Dose-Response Models in Biological Control of Plant Pathogens: An Empirical Verification

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ABSTRACT

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Two models, based on the hyperbolic saturation relationship and probit function, that relate the densities of the biocontrol agent and pathogen with disease response were developed. The hyperbolic saturation (HS) and probit (PB) models and a modification of the negative exponential (MNE) model recently proposed by Johnson were evaluated. In the NE model, parameters c and k supply information on the efficiency of the pathogen and biocontrol agent, respectively, and provide endpoint values such as the maximum proportion of pathogen inoculum inactivated by the biocontrol agent (B). The HS model gives information on the 50% effective dose (ED_{50}) for both the pathogen (K_x) and the biocontrol agent (K_z), asymptotic disease levels without biological control (Y_{max}), and the maximum proportion of pathogen the biocontrol agent can inactivate (I_{max}). The PB model provides information on the relative virulence of the pathogen (τ) and relative efficiency of the biocontrol agent (σ) and on the ED_{50} for the pathogen (λ) and biocontrol agent (μ). Two pathosystems (an aerial and a root disease) and two types of biocontrol agent (antagonistic bacteria and nonpathogenic isolates of the pathogen) were compared. The data from Mandeel and Baker on biological control of *Fusarium oxysporum* f. sp. *cucumerinum* on cucumber with nonpathogenic isolates as well as our data on biological control of *Stemphylium vesicarium* on pear with selected isolates of *Pseudomonas* and *Erwinia* spp. were used. Data sets of the effect of several densities of the biocontrol agent and pathogen on disease levels were fitted to the models by nonlinear regression. Estimated parameters permitted quantitative comparisons among biocontrol-pathogen-host systems. The most valuable parameters obtained from the data sets that fitted adequately to the models were the proportion of pathogen potentially inactivated by the biocontrol agent, the relative efficiency and ED_{50} of the biocontrol agent, and the ED_{50} biocontrol/pathogen ratio. The values of B ranged from 0.79 to 0.98, and the values of I_{max} ranged from 0.96 to 1.04, indicating that a high proportion of the pathogen inoculum was susceptible to inactivation by the biocontrol agents. The values of σ ranged from 0.7 to 1.4 and were consistent with the independent action of the biocontrol agents on the pathogen. The mean ED_{50} for the biocontrol agent (HS and PB models) for the fungus-fungus pathogen-biocontrol system was 2×10^3 CFU/g of soil, and for the bacteria-fungus pathogen-biocontrol system, it was 6×10^6 CFU/ml. The ED_{50} biocontrol/pathogen ratio ranged from 1 to 10 for the fungus-fungus biocontrol-pathogen system and from 77 to 435 for the antagonistic bacteria-fungus system, indicating the existence of a lower number of targets for fungus-fungus competition for common sites than with bacteria-fungus antagonism.

Biological control of plant pathogens has been the focus of many studies in plant protection that search for alternative or complementary methods to the use of antimicrobial chemicals (1, 2). Biological control of soilborne and aerial plant and postharvest diseases depends greatly on pathogen and biocontrol agent densities (1,2,3,4,5,11,12,13,15,20). Consequently, during screening procedures, the most efficient isolates of biocontrol candidates are retained from controlled environment or greenhouse trials based on infectivity titration experiments with the pathogen at several densities of the biocontrol agent. An example of this kind of work is given by Mandeel and Baker (13), who studied the effect of the density of nonpathogenic *Fusarium oxysporum* Schlechtend.:Fr. strains C5 and C14 on the biocontrol of *F. oxysporum* f. sp. *cucumerinum* J.H. Owen on cucumber.

There is a need for objective means to fit data on infectivity titration of pathogens and biocontrol agents to suitable models that may provide dose-response surfaces and parameters describing the virulence of the pathogen and the efficiency of the biocontrol agent. In addition, the model parameters may give information on the dose range of the biocontrol agent needed to provide reliable, economical biological control.

Johnson (10) proposed a model to explain dose-response relationships in inundative biological control based on the negative exponential equation that relates disease to the density of a pathogen (13,19). Johnson (10) used data interpolated from regression lines taken from Mandeel and Baker (13) and concluded that the model described the effect on disease levels of different densities of the biocontrol agents at a constant pathogen dose. However, Johnson (10) assumed equal values for the efficiency of biocontrol agents *F. oxysporum* C5 and C14 and iteratively estimated the maximum proportion of pathogen rendered ineffective by the biocontrol agent. Unfortunately, information on goodness-of-fit for the model was not provided.

Models based on hyperbolic saturation of the host by the pathogen (9,18) and on the lognormal probability distribution (7,16) were used to relate the dose of several fungal and bacterial pathogens to disease levels. These models could be adapted to biocontrol studies and are interesting because they provide valuable parameters, such as the 50% effective dose (ED_{50}) and the relative efficiency, that are useful in comparing dose-response relationships.

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This paper evaluates Johnson's (10) model and proposes and evaluates two models, based on hyperbolic saturation and probit function, that relate the dose of biocontrol agent and pathogen to disease. The raw data values from Mandeel and Baker (13) as well as data obtained by us on the biological control of *Stemphylium vesicarium* (Wallr.) E. Simmons on pear (*Pyrus communis* L.) by selected isolates of *Pseudomonas* and *Erwinia* spp. were used.

MODELS

Negative exponential model. The most common type of pathogen dose-disease relationship is based on the negative exponential function (10,13,19). The model is based on the assumption that the efficiency of inoculum is maximum at very low pathogen densities and that multiple infections occur at high densities. Due to multiple infections, the amount of disease produced per unit of inoculum decreases linearly as disease proportion increases. The following differential equation summarizes this effect:

$$dy/dx = k(A - y) \tag{1}$$

where y is the proportion of disease, x is the inoculum density of the pathogen, k is a constant of efficiency of the inoculum to produce infections, and A is the proportion of plants or host tissue susceptible to infection. Integration of equation 1, if no disease exists at 0 inoculum dose and not all plants or plant tissue are susceptible to the disease, gives the following equation:

$$y = A(1 - e^{-kx}) \tag{2}$$

A model for biological control of plant pathogens based on the negative exponential function proposed by Johnson (10) assumes that the efficiency of the biocontrol agent is maximum at low densities and that multiple inactivations of the pathogen occur at high densities. Consequently, the amount of inactivated pathogen per unit of biocontrol agent decreases at high densities of the biocontrol agent. The model has the following form:

$$y = 1 - e^{-kx[(1-B)+Be^{-cz}]} \tag{3}$$

where B represents the maximum proportion of the pathogen population that can be rendered ineffective by the biocontrol agent, z is the density of the biocontrol agent, and c is a constant for the efficiency of the biocontrol agent in rendering the pathogen ineffective. Because under certain conditions not all plants or plant tissues are susceptible to the disease, an asymptote (A) can be included to give the general model:

$$y = A\left\{1 - e^{-kx[(1-B)+Be^{-cz}]}\right\} \tag{4}$$

Hyperbolic saturation model. Another model of pathogen-dose disease relationship is based on the fact that saturation of host targets for infection occurs at high inoculum densities. The approach has been used by Schneider (18) and Fukui et al. (9) and follows a hyperbolic saturation relationship commonly used in Michaelis-Menten enzyme kinetics and Monod substrate-uptake kinetics by microorganisms. The equation is as follows:

