Relationship of Infection and Damping-Off of Soybean to Inoculum Density of *Pythium ultimum*

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ABSTRACT

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Soybean seeds were planted in pasteurized soil artificially infested with 0.1 to 600 sporangia of *Pythium ultimum* per gram of soil (spg). Five variables were evaluated as functions of inoculum density (*ID*): disease incidence (*DI*), infection of seeds 2 days after planting (seed infection), infection of seed coats 2 days after planting (coat infection), colonization of heat-killed seeds 1 day after planting (dead seed colonization), and colonization of selective medium in soil microbiological sampling tubes 1 day after insertion into soil (tube colonization). Five types of regression models were fitted to each data set after transformation of data by the multiple infection transformation, $-\ln(1-Z)$, in which Z = the proportion of affected units. *DI* was not significantly different at *ID*s of from 10 to 600 spg. Data for *DI* at lower *ID*s and seed infection were fitted to equations that indicated the existence of a spermoplane effect (sensu R. Baker), the existence of limiting sites (sensu J. E. Vanderplank), and a nonlinear relationship between the variables and *ID*. Data for coat infection, dead seed colonization, and tube colonization were fitted to equations that indicated the existence of a spermosphere effect (sensu Baker), the existence of no limiting sites (sensu Vanderplank), and a linear relationship between the variables and *ID*. Calculated spermosphere widths were approximately 8, 130, and 60 times the average radius of *P. ultimum* sporangia for seed infection, coat infection, and dead seed colonization, respectively. The results indicate that the relationships of *DI* and seed infection to *ID* are probably not true spermoplane effects (in which only propagules that actually touch the seed surface can initiate infections), but are situations in which some other factor affects the relationship of the variable and *ID*. This other factor seems to be associated with host viability, and may be related to unequal susceptibility within the host population.

Additional key words: epidemiology, *Glycine max,* seed vigor.

Relationships between inoculum density (*ID*) and disease incidence (*DI*) have been investigated for a number of diseases caused by soilborne plant pathogens; eg, *Rhizoctonia solani* (5,21,27), *Fusarium* spp. (12,16), *Pythium* spp. (20,25), *Phytophthora* spp. (20), and a number of others.

The predominant hypothesis with which *ID-DI* experiments have been interpreted has been that of Baker and his co-workers (2–4). This hypothesis postulates that rhizosphere and rhizoplane effects are distinct phenomena that can be modeled by different equations. It is further proposed that whether a rhizosphere or rhizoplane effect is in operation in a particular situation can be determined by examination of the exponent in an equation of the form:

$$S = k\ ID^b \qquad (1)$$

in which S is number of successful infections, *ID* is inoculum density, and k and b are constants. A value of $b = 1$ is taken to indicate a rhizosphere effect and a value of $b = 2/3$ is taken to indicate a rhizoplane effect. Baker et al (4) propose that values of b be determined by calculation of the slope of a linear equation regressed on a log-log transformation of a multiple-infection transformation (10) of *DI* data against *ID* (the log-log method).

The general use of such a log-log transformation for the fitting of equations of the form $y = kx^b$ (the allometric equation) to data has been criticized by Zar (28) because after the transformation the residuals often are not normally distributed and do not have a variance that is constant for different values of x. If this is the case, values of b and k can be calculated that do not describe the equation giving the best fit to the nontransformed data. Because the hypothesis of Baker et al places considerable emphasis on the value of b, such a miscalculation could result in the misinterpretation of data. The specific use of the log-log transformation in *ID-DI* research has been criticized by Vanderplank (26) and Grogan et al (11). These criticisms of the use of log-log transformations may be overcome by the fitting of equation 1 directly to data by iterative methods (28). Computer programs for such iterative methods are available in commonly used statistical analysis packages (13). However, for the type of data often obtained in *ID-DI* experiments, the basis for the criticisms (principally nonequal variances of the residuals) is not wholly addressed by using iterative methods. If data on incidence of disease, infection, or colonization are recorded and are transformed by the multiple-infection transformation (10), then the variance of the dependent variable can be shown by propagation-of-error methods to be a function of the value of that variable (6):

$$Var\ (Y) \propto \frac{1 - e^{-Y}}{e^{-Y}} \qquad (2)$$

in which Y is the multiple infection transformation of the incidence data, $Var\ (Y)$ is the variance of Y evaluated at Y, and e is the base of the natural logarithms. Similarly, for data that are subjected to a further logarithmic transformation:

$$Var\ (Y') \propto \frac{(e^{eY'} - 1)}{e^{2Y'}} \qquad (3)$$

in which Y' is the natural log transformation of the multiple-infection transformation of incidence data, $Var\ (Y')$ is the variance of Y' evaluated at Y', and e is the base of the natural logarithms. In practice, equations 2 and 3 are used most appropriately in a procedure of iterative weighting, in which weight = the reciprocal of equation 2 or 3 evaluated at each *ID* using Y or Y' calculated by using the regression equation fitted in the previous iteration. This method is appropriate for the fitting of most types of regression models to multiple-infection transformation-transformed *DI* data, and provides a statistically sound method of fitting models of the form of equation 1 by either the iterative or log-log methods.

