

# Dose-Response Relationships and Inundative Biological Control

Kenneth B. Johnson

Department of Botany and Plant Pathology, Oregon State University, Corvallis 97331-2902.

Technical paper 10,290 of the Oregon Agricultural Experiment Station.

Accepted for publication 25 May 1994.

Biological control of plant disease is currently receiving increased research effort owing to the desire to enhance the sustainability of agricultural production systems and to reduce the use of chemical pesticides in these systems. Much of the current research on biological control is focused on understanding the mechanisms by which biological agents reduce the impact of pathogen populations (e.g., antibiosis [6,22], competition [6,16, 20], hyperparasitism [6], and induced resistance [6,17]). Development of a theory defining epidemiological parameters that may govern the efficacy of biological control of plant disease has received less research effort (1,5). This is particularly true for systems in which biological control agents are introduced inundatively (i.e., artificially in high concentration) to soil or phyllosphere environments.

The purpose of this letter is to propose a model that defines parameters that affect the efficacy and expected behavior of inundatively applied biological control agents. The model was developed deductively based on an analogy to the most common dose-response relationship observed for plant pathogen-plant host interactions.

**Pathogen dose-plant disease response.** Inoculum of a plant pathogen that is randomly distributed can produce a disease response governed by the equation

$$y = 1 - \exp(-kx), \quad (1)$$

where  $x$  is the inoculum density of the pathogen,  $k$  is a constant that governs the efficiency of the inoculum, and  $y$  is the proportion of disease. Van der Plank (21) considered this equation the most common type of pathogen dose-disease response relationship; Baker (4) used this equation to define mathematically the concept of inoculum potential. With equation 1, the amount of disease produced per unit of inoculum diminishes with increasing inoculum density. This occurs because some plant tissue is infected more than once; Gregory's (12) multiple infection transformation,  $-\ln(1 - y)$ , linearizes equation 1.

Equation 1 assumes 100% of the plants or plant tissue is susceptible to infection. Researchers have observed, however, that some host tissue may be unavailable for infection (3,15,18,19). An asymptote,  $L$ , can be introduced to a dose-response relationship to exclude unavailable tissue:

$$y = L[1 - \exp(-kx)], \quad (2)$$

where  $L$  is defined as the proportion of host tissue available for infection. The effect of  $L$  on the dose-response relationship is shown in Figure 1.

**Deductive model for biological control.** I propose that the effect of a biological control agent on a plant pathogen population will follow dynamics analogous to the pathogen dose-disease response relationship described above. The equation that defines

the effect of a biological agent on a pathogen population is

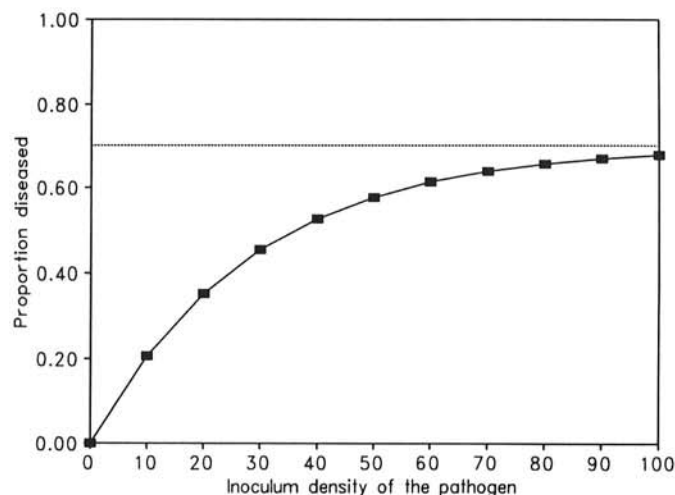
$$x_i/x = 1 - \exp(-cz), \quad (3)$$

where  $z$  is the density of the biocontrol agent,  $c$  is a constant that governs the efficiency of the biological control agent, and  $x_i/x$  is the proportion of inoculum units of the pathogen population rendered ineffective by the biocontrol agent. The variable  $x_i/x$  is intended to be consistent with accepted definitions for biological control (11) and also is intended to include the various mechanisms by which inoculum of a pathogen can be rendered ineffective. The equation assumes, however, that the mechanism by which a biological control agent affects a pathogen is independent of its own density and that the proportional efficacy obtained by use of a biological control agent,  $x_i/x$ , remains constant over a range of pathogen densities. This second assumption is most likely to hold true when the ratio of the density of the biological control agent to the inoculum density of the pathogen is large.