$$y = Y_{max}\, x/(x + K_x) \tag{5}$$

where Y_{max} is the maximum disease proportion the pathogen can produce and K_x is a half-saturation constant corresponding to the pathogen dose producing half the maximum disease proportion. A biocontrol model can be derived based on the assumption that the biocontrol agent or its released antagonistic compounds bind the pathogen cell (the substrate) after a hyperbolic saturation relationship. The equation that gives the proportion of inactivated pathogen (x_i/x) is

$$x_i/x = I_{max}\, z/(z + K_z) \tag{6}$$

where z is the biocontrol agent density, I_{max} is the maximum proportion of pathogen the biocontrol agent can inactivate, and K_z is the dose of biocontrol agent that produces an inactivation of $I_{max}/2$. Therefore, $1 - (x_i/x)$ is the proportion of active pathogen remaining in the presence of a given biocontrol agent density. Transformation of equation 6 to indicate the proportion of active pathogen remaining and substitution in equation 5 gives

$$y = Y_{max}\frac{x\left(1 - \dfrac{I_{max}\, z}{z + K_z}\right)}{x\left(1 - \dfrac{I_{max}\, z}{z + K_z}\right) + K_x} \tag{7}$$

Probit model. A third pathogen dose-response relationship is based on the lognormal probability distribution and assumes that the efficiency of the pathogen is low at low densities, maximum at intermediate densities, and decreases at high concentrations. The equation describing this effect is:

$$dy/dw = \left[1/\sqrt{2\pi}\right]\left[e^{-(1/2)w^2}\right] \tag{8}$$

where $w = (u - \lambda)/\tau$, $u = \log_{10} x$, λ, and τ are the mean and standard deviation of the probit (normal) distribution. Integration of equation 8 gives the cumulative probability distribution or probit function (8) that relates the probability of disease to the inoculum density of the pathogen:

$$P_D(x) = \phi[(u - \lambda)/\tau] = \int_{-\infty}^{(u-\lambda)/\tau}\left[1/\sqrt{2\pi}\right]\left[e^{-(1/2)w^2}\right]dw \tag{9}$$

where ϕ denotes the cumulative distribution function for the standard normal. The parameter λ is equivalent to ED_{50}, and τ is a measure of the efficiency of the inoculum. The function has been used by Ercolani (7) and Rouse et al. (16) to relate the dose of plant pathogenic bacteria with disease levels in plants.

A probabilistic model of biological control can be derived based on the probit model and on the assumption that the pathogen population is inactivated in relation to the density of the biocontrol agent. The model assumes that the efficiency of the biocontrol agent in inactivating the pathogen is low at low densities, maximum at intermediate densities, and decreases at high densities of the biocontrol agent. The equation describing the proportion of inactivated pathogen in the presence of a given biocontrol agent density is:

$$P_I(z) = \phi[(t - \mu)/\sigma] = \int_{-\infty}^{(t-\mu)/\sigma}\left[1/\sqrt{2\pi}\right]\left[e^{-(1/2)v^2}\right]dv \tag{10}$$

where $v = (t - \mu)/\sigma$, t is the $\log_{10}$ of the density of the biocontrol agent, and μ and σ are the mean (ED_{50}) and standard deviation (efficiency of inactivation) of the biocontrol dose-pathogen inactivation curve, respectively. The probability that the pathogen remains active is $1 - P_I(z)$. Therefore, the probability of disease depends on the probability that the host is infected by the amount of active pathogen and the probability that the pathogen is active in the presence of the biocontrol agent. Transformation of equation 10 to indicate the proportion of active pathogen remaining and substitution in equation 9 results in:

$$y = P_D(x, z{:}\lambda, \tau, \mu, \sigma) = \phi\left[\left(u + \log_{10}\{1 - \phi[(t - \mu)/\sigma]\} - \lambda\right)/\tau\right] \tag{11}$$

which relates the probability of disease to the pathogen and biocontrol agent densities.

MATERIALS AND METHODS

Plant material and preparation of inoculum of *S. vesicarium*. Two- to three-year-old self-rooted pear plants (cultivar Conference) were obtained by micropropagation, cultivated in plastic containers, and used for the leaf-infection experiments. A highly

virulent isolate of *S. vesicarium*, EPS26, was used for inoculations (14). The fungus was grown in petri dishes on V8 agar at 20°C under a photo period of 12 h with fluorescent light at 150 $\mu E\ m^{-2}\ s^{-1}$. Conidial suspensions were obtained from 8- to 10-day-old cultures by washing the agar surface with a diluted solution of Tween 20 in distilled water (1 drop of Tween 20 per liter). The conidial suspension was filtered through two layers of cheesecloth to separate conidia from mycelial fragments. Conidial concentration was assessed with a hemacytometer and adjusted by dilution to 1×10^6 conidia per ml. Conidial germination was assessed after 2 h of incubation at 20°C in the hemacytometer chamber, and suspensions with less than 90% conidia germination were discarded.

Inhibition of infection of detached pear leaves. A detached pear leaf assay was used for determining inhibition of infection of conidia of *S. vesicarium* by antagonistic bacterial isolates. Young pear leaves were collected from the shoot tips of plants. The leaves were surface-disinfested by immersion for 5 min in a diluted solution of NaOCl (0.1% Cl), washed two times with sterile distilled water, and excess water was removed under an air stream. The leaves were pretreated by immersion in bacterial suspension for 10 min. Bacterial suspensions were prepared by resuspending cultures grown on Luria-Bertani agar at 22°C for 48 h in sterile distilled water. Inoculated leaves were left under an air stream to remove excess water, placed in transparent plastic boxes filled with moistened paper towels, and incubated overnight in the dark at 22°C. The conidial suspension (6 10-µl drops) was deposited on the abaxial surface of each leaf, and the leaves were incubated at 22°C under a 12-h photoperiod (150 $\mu E\ m^{-2}\ s^{-1}$) for 3 days in a controlled environment chamber (model PGR-15, Conviron Winnipeg, Manitoba, Canada). The range of biocontrol agent concentrations studied was from 1×10^4 to 10^9 CFU/ml. The range of pathogen concentrations used was from 1×10^3 to 10^6 conidia per ml (see Figs. 6 and 7 below). The experimental design was completely randomized for each combination of pathogen-biocontrol agent dose and consisted of three replicates of three leaves per replicate. For each bacterial isolate, the experiment was repeated at least once. Disease levels for each inoculated leaf area were assessed according to the following scale: 0, no infection; 1, pin-point lesions; 2, pin-point infections that coalesce; 3, necrosis affecting more than three-quarters of the drop area. Disease severity for each leaf was calculated as the sum of the rating for the six inoculated drops and was expressed as a percentage of the maximum attainable severity (18 units).

Data analysis. Regression and parameter estimation was performed by a nonlinear-least-squares procedure based on the Newton-Raphson method (6,17) using the NLIN procedure of the Statistical Analysis System (17). Initial estimates of the model parameters were obtained by means of a grid of values (factorial design) and evaluation of the sum of squares. The grid point at which the smallest sum of squares was found was used as the starting point for the iterative parameter estimation procedure. Because the models evaluated were nonlinear in their parameters, the usual *F* tests for linear regression and lack of fit were not adequate. Therefore, model performance was determined by comparison of the mean square error (MSE) and the asymptotic standard error (ASE) for the estimated parameters and by visual inspection of model predictions compared to observed values (plots of the residuals against predicted or observed values and against pathogen or biocontrol agent densities) (6).