The hypothesis of Baker et al (4) has also been criticized on

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theoretical grounds (11,15,26). Leonard (15) notes that if the rhizosphere/rhizoplane model is not valid, then fitting of equation 1 to *ID-DI* data (after multiple infection transformation) should produce values of $b \approx 1$, unless some other factor interferes with the *ID-DI* relationship. He suggests that some of these interfering factors might include nonrandom distribution of inoculum, unequal susceptibility of hosts, and an unequal probability that different infections will result in the same amount of disease. Grogan et al (11) note that equation 1 describes a straight line when $b = 1$, and a line curving to the right when $b < 1$. They point out that values of $b \approx 2/3$ could be obtained in regressions of equation 1 on data that display a plateau (ie, above a certain *ID*, *DI* does not increase as *ID* is increased). The fact that values of $b \approx 2/3$ have been reported for *ID-DI* relationships for a number of host-pathogen systems (12,20,21) indicates either that one or more of the above-mentioned or other factors may operate in many disease situations, or that the rhizoplane effect of Baker et al (4) is present.

Vanderplank (26) proposed that *ID-DI* relationships be modeled by an equation of the form:

$$y = N(1 - e^{-a \cdot ID}) \quad (4)$$

in which y = infections, ID = inoculum density, N = the number of susceptible sites, and a = a measure of site susceptibility. Vanderplank proposed that sites should be concluded to be limiting to disease when the value of $a \cdot ID$ is relatively large, and that sites should be considered to be not limiting when $a \cdot ID$ is relatively small (ie, $a \cdot ID \leq 0.01$). A more specific indicator might be the value of the expression, $e^{-a \cdot ID}$, evaluated at the maximum ID (ID_{max}) used in the fitting of an equation of the form of equation 4. It can be shown that:

$$e^{-a \cdot ID_{max}} = \frac{\text{slope of } y = N(1 - e^{-a \cdot ID}) \text{ at } ID_{max}}{\text{slope of } y = N(1 - e^{-a \cdot ID}) \text{ as ID} \rightarrow 0^{+}}$$

and

$$e^{-a \cdot ID_{max}} = \frac{N - y \text{ evaluated at } ID_{max}}{N}$$

Thus, the value of $e^{-a \cdot ID_{max}}$ indicates both the extent to which the slope of the regression line changes over the range of the data and how closely the regression line approaches the limiting value of N in the range of the data. A value of $e^{-a \cdot ID_{max}} \approx 1$ would indicate a situation in which sites are not limiting. A value of $e^{-a \cdot ID_{max}} \approx 0$ would indicate that sites are limiting. Baker et al (2–4) also recognize the importance of limiting sites in some situations. They do not propose any specific equation for the modeling of situations where sites are limiting. However, they do suggest that if a true rhizoplane effect exists, then the value of b in a regression equation of the form of equation 1 should be 2/3, even when low ranges of *ID* values are used for calculation; but that if a value of $b \approx 2/3$ has been calculated because limiting sites are present (ie, data points on the transitional or plateau sections of the curve have been included in the regression), then the value of b should be near 1 when data from only low *ID*s are used (3).

Another type of analysis of *ID-DI* data has been proposed by Gilligan (9) and modified by Ferriss (7). The method involves the calculation of the width of the rhizosphere from *ID-DI* data and physical parameters of the affected plant part. Although an exact rhizosphere width cannot be calculated, in most cases a minimum rhizosphere width can be calculated if the inoculum density of the pathogen has not been underestimated. Calculation of rhizosphere width allows a determination of whether a rhizosphere or rhizoplane effect is in operation that is independent of the value of b obtained by regression. Thus, it should be possible to determine the applicability of the hypothesis of Baker et al (2,4) to a particular host-pathogen system by comparison of b obtained by regression with calculated values of rhizosphere width. If $b \approx 2/3$ and a large rhizosphere width were to be calculated, it could be concluded that the value of b is not necessarily related to the existence of rhizosphere or rhizoplane effects as postulated by the hypothesis of Baker et al (2,4) and it would be important to determine experimentally what factors result in the types of *ID-DI* relationships identified as rhizosphere and rhizoplane effects by the hypothesis of Baker et al (2,4).

The objectives of the research reported here were to determine experimentally the relationships between various disease variables and the inoculum density of *Pythium ultimum* Trow in soil, and to evaluate the suitability of various mathematical models for the interpretation of those relationships.

MATERIALS AND METHODS

The *P. ultimum* isolate used in these experiments was isolated from a rotted soybean seed. To prepare inoculum, plugs from the margins of colonies on clarified V-8 agar (18) were incubated in autoclaved, deionized water for 7–14 days. Plugs and associated mats were washed with excess cold (5 C) autoclaved water, and suspended in cold water. The suspension was comminuted 1 min in a Waring Blendor, sonicated, and centrifuged to pellet the suspended spores and mycelium. The pellet was resuspended in cold autoclaved water, and a hemacytometer was used to determine spore density. Sporangia larger than 15 μm in diameter ("large sporangia"), sporangia smaller than 15 μm in diameter, and oospores were counted separately. Proportions of large sporangia, small sporangia, and oospores were from 60 to 88%, from 3 to 9%, and from 9 to 31% of the total spore population, respectively. To determine the population of viable propagules, samples of the inoculum suspension were plated on a *Pythium*-selective medium as described below. Plate counts corresponded closely to counts of large sporangia.