As in equation 1, an asymptote can be added to equation 3:

$$x_i/x = A[1 - \exp(-cz)], \quad (4)$$

where the asymptote,  $A$ , represents the maximum proportion of a pathogen population that can be rendered ineffective by a biological control agent. Biological factors that may influence the magnitude of  $A$  include the degree to which a biological agent is adapted to the same ecological niche as the plant pathogen (10), the existence of a refuge that may protect some of the pathogen propagules from the influence of a biological agent (14), and



**Fig. 1.** Proportion of plant tissue diseased ( $y$ ) in response to the inoculum dose of a pathogen ( $x$ ). The curve is described by:  $y = L[1 - \exp(-kx)]$ ; the value of the efficiency constant,  $k$ , was assumed to be 0.035, and the proportion of host tissue available for infection,  $L$ , was assumed to be 0.70. Units for inoculum density of the pathogen were chosen arbitrarily.

the degree to which the spatial distributions of the pathogen and a biological agent readily coincide (7).

The amount of disease obtained in a pathosystem in which all host tissue is susceptible and all pathogenic inoculum is potentially affected by a biological control agent is given by

$$y = 1 - \exp[-kx \times \exp(-cz)], \quad (5)$$

where the term,  $\exp(-cz)$ , is taken from equation 3 and is defined as the proportion of pathogenic inoculum that remains effective (i.e.,  $1 - x_i/x$ ). In equation 5, reductions in disease are functionally dependent on the density of the biological control agent (Fig. 2A). Equation 5 predicts that reductions in disease will be significant over a relatively wide range of pathogen densities as long as the density of the biological control agent remains high (Fig. 2A).

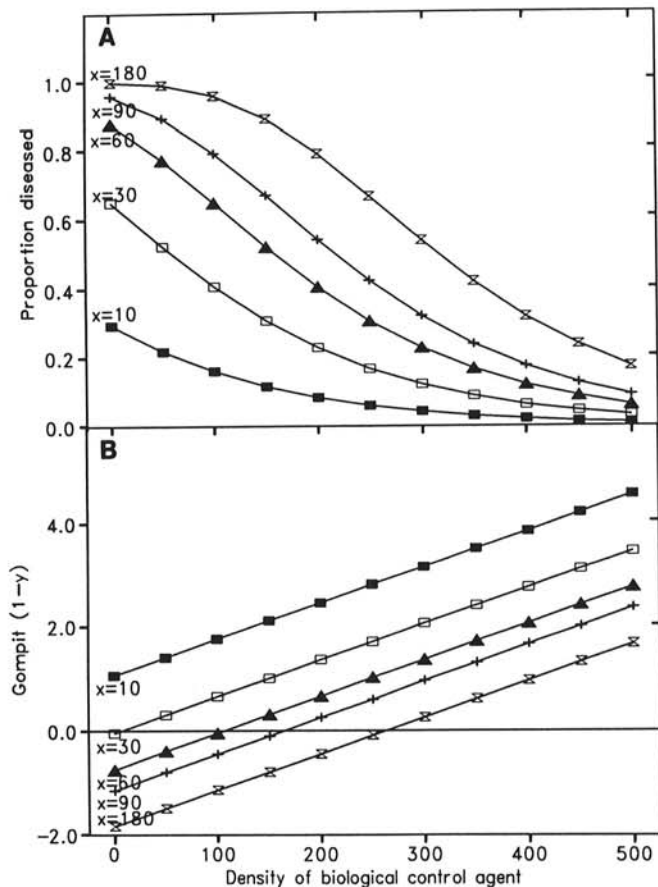
For specific inoculum densities of the pathogen, equation 5 can be linearized by taking the Gompertz transformation:  $-\ln[-\ln(1 - y)]$  (termed "gompit (1 - y)" [8]). The slope of gompit (1 - y) plotted on the density of the biological control agent is an empirical estimate of the value of the efficiency constant,  $c$  (Fig. 2B).

