Analysis of Monocyclic Pathosystems with *Erwinia-Lycopersicon* as the Model

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ABSTRACT

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The progress of soft rot in tomatoes (*Lycopersicon esculentum*) following a single inoculation with *Erwinia carotovora* subsp. *carotovora* (Ecc) was typical of simple interest disease. The progress curves and curve segments were fit well by the monomolecular model. The curves were also fit to the Weibull model; >63% of the curves had values for the scale parameter near the idealized for simple interest disease (c = 1.0). Different inoculum concentrations, cultivar resistances, host maturities, storage temperatures, and predisposition affected length of latency, rate of disease development, and graphically asymptotic maximum disease. In nature, disease caused on tomato by Ecc may be of the polycyclic compound interest type, but in monocyclic experimentation the progress curves and responses to infection stimuli were like those of simple-interest-type plant diseases. Since the Ecc-tomato pathosystem is easy to handle and has rapid symptom expression, it could be a versatile, general model system to examine pathosystem interrelationships for simple interest diseases.

Additional key words: epidemic analysis.

In the investigation of plant disease, researchers frequently conduct single disease cycle (monocyclic) experimentation (26) to examine specific components of the pathosystem. The disease is then assessed either at a single point in time, or in some cases, the assessments are made at a series of time steps. Epidemiologically, these monocyclic tests can be likened to the development of simple interest diseases (root rots, wilts, damping-off, etc.) sensu Vanderplank (25). The progress curves for such monocyclic tests of compound interest diseases range from monomolecular (8) to asymmetrically sigmoidal (8,22). In natural pathosystems, some compound interest diseases proceed by a series of monocyclic occurrences. The progress curve then may have the appearance of a monocyclic disease (1,18,20). The progress of simple interest disease over time has not received the attention that has been given to compound interest diseases. Many of the simple interest diseases develop so slowly that specific advances and declines in disease progress cannot always be directly associated with the causal changes in environmental conditions or host susceptibility. A simulation model has been developed to test these relationships (9). If disease development from a single inoculation is indeed comparable to the progress of simple interest disease, then a rapidly developing disease could serve as a useful model for the simple interest type. In this sense, we report studies of the progress of monocyclic infection of tomato fruit by *Erwinia carotovora* subsp. *carotovora* (Jones, 1901) Bergey et al 1923 (Ecc).

MATERIALS AND METHODS

Tomatoes. Hand-harvested tomatoes (*Lycopersicon esculentum* L.) grown in Florida were inoculated at various stages of ripeness, which was controlled by selection of fruit with the desired color characteristics. Lesions rarely resulted from wounding unwashed fruit with sterile needles; natural populations of soft rot bacteria were either absent, or in such low number that they were inconsequential.

Inocula. Florida isolates of Ecc strains SR-1 (4) or SR-12 (7) were grown in nutrient broth as shake cultures for 24 hr at room temperature (~26 C). The bacteria were harvested by low-speed centrifugation. The resulting pellets were suspended as described previously (4,7). Appropriate dilutions were made from the stock suspensions to prepare the inoculum for a given test.

Inoculation technique. Each fruit was wound-inoculated in four punctures in each of four locations by a four-pin instrument that was dipped into a dilute suspension of Ecc (ranging from 10^2 to 10^9 cells per milliliter) before being thrust into the fruit. The pins were 2–3 mm apart and protruded by ~2 mm; each pin formed a wound ~1.6 mm^3 in volume. This technique has resulted in uniform deposition of bacteria in the puncture (3). The fruits being tested were arranged on plastic trays in a single layer with 30 fruits per tray, inoculated and stored at high humidity (>80%) and at temperatures ranging 12.7 to 30 C.

Observations. At each observation, the number of fruits with lesions was recorded and the affected fruits were discarded to avoid sources for secondary spread. Lesions at positions other than at the wound site, or clearly caused by fungi, were disregarded. The latent period was the time elapsed between inoculation and the appearance of symptoms on one or more fruit. Since bacteria would then be present on the lesion surface and could be disseminated, this definition of latent period is similar to that of Zadoks and Schein (27) and Bartz (3). The incidence values represent the proportion of available fruits that were affected with bacterial soft rot in at least one of the four punctures at each inoculated wound site. Because of the rapid and confluent decay, accurate determination of the number of punctures with rot at the same wound site was not possible. The rapid decay also precluded evaluation by severity. Rot development following inoculations over fruit locules was more rapid than if inoculum was placed over interlocular walls. Thus, disease development in groups of fruit with the chance occurrence of predominantly locular inoculations would have been falsely interpreted as more severe. Rates of lesion area enlargement (7) or volume of rotted tissue (J. A. Bartz, *unpublished*) have not been useful criteria for determining treatment differences within this pathosystem.

