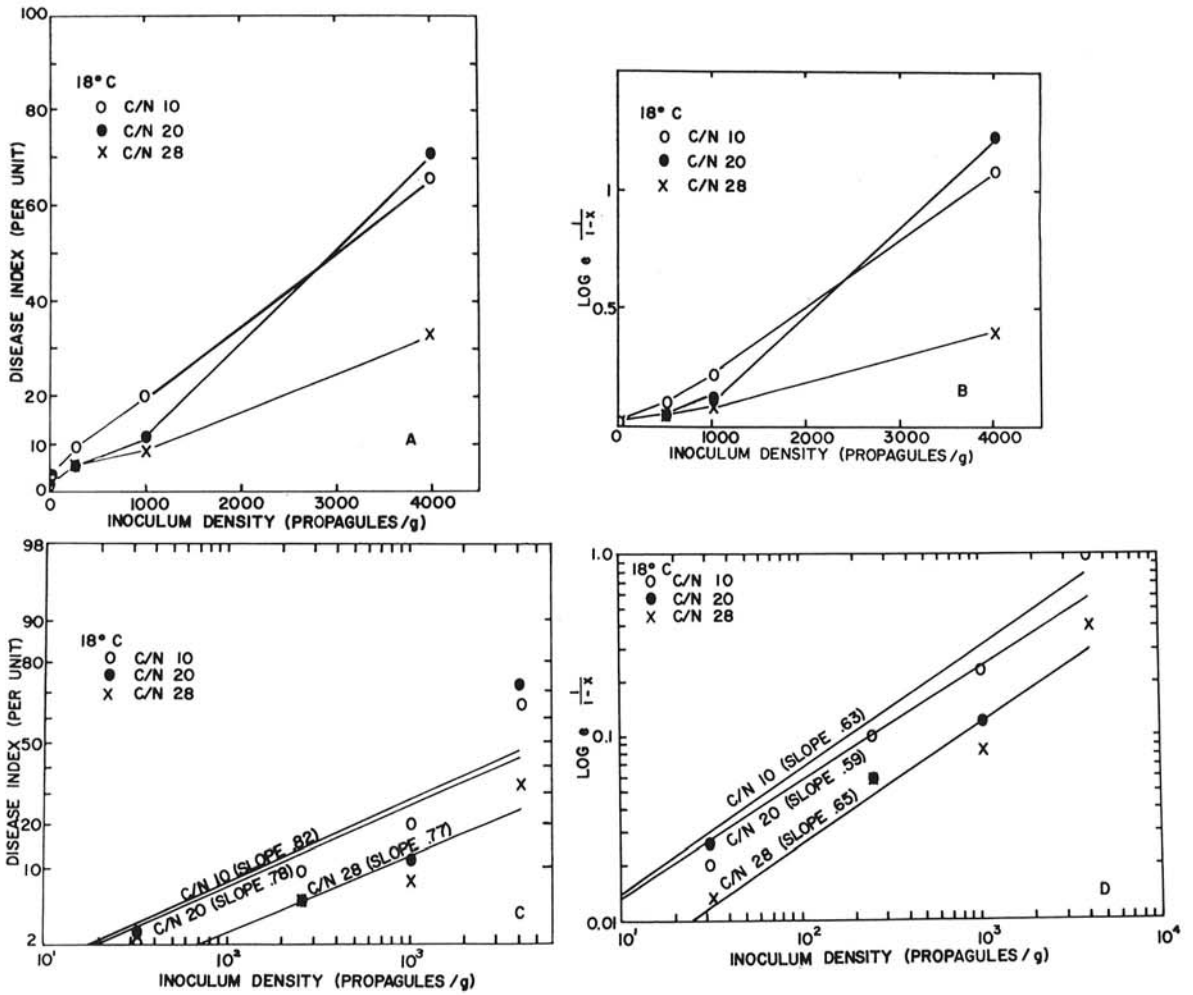
Examples of models with moving infection courts.— Extremely critical work has been done by investigators involving inoculum density relationships of club root. Some time ago, Samuel & Garrett (32) counted the number of infected root hairs as a function of pH and population of the pathogen. At the highest pH used, there are not enough points for analysis. But on the log-probit analysis, the slopes are 0.64 at pH 5.8 and 0.71 at pH 6.2. These are essentially parallel. On a log-log basis corrected for multiple infections, the slopes are 0.70 and 0.37. The latter appears to have points on the transitional slope.