Equation 5 can be expanded to account for the effects of the asymptote,  $A$ , on disease:

$$y = 1 - \exp\{-kx[1 - A [1 - \exp(-cz)]]\},$$

which rearranges to

$$y = 1 - \exp\{-kx[(1 - A) + A \times \exp(-cz)]\}. \quad (6)$$

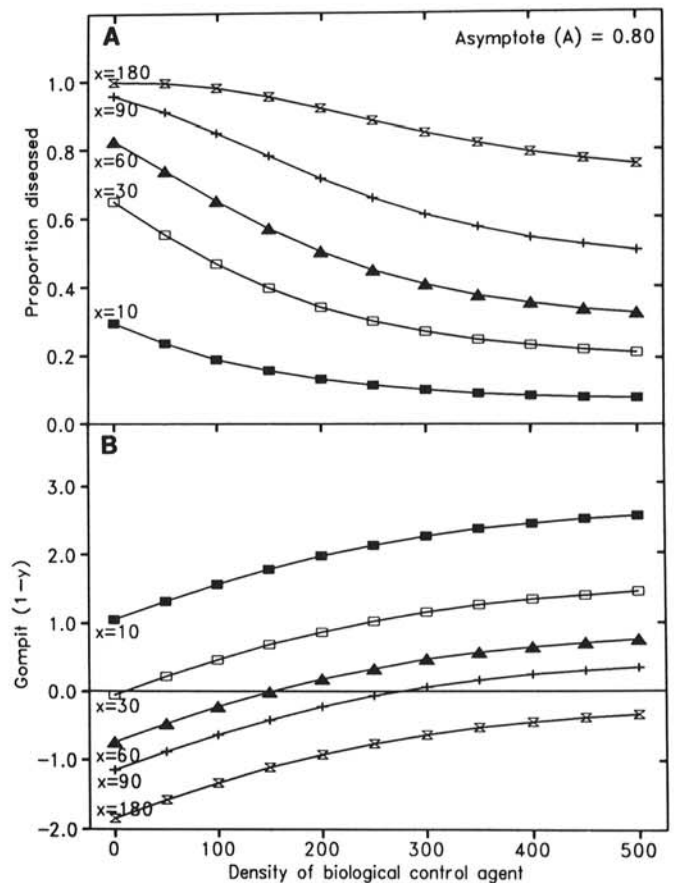


**Fig. 2. A**, Proportion of disease ( $y$ ) in response to the density of a biological control agent,  $z$ , at five inoculum densities of the pathogen,  $x$ . The curves are described by:  $y = 1 - \exp[-kx \times \exp(-cz)]$ , where the values of the efficiency constants,  $k$  and  $c$ , were assumed to be 0.035 and 0.007, respectively. **B**, Gompertz transformation of the curves in **A**; the slopes of the lines are equivalent to the value of the efficiency constant,  $c$ .

Compared to equation 5, equation 6 predicts that the amount of disease reduction obtained by a biological control agent is influenced more by the inoculum density of the pathogen (Fig. 3A). Gompertz transformation of equation 6 results in a series of parallel but nonlinear curves for fixed inoculum densities of the pathogen (Fig. 3B). At high densities of the biological control agent, the slopes of these curves are significantly reduced compared to the slopes obtained after transformation of equation 5 (Fig. 2B). Curves depicted in Figure 3B, however, cannot be used to estimate the efficiency constant,  $c$ ;  $c$  is estimated by plotting gompit  $\{(1 - y)/\exp[-kx(1 - A)]\}$  on the density of the biocontrol agent,  $z$ .