Fruit treatments. In some tests, several factors were examined for possible predisposing effects. Mature-green fruit were chilled in 7 C storage for 5 days. Previously, this treatment increased the susceptibility of tomatoes to bacterial soft rot (4). An aqueous spray of 800 ppm a.i. ethrel ([2-chloroethyl] phosphonic acid) (Amchem Products, Inc., Ambler, PA 19002), which stimulates postharvest ripening of harvested fruit by decomposing to ethylene, was applied to the plants about 10 days before the anticipated date of harvest.

Curve analysis. Disease progress curves were fit to both the monomolecular (simple interest) model and the Weibull model. The monomolecular model is $y = 1 - \exp(-kt)$, in which y =

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disease proportion in the range $0 < y < 1$, k = rate, and t = time (1,16). The Weibull model is $y = 1 - \exp\{-[(t-a)k]^c\}$ in which y, k, and t are as above, a is a position parameter, and c is a shape parameter (15,24). The Weibull model is frequently expressed as $y = 1 - \exp\{-[(t-a)/b]^c\}$ (15,17,19) with the scale parameter b as the inverse of rate. In our Weibull equation above, $k = 1/b$. The Weibull model has been used to differentiate epidemiological types based on the variation in the shape parameter (c) from 1.0 for simple interest diseases to 3.6 for compound interest diseases (19,23). Considerable variation in the shape parameter for epidemic curves has been reported (10,11). Because parameter estimation frequently is difficult when there are three unknowns (23, and R. D. Berger, *unpublished*), the t values were also rescaled to make the scale parameter, a, equal zero. Estimation of the two remaining parameters (k and c) then was more accurate in the reduced Weibull model equation [$y = 1 - \exp(-(kt)^c)$]. The curve fitting was done by using the least squares program for nonlinear models (NLIN procedure of SAS [2]). Computing was done at the Northeast Regional Data Center of the State University System of Florida, Gainesville.

TABLE 1. *Erwinia carotovora* var. *carotovora* on tomato fruit. Parameters for disease progress curves fit to two growth models

	Model				
	Monomolecular		Weibull		
Cultivar/treatment[a]	k[b]	RSS[c]	c[d]	k[d]	RSS
Florida MH-1/1×10^9	0.16	0.03	1.01	0.16	0.06
Florida MH-1/1×10^8	0.14	0.03	1.01	0.14	0.04
Florida MH-1/1×10^7	0.03	0.01	1.04	0.03	0.01
Floradel/1×10^8	0.25	0.001	0.99	0.25	0.001
Floradel/1×10^6	0.17	0.01	1.01	0.17	0.012
Floradel/1×10^4	0.09	0.007	1.35	0.09	0.004
Floradel/1×10^2	0.01	0.001	1.01	0.01	0.001
Florida MH-1/30 C	0.28	0.06	1.38	0.23	0.03
Florida MH-1/21 C days 0–14	0.06	0.04	1.28	0.05	0.04
Florida MH-1/21 C days 6–14	0.07	0.007	1.01	0.08	0.007
Florida MH-1/12.8 C days 8–14	0.08	0.005	1.00	0.08	0.006
Walter/1×10^6	0.14	0.03	1.03	0.14	0.03
Florida MH-1/1×10^6	0.13	0.05	1.58	0.13	0.005
Homestead/1×10^6	0.09	0.03	1.76	0.10	0.004
Walter/red	0.29	0.004	1.11	0.28	0.002
Walter/pink	0.20	0.01	1.13	0.20	0.008
Walter/green	0.06	0.005	0.93	0.06	0.006
Walter/ethrel/days 2–17	0.09	0.07	1.93	0.10	0.03
Walter/no ethrel/days 2–17	0.03	0.03	1.11	0.03	0.03
Walter/ethrel/days 2–10	0.04	0.0001	0.60	0.02	<0.001
Walter/no ethrel/days 2–10	0.004	<0.0001	1.00	0.01	0.001
Walter/ethrel/days 10–17	0.19	0.008	1.36	0.19	0.005
Walter/no ethrel/days 10–17	0.07	0.003	1.64	0.07	0.001
Walter/chilled	0.11	0.01	1.02	0.11	0.01
Walter/not chilled	0.13	0.02	1.04	0.13	0.02
Homestead/chilled	0.09	0.02	0.85	0.10	0.02
Homestead/not chilled	0.06	0.03	1.93	0.06	0.01
Simple interest-idealized[e]	0.20	0.00	1.00	0.20	0.00
Compound interest-idealized[f]	0.096	0.376	4.64	0.05	0.003