MacFarlane (24) added various levels of inoculum of *P. brassicae,* and recorded both the number of infections in root hairs and resultant clubbing. At relatively low inoculum densities (10^1-10^4 spores/ml) in Experiment 4, the number of root hair infections increased with a slope of 0.60-0.63, depending on whether four or the first three points were used (Table 2). In the other three experiments at higher inoculum densities (10^3-10^6 spores/ml), the values of slopes

were lower (0.31-0.50). In all cases, slopes increased when the first three points were used instead of four or five. This indicates that the points are on or approaching the transitional slope, and that the 0.63 value may be nearest to the logarithmic portion.

In Table 3, disease resulting from these experiments

TABLE 2. Slopes (log-log) computed from data of MacFarlane (24) on relationships between number of root hair infections at different spore concentrations of *Plasmodiophora brassicae* in soil

Experiment	No. points[a]		
	3	4	5
1	0.50 (0.96)		0.31 (0.93) +
2	0.47 (0.98)	0.43 (0.99) +	
3	0.40 (0.99) +	0.31 (0.97) +	
4	0.63 (1.00) +	0.60 (1.00) +	

[a] In each case the points include the lowest levels of inoculum. Numbers in parentheses are correlation coefficients; those marked + are significantly positive at the 5% level of significance.
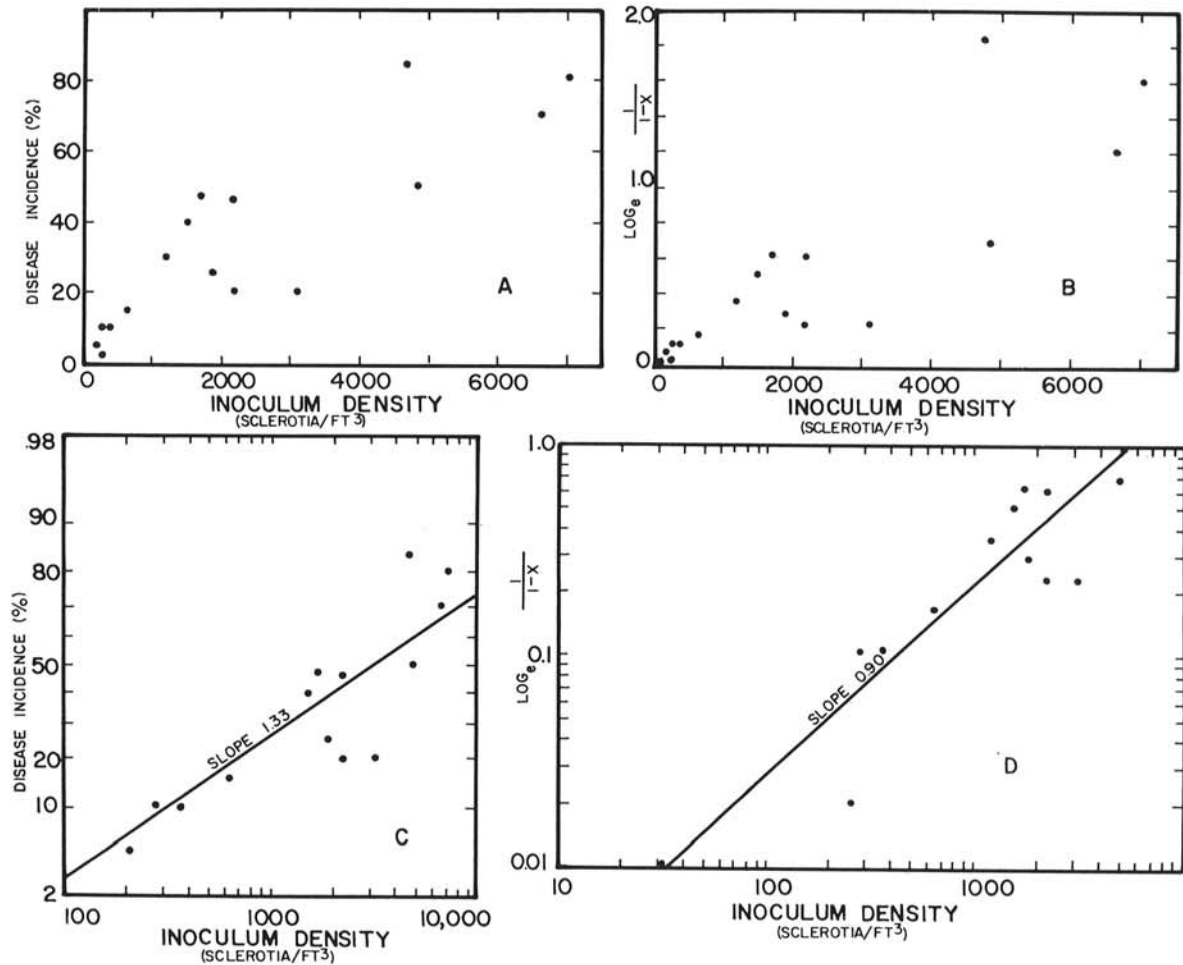
Fig. 10.. Analyses of data of Leach & Davey (23) for Sclerotium rot of sugar beets. **A)** Arithmetic plot; **B)** semi-logarithmic transformation; **C)** log-probit transformation; **D)** log-log transformation. The correlation coefficients are significantly positive at the 5% level of significance in the last two transformations.