**Model sensitivity.** Equation 6 contains two efficiency parameters/variables ( $k$  and  $c$ ), two density variables ( $x$  and  $z$ ), and an asymptote parameter/variable ( $A$ ) that can influence the amount of disease control obtained by use of a biological agent. The results of a sensitivity analysis of equation 6 (Fig. 4A and B) showed that incremental 10% reductions in the value of the asymptote ( $A$ ) had a greater effect on the efficacy of a biological control agent than similar reductions in the value of the efficiency constant ( $c$ ). Further, as noted above, incremental reductions in the value of  $A$  increased the effect of pathogen inoculum density on the amount of disease control obtained (Fig. 4A). Proportional reductions in the density of the biological control agent,  $z$ , gave results analogous to the proportional reductions in  $c$  (Fig. 4B).

**Empirical verification.** The biological control literature contains limited data to evaluate equation 6. Few studies have been designed to evaluate disease control over a wide range of densities of a biological control agent, and direct measurement of the variable  $x_i/x$  is difficult in many experimental systems. A recent study



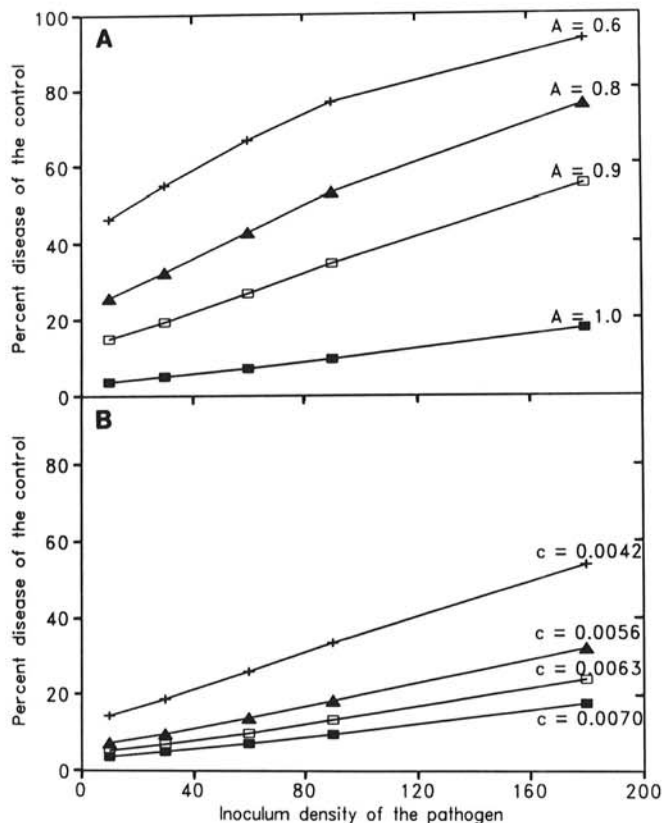
**Fig. 3. A**, Proportion of disease,  $y$ , in response to the density of a biological control agent,  $z$ , at five inoculum densities of the pathogen,  $x$ . The curve is described by:  $y = 1 - \exp\{-kx[(1 - A) + A \times \exp(-cz)]\}$ , where the value of the asymptote parameter,  $A$ , is 0.8 and the values of the efficiency constants,  $k$  and  $c$ , were assumed to be 0.035 and 0.007, respectively. **B**, Gompertz transformation of the curves in **A**.

by Mandeel and Baker (17), however, examined the effect of inoculum density of *Fusarium oxysporum* f. sp. *cucumerinum* on development of Fusarium wilt of cucumber in soils amended with isolates C5 or C14 of nonpathogenic *F. oxysporum*. These nonpathogenic isolates are thought to compete with pathogenic strains for nutrients and infection sites or to induce resistance in the host to infection by the pathogen (6). In the study, densities of C5 and C14 ranged from 0 to 50,000 cfu/g, and disease, expressed as  $-\ln(1 - y)$ , was regressed on the inoculum density of the pathogen (0–2,000 cfu/g) for each density of C5 or C14.