[a] Inoculum concentrations, storage temperatures, fruit maturity, fruit treatment, or curve segment.
[b] Rate parameter from the fit to the model: $y = 1 - \exp(-kt)$, in which y is disease proportion in the range $0 < y < 1$, t = time, and k = rate.
[c] Residual sum of squares from curve fit to the respective model using the NLIN procedure of SAS (2).
[d] Parameters from the fit to the model: $y = 1 - \exp(-((t-a)k)^c)$, in which y = disease proportion, t = time, a = position parameter, c = shape parameter, and k = rate.
[e] Curve generated from the model: $y = 1 - \exp(-kt)$, in which $k = 0.2$.
[f] Curve generated from the logistic model: $y = 1/(1 + B\exp(-kt))$, in which $B = 1/y_0$ ($y_0 = 0.001$), and $k = 0.4$.

RESULTS

General curve shapes. Disease progress curves for the many tests had several shapes: monomolecular growth (inverted J-shape), bimodal monomolecular growth, nearly linear, and asymmetrical sigmoid. Since most of the curves resembled the monomolecular growth for a major portion of their run, this curve shape was assumed to represent the generalized progression of the disease. The curves (or their major segments) fit the monomolecular model; ie, all curves had residual sums of squares <0.07 (Table 1). For the 27 curves and curve segments, the shape parameter of the Weibull model was in the range $0.6 < c < 1.9$. For this parameter, 63% of the curves had an estimated $c = 1.0 \pm 0.15$ and 85% of the curves had $c = 1.0 \pm 0.5$. These estimates were close to the idealized Weibull parameter value ($c = 1.0$) for simple interest disease. A progress curve was generated with the monomolecular model [$k = 0.2$] and this curve was then fitted to the Weibull model. A shape value of $c = 1.0$ was estimated for the generated monomolecular curve. Understandably, the fit of this curve to the Weibull model was

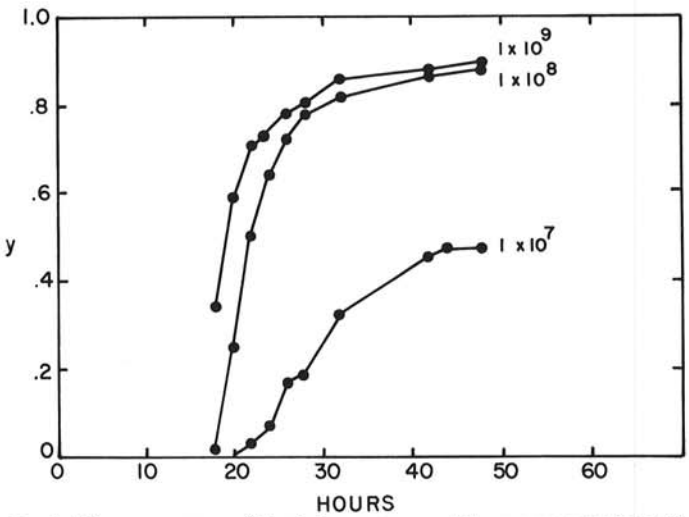

Fig. 1. Disease progress of *Erwinia carotovora* at three concentrations of inoculum on Walter tomato fruit.