Maury silt loam soil was collected from a field in which soybeans had been grown the previous season. The soil was shredded, sifted through a 4-mm sieve, and pasteurized by treatment of 1-kg bags of soil in a microwave oven (Kenmore Model 99601, Sears Roebuck and Co., Chicago, IL 60684) at full power for 135 sec. Treated soil contained approximately 500 fungal propagules per gram, and 5×10^6 bacterial propagules per gram; and did not contain detectable populations of *Pythium* spp., *Fusarium* spp., or *Rhizoctonia* spp. Analysis of the soil by the University of Kentucky Soil Testing Laboratory indicated soil pH = 6.1, cation exchange capacity = 16.6 meq/100 g, organic matter = 2.9%, and nutrient contents of 22 μg P, 120 μg K, 1,640 μg Ca, 84 μg Mg, 3.3 μg Mn, and 1.2 μg Zn per gram of soil. Inoculum was added to soil which was at approximately −0.2 bars soil matric potential. Soil matric potential was determined by comparison of soil moisture content (determined gravimetrically) with a soil moisture characteristic curve for shredded, sifted Maury silt loam soil. Infested soil was mixed for 1 min in a tabletop food mixer (model N-50, Hobart Corp., Troy, OH 45374). Spore densities were adjusted before the addition of inoculum to soil, so that all *ID* levels received the same amount of water in which the inoculum was suspended. To determine if populations of *P. ultimum* increased in infested soil, samples of infested soil were plated on a *Pythium*-selective medium (as described below) 0, 2, or 6 days after soil infestation. Recovered populations were consistently less than 50% of populations of large sporangia calculated to have been added to the soil. The proportion of the calculated population recovered was not significantly different for different inoculum densities.

Soybean (*Glycine max* (L.) Merr. 'Williams') seeds from a medium quality seed lot (87% standard germination [1]) were used. Seeds with visible symptoms of disease or visibly cracked seed coats were discarded before use. In some experiments, seeds were killed by incubation at 13% moisture for 7 days at 55 C. Fifteen seeds were placed on the surface of 200 g of soil in a 16 × 2 × 5-cm plastic tray, and were covered with 200 g of soil. Trays were watered from above to approximately −0.02 bars soil matric potential with deionized water, covered with aluminum foil to reduce evaporation, and incubated at 15 C for 48 hr. After incubation trays were treated two ways. Some trays were uncovered and placed in a growth chamber at 25 C with 12 hr light and dark cycles, and 3,300 lx at the level of the plants. Trays were watered daily to approximately −0.02 bars soil matric potential. Number of established plants (emerged and with expanded true leaves) was recorded 14 days after planting. For

other trays, seeds were washed from the soil 2 days after planting and surface sterilized for 30 sec in 0.25% NaOCl. To determine infection, seed coats were separated from the seeds aseptically, and both were plated separately on a selective medium that contained 18 g of Difco cornmeal agar, 100 mg pentachloronitrobenzene, 10 mg rose bengal, 5 mg rifampacin, and 5 mg pimaricin per liter (19). Colonization of heat-killed seeds was determined 1 day after planting by a similar procedure. Seed coats were removed from heat-killed seeds before plating. Soil microbiological sampling tubes were prepared by loading 50 μl micropipettes (484 μm inside diameter) with selective medium. Sampling tubes were inserted approximately 15 mm into soil with its matric potential adjusted to approximately −0.02 bars. Colonization was determined by microscopic examination after 24 hr incubation in soil.

DI was calculated as $(C - E)/C$, in which C = the average number of established plants per tray in uninfested soil, and E = the number of established plants in a tray containing infested soil (14). Prior to regression analysis, data for each dependent variable were transformed by the multiple infection transformation, $-\ln(1 - Z)$ (10), in which Z = proportion of affected units. Regression analysis of the multiple infection transformation of each dependent variable against *ID* was performed by using the computer procedures GLM and NLIN of the Statistical Analysis System (13). Values of inoculum density used in the regression analysis were calculated from hemacytometer counts on the basis of large sporangia per gram of dry soil. Five models were fitted to each data set: a standard quadratic equation ($y = k_1 + k_2x + k_3x^2$), a quadratic equation tied to the origin ($y = k_1x + k_2x^2$), the limiting site equation ($y = N(1 - e^{-ax})$) as proposed by Vanderplank (26), the allometric equation ($y = kx^b$) fitted by linear regression of log y against log x (log-log method), and the allometric equation ($y = kx^b$) fitted by an iterative method. Each regression was performed using three iterations of weighted regression, with initial values of the parameters obtained from a nonweighted regression. For each *ID* in each model, the weight used was equal to the reciprocal of equation 2 or 3 evaluated at Y or Y'; with Y or Y' = the predicted value of the variable for that *ID* obtained by using the equation for the model that was fitted in the previous iteration.

Values for spermosphere width were calculated from the appropriate regression of the limiting site equation or the quadratic equation tied to the origin and an equation proposed by Ferriss (4). In all spermosphere-width calculations it was assumed that the effective *ID* in soil was equal to the *ID* calculated from hemacytometer counts of large sporangia. To obtain the average radius of an imbibed soybean seed, 100 seeds from the seed lot used in the *ID* experiments were imbibed for 24 hr, their volume was determined by water displacement, and their average radius calculated from their average volume. It was assumed that the shape of a soybean seed was a sphere.

All data presented, except that for sampling tube colonization, is from a minimum of two independent performances of each experiment. Data presented for sampling tube colonization is from a single experiment; however, similar types of relationships were obtained in two other tube-colonization experiments.