To evaluate equation 6, the estimated regression values for  $-\ln(1 - y)$  at 2,000 cfu/g of *F. o. cucumerinum* were selected from Figure 1 of Mandeel and Baker (17) and plotted against the density of C5 or C14 (Fig. 5A). A transformed version of equation 6,

$$-\ln(1 - y) = kx[(1 - A) + A \times \exp(-cz)], \quad (7)$$

was then fit to the estimated regression values of  $-\ln(1 - y)$  obtained for each biological control agent. A value for  $kx$  was estimated from Figure 5A, where the densities of C5 and C14 were zero (at  $z = 0$ ,  $kx = -\ln(1 - y) = 0.74$ ). Values for  $A$  were taken to be 0.001 less than the values of  $1 + \ln(1 - y)/kx$  at the highest density of the biological control agents (50,000 cfu/g); values of  $c$  were then estimated iteratively. The curves shown in Figure 5A summarize the effects of C5 and C14 on  $-\ln(1 - y)$ ; each curve has the same value for  $c$  (0.00015) and different values for  $A$  (for C5  $A = 0.582$ ; for C14  $A = 0.893$ ). The different values of  $A$  indicate that *F. oxysporum* isolate C14

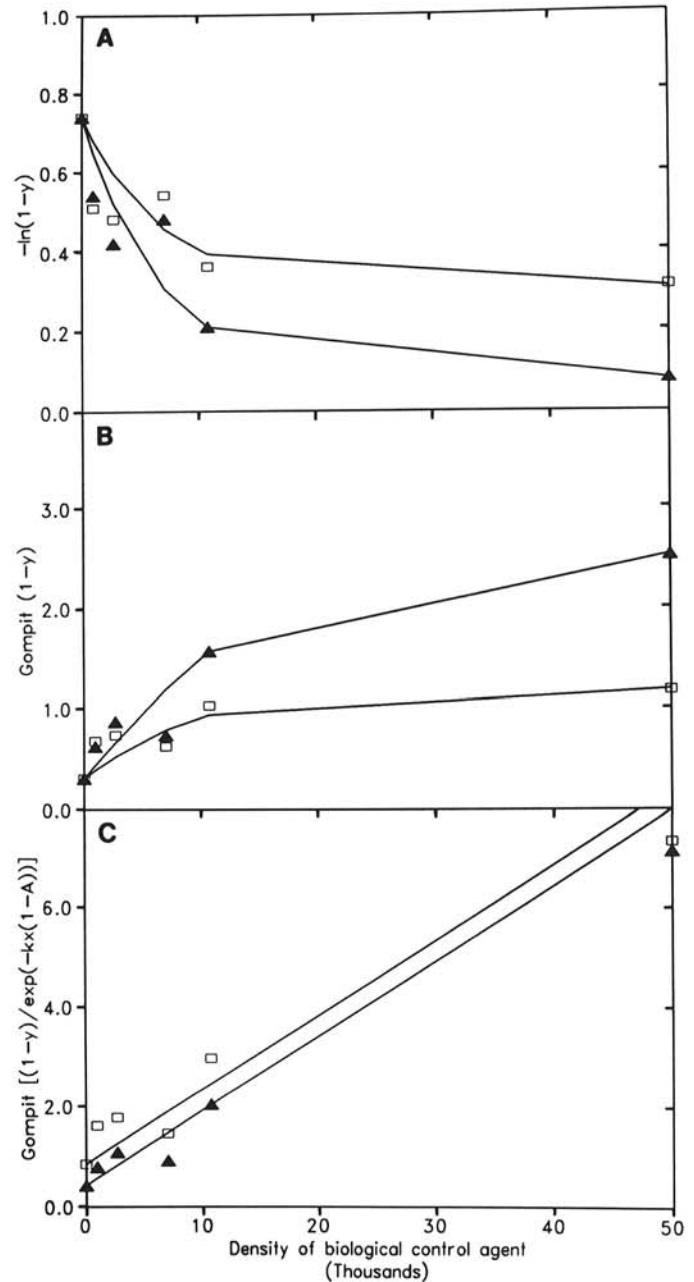


**Fig. 4.** A, Effect of the inoculum density of the pathogen,  $x$ , on percent disease of the control as influenced by the presence of a biological control agent,  $z$ . Results are based on the equation:  $y = 1 - \exp\{-kx[(1 - A) + A \times \exp(-cz)]\}$ , where  $y$  is the proportion diseased,  $k$  is an efficiency constant with a value of 0.035, and  $A$  and  $c$  are parameters that affect the efficiency of the biological control agent. The density of the biological control agent,  $z$ , was 500 units. For each value of  $x$ , percent disease of the control was obtained by dividing the value of  $y$ , when  $z = 500$ , by the value of  $y$ , when  $z = 0$ , then multiplying by 100. A, Results obtained by varying the value of  $A$  when  $c = 0.007$ ; B, results obtained by varying the value of  $c$  when  $A = 1.0$ .

has the ability to render a higher proportion of inoculum of *F. o. cucumerinum* ineffective than does isolate C5.