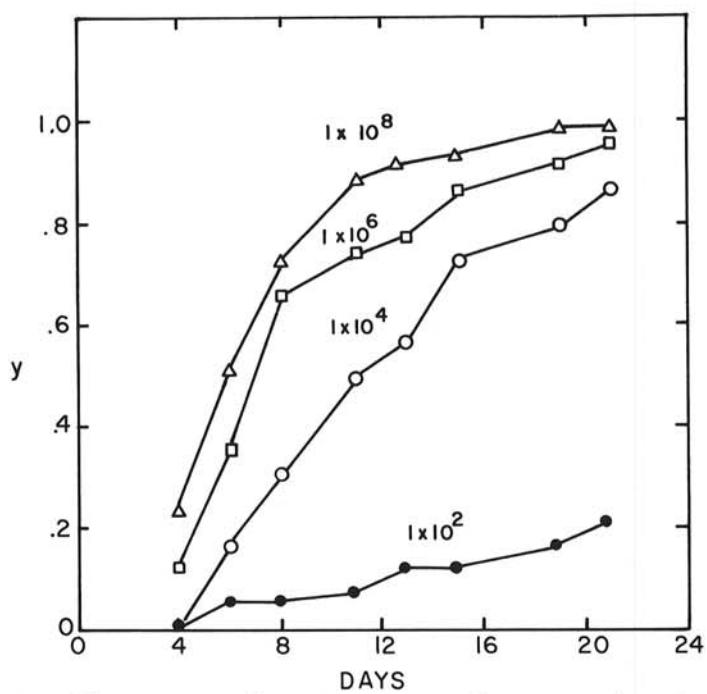

Fig. 2. Disease progress of *Erwinia carotovora* at four concentrations of inoculum on Floradel tomato fruit.

perfect. With $c = 1.0$ and $a = 0$, the Weibull model equation is reduced to the monomolecular model equation.

A progress curve was also generated with the logistic model [$y = 1/(1 + B\exp(-kt))$; $k = 0.4$] and fitted to both the monomolecular and Weibull models. The fit of the sigmoidal logistic curve to the monomolecular model was poor (high residual sums of squares). The fit of the logistic curve to the Weibull model was good (low residual sums of squares) and a shape value of $c = 4.64$ was estimated.

Based upon the goodness of fit of most curves to the monomolecular model and to the idealized Weibull shape parameter and the avoidance of secondary spread of disease in the experimentation, the monocyclic disease development of Ecc on tomato was considered to be simple interest type.

Inoculum concentration. As the concentration of bacteria in the initial inoculum increased, three major effects occurred in progress of the disease that developed: the latent period became shorter; the rate of disease progress increased; and the upper asymptote was higher (Table 1, Figs. 1 and 2). The disease progress curves for all inoculum concentrations in the several tests were characteristic of the monomolecular type.

Cultivar resistance. The disease progress curves for cultivars with differential resistance were similar to those for differing inoculum concentration. That is, with increased cultivar resistance (Walter = susceptible, Florida MH-1 = intermediate, and Homestead-24 = resistant) the latent period was 1–2 days longer, rates were slower, and the curves approached a lower asymptote (Table 1, Fig. 3).

Host maturity. The differences in host maturity (ripeness) at the time of inoculation also affected the disease progress (Table 1, Fig. 4). Fruits that were red (ripe) when inoculated were the most susceptible, showing the most rapid rate and highest asymptote. Fruits that were green at inoculation had the least rapid rate and the lowest asymptote. Pink fruits were intermediate between red and green fruit for both parameters. The effect of fruit ripeness on latent period was not characterized because disease incidence was not recorded until rot had begun in all treatments.

Temperature. The temperature at which inoculated fruits were stored greatly affected the disease progress (Fig. 5). Symptoms appeared early (<24 hr) in fruit stored at high temperature (30 C), the disease had a fast rate of increase, and reached 100% incidence in 11 days. Fruit stored at cooler temperatures (21 and 12.8 C) had delayed initial symptoms (4 and 8 days, respectively), slower rates of increase, and had not approached an asymptote even after 20 days.

Predisposition. Exposure of plants or their parts to certain environmental conditions may predispose them to disease. The preinoculation treatment of tomato plants with ethrel affected postharvest disease progress very much like natural ripening. Inoculated green fruits from plants that received a preharvest ethrel treatment had a shorter latent period, a more rapid rate of disease increase, and higher asymptote than fruits that were not treated (Fig. 6). The resultant curve was interpreted as being bimodal monomolecular.