RESULTS

Plots of the dependent variables as functions of *ID* are presented in Fig. 1. Each data point represents the multiple infection transformation of the proportion of affected plants, seeds, or sampling tubes in a tray containing 15 plants, 15 seeds, or 25 sampling tubes. The regression line in each figure is that which corresponds to the allometric equation ($y = kx^b$) fitted by the iterative method. *DI* tended to reach a maximum value of approximately 1.5 (corresponding to 78% damping-off) at *ID*s of 10 sporangia per gram (spg) and above. *DI*s were not significantly different ($P = 0.05$) at *ID*s of from 10 to 600 spg. At lower *ID*s (0.5–10 spg), the data were fitted by an allometric equation with an exponent of 0.656 ± 0.146 (95% confidence interval). Unlike *DI*, seed infection increased with increasing *ID*, but did not reach a plateau. The regression of the seed infection data yielded a curve with an exponent of 0.563 ± 0.085. Seed coat infection, colonization of heat-killed (dead) seeds, and sampling tube colonization all increased approximately linearly with *ID* until 100% colonization was attained. Exponent values near 1.0 were obtained for each of these variables.

Values of the exponent of the allometric equation tended to be larger when determined by log-log regression, compared with when determined by the iterative regression (Table 1). However, both types of regressions identified the data sets for seed infection at 1–200 spg, 1–60 spg, and 1–20 spg and *DI* at 0.5–600 spg and 0.5–10 spg as having exponent values significantly less ($P = 0.05$) than 1.

These same data sets were identified as being nonlinear by a test of whether the coefficient of the squared term in the two-term quadratic equation differed significantly ($P = 0.05$) from zero; however, two of the data sets were not identified as being significantly nonlinear when the three-term quadratic equation was used. With the exception of the seed infection at 1–20 spg data set, these same data sets had the lowest values of $e^{-a \cdot ID_{max}}$ in the regressions of the limiting site equation. In general, as the range of *ID*s used in the regressions of *DI* and seed infection was decreased, the significance level for the coefficient of the squared term $\neq 0$ in the two-term quadratic equation increased, and the value of $e^{-a \cdot ID_{max}}$ increased. For all data sets except *DI* at 0.5–2 spg and 0.5–5 spg, the use of a weighted regression decreased the magnitude of the discrepancy between the values of b calculated by the log-log and iterative methods (Ferriss, *unpublished*).

Calculated values of spermosphere width for each variable were similar whether the calculation was based on the limiting site equation, or the two-term quadratic equation (Table 2). Calculated spermosphere width was near 0.6 mm for dead seed colonization, near 1.3 mm for seed coat infection, and over 80 μm for seed infection.

DISCUSSION

If the results presented herein were interpreted strictly on the basis of the hypothesis of Baker et al (2,4) (ie, based on the value of b in $y = kx^b$), then the following conclusions could be drawn: a "spermoplane effect" is in operation for disease incidence at 0.5–10 spg, and for seed infection at all *ID* ranges except 1–10 spg; a spermosphere effect is in operation for coat infection, dead seed colonization, and tube colonization; it cannot be determined from the data which effect is in operation for *DI* at 0.5–5 spg or 0.5–2 spg, and seed infection at 1–10 spg; and the plateau section of the curve is included in the data for *DI* at 0.5–600 spg. With two exceptions (*DI* at 0.5–2 spg and 0.5–5 spg), all of these conclusions would be drawn regardless of the method used to fit the equation (ie, log-log or iterative, weighted or unweighted).

The existence of both spermosphere and spermoplane effects within the same system presents a contradiction. One possible explanation would be that *P. ultimum* behaves differently in response to different substrates. Thus, it might be that a seed coat or dead seed can be colonized by propagules which are at some distance from it initially, while a viable seed can only become infected if a propagule actually makes contact. This explanation requires the assumption that infection of seeds and seed coats are independent processes. Such an assumption precludes the possibility that a propagule can germinate in response to seed coat exudate, grow toward and colonize the seed coat, and then penetrate the seed coat and attack its contents. Such penetration of the seed coat is supported by the observation that *P. ultimum* growth from plated seeds often emerged from areas of the cotyledons that had been covered by the seed coat before its excision. Similarly, Stanghellini and Hancock (24) observed that pinto bean seeds were infected by *P. ultimum* prior to the emergence of the radicle. Thus, this explanation is supported only by the calculated values of b. Another possible explanation for values of $b \approx 2/3$ and 1 within the same system would be that values of b, which are not significantly different from 2/3 have been calculated because sections of the plateau or "transitional" sections of the curve have been included in the calculations. Baker and Drury (3) recommend that this possibility be tested for by

examination of values of b for data sets resulting from progressively lower IDs. They suggest that if a true rhizoplane or spermoplane effect exists, then the value of b should be near 2/3 regardless of the section of the curve that is being examined. However, such a situation could also exist if the true behavior of the data was described by a multiparameter Poisson equation ($y = N_1(1-e^{-a}1^x)+N_2(1-e^{-a}2^x)+\ldots N_i(1-e^{-a}i^x)$) as proposed by Vanderplank (26). In any case, although values of b are close to 2/3 for low IDs of seed infection and DI, the confidence intervals for b include both 2/3 and 1 in most cases. Thus, a definite conclusion cannot be reached by this method. Another method of testing if the inclusion of the transitional section of the curve is responsible for values of $b \approx 2/3$ would be to examine calculated spermosphere width at low IDs (ie, as $ID \rightarrow 0^+$). Because pathogen propagules are considered to be points in the spermosphere width model, then if a true spermoplane exists, calculated spermosphere width should be equal to or less than propagule radius. If inclusion of the transitional section of the curve has resulted in a calculated value of