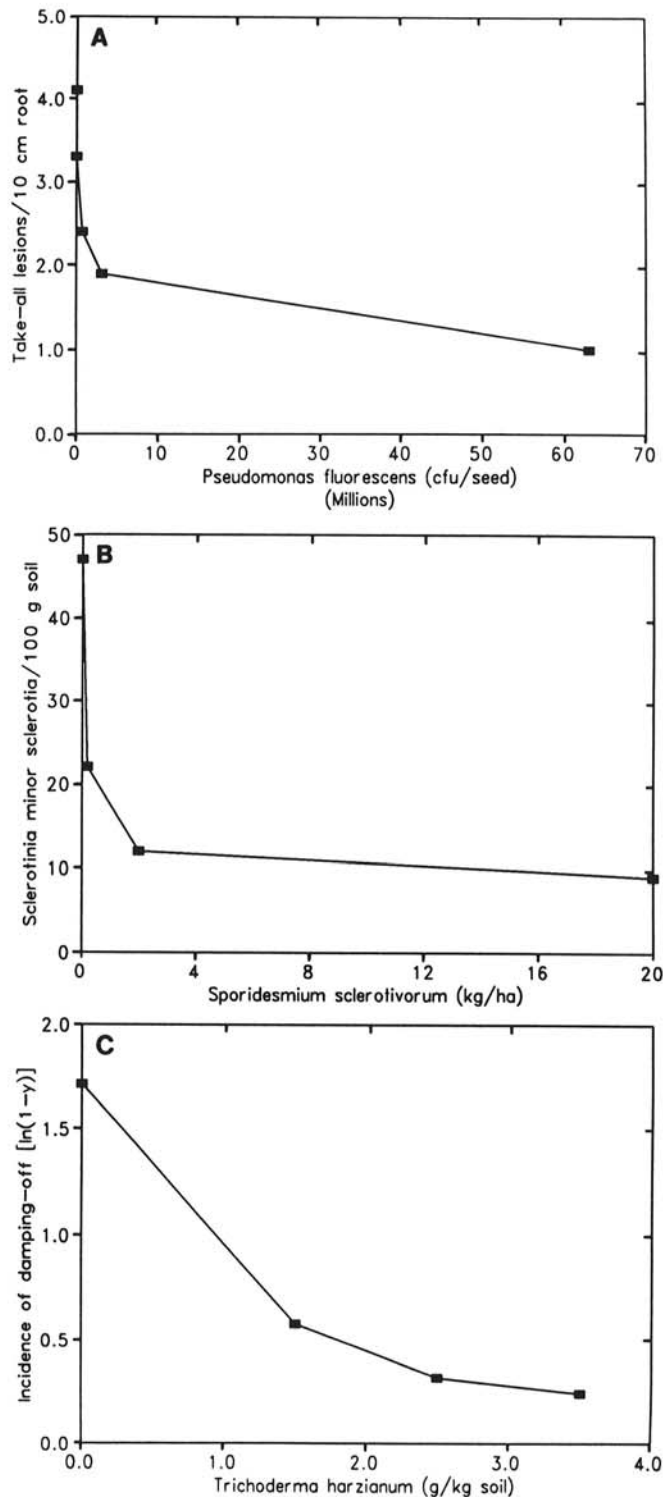
The data obtained from the study of Mandeel and Baker (17) also were transformed with gompit  $(1 - y)$  and gompit  $\{(1 - y)/\exp[-kx(1 - A)]\}$ . Plots of the Gompertz-transformed data on the density of C5 and C14 are presented in Figure 5B and C along with the appropriate curves generated from the estimated values for  $kx$ ,  $A$ , and  $c$ . The transformed data generally agreed with the curves derived from equation 6 (Fig. 5B and C).