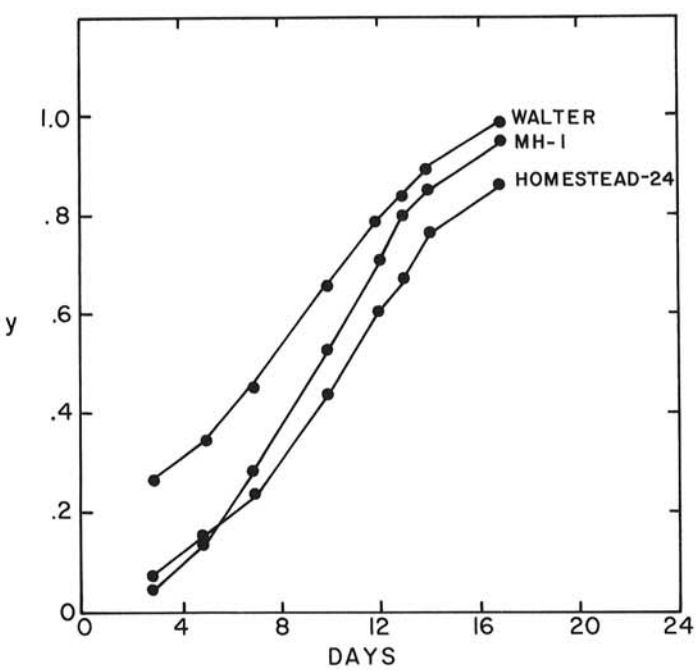

Fig. 3. Disease progress of *Erwinia carotovora* on fruit of three tomato cultivars. Values are the average of three concentrations of inoculum (10^5, 10^6, and 10^7 cells per milliliter) and three tests.

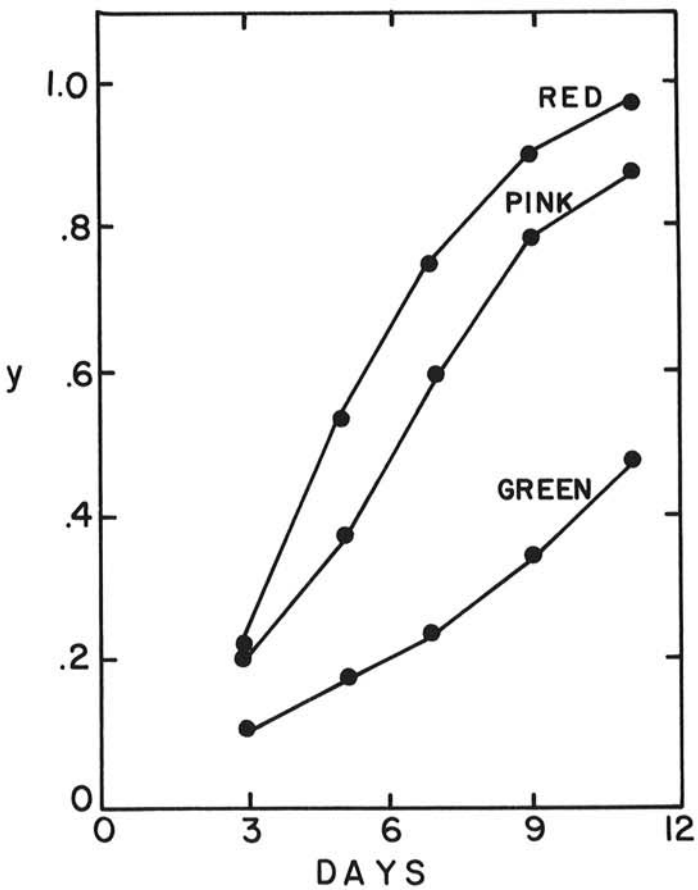

Fig. 4. Disease progress of *Erwinia carotovora* on fruit of three maturities at time of inoculation. Values are the average of four tests.

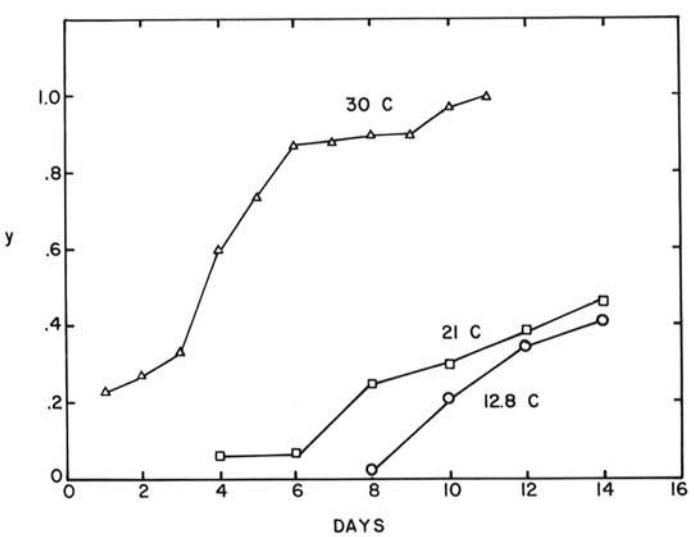

Fig. 5. Disease progress of *Erwinia carotovora* on Florida MH-1 tomato fruit stored at three temperatures.

