Temporal Analysis of Two Viruses Increasing in the Same Tobacco Fields

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ABSTRACT

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Virus epidemics caused by tobacco etch virus (TEV) and tobacco vein mottling virus (TVMV) were monitored in six experimental fields (about 3,300 plants) from 1983–1985. Fields were arranged in pairs, with one field in each pair treated with insecticides to control aphid colonization. Proportions of plants with symptoms due to infection by TEV (y_1) and TVMV (y_2) were determined at least weekly. Disease progression was quantified by fitting the Lotka-Volterra linked differential equations to the individual virus data and the logistic equation to the combined virus data. The Lotka-Volterra equations, which have parameters representing rates of increase (r_1 and r_2), maximum disease levels (K_1 and K_2), and inhibitory effects of incidence of one virus on the increase of the other (a_{12} and a_{21}), provided excellent fits. The estimated rate of increase for TVMV (r_2) was significantly ($P = 0.05$) different from that of TEV (r_1) in nine cases. Rates ranged from 0.09 to 0.28 per day. Maximum disease levels were as high as 1.0 in some 1984 fields and as low as 0.01 in 1983. Estimated K_1 often was less than or equal to K_2. The competition coefficients were not significantly different from 0 in $> 70\%$ of the epidemics, indicating that neither virus, at the population level, had a consistent inhibitory effect on increase of the other. The logistic equation also precisely described the combined virus data. Except for one field, rates (r) of combined virus increase were between 0.13 and 0.26 per day.

Additional key words: comparative epidemiology, differential equations, disease progress curves, *Nicotiana tabacum*, quantitative epidemiology.

Since the publication of "Plant Diseases: Epidemics and Control" in 1963 (21), many plant virus disease epidemics have been described and compared with the logistic and related models (9,10,19,20). Usually, diseases or viruses are individually considered, i.e., disease intensity due to a single virus (or other pathogen) is modeled as a function of time. In many cases, however, more than one virus disease increases concomitantly over time in a crop. For instance, epidemics caused by both tobacco etch virus (TEV) and tobacco vein mottling virus (TVMV) are common in burley tobacco (*Nicotiana tabacum* L.) (2,13). One could analyze disease progress corresponding to each pathogen separately, but such an approach fails to incorporate the potential inhibitory feedback effects of one disease on another. Separate analyses also neglect the correlation in intensity of the two or more diseases (23).

An alternative to separate analyses is to simultaneously model disease increase due to each virus with a set of linked differential equations (5,12). In ecology, the Lotka-Volterra competition equations have been used for over 50 yr to describe population growth of two or more competing species (11). Essentially an expansion of the logistic model, the Lotka-Volterra equations can be used to analyze epidemics of two or more diseases (5,18). In this study, we explored the use of the Lotka-Volterra equations for analyzing and comparing virus disease epidemics of tobacco, caused by TEV and TVMV (both potyviruses), in Kentucky over a 3-yr period. The logistic model was used to describe the increase of the combined virus incidence. Insecticide treatments were used to achieve differences in vector colonization and, potentially, differences in virus disease dynamics.

MATERIALS AND METHODS

Field data collection. Six field sites were selected on the University of Kentucky "South Farm" near Lexington. Three pairs of plots were established; these were paired for their similarity with regard to exposure, slope and, to the greatest extent possible, surrounding vegetation. The greatest distance between any two

paired plots was 825 m and the least 230 m. Standard procedures used in the cultivation of burley tobacco were followed unless otherwise noted.

Tobacco cultivar Burley 21 was used in 1983 and cultivar Kentucky 14 in 1984 and 1985; these cultivars reacted identically with regard to virus susceptibility, aphid colonization, and as sources of virus for aphid transmission in greenhouse experiments (Pirone, *unpublished*). The first two field pairs (A and B) had 22 rows of 150 plants each, whereas the C field pairs had 50 rows of 60 plants each. Distance between rows was 102 cm and plant spacing was 46 cm, except in 1985 when it was 41 cm due to an error in calibration of the transplanter. Dates of transplanting were 8–13 June 1983; 7 and 8 June 1984; and 23–28 May 1985. Maps of each field were constructed to mark the location of each plant and that of missing plants. All transplanting was completed at least 2 wk before the first TEV or TVMV infected plant was found each season.