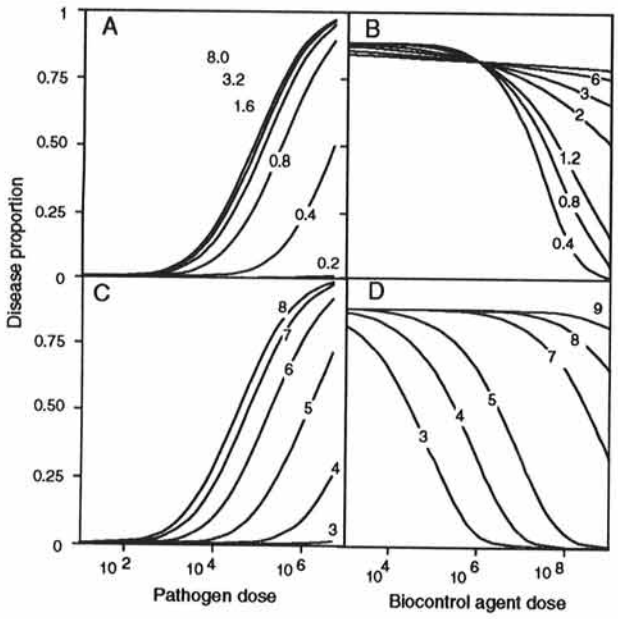

Fig. 1. Effect of **A and B,** σ and **C and D,** µ probit model parameters from equation 11 on disease pathogen-dose curves at a fixed biocontrol agent dose of 1×10^8 CFU/ml (**A and C**) and on disease biocontrol agent-dose curves at a fixed pathogen dose of 5×10^5 conidia per ml (**B and D**). Fixed values of λ and τ for the pathogen of 4.5 and 1, respectively, were used for calculations.

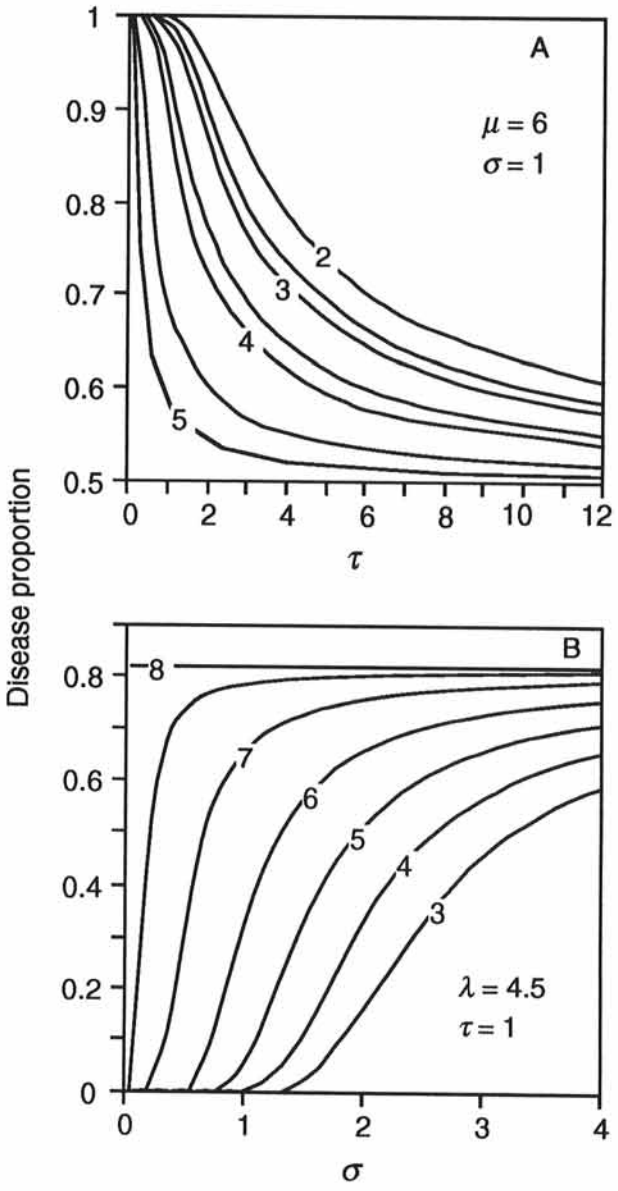

Fig. 2. Dependence of disease levels on **A,** τ at different λ for the pathogen and **B,** σ at different µ for the biocontrol agent as predicted by the probit model. The pathogen and biocontrol agent doses were 5×10^5 conidia per ml and 1×10^8 CFU/ml, respectively.

RESULTS

Effect of fixing model parameters on disease-dose relationships. The effect on disease of fixing parameters at various values for the probit model from equation 11 is shown in Figures 1 and 2. At constant μ, decreases in σ increased the apparent ED_{50} of the pathogen (Fig. 1A), increased the slope of the disease biocontrol agent-dose curves (Fig. 1B), and consequently increased biocontrol levels. Also, decreases in μ at constant σ increased the apparent ED_{50} for the pathogen in the disease pathogen-dose curves (Fig. 1C). For this model, increases of dose or decreases of μ for the biocontrol agent decreased the amount of effective pathogen and increased the apparent ED_{50} (λ_{ap}) of the pathogen. This conclusion can be obtained by rearranging equation 11 to give $y = \phi[(u - \lambda_{ap})/\tau]$, where $\lambda_{ap} = 1 - \log_{10}\{1 - \phi[(t - \mu)/\sigma]\}$. The effect on disease levels of changes in model parameters τ and λ for the pathogen and of σ and μ for the biocontrol agent are shown in Figure 2. For low values of τ, disease levels were strongly influenced by the λ of the pathogen (Fig. 2A). Similarly, at low values of σ, disease control was strongly influenced by the μ of the biocontrol agent (Fig. 2B).

The analysis of the HS model from equation 7 is shown in Figures 3 and 4. At constant K_z, increases in I_{max} increased the apparent ED_{50} of the pathogen (Fig. 3A), increased the slope of the disease biocontrol agent-dose curves (Fig. 3B), and, consequently, increased biocontrol levels. Also, at constant I_{max}, decreases in K_z increased the apparent ED_{50} for the pathogen in the disease pathogen curves (Fig. 3C). With this model, increases of the dose or decreases of the K_z of the biocontrol agent decreased the effective amount of pathogen (x_{ef}), which is reflected in the graphs as a change in the apparent ED_{50} for the pathogen. This conclusion can be obtained by rearranging equation 7 to give $y = Y_{max}\, x_{ef}/(x_{ef} + K_x)$, where $x_{ef} = x - x\, z\, I_{max}/(z + K_z)$. The effect on disease levels of changes in model parameters Y_{max} and K_x for the pathogen and of K_z and I_{max} for the biocontrol agent are shown in Figure 4. For high values of Y_{max}, disease levels were strongly influenced by the K_x of the pathogen (Fig. 4A). Similarly, at high values of I_{max}, disease biocontrol was strongly influenced by the K_z of the biocontrol agent (Fig. 4B).