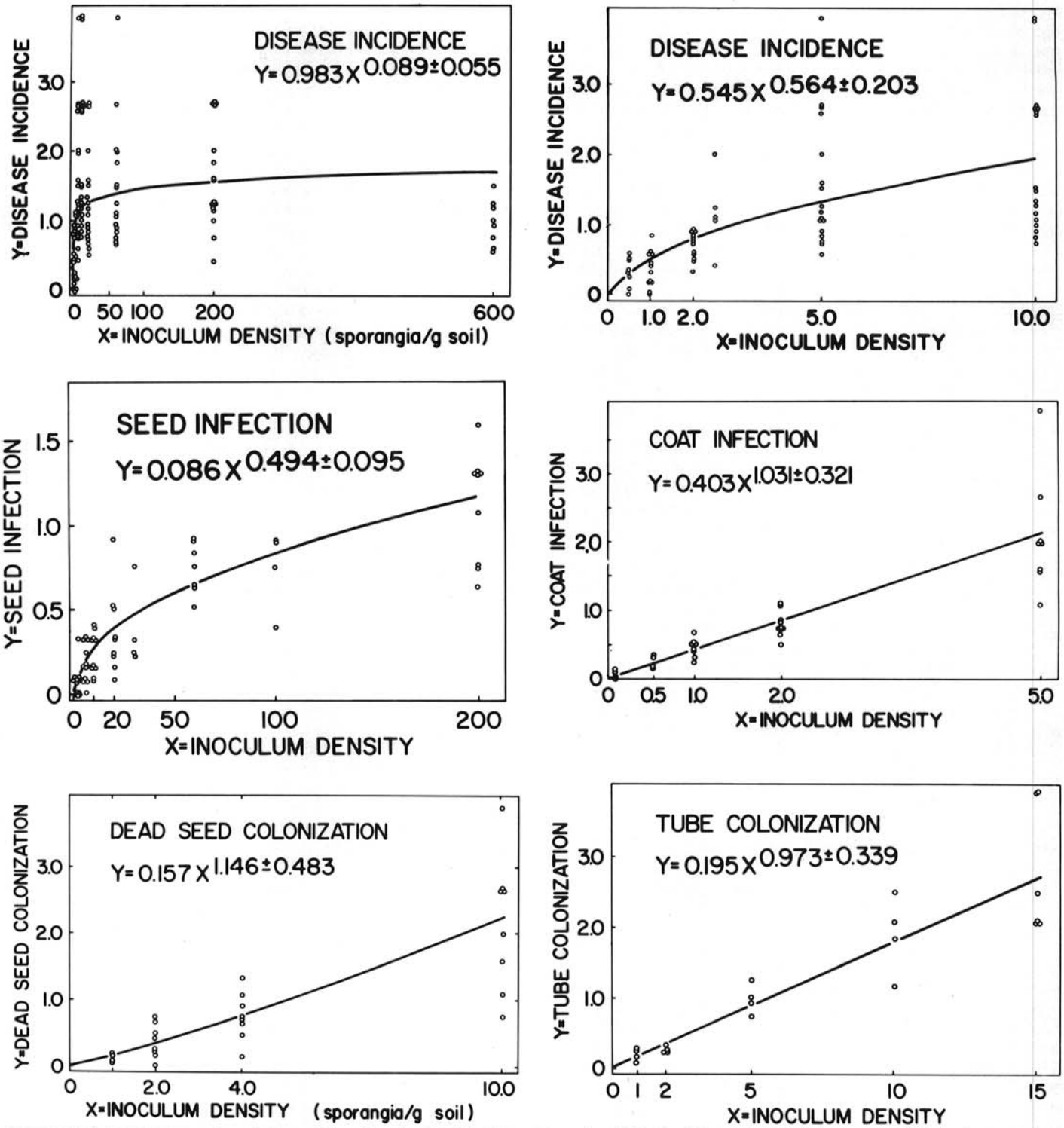

Fig. 1. Relationships between six variables and inoculum density of *Pythium ultimum* in soil. Each point represents data from a tray of 15 plants, except that for tube colonization each point represents data from 25 soil sampling tubes. Disease incidence was calculated as $(C-E)/C$, in which C = the average number of established plants per tray of uninfested soil, and E = number of established plants in a tray containing infested soil. Values on the vertical axis represent a transformation of raw data by the equation $Y=-\ln(1-Z)$, in which Z = proportion of affected units. Regression lines represent an equation of the form $y = kx^b$ regressed on transformed data by an iterative method. Values of $b \pm d$ delimit a 95% confidence interval for the value of b.