The exposure of tomato fruit to chilling temperature of 7 C for 5 days predisposed them to bacterial soft rot. Inoculated, chilled fruits had a shorter latent period (2 vs 3 days) than fruits that were not chilled (Fig. 7).

Susceptibility changes in time. Fruits that were green at inoculation gradually ripened over time. The susceptibility of these fruit to soft rot then increased with time. Thus, disease reached an apparent low asymptote ($y_1 \cong 0.2$) in green fruit for storage up to $\cong$ 6 days. Disease then increased with longer storage times to reach a higher asymptote ($y_1 \cong 0.8$). The resultant curves (21 C curve in Fig. 5, and the control curve in Fig. 6) for disease were interpreted as bimodal monomolecular growth. The first mode portrayed the response of green fruit to inoculation, the second mode characterized the changes in susceptibility brought on by the ripening process.

DISCUSSION

The monocyclic experimentation with Ecc on tomato fruit resulted in disease progress curves that were fit well by the monomolecular growth model and conform to simple interest disease progress curves as described by Vanderplank (25). This conclusion was reinforced by the goodness of fit of many curves to the idealized shape parameter value ($c = 1.0$) of the Weibull model for simple interest diseases (19). The progress curves of simple and compound interest diseases in monocyclic experimentation are, in reality, the curves of differential latent periods. Not all of the host units have initial symptom expression at the same instant in time. Therefore, the progress curve is the expression of the variability among host units during latent periods of disease. The length of latent period for each host unit is determined by the conditions of the experiment and the biological variation among hosts. In contrast, the development of soilborne diseases such as root rots, wilts, and damping-off in the field are a combination of the differential latent periods and differential times of infection. Often, the infection of individual hosts in the field may take place over an extended time period as growing roots encounter inoculum in the soil. Progress curves of these latter diseases are also monomolecular (25) although other curve shapes are sometimes found (10,11).

Our fit of a logistic-generated sigmoid curve (compound interest) to the Weibull model yielded a shape value of $c = 4.64$. This is somewhat higher than the value ($c = 3.6$) reported by Pennypacker et al (19) for such curves. The Weibull shape value for sigmoidal curves varies with initial values (y_o), maximum values (y_{max}), and rated (k) (Berger, *unpublished*).

A generalized progress model for disease in monocyclic experiments and for simple interest disease is proposed (Fig. 8) in which three major epidemic parameters are affected by varying the components in the pathosystem. Components such as increased inoculum concentration, predisposition, varietal susceptibility, host maturity, more favorable environment (including lack of antagonism), and pathogen aggressiveness can shorten the latent period, increase the rate of disease progress, and cause the disease progress curve to approach a higher asymptote. Evidence to support the realism of this generalized model for several simple interest diseases exists in the literature (12–14, 21).

A mathematical model could be derived to quantify the interactions between components in the system. Such a model also must consider the inherent variability that exists in seemingly similar source material because of host nutrition (6), subtle differences in host maturity (5), resistance (4), and predisposition resulting from certain harvesting and postharvest handling practices. In the Ecc-Lycopersicon pathosystem, a base value of probable susceptibility can be gauged by inoculating a small test sample with a high inoculum concentration ($\geqslant 10^9$ cells per milliliter). Results can be obtained in 24–48 hr and these responses can be used to establish the treatment levels needed to test the interactions. The Ecc-Lycopersicon system is easily handled by routine laboratory methods. Thus, this pathosystem can serve as an efficient model to define component interactions for simple interest diseases.