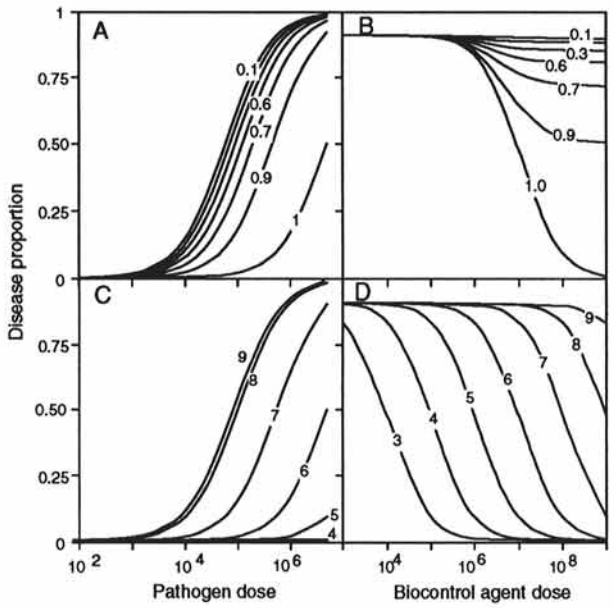

Fig. 3. Effect of the **A and B,** I_{max} and **C and D,** K_z hyperbolic saturation model parameters from equation 7 on disease pathogen-dose curves at a fixed biocontrol agent dose of 1×10^8 CFU/ml (**A and C**) and on disease biocontrol agent-dose curves at a fixed pathogen dose of 5×10^5 conidia per ml (**B and D**). Fixed values of K_x and Y_{max} of 5×10^4 conidia per ml and 1, respectively, were used for calculations.

Empirical verification. The negative exponential (NE), modified negative exponential (MNE), hyperbolic saturation (HS), and probit (PB) models were verified. The regression analysis of disease incidence on the densities of the pathogen and biocontrol agents with raw data from Mandeel and Baker's (13) Figure 1 on the effect of the density of nonpathogenic *F. oxysporum* strains C5 and C14 on biocontrol of *F. oxysporum* f. sp. *cucumerinum* on cucumber are presented in Tables 1 through 4 and Figure 5. Regressions also were performed with our data on the biocontrol of *S. vesicarium* on pear by selected isolates of *Pseudomonas* and *Erwinia* spp. (Tables 1 through 4 and Figure 6).

The values of the model parameters related to the virulence of the pathogens (k, K_x, λ) were used to compare the consistency of parameter estimations within the two sets of biocontrol trials performed for each pathogen. For *S. vesicarium* on pear leaves, only small differences were detected in estimated parameters for the pathogen between the two biocontrol trials performed with *Pseudomonas* EPS288 and *Erwinia* EPS5001. For the NE model, the values of k were 1.9 and 1.4×10^{-5}. For the MNE model, the values

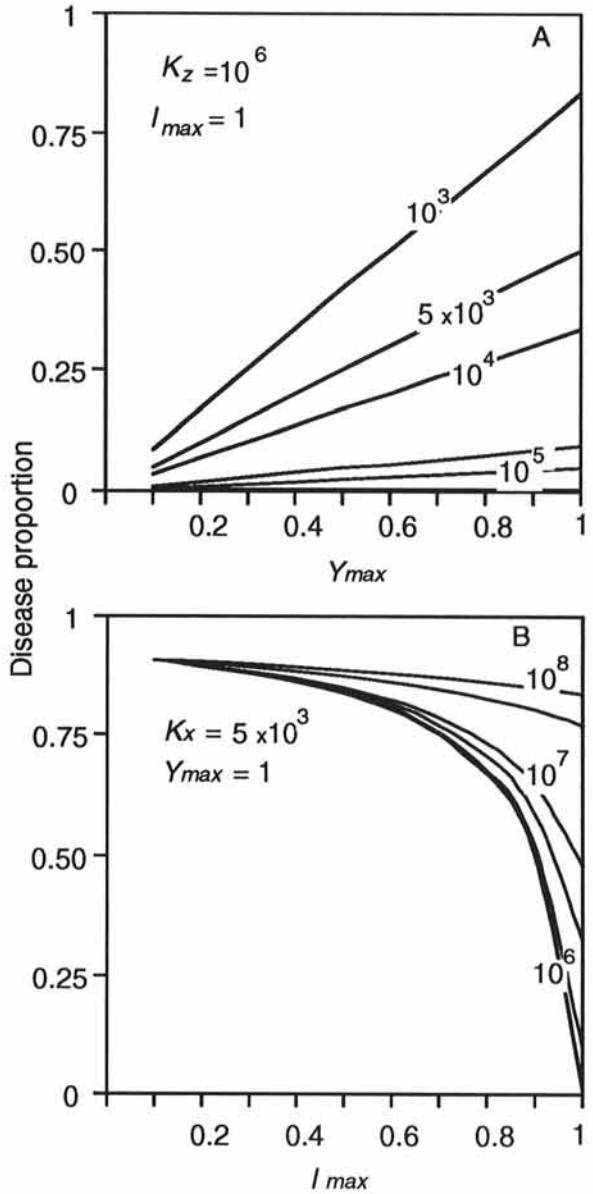

Fig. 4. Dependence of disease levels on **A,** Y_{max} at different half-saturation pathogen doses (K_x) and **B,** I_{max} at different 50% effective dose (ED_{50}) for the biocontrol agent (K_z) as predicted by the hyperbolic saturation model. The pathogen and biocontrol agent doses were 5×10^5 conidia per ml and 1×10^8 CFU/ml, respectively.

TABLE 1. Estimated parameters and goodness-of-fit for the negative exponential model (equation 3) for biocontrol of *Fusarium oxysporum* f. sp. *cucumerinum* on cucumber by different nonpathogenic isolates[a] and biocontrol of *Stemphylium vesicarium* by antagonistic bacteria[b,c]

Pathogen-biocontrol system	*DFE*	Parameter			*MSE*
		k	*B*	*c*	
S. vesicarium-*Erwinia* EPS5001	16	1.37×10^{-5} (0.38×10^{-5})	0.970 (0.016)	9.32×10^{-8} (5.00×10^{-8})	0.01870
S. vesicarium-*Pseudomonas* EPS288	21	1.85×10^{-5} (0.37×10^{-5})	0.954 (0.013)	3.75×10^{-8} (0.81×10^{-8})	0.00940
F. oxysporum f. sp. *cucumerinum*-*F. oxysporum* C14	27	5.19×10^{-4} (0.81×10^{-4})	0.925 (0.109)	1.02×10^{-4} (0.44×10^{-4})	0.01853
F. oxysporum f. sp. *cucumerinum*-*F. oxysporum* C5	27	5.98×10^{-4} (1.39×10^{-4})	0.654 (0.163)	1.82×10^{-4} (1.46×10^{-4})	0.03526

[a] Data shown in Figure 5 is taken from raw data in Mandeel and Baker (13).
[b] Data shown in Figure 6.
[c] *DFE* = degrees of freedom for the error; *MSE* = mean square error. The asymptotic standard errors for the parameter estimates are given in parentheses.