TABLE 1. Values of measures of linearity for three types of models for regressions of variables as functions of inoculum density

Variable[a]	b in $y = kx^b$ (value ± 95% confidence interval)		Significance level for coefficient of the squared *term* ≠ 0 in quadratic equations[d]		Value of e^{-ax} in $y = N(1-e^{-ax})$ for x = maximum *ID* used in the regression
	log-log[b]	Iterative[c]	Two-term	Three-term	
Disease incidence (0.5–600 spg)	0.175 ± 0.064	0.129 ± 0.058	<0.0001	0.0062	$<10^{-99}$
Disease incidence (0.5–10 spg)	0.705 ± 0.172	0.656 ± 0.146	<0.0001	0.0116	0.0970
Disease incidence (0.5–5 spg)	0.872 ± 0.251	0.774 ± 0.205	0.0630	0.8561	0.3808
Disease incidence (0.5–2 spg)	0.926 ± 0.553	0.618 ± 0.356	0.1169	0.2634	0.4771
Seed infection (1–200 spg)	0.601 ± 0.089	0.563 ± 0.085	<0.0001	<0.0001	0.0055
Seed infection (1–60 spg)	0.748 ± 0.145	0.748 ± 0.179	0.0036	0.3645	0.1705
Seed infection (1–20 spg)	0.647 ± 0.257	0.664 ± 0.246	0.0135	0.1650	0.5599
Seed infection (1–10 spg)	0.775 ± 0.356	0.755 ± 0.348	0.1618	0.4718	0.7480
Coat infection (0.1–5 spg)	0.995 ± 0.146	0.999 ± 0.150	0.8904	0.7503	0.9512
Dead seed colonization (1–10 spg)	1.249 ± 0.371	1.174 ± 0.285	0.2690	0.9934	0.9970
Tube colonization (1–15 spg)	1.090 ± 0.172	1.085 ± 0.167	0.4613	0.9106	0.9993

[a] In all regressions data were transformed by the multiple-infection transformation before analysis. All regressions were weighted. spg = Sporangia per gram of soil.
[b] Value = slope of a linear regression of ln(variable) on ln(*ID*).
[c] Value determined by direct fitting of the equation by an iterative method.
[d] Probability of a larger value of t in a t-test of the null hypothesis that coefficient of the squared term = 0 for two-term ($y = k_1x + k_2x^2$) and three-term ($y = k_i + k_2x + k_3x^2$) quadratic equations.

$b \approx 2/3$, then calculated spermosphere width should be significantly greater than propagule radius. For the *P. ultimum*-soybean data mean calculated spermosphere widths were at least seven times the approximate mean radius of a sporangium of *P. ultimum* (10 μm) for seed infection (ie, 7.0 = 70 μm ÷ 10 μm), and were at least 34 times the mean radius for the other variables (Table 2). These results were obtained whether the Poisson or two-term quadratic regressions were used in the calculations, and whether or not the regressions used had been fitted to data sets which included data from small or large ranges of *ID*. If these calculated spermosphere widths are assumed to be accurate, then their relatively large size would indicate that a spermosphere effect exists for all the variables, and values of $b \approx 2/3$ have been calculated because transitional sections of the curves have been included in the data. However, the accuracy of the spermosphere width calculation is dependent on the accuracy with which the parameters used in its calculation have been estimated. The two primary factors which influence the accuracy of the spermosphere-width calculation are the fitted regression equation and the seed size. Assuming that the regression equation used fits the recorded data, an overestimation of spermosphere width would result if either *ID* had been underestimated or infection had been overestimated. For spermosphere width to be overestimated by the factor by which the minimum calculated spermosphere width for seed infection exceeds the radius of a *P. ultimum* sporangium, such overestimation or underestimation would have to be by a factor of 8.5. For an error in the determination of seed size to result in a similar overestimation of spermosphere width, the actual seed radius would have to be 2.93 times that used in the calculation. Errors of that magnitude and direction in the determination of seed infection, *ID*, and seed size are unlikely. In particular, it should be noted that because the population assays indicated that the population of viable propagules declined after the propagules were added to the soil, the values for *ID* which were used for fitting of the regression equations probably overestimated the actual *ID*. Such an overestimation of *ID* would result in an underestimation of spermosphere width. Thus, the minimum calculated spermosphere widths seem to be valid, assuming that the spermosphere width equation itself is valid. Additionally, the calculated spermosphere width for coat infection (~1.3 mm) is well within the range of distances from large legume seeds at which *P. ultimum* propagules have been reported to germinate. Stanghellini and Hancock (24) observed germination of *P. ultimum* sporangia at distances of up to 12 mm from germinating bean seeds. Shlub and Schmitthenner (22) observed approximately 15% germination of *P. ultimum* sporangia at 2.5–5 mm from soybean seeds at 5 hr after planting. Singh (23) reported that populations of *P. ultimum* in naturally infested soil had doubled at 10–20 mm from pea seeds 48 hr after planting. Although the proportion of spores which germinate at these maximum distances that actually produce seed infections is not

TABLE 2. Calculated minimum spermosphere widths for seed infection, coat infection, and dead seed colonization

Variable[b]	Calculated minimum spermosphere width (mm)[a]		
	From $y = N(1-e^{-a(ID)})$	From $y = k_1(ID) + k_2(ID)^2$	
		Mean	95% Confidence interval[c]
Seed infection (1–200 spg)	0.114	0.084	(0.070–0.099)
Seed infection (1–10 spg)	0.134	0.164	(0.085–0.232)
Coat infection	1.365	1.342	(1.089–1.574)
Dead seed colonization	0.732	0.605	(0.354–0.834)