is analyzed. In one case, the percentage of plants with clubs was relatively high (33%) at the lowest inoculum level and the slope (log-log) was 0.43. The other slopes ranged from 0.56-0.67, when the three points in each case most likely to be on the log-log slope were considered.

Data collected by Colhoun (10), concerned with the effect of various inoculum densities of *Plasmodiophora* *brassicae* and light intensities on club root development, are presented in Fig. 11. Some of the data appear to contain a substantial number of points on the transitional slope [Colhoun (10) Table 1, col. 1; Table 2, col. 1]. Thus, the usable points on these yield relatively flat slopes (Fig. 11-C, D). The others yield slopes of 0.61 to 0.68. There is no evidence for synergism.

TABLE 3. Slopes computed from data of MacFarlane (24) for relationships between percentage of plants with severe tap root clubs at various spore concentrations of *Plasmodiophora brassicae* (percentages corrected for multiple infections)

Experiment	No. plants[a]			
	3		4	
	log-probability	log-log	log-probability	log-log
2 (low nutrient)	0.98 (1.00) +	0.66 (0.94)	0.81 (0.97) +	0.50 (0.95) +
2 (High nutrient)	1.36 (1.00) +	0.67 (0.98)		
3 (Early, low nutrient)	1.01 (0.98)	0.43 (0.98)		
3 (Late, low nutrient)	1.30 (0.98)	0.60 (0.99) +		
3 (Late, high nutrient)	1.15 (1.00) +	0.61 (0.99) +	1.08 (1.00) +	0.50 (0.97) +
4	1.42 (0.98)	0.56* (0.98)	1.63 (0.99) +	0.96 (0.94) +