TABLE 2. Estimated parameters and goodness-of-fit for the modified negative exponential model (equation 4) for biocontrol of *Fusarium oxysporum* f. sp. *cucumerinum* on cucumber by different nonpathogenic isolates[a] and biocontrol of *Stemphylium vesicarium* by antagonistic bacteria[b,c]

Pathogen- biocontrol system	*DFE*	Parameter				*MSE*
		A	*k*	*B*	*c*	
S. vesicarium-*Erwinia* EPS5001	15	0.973 (0.013)	2.08×10^{-5} (0.84×10^{-5})	0.796 (0.049)	9.55×10^{-8} (5.00×10^{-8})	0.00794
S. vesicarium-*Pseudomonas* EPS288	20	0.958 (0.051)	2.02×10^{-5} (0.60×10^{-5})	0.954 (0.014)	3.54×10^{-8} (0.88×10^{-8})	0.00954
F. oxysporum f. sp. *cucumerinum*-*F. oxysporum* C14	26	0.482 (0.047)	3.36×10^{-3} (1.18×10^{-3})	0.976 (0.032)	1.67×10^{-4} (0.55×10^{-4})	0.01434
F. oxysporum f. sp. *cucumerinum*-*F. oxysporum* C5	26	...	...	...	...	FC

[a] Data shown in Figure 5 is taken from raw data in Mandeel and Baker (13).
[b] Data shown in Figure 6.
[c] *DFE* = degrees of freedom for the error; *MSE* = mean square error. The asymptotic standard errors for the parameter estimates are given in parentheses. FC = failed to converge.

TABLE 3. Estimated parameters and goodness-of-fit for the hyperbolic saturation model (equation 7) for biocontrol of *Fusarium oxysporum* f. sp. *cucumerinum* on cucumber by different nonpathogenic isolates[a] and biocontrol of *Stemphylium vesicarium* by antagonistic bacteria[b,c]

Pathogen-biocontrol system	*DFE*	Parameter				*MSE*
		Y_{max} (maximum disease proportion)	K_x (ED_{50} pathogen)[d]	I_{max} (maximum pathogen proportion inactivated)	K_z (ED_{50} biocontrol)[d]	
S. vesicarium-*Erwinia* EPS5001	15	0.863 (0.072)	3.72×10^4 (2.35×10^4)	1.028 (0.053)	6.34×10^6 (5.52×10^6)	0.01068
S. vesicarium-*Pseudomonas* EPS288	20	1.042 (0.073)	3.38×10^4 (1.91×10^4)	0.967 (0.024)	8.07×10^6 (6.40×10^6)	0.01035
F. oxysporum f. sp. *cucumerinum*-*F. oxysporum* C14	26	0.610 (0.089)	2.81×10^2 (1.79×10^2)	1.037 (0.048)	2.80×10^3 (2.29×10^3)	0.01325
F. oxysporum f. sp. *cucumerinum*-*F. oxysporum* C5	26	0.506 (0.067)	1.57×10^2 (0.94×10^2)	0.890 (0.224)	9.98×10^3 (9.90×10^3)	0.02401

[a] Data shown in Figure 5 is taken from raw data in Mandeel and Baker (13).
[b] Data shown in Figure 6.
[c] *DFE* = degrees of freedom for the error; *MSE* = mean square error. The asymptotic standard errors for the parameter estimates are given in parentheses.
[d] Densities for *S. vesicarium* and antagonistic bacteria are conidia per ml and CFU/ml, respectively. Densities for *F. oxysporum* and nonpathogenic isolates are CFU/g of soil.

of k were 2.1 and 2.0×10^{-5}. For the HS model, the values of K_X were 3.4 and 3.7×10^4 conidia per ml. For the PB model, the values of λ were 4.8 and 4.4 $\log_{10}$ conidia per ml. For *F. oxysporum* f. sp. *cucumerinum*, small differences were observed in estimated parameters for the pathogen between the two biocontrol trials performed with *F. oxysporum* C14 and C5. For the NE model, the values of k were 5.2 and 5.9×10^{-4}. For the HS model, the values of K_X were 2.8 and 1.6×10^2 CFU/g of soil. For the PB model, the values of λ were 2.9 and 3.0 $\log_{10}$ CFU/g of soil.

In the models, the *ASE* for the parameter estimates were large for the efficiency parameters of the pathogens (k, K_x, and λ) and biocontrol agents (c, K_z, and μ). Also, the asymptotic correlation coefficients between the parameters were high for some data sets (data not shown), indicating a certain degree of overparameterization of the models. However, the *ASE* were always lower than the corresponding parameter estimates. Because our interest was mainly on the biocontrol agent, these particular *ASE* were used together with the *MSE* as criteria for goodness-of-fit of the models.

In the biocontrol-pathosystems, including *Erwinia* EPS5001, *Pseudomonas* EPS288, and *F. oxysporum* C14, the NE, MNE, HS, and PB models described the experimental data relatively well. The NE model proposed by Johnson (10) gave the highest *MSE* for *Erwinia*

TABLE 4. Estimated parameters and goodness-of-fit for the probit model (equation 11) for biocontrol of *Fusarium oxysporum* f. sp. *cucumerinum* on cucumber by different nonpathogenic isolates[a] and biocontrol of *Stemphylium vesicarium* by antagonistic bacteria[b,c]

Pathogen-biocontrol system	*DFE*	Parameter				*MSE*
		λ ($\log_{10}$ ED_{50} pathogen)[d]	τ (pathogen efficiency)	μ ($\log_{10}$ ED_{50} biocontrol)[d]	σ (biocontrol efficiency)	
S. vesicarium-*Erwinia* EPS5001	15	4.812 (0.212)	0.901 (0.153)	6.740 (0.723)	0.660 (0.366)	0.01426
S. vesicarium-*Pseudomonas* EPS288	20	4.444 (0.229)	0.617 (0.087)	6.563 (0.954)	1.383 (0.438)	0.01160
F. oxysporum f. sp. *cucumerinum*-*F. oxysporum* C14	26	2.948 (0.154)	1.137 (0.231)	2.959 (0.426)	0.889 (0.263)	0.01216
F. oxysporum f. sp. *cucumerinum*-*F. oxysporum* C5	26	3.007 (0.321)	1.589 (0.452)	2.922 (1.521)	1.443 (1.095)	0.02117

[a] Data shown in Figure 5 is taken from raw data in Mandeel and Baker (13).
[b] Data shown in Figure 6.
[c] *DFE* = degrees of freedom for the error; *MSE* = mean square error. The asymptotic standard errors for the parameter estimates are given in parentheses.
[d] Densities for *S. vesicarium* and antagonistic bacteria are conidia per ml and CFU/ml, respectively. Densities for *F. oxysporum* and nonpathogenic isolates are CFU/g of soil.