[a] Minimum spermosphere width was calculated for $ID \rightarrow 0^+$ using fitted regression equations of the forms $y = N(1-e^{-a(ID)})$ or $y = k_1(ID) + k_2(ID)^2$, and the equation $W = ((3\ L/4\pi\ BC) + r^3)^{1/3} - r$ in which W = minimum spermosphere width, B = soil bulk density (9.009×10^{-4} g/mm^3), C = propagule competence (assumed to be 1), r = average radius of an imbibed soybean seed (4.519 mm), and L = the limit of the regression equation *divided by ID* as $ID \rightarrow 0^+$ ($L = Na$ for $y = N(1-e^{-a(ID)})$, and $L = k_1$, for $y = k_1(ID) + k_2(ID)^2$, in which y = value of variable after multiple-infection transformation, ID = inoculum density (sporangia per gram) and N, a, k_1, and k_2 = constants) (7).
[b] spg = Sporangia per gram of soil.
[c] 95% Confidence interval for mean spermosphere width based on the 95% confidence interval for the value of k_1 in the regression equation of the form $y = k_1(ID) + k_2(ID)^2$.

known, the reports of their germination provide strong supportive evidence that *P. ultimum* infection of seeds is a spermosphere, rather than a spermoplane, phenomenon.

The existence of this wide spermosphere can be taken to indicate that the calculated values of $b \approx 0.67$ are due to some factor other than the existence of a spermoplane effect. If the data are interpreted on the basis of the hypothesis proposed by Vanderplank (26), then the existence of limiting sites is indicated for seed infection and *DI*, while no limiting sites are indicated for coat infection and dead seed colonization. This interpretation allows a simple classification of the results. However, this classification can be considered, at most, to be a preliminary interpretation of the results. The physical nature of infection sites is not defined by Vanderplank (26); consequently, in order to establish that sites are indeed limiting, the actual nature of the sites must be established. One possible class of physical sites could be cracks in the soybean seed coat, which allow *P. ultimum* to breach the seed coat and colonize the seed. However, Schlub and Schmitthenner (22) found that the existence of visible seed coat cracks on soybean seeds had no effect on susceptibility to *P. ultimum*. It is possible that some other classes of specific infection sites are present on soybean seeds; however, the description of such sites must await further investigation. It is also possible that the "site" indicated by Vanderplank's equation is not an actual physical site, but rather an *ID*-dependent factor which influences the efficiency of inoculum. This factor could be one of those suggested by Leonard (15); ie, nonrandom distribution of inoculum and unequal susceptibility of hosts. For the *P. ultimum*-soybean data it is unlikely that nonrandom distribution of inoculum could have resulted in the "limiting site" relationships observed for seed infection and *DI*. If such were the case, "limiting site" relationships would also be expected to have been observed for coat infection, dead seed colonization, and sampling tube colonization; however, sites were not indicated to be limiting for these variables. Unequal susceptibility of hosts seems to be the most likely explanation for the observations. "Limiting site" relationships were observed for those variables associated with a living seed (seed infection and *DI*), while killing of seeds by heat treatment resulted in a "no limiting site" relationship. Soybean seed lots vary in their susceptibility to damping-off; and the "cold test," in which seeds are incubated in *Pythium*-infested soil, is a common method of evaluation of this variation (17). It is likely that seeds within a single seed lot vary in susceptibility to *P. ultimum* just as seeds in different seed lots vary in susceptibility. This variation within seed lots could be due to such factors as seed maturity at harvest, mechanical damage, or infection by other fungi. Whatever the cause of the variation, the effect would be the same: some seeds would be infected easily by even low populations of *P. ultimum,* while other seeds would be relatively resistant to infection. A case in which a certain portion of a seed lot could not be damped-off no matter what the *ID* of the pathogen would result in a relationship such as was observed for disease incidence in these experiments (Fig. 1A). Similarly, Schlub and Schmitthenner (22) reported that soybean seedling disease was not significantly different for nonscarified seeds planted in soil containing 15 or 130 *P. ultimum* propagules per gram of soil; and Flentje and Saksena (8) reported that damping-off of pea was not significantly different in soil amended with 1× or 3× rates of *P. ultimum* inoculum when the soil was at relatively high moisture contents, but was significantly higher at the higher *ID* in dryer soil. Thus, the research reported herein supports the hypothesis that, above a certain minimum level, *ID* of *P. ultimum* may not be as important in determining the severity of soybean seedling disease as are other factors such as soil moisture, soil temperature, and seed quality.

The statistical analysis used in this paper was somewhat more extensive than that used in previous reports of *ID-DI* relationships for soilborne plant pathogens. However, a comparison of the results obtained using the different types of analysis indicates that this extensive analysis was probably not necessary. The existence of two distinct types of *ID*-variable relationships was discerned whether the data were analyzed on the basis of the hypothesis of Baker et al (2,4), the hypothesis of Vanderplank (26), or a test of linearity using quadratic equations. In one type of relationship, an increase in *ID* resulted in a proportional increase in the variable. This type corresponded to the spermosphere effect of Baker et al (2,4), the no limiting sites situation of Vanderplank, and a linear relationship as indicated by examination of a regression of a quadratic equation tied to the origin. In the second type of relationship, an increase in *ID* resulted in a less than proportional increase in the variable. This type corresponded to the spermoplane effect of Baker et al (2,4), the limiting site situation of Vanderplank (26), and a nonlinear relationship as indicated by the quadratic regression. A third type of relationship, in which an increase in *ID* above a certain level did not result in a significant increase in the variable (ie, a plateau), was not indicated by examination of parameter values for any of the types of regressions, but could be discerned by examination of a graph of the data and could be confirmed by analysis of variance. Significantly, the first and second types of relationships could not be discriminated unless the multiple-infection transformation was used (*unpublished*). For most data sets, use of weighted versus unweighted regression did not alter the implications of the regressions (*unpublished*). The close correspondence between the results obtained using the different types of regressions indicates that regression analysis alone is probably not sufficient to determine which hypothesis (ie, that of Baker et al [2,4] or Vanderplank [26]) best describes the relationship of a particular disease to *ID*. However, the correspondence also indicates that it may be relatively unimportant which model is fitted to a data set as long as a good fit is obtained, and it is recognized that any conclusions drawn from the regression are tentative (ie, whether a rhizosphere effect exists, limiting sites are present, etc.). Although the fitting of a regression equation to an *ID-DI* data set is an important step in the development of an understanding of an *ID-DI* relationship, for most systems determination of the mechanism responsible for a particular relationship will require further investigation.