Biological control agents that have been proposed to utilize



**Fig. 5.** Effect of nonpathogenic *Fusarium oxysporum* isolates C5 (□) and C14 (▲) on the proportion of cucumber plants diseased,  $y$ , with Fusarium wilt 55 days after planting. Density of the pathogen, *F. o. cucumerinum*,  $x$  was 2,000 cfu/g. Data points were obtained from Mandeel and Baker (17); curves were fit to the data based on the equation:  $y = 1 - \exp\{-kx[(1 - A) + A \times \exp(-cz)]\}$ , where  $z$  is the density of isolate C5 or C14,  $k$  and  $c$  are efficiency constants with values of 0.00037 and 0.00015, respectively, and  $A$  is an asymptote parameter with values of 0.582 for C5 and 0.893 for C14. A, Data points transformed with  $-\ln(1 - y)$ ; curves represent  $kx[(1 - A) + A \times \exp(-cz)]$ . B, Data points transformed with gompit  $(1 - y)$ ; curves represent  $-\ln\{kx[(1 - A) + A \times \exp(-cz)]\}$ . C, Data points transformed with gompit  $\{(1 - y)/\exp[-kx(1 - A)]\}$ ; curves represent  $-\ln(kx \times A) + cz$ .

the mechanisms of antibiosis or hyperparasitism also show non-linear, asymptotic curves for the effect of the dose of a biocontrol agent on pathogen density or disease response (2,9,13; Fig. 6A-C). If the dependent variables in Figure 6 are scaled proportionately,



**Fig. 6.** Dose-response relationships describing the effect of three biological control agents on disease or pathogen populations. **A.** Effect of *Pseudomonas fluorescens* strain 2-79RN<sub>10</sub> applied to wheat seed on number of take-all lesions induced by *Gaeumannomyces graminis* var. *tritici* on roots of wheat seedlings; data were taken from Figure 2 of Bull et al (9). **B.** Effect of *Sporidesmium sclerotivorum* applied onto diseased lettuce plants on subsequent survival of sclerotia of *Sclerotinia minor* in soil; data were taken from Figure 1 of Adams and Fravel (2). **C.** Effect of *Trichoderma harzianum* grown on wheat bran and incorporated into soil on damping-off of eggplant caused by *Rhizoctonia solani*; data were taken from Figure 2 of Hadar et al (13).

estimates of the asymptote parameter,  $A$ , range from 0.76 (Fig. 6A) to 0.86 (Fig. 6B). The study of Bull et al (9) and of Adams and Fravel (2) each had the various doses of the biological control agent arranged on a logarithmic scale (Fig. 6A-B), whereas the study of Hadar et al (13) scaled the doses of *Trichoderma harzianum* arithmetically (Fig. 6C). Based on the curves shown in Figure 6, experiments designed to have both arithmetic and logarithmic scaling of the dose of the biological control agent would probably result in the most precise estimations of  $A$  and  $c$ .

**Conclusions.** The dose-response relationship (model) proposed in this letter (equation 6) states that the degree of disease control obtained with a biological agent depends on the density of the agent, the density of the pathogen, how efficiently individual units of the agent render units of the pathogen ineffective, and on the proportion of the pathogen population that is potentially affected by the agent. In addition, the model proposes a conceptual framework within which these factors interact and sets expectations for disease control in systems in which the parameter values have been determined. Evaluation of the dose-response model with empirical data suggested that the concepts contained within equation 6 are valid for analyzing the efficacy of biological control, and this analysis demonstrated graphic tools that aid estimation of model parameters. A sensitivity analysis of the model highlighted the importance of the asymptote parameter,  $A$ , which determines the proportion of pathogenic inoculum potentially affected by a biological control agent. The magnitude of  $A$  greatly influenced expected disease control, particularly at moderate-to-high inoculum doses of the pathogen. Further research is needed on various biological control systems to verify the dose-response relationships outlined in this letter and to determine the commonality of asymptotes that limit the efficacy of biological control agents and the factors that determine their magnitude.