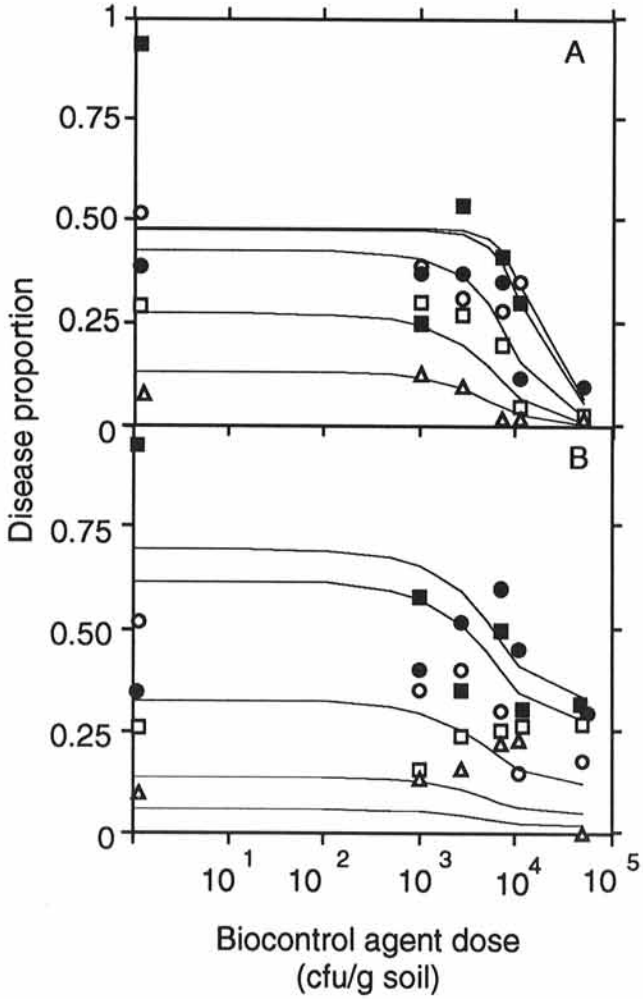

Fig. 5. Infectivity titration of *Fusarium oxysporum* f. sp. *cucumerinum* on cucumber plants with nonpathogenic isolates **A,** C14 and **B,** C5 of *F. oxysporum*. Data on $\ln[1/(1-y)]$ were taken directly from the raw data points of Figure 6 in Mandeel and Baker (13) and back-transformed to disease proportion values. The pathogen doses used were 100 (△), 250 (□), 650 (●), 1,600 (○), and 2,000 (■) CFU/g of soil. The lines represent predictions by the modified negative exponential model (equation 4) for C14 and by the probit model (equation 11) for C5. Goodness-of-fit and estimated parameters of the models from equations 3, 4, 7, and 11 are shown in Tables 1, 2, 3, and 4, respectively.

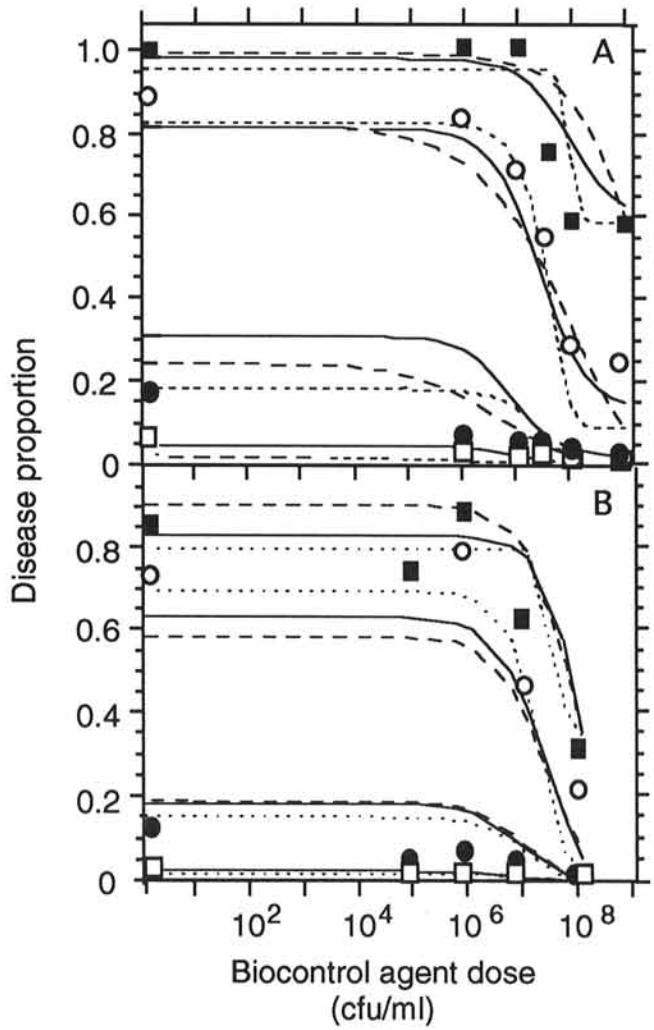

Fig. 6. Infectivity titration of *Stemphylium vesicarium* on detached pear leaves treated with different doses of **A,** *Pseudomonas* EPS288 and **B,** *Erwinia* EPS5001. The pathogen densities used were 1×10^3 (□), 1×10^4 (●), 1×10^5 (○), and 1×10^6 (■) conidia per ml. The lines represent predictions by the modified negative exponential (equation 4) (.....), hyperbolic saturation (equation 7) (____), and probit (equation 11) (- - -) models. Data represent the mean of two experiments. Goodness-of-fit and estimated parameters of the models from equations 3, 4, 7, and 11 are shown in Tables 1, 2, 3, and 4, respectively.

EPS5001 and *F. oxysporum* C14 and similar values to the other models in the case of *Pseudomonas* EPS288. For *F. oxysporum* C14 the MNE, HS, and PB models gave similar *MSE*, and the PB model produced the lowest *ASE* for the biocontrol agent parameters. For *Pseudomonas* EPS288, *MSE* values were similar in all models, and the *ASE* values for the biocontrol agent parameters were lowest in the PB model. For *Erwinia* EPS5001, the lowest values of *MSE* were obtained with the MNE and HS models, and the *ASE* values were lowest in the PB model. None of the models adequately fit the data values for *F. oxysporum* C5 as judged by the larger *MSE* and *ASE* values and the fact that the MNE model failed to converge. This situation was probably due to high variability in the experimental data.

No consistent patterns for any of the models were detected by plotting residuals against predicted or observed disease values or against pathogen or biocontrol agent dose for *F. oxysporum* C14, *Erwinia* EPS5001, and *Pseudomonas* EPS288. A data point of *F. oxysporum* C14 corresponding to pathogen densities of 2,000 CFU/g of soil and without biocontrol agent (reference 13, Fig. 3) was clearly an outlier. In this case, the values of the estimated parameters changed only slightly when this point was excluded from the analysis. The NE, HS, and PB models gave a significant linear relationship between residuals and observed or predicted disease levels for *F. oxysporum* C5, indicating a bias in prediction with these data sets.

Estimation of model parameters from a single biocontrol agent dose curve at fixed pathogen density. Figure 7 shows the effect of the treatment of pear leaves with different densities of *Pseudomonas* EPS381 on disease levels at a fixed dose of 1×10^5 conidia per ml of *S. vesicarium*. The HS and MNE models fit the data sets adequately on the basis of the *MSE* (0.0016 and 0.0024, respectively) and the *ASE* for the parameter estimates. Also, estimated parameters were in the range of values obtained for *Erwinia* EPS5001 and *Pseudomonas* EPS288 with combinations of pathogen and biocontrol agent densities (Tables 2 and 3). The probit model failed to converge, probably because of the lack of data values at biocontrol concentrations higher than 10^9 CFU/ml.