LITERATURE CITED

1. Association of Official Seed Analysts. 1978. Rules for testing seeds. J. Seed Technol. 3:1-126.
2. Baker, R. 1971. Analyses involving inoculum density of soilborne plant pathogens in epidemiology. Phytopathology 61:1280-1292.
3. Baker, R., and Drury, R. 1981. Inoculum potential and soilborne pathogens: The essence of every model is within the frame. Phytopathology 71:363-372.
4. Baker, R., Maurer, C. L., and Maurer, R. A. 1967. Ecology of plant pathogens in soil. VIII. Mathemetical models and inoculum density. Phytopathology 57:662-666.
5. Benson, D. M., and Baker, R. 1974. Epidemiology of *Rhizoctonia solani* pre-emergence damping-off of radish: Influence of pentachloronitrobenzene. Phytopathology 64:38-40.
6. Deming, W. E. 1943. Statistical adjustment of data. John Wiley, New York.
7. Ferriss, R. S. 1981. Calculating rhizosphere size. Phytopathology 71:1229-1231.
8. Flentje, N. T., and Saskena, H. K. 1964. Pre-emergence rotting of peas in south Australia III. Host-pathogen interaction. Aust. J. Biol. Sci. 17:665-675.
9. Gilligan, C. A. 1979. Modeling rhizosphere infection. Phytopathology 69:782-784.
10. Gregory, P. H. 1948. The multiple-infection transformation. Ann. Appl. Biol. 35:412-417.
11. Grogan, R. G., Sall, M. A., and Punja, Z. K. 1980. Concepts for modeling root infection by soilborne fungi. Phytopathology 70:361-363.
12. Guy, S. O., and Baker, R. 1979. Inoculum potential in relation to biological control of Fusarium wilt of peas. Phytopathology 67:72-78.
13. Helwig, J. T., and Council, K. A. (eds.). 1979. SAS Users Guide. Statistical Analysis System Institute Inc., Raleigh, NC. 495 pp.
14. Henis, Y., Ghaffar, A., and Baker, R. 1979. Factors affecting suppressiveness to *Rhizoctonia solani* in soil. Phytopathology 69:1164-1169.
15. Leonard, K. J. 1980. A reinterpretation of the mathematical analysis of rhizoplane and rhizosphere effects. Phytopathology 70:695-696.
16. Louvet, J., Rouxel, F., and Alabouvette, C. 1976. Studies on disease suppressiveness of soils. I. Proof of the microbiological nature of the suppressiveness of a soil to Fusarium wilt of muskmelon. Ann. Phytopathol. 8:424-436.

17. McDonald, M. B., Jr. 1976. Standardization of germination and vigor tests in soybeans. Proc. 6th Soybean Seed Res. Conf. 14-22.
18. Miller, P. M. 1955. V-8 juice agar as a general purpose medium for fungi and bacteria. Phytopathology 45:461-462.
19. Mircetich, S. M., and Kraft, J. M. 1973. Efficiency of various selective media in determining *Pythium* populations in soil. Mycopathol. Mycol. Appl. 50:151-161.
20. Mitchell, D. J. 1978. Relationships of inoculum levels of several soilborne species of *Pythium* and *Phytophthora* to infection of several hosts. Phytopathology 68:1754-1759.
21. Rouse, D. I., and Baker, R. 1978. Modeling and quantitative analysis of biological control mechanisms. Phytopathology 68:1297-1302.
22. Schlub, R. L., and Schmitthenner, A. F. 1978. Effects of soybean seedcoat cracks on seed exudation and seedling quality in soil infested with *Pythium ultimum*. Phytopathology 68:1186-1191.
23. Singh, R. S. 1965. Development of *Pythium ultimum* in soil in relation to presence and germination of seeds of different crops. Mycopathol. Mycol. Appl. 27:155-160.
24. Stanghellini, M. E., and Hancock, J. G. 1971. Radial extent of the bean spermosphere and its relation to the behavior of *Pythium ultimum*. Phytopathology 61:165-168.
25. Stasz, T. E., and Harman, G. E. 1980. Interactions of *Pythium ultimum* with germinating resistant or susceptible pea seeds. Phytopathology 70:27-31.
26. Vanderplank, J. E. 1975. Principles of Plant Infection. Academic Press, New York. 216 pp.
27. Warren, L. 1975. Effect of inoculum concentration on resistance of lima bean to *Rhizoctonia solani*. Phytopathology 65:341-345.
28. Zar, J. H. 1968. Calculation and miscalculation of the allometric equation as a model in biological data. Bioscience 18:1118-1120.