[a] Except in case marked "*" the points include the lowest levels of inoculum. Numbers in parentheses are correlation coefficients; those marked "+" are significantly positive at the 5% level of significance.
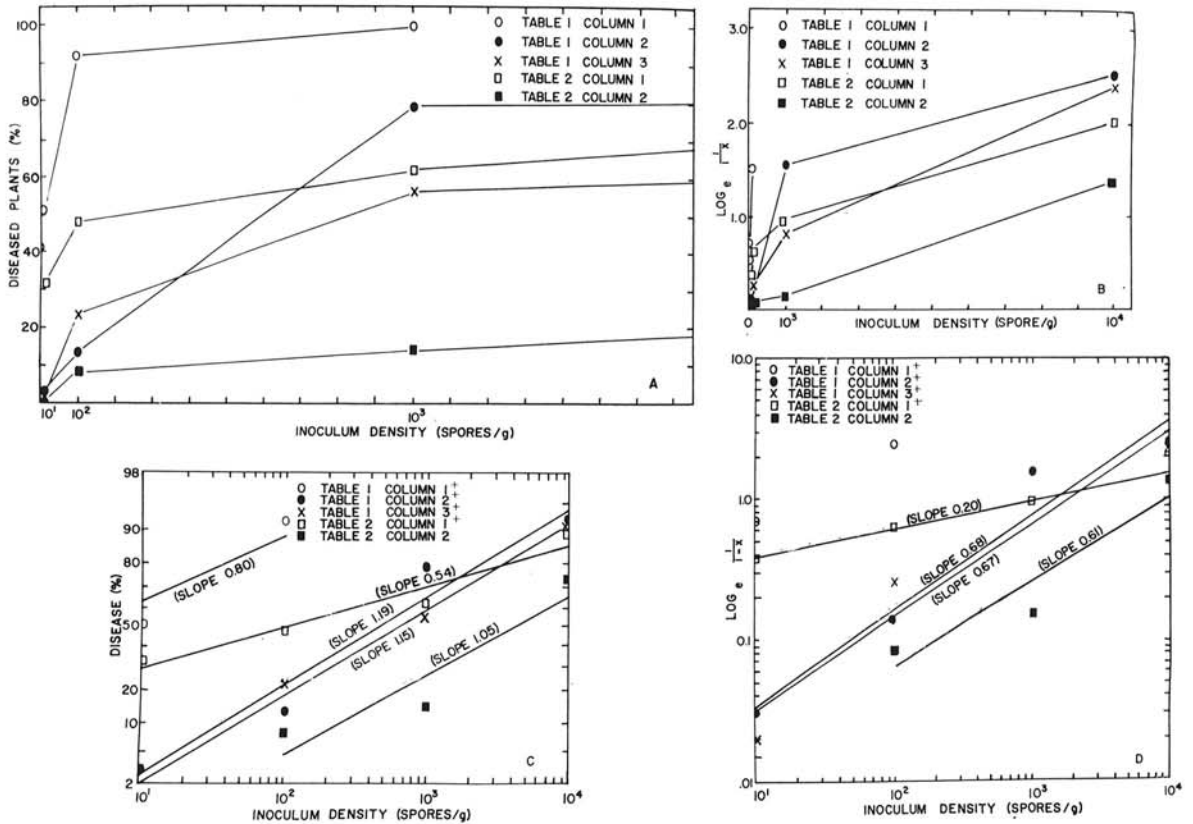
Fig. 11. Analyses of data in tables of Colhoun (10) for club root of crucifers. **A)** Arithmetic plot; **B)** semilogarithmic transformation; **C)** log-probit transformation; **D)** log-log transformation. The correlation coefficients are significantly positive at the 5% level of significance for those marked + in the last two transformations.

While detailed studies of infection courts of *Verticillium* have not been done for all hosts, the pathogen is thought to enter through the root tip (14). Lacy & Horner (22) applied various inoculum densities of this pathogen to mint varieties of low, medium, and high resistance. A substantial number of points lie on the transitional slope. Slopes are relatively flat as a result.

DISCUSSION.—Three analyses are suggested in the literature for treatment of inoculum density-disease data: the semilogarithmic transformation correcting for multiple infections; the logarithmic-probability transformation; and the log-log transformation treating successful infections as a function of inoculum density.

The semilogarithmic transformation should transform data so that there is a linear relationship between disease and inoculum density. But it only does this successfully in the logarithmic portions of the slopes in Fig. 6, 7, 8, and 9. Thus, it has limited significance by itself.

The logarithmic-probability transformation provides a linear relationship between log concentration and probit of disease. In some cases, it may be superior to others in compensating for relatively low proportions of disease and points on the transitional slope. From this analysis, one is able to deduce evidence suggesting synergism but, to detect it, one must have

adequate data about the ED_{50} point. There appeared to be evidence for synergism in Rhizoctonia damping-off of radishes (Fig. 6) and Rhizoctonia stem rot of beans (Fig. 8).