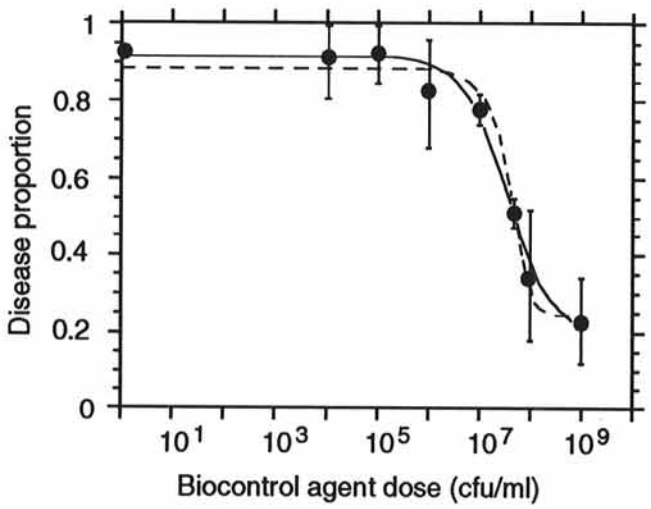

Fig. 7. Infectivity titration of *Stemphylium vesicarium* at a fixed dose of 1×10^5 conidia per ml on detached pear leaves treated with different doses of *Pseudomonas* EPS381. Each value represents the mean of three experiments and the confidence interval. The lines represent predictions by the modified negative exponential (MNE) (equation 4) (- - -) and hyperbolic saturation (HS) (equation 7) (___) models. A value for A and Y_{max} of 0.95 was used for fitting data to model equations. The estimated parameters for the HS model were I_{max} = 0.988 (*ASE* [asymptotic standard error] = 0.006), $K_z = 2.00 \times 10^6$ CFU/ml ($ASE = 1.20 \times 10^6$), and $K_x = 4.6 \times 10^3$ conidia per ml ($ASE = 2.30 \times 10^3$), and the mean square error (*MSE*) was 0.0016. The estimated parameters for the MNE model were $B = 0.884$ ($ASE = 0.026$), $c = 3.17 \times 10^{-8}$ ($ASE = 0.54 \times 10^{-8}$), $k = 2.66 \times 10^{-5}$ ($ASE = 0.33 \times 10^{-5}$), and the *MSE* was 0.0024.

DISCUSSION

Few studies in the biocontrol literature have been performed to evaluate disease control over a range of biocontrol agent densities (1,5,15,20) and works that include the combined effects of a wide range of pathogen and biocontrol agent densities are even less abundant (11,13). Johnson (10) proposed a nonlinear model based on the negative exponential function to explain the combined effects of pathogen and biocontrol agent densities on disease levels and to fit the data of Mandeel and Baker (13) to estimate model parameters with graphic tools based on a combination of visual estimation for some parameters and iterative methods for others. The model proposed by Johnson (10) is useful from a theoretical point of view; however, the methodology used for model evaluation does not provide objective criteria for goodness-of-fit and parameter estimation and is not applicable to obtain response surfaces for the combined effects of the pathogen and biocontrol agent on disease levels. Some examples of the limitations of existing methods for fitting data on dose-response relationships in biological control of plant diseases recently have been provided (11,14). In the work of Raaijmakers et al. (15) on biocontrol of Fusarium wilt of radish by *Pseudomonas* spp. the authors took advantage of the theoretical relationships proposed by Johnson (10) and used simple linear regression analysis to fit their data. In the study of Korsten et al. (11) on biological control of postharvest diseases of avocado with *Bacillus subtilis*, highly complicated polynomial regression equations were used to fit data on combined effects to disease levels of several pathogens and the biocontrol agent. The models proposed and evaluated in our work and the methodology used to fit data to the models by nonlinear regression procedures are useful as tools for describing quantitative responses in biocontrol systems.

Our models were relatively sophisticated because their development was based on a "mechanistic" approach oriented to build in parameters in a meaningful way and to construct functions with some underlying biological plausibility. The MNE model is a generalization of the model proposed by Johnson (10). In the MNE model, parameters c and k supply information on the efficiency of the pathogen and biocontrol agent, respectively, and provide endpoint values, such as the maximum proportion of pathogen inoculum inactivated by the biocontrol agent (B) and asymptotic disease levels without biocontrol (A), but do not contain information on curve positioning. The HS model gives information on the ED_{50} for both the pathogen (K_x) and the biocontrol agent (K_z) (curve positioning), on asymptotic disease levels without biocontrol (Y_{max}), and on the maximum proportion of the pathogen the biocontrol agent can inactivate (I_{max}) but not on the slope of the dose-response relationship. The PB model provides information on the relative virulence of the pathogen and the efficiency of the biocontrol agent (τ and σ of the dose-response curves) and ED_{50} for the pathogen and biocontrol agent (curve positioning parameters μ and λ) but assumes that at a higher dose of the biocontrol agent full control of the disease is achieved.

The three models fit relatively well to the data used in our study, as was evident when comparing the experimental error in estimating disease proportion with the model error. For a given model, a measure of mean absolute error is provided by the rooted *MSE* (*RMSE*). The *RMSE* can be divided by the maximum attainable disease values (1.0) and be expressed as a percent to provide a measure of the mean relative model error (*MRME*). The *MRME* values for *F. oxysporum* C14, *Erwinia* EPS5001, and *Pseudomonas* EPS288, were 9 to 12% for the MNE model, 10 to 12% for the HS model, and 11 to 12% for the PB model. A measure of the experimental error (*EE*) in estimating disease proportion is given for *Pseudomonas* EPS381 by the confidence intervals from Figure 7, which ranged from 10 to 30% of the maximum disease values attainable. It can be concluded that the *MRME* is within the range of the *EE*. Therefore, the three models fit the data well,

but it was not possible to discriminate between them in terms of the best goodness-of-fit.

A limitation of our models is the large *ASE* for the parameter estimates and the high asymptotic correlation coefficients (*ACC*) between the parameters. The high *ASE* and *ACC* are an indication of over parameterization that produces some difficulties in estimating efficiency parameters related to the pathogen and biocontrol agent, such as *k and c* (MNE model), μ *and* λ (PB model), and K_x and K_z (HS model). One solution to the problem of over parameterization and low precision in estimating parameters may be to model the data with fewer parameters; one or more parameters in the model may be fixed at values predetermined by prior information, and the remainder of the parameters may then be more precisely estimated. Additional factors, not intrinsically related to the models, that can increase the values of the *ASE* are experimental errors associated with the measurement of microbial densities and disease levels. These errors may be particularly important at pathogen and biocontrol densities near the inflection point of the dose-response curves, due to the fact that in this region small changes in the concentration of biocontrol agent or pathogen produce strong changes in disease levels. Consequently, enough data points (e.g., at least five to six times as many data points as model parameters), linearly scaled doses of biocontrol agent for the turn-point region of the dose-response curves, and accurate measures of disease levels would contribute to better estimations of model parameters.