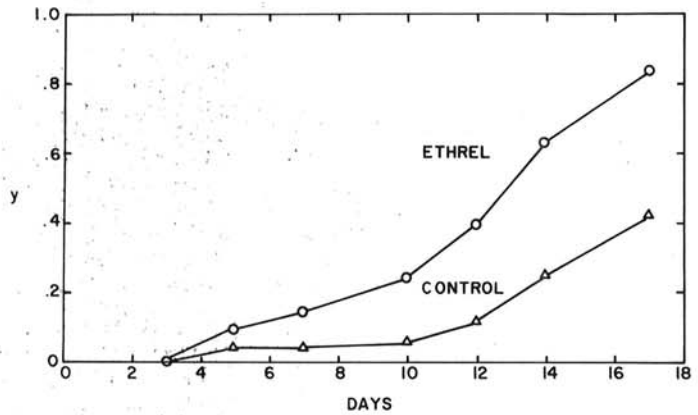

Fig. 6. Disease progress of *Erwinia carotovora* on Walter tomato fruit following preharvest treatment of plants with ethrel.

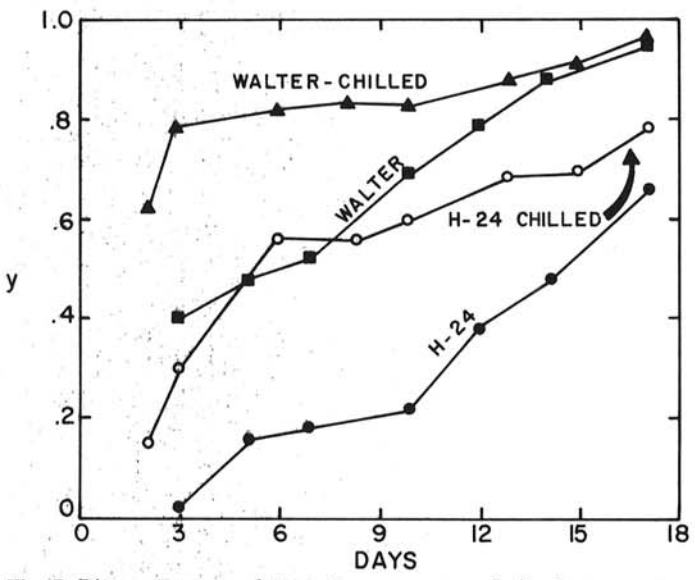

Fig. 7. Disease progress of *Erwinia carotovora* on fruit of two tomato cultivars (Walter and Homestead-24) following chilling at 7 C for five days. Values are the average of three inoculum concentrations (10^5, 10^6, and 10^7 cells per milliliter).

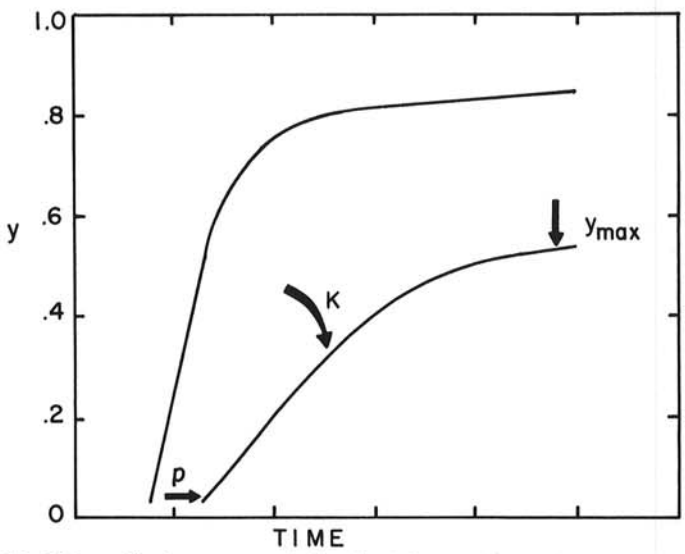

Fig. 8. Generalized progress model for simple interest disease (upper curve). Latent period (p) is delayed, infection rate (k) is slowed, and the asymptote (y_{max}) is lowered by less favorable conditions of host, pathogen, and environment (lower curve).