#### LITERATURE CITED

- Adams, P. B. 1990. The potential of mycoparasites for biological control of plant diseases. *Annu. Rev. Phytopathol.* 28:59-72.
- Adams, P. B., and Fravel, D. R. 1990. Economical biological control of *Sclerotinia* lettuce drop by *Sporidesmium sclerotivorum*. *Phytopathology* 80:1120-1124.
- Analitis, S. 1973. Zur Methodik der Analyse von Epidemien dargestellt am Apfelschorf (*Venturia inequalis* (Cooke) Aderh.). *Acta Phytomed.* 1:1-75.
- Baker, R. 1978. Inoculum potential. Pages 137-157 in: *Plant Disease: An Advanced Treatise*, vol. 2. J. G. Horsfall, Jr. and E. B. Cowling, eds. Academic Press, New York.
- Baker, R. 1990. An overview of current and future strategies and models for biological control. Pages 375-388 in: *Biological Control of Soil-borne Plant Pathogens*. D. Hornby, ed. CAB International, London.
- Baker, R. 1991. Four horses of biological control. Pages 1-16 in: *Biological Control of Plant Disease*. Miscellaneous Publication. 72-1991, Minnesota Agricultural Experiment Station, St. Paul.
- Barclay, H. J. 1992. Modelling the effects of population aggregation on the efficacy of insect pest control. *Res. Popul. Ecol.* 34:131-141.
- Berger, R. D. 1981. Comparison of the gompertz and logistic equations to describe plant disease progress. *Phytopathology* 71:716-719.
- Bull, C. T., Weller, D. M., and Thomashow, L. S. 1991. Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. *Phytopathology* 81:954-959.
- Colwell, R. K., and Fuentes, E. R. 1975. Experimental studies of the niche. *Annu. Rev. Ecol. Syst.* 6:281-310.
- Cook, R. J., and Baker, K. F. 1983. *The Nature and Practice of Biological Control of Plant Pathogens*. American Phytopathological Society, St. Paul, MN.
- Gregory, P. H. 1948. The multiple infection transformation. *Ann. Appl. Biol.* 35:412-417.
- Hadar, Y., Chet, I., and Henis, Y. 1979. Biological control of *Rhizoctonia solani* damping-off with wheat bran culture of *Trichoderma harzianum*. *Phytopathology* 69:64-68.
- Hawkins, B. A., Thomas, M. B., Hochberg, M. E. 1993. Refuge theory and biological control. *Science* 262:1429-1432.
- Kranz, J. 1977. A study on the maximum severity in plant diseases. Pages 169-173 in: *Travaux dédiés à G. Viennot-Bourgin*, Paris.

16. Loper, J. E. 1990. Molecular and biochemical basis for activities of biological control agents: The role of siderophores. Pages 735-747 in: *New Directions in Biological Control*. R. Baker and P. E. Dunn, eds. Alan R. Liss, Inc., New York.
17. Mandeel, Q., and Baker, R. 1991. Mechanisms involved in biological control of *Fusarium* wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum*. *Phytopathology* 81:462-469.
18. Neher, D. A., and Campbell, C. L. 1992. Underestimation of disease progress rates with the logistic, monomolecular, and gompertz models when maximum disease intensity is less than 100 percent. *Phytopathology* 82:811-814.
19. Park, E. W., and Lim, S. M. 1985. Empirical estimation of the asymptotes of disease progress curves and the use of the Richards generalized rate parameters for describing disease progress. *Phytopathology* 75:786-791.
20. Paulitz, T. C. 1990. Biochemical and ecological aspects of competition in biological control. Pages 713-724 in: *New Directions in Biological Control*. R. Baker and P. E. Dunn, eds. Alan R. Liss, Inc., New York.
21. Van der Plank, J. E. 1975. *Principles of Plant Infection*. Academic Press, New York.
22. Weller, D. M., and Thomashow, L. S. 1990. Antibiotics: Evidence for their production and site where they are produced. Pages 703-711 in: *New Directions in Biological Control*. R. Baker and P. E. Dunn, eds. Alan R. Liss, Inc., New York.