We analyzed two pathosystems (an aerial and a soilborne disease) and two biocontrol systems (antagonistic bacteria and nonpathogenic isolates of the pathogen). The combined use of the three models provided parameters that permitted comparisons between the *F. oxysporum* and *S. vesicarium* pathosystems. Among the most interesting parameters were the relative efficiency (σ) and the ED_{50} of the biocontrol agent inactivating the pathogen (K_z and μ) and the ED_{50} ratio of biocontrol to pathogen (K_z/K_x) (Table 5). The ED_{50} for *S. vesicarium* (without the biocontrol agent) was about 40 times higher (approximately 4×10^4 conidia per ml) than for the pathogenic isolate of *F. oxysporum* (approximately 1×10^3 CFU/g of soil). The K_z/K_x for the antagonistic bacteria, *S. vesicarium*, was in the range of 148 to 434 CFU per conidium, whereas for the nonpathogenic isolates of *F. oxysporum* the ratio was in the range of 1 to 10 CFU/CFU. Mandeel and Baker (13) showed that the mechanism of biological control was mainly due to competition, whereas in our pathosystem biological control was related to inhibition of germination and to direct interaction of the antagonistic bacteria with conidia (E. Montesinos and A. Bonaterra, *unpublished data*). Therefore, the number of targets to be counteracted by the biocontrol agent are lower in the *F. oxysporum* fungus-fungus competition for common rhizosphere or infection sites than in the bacterial-fungus antagonism in which several bacteria are needed to inactivate a pathogen cell (the conidium). Adams (1) reviewed the efficiency of several mycoparasites as biocontrol agents and considered the ratio of the number of propagules of the mycoparasite required to obtain disease control to the typical inoculum density of a plant pathogen in production fields. Adams (1) showed that efficiency values ranged from 0.2 to 5×10^4 propagules of fungus mycoparasite per propagule of pathogen. Our results of the ED_{50} biocontrol agent/pathogen ratio for the four biocontrol-pathogen-host systems studied with the models proposed agreed with the values shown by Adams for *Talaromyces flavus-Verticillium dahliae*, *Sporidesmium sclerotivorum-Sclerotinia minor*, *Laetisaria arvalis-Pythium ultimum*, *P. nunn-P. ultimum*, and *Trichoderma* spp.*-Rhizoctonia solani*.

The parameters obtained from the HS and PB models also permitted the establishment of comparisons between the efficiency of a series of biocontrol agents within the same pathosystem. The ED_{50} ratios K_z/K_x and $10^{\mu}/10^{\lambda}$ were useful for this purpose (Table 5). The lower the values of the ED_{50} ratio of biocontrol to pathogen, the higher is the efficiency of biocontrol. In the *F. oxysporum* pathosystem, isolate C14 seems to be more efficient than C5, and this agreed with Mandeel and Baker's conclusions (13), but the fitting of the data on isolate C5 to the models was poor. Also, the *Erwinia* isolate studied in the present work was more efficient than *Pseudomonas* in the control of leaf infection by *S. vesicarium*.

In the systems studied that fit the data, the values of B ranged from 0.79 to 0.98, and I_{max} ranged from 0.97 to 1.04, indicating that a high proportion of the pathogen inoculum was inactivated by the biocontrol agents. As was suggested by Johnson (10), this may indicate similarities in the ecological niche of the pathogen and biocontrol agent, the lack of a "refuge" that may protect the pathogen from the influence of the biocontrol agent, or a coincidence in the spatial distributions among the pathogen and biocontrol agent.

The results of fitting the PB model to the data presented in our study are consistent with the hypothesis of independent action of both the pathogen cells on the host and the biocontrol agent cells on the pathogen, because values for the slopes of the PB model were lower than 2 (τ from 0.6 to 1.1 for the pathogens and σ from 0.7 to 1.4 for the biocontrol agents). Peto (cited in reference 16) demonstrated mathematically that the slope of the probit function should be approximately 2 (using the $\log_{10}$ density) if the pathogen cells are functioning independently during the infection process. Ercolani (7) showed that in compatible interactions between pathogenic bacteria and plants, the slope of the probit function was lower than 2, which agreed with the expected values in a case of independent action of pathogen cells. In incompatible interactions, Ercolani (7) found slopes higher than 2, suggesting a cooperative effect among the pathogen cells. Rouse et al. (16) also showed that the slope values of the probit function obtained by fitting field data on infection of bean leaflets by *Pseudomonas syringae* were not significantly different from 2 and suggested the existence of independent action of the pathogen cells.

Obtaining response surfaces for a given pathogen-biocontrol-host system requires considerable experimental work involving the combined effect of a wide range of biocontrol agent and pathogen densities. Often the objective is limited to comparing the potential of several biocontrol agents at a fixed pathogen dose. In this case, parameters describing the efficiency of the biocontrol agent can be obtained by fitting data to equations 4 and 7, assuming A and Y_{max} to be the maximum disease value observed experimentally. This is usually applicable to the NE and HS models because they contain asymptotic parameters that can be determined easily from graphs of the data. However, because the PB model is a four-parameter model with no asymptote parameters, it usually fails to converge, and the method is not applicable in most cases for a

TABLE 5. Efficiency of several biocontrol agents calculated as the ED_{50} biocontrol agent/pathogen ratio with parameters from the hyperbolic saturation (equation 7) and probit (equation 11) models

Pathogen-biocontrol system	K_z/K_x	μ/λ[a]
S. vesicarium-*Erwinia* EPS5001, on pear	148.4[b]	77.3
S. vesicarium-*Pseudomonas* EPS288, on pear	238.8[b]	131.3
S. vesicarium-*Pseudomonas* EPS381, on pear	434.8[b]	FC[c]
F. oxysporum f. sp. *cucumerinum*-*F. oxysporum* C14, on cucumber	9.9[d]	1.0

[a] The values of μ and λ originally expressed as $\log_{10}$ of the dose were back-transformed to calculate the biocontrol agent/pathogen ratio.
[b] CFU per conidium.
[c] Failed to converge.
[d] CFU of biocontrol agent per CFU of pathogen.

single pathogen dose tested. An example of the usefulness of biocontrol titration studies at a single pathogen dose is given by Cartwright and Benson (5) on the biocontrol of Rhizoctonia stem rot of poinsettia. The apparent ED_{50} taken directly from graphs was around 10^6 CFU/ml for *Pseudomonas cepacia* and somewhere between 10^6 and 10^7 conidia per ml for *Paecilomyces lilacinus*. Unfortunately, the pathogen densities in these experiments were not provided, and calculation of the ED_{50} ratio of biocontrol to pathogen by fitting the data to our model equations with nonlinear regression was not possible. However, the apparent ED_{50} values for *Pseudomonas cepacia* agreed with those obtained by us with *Pseudomonas* and *Erwinia* spp. (approximately 6.0×10^6 CFU/ml). The problem with this common approach is that at low pathogen densities overcontrol occurs, whereas at high pathogen densities a lack of control may be observed. The methods we developed with the combined effects of pathogen and biocontrol agent permit assessment by an objective means, the ED_{50} of the pathogen and biocontrol agent, and efficiency values, the ED_{50} biocontrol agent/pathogen ratio. These parameters have the advantage of being independent on the pathogen densities and, therefore, can be used to establish comparisons among biocontrol agents and host plants under diverse environmental conditions.

Finally, the concepts contained implicitly in the model equations and the parameters obtained by fitting data to the models may provide evidence of certain underlying biological mechanisms during the biocontrol process. However, direct analysis of the mechanisms by means of controlled experiments designed to test specific hypotheses is the appropriate approach for inferring biological mechanisms. Experience with the models in additional aerial and root pathogens and in other pathosystems (e.g., postharvest diseases) may lead to more thorough knowledge of the biological significance of the parameters.

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