LITERATURE CITED

1. Analytis, S. 1973. Zur Methodik der Analyse von Epidemien dargestellt am Apfelschorf (*Venturia inaequalis* (Cooke) Aderh.) Acta Phytomed. 1:1-76.
2. Barr, A. J., Goodnight, J. H., Sall, J. P., and Helwig, J. T. 1976. A User's Guide to SAS 76. (Statistical Analysis Systems) Institute Inc., Raleigh, NC. 329 pp.
3. Bartz, J. A. 1981. Variation in latent period of bacterial soft rot in tomato fruit. Phytopathology 71:1057-1062.
4. Bartz, J. A., and Crill, J. P. 1972. Tolerance of fruit of different tomato cultivars to soft rot. Phytopathology 62:1085-1088.
5. Bartz, J. A., and Crill, J. P. 1978. The postharvest susceptibility of green, pink, and red tomato fruit to *Erwinia carotovora* var. *carotovora*. Plant Dis. Rep. 62:582-585.
6. Bartz, J. A., Geraldson, C. M., and Crill, J. P. 1979. Nitrogen nutrition of tomato plants and susceptibility of the fruit to bacterial soft rot. Phytopathology 69:163-166.
7. Bartz, J. A., Crill, J. P., and John, C. A. 1975. Inheritance of tolerance to *Erwinia carotovora* in Florida MH-1 tomato. Phytopathology 65:1146-1150.
8. Berger, R. D. 1975. Disease progress in early epidemic stages. Research Report to the Director, Fla. Agric. Exp. Stn., Belle Glade. 49 pp.
9. Bloomberg, W. J. 1979. Model simulations of infection of Douglas-fir seedlings by *Fusarium oxysporum*. Phytopathology 69:1072-1077.
10. Campbell, C. L., Pennypacker, S. P., and Madden, L. V. 1980. Progression dynamics of hypocotyl rot of snapbean. Phytopathology 70:487-494.
11. Campbell, C. L., and Powell, N. T. 1980. Progression of diseases induced by soilborne pathogens: Tobacco black shank. Prot. Ecol. 2:177-182.
12. Chet, I., and Baker, R. 1981. Isolation and biocontrol potential of *Trichoderma hamatum* from soil naturally suppressive to *Rhizoctonia solani*. Phytopathology 71:286-290.
13. Crowe, F. J., Hall, D. H., Greathead, A. S., and Baghott, K. G. 1980. Inoculum density of *Sclerotium cepivorum* and the incidence of white rot of onion and garlic. Phytopathology 70:64-69.
14. Elad, Y., Chet, I., and Katan, J. 1980. *Trichoderma harzianum*: A biocontrol agent effective against *Sclerotium rolfsii* and *Rhizoctonia solani*. Phytopathology 70:119-121.
15. Hastings, N. A. J., and Peacock, J. B. 1975. Statistical Distributions: A Handbook for Students and Practitioners. John Wiley & Sons, New York. 130 pp.
16. Jowett, D., Browning, J. A., and Haning, B. C. 1974. Non-linear disease progress curves. Pages 115–136 in: J. Kranz, ed. Epidemics of Plant Diseases: Mathematical Analysis and Modeling. Springer, New York. 170 pp.
17. Madden, L. V. 1980. Quantification of disease progression. Prot. Ecol. 2:159-176.
18. Pearson, R. C. 1980. Benomyl-resistant strains of *Uncinula necator* on grapes. Plant Dis. 64:677-680.
19. Pennypacker, S. P., Knoble, H. D., Antle, C. E., and Madden, L. V. 1980. A flexible model for studying plant disease progression. Phytopathology 70:232-235.
20. Royce, D. J., and Ries, S. M. 1978. Detection of *Cytospora* species in twig elements of peach and its relation to the incidence of perennial canker. Phytopathology 68:663-667.
21. Scher, F. M., and Baker, R. 1980. Mechanism of biological control in a Fusarium-suppressive soil. Phytopathology 70:412-417.
22. Shaner, G. 1980. Probits for analyzing latent period data in studies of slow rusting resistance. Phytopathology 70:1179-1182.
23. Stack, R. W. 1980. Disease progression in common root rot of spring wheat and barley. Can. J. Plant Pathol. 2:187-193.
24. Stauffer, H. B. 1979. A derivation for the Weibull distribution. J. Theor. Biol. 81:55-63.
25. Vanderplank, J. E. 1963. Plant Diseases: Epidemics and Control. Academic Press, New York. 349 pp.
26. Zadoks, J. C. 1972. Methodology of epidemiological research. Annu. Rev. Phytopathol. 10:253-276.
27. Zadoks, J. C., and Schein, R. D. 1979. Epidemiology and Plant Disease Management. Oxford University Press, New York. 427 pp.