It has been suggested by Horsfall & Dimond (12, 20) that the slope of the inoculum potential curve on log-probit analysis gives information on the mechanism of disease induction. Differences in slopes would indicate that the biochemical processes of infection may differ, for example, if some environmental factor (like pH) is changed. Similarly, data involving two or more host varieties differing metabolically in resistance mechanisms would have characteristically different slopes. Inoculum potential can be considered to be measured in terms of energy (1). Basically then, as units of energy are added, the hypothesis suggests that biochemical processes differ on a per unit basis for the same host-pathogen interactions in different environments or for different hosts. Certainly the values of slopes in the lag, synergistic, or transitional portions are different when compared with the log-log phase (Fig. 5), but there are other reasons for this (discussed previously). All the log-probit and log-log analyses in this paper, however, would suggest that position but not slope of the curves changes with various treatments altering environment (Fig. 6, 9, 11), comparing fungicides (Figs. 6, 7), or different

host varieties (21). The example used by Horsfall & Dimond (20) of a soil-borne pathogen to substantiate their hypothesis was suggested from the paper by Samuel & Garrett (32) for *P. brassicae*. They state that the data ". . . show that as pH is increased the slope of the curve becomes steeper". Regression analyses, however, show that for pH 5.8 the slope is 0.64 and for pH 6.2 the slope is 0.70 on log-probit. These are the only curves in Samuel & Garrett's paper containing three points or more, and they are essentially parallel.

Figures 2 and 4 demonstrate theoretical relationships between the log-log and log-probit analyses. In some instances (for example, Fig. 6 and 8), the relationships of the slopes of the two different analyses coincide with one another according to theory, but in many other instances, similarities are not readily apparent. Slopes on the log-probit curves, however, must have considerable data about the ED_{50} point, as this is where computations for the relations are centered. Also, the assumptions in the two analyses are not the same.

Models based on logarithmic transformations of successful infections and inoculum density should not only contribute evidence for synergism, but give some indication of the extent and nature of the host influence. Thus, the data suggest a rhizosphere effect for Rhizoctonia damping-off of radishes (Fig. 6), perhaps Pythium damping-off of peas (Fig. 7), and Rhizoctonia stem rot of beans (Fig. 8) because slopes in the log-log transformation are near 1.0.

The substantial exudations of nutrients from seeds (33), and the capabilities of these fungi for some growth in soil (15), would make this conclusion tenable. In contrast, a rhizoplane influence was apparent for bean root rot (Fig. 9). Relatively little exudation has been detected from bean hypocotyls, and chlamydospores of *F. solani* f. *phaseoli* germinate only in the presence of sufficient nutrients (2, 33). Thus, a rhizoplane influence is indeed likely.

Details of all the steps in the life cycle of *P. brassicae* are not completely known (21). Certainly spores germinate, producing biflagellate amoeboid zoospores infecting root hairs. The root hairs thus become infection courts which are produced in soil, become infected, and are transitory while new hairs are being formed behind the advancing root tip. This indeed is a motile infection court, and analysis of data on exponential slopes of the data of Samuel & Garrett (32), Tables 3 and 4, and in Fig. 11 would indicate such a situation.

These host-pathogen relationships appear to confirm the idea that the log-log slopes of curves in geometrical models have meaning in terms of the extent and nature of the hosts' influence on propagules of soil-borne pathogens. The others yield slopes of lower values than predicted, but in these there is evidence that substantial portions of the data contain points on transitional slopes. Recently, other investigators have also contributed data confirming the models (9, 19, 35).

From this analysis of the curves and the literature pertaining to inoculum density, it is apparent that these

relationships should form an important basis for interpreting data and for studies of the epidemiology of any given soil-borne pathogen. Certainly in such studies, the inoculum and infection court should be in an ecological environment correlating with field conditions for data to have significance. There should be adequate points so that the various phases of the slopes may be detected. In many instances, points obtained by researchers have mostly been on the transitional slope, and were derived from inoculum levels substantially above those found in nature (5). If a log-probit analysis is anticipated, benefits would result from using as many points as possible about ED_{50}. Finally, there is one question not answered but no less important. That is, what phase of the curve is usual for pathogens at inoculum levels under field conditions? We might predict that the answer will not be simple; that pathogens may reach levels according to ecological and geometric restrictions that would permit relationships to be on the logarithmic, synergistic, or transitional slopes, or on the plateau. Once equilibrium is reached, however, it would not be surprising to find mutual characteristics for groups of pathogens which could allow generalizations and models to be constructed.

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