One field of each pair was treated with insecticide to suppress colonization by aphids. Disulfoton (Disyston 15 G) was applied at 4.5 kg a.i. per hectare immediately before transplanting. Acephate (Orthene 75% EC) was applied at 0.84 kg a.i. per hectare at 2-wk intervals or more often if there was evidence of the initiation of aphid colonies. Dates of application were: 13 and 24 June, 12 and 27 July, and 10 August 1983; 8 and 22 June, 2, 9, 18, 24, and 31 July, and 13 August 1984; 21 and 28 June, 5, 12, 19, and 30 July, and 9 and 16 August 1985. The insecticide was applied to the fields of each pair on an alternate year basis, i.e., fields treated in 1983 and 1985 were untreated in 1984, and vice versa. Fields treated with insecticide are indicated with an I (e.g., A-I), and those not treated with an N (e.g., A-N).

Aphids were collected from the first (A) pair of fields. Horizontal ermine-lime traps containing a mixture of ethylene glycol and water were placed in the center and near the corners of each field, a total of five in each field. One of the top leaves of a specific plant, usually one immediately adjacent to a trap, was also used for collection of aphids for comparison with the trap catch. Aphids usually were removed from the trap or plant each day, placed in vials containing 95% ethyl alcohol, and identified later.

The first pair of fields was monitored for virus-infected plants approximately three times a week and the others approximately once a week. Plants were marked when symptoms first appeared and the infecting virus was recorded. Symptoms caused by TEV and TVMV are distinctive enough to allow visual discrimination between these viruses and also to distinguish them from the other viruses that sometimes occurred in those plots (tobacco streak virus, tobacco ringspot virus, peanut stunt virus). For the first several weeks of each season, the accuracy of identification was checked by assay of random samples of 10–20 diseased plants with antisera specific to TEV and TVMV. For these small samples there was complete agreement between visual assessments and serology. More extensive testing likely would have indicated a nonzero but very low error rate in detecting single infections. For the balance of the season only visual assessment was used. If plants were infected by both viruses, symptoms only could be observed for the first infection. It was not possible, therefore, to visually determine the number of plants infected by both viruses. Spatial pattern of virus-infected plants in each field was analyzed and will be described in a separate paper.

Data analyses. Let y_i represent the proportion of plants infected by TEV ($i=1$) and TVMV ($i=2$), respectively. The absolute rate of increase of disease then can be represented by dy_i/dt. When both viruses are present in the same field, disease increase can be expressed by the following Lotka-Volterra competition equations:

$$dy_1/dt = r_1 y_1 (K_1 - y_1 - a_{12} y_2)/K_1 \quad (1)$$

$$dy_2/dt = r_2 y_2 (K_2 - y_2 - a_{21} y_1)/K_2 \quad (2)$$

in which: r_i, K_i, and a_{ij} ($i \neq j$) are unknown parameters; r_i represents the rate parameter for TEV ($i=1$) or TVMV ($i=2$); K_i represents the maximum disease incidence of each virus when the other is not present; and a_{ij} is a competition coefficient that represents the inhibitory effect of virus j incidence on the increase of virus i. Both r_1 and r_2 are relative rate parameters. For instance, r_1 expresses the rate at which y_1 increases to K_1, or equivalently, the rate at which y_1/K_1 increases to 1. An absolute rate parameter was obtained by multiplying the relative rate parameter by the maximum disease level parameter producing a scaled version of Richard's weighted mean growth rate (16). Thus, two derived parameters, r_1K_1 and r_2K_2, were calculated for each epidemic. Although the y's were measured as proportions here, they also could be measured as numbers of infected plants, with corresponding scale changes in the K_is. When a_{ij} equals 0, there is no inhibitory effect of virus j on virus i, and the equation for i reduces to the classic Verhulst-Pearl logistic equation (11). If a_{ij} equals 0 and K_i equals 1 (i.e., 100% disease incidence), the logistic equation for i is identical to Vanderplank's (21) model of compound-interest disease. Because the Lotka-Volterra equations are based on the logistic, they have most of the same implicit and explicit assumptions as the logistic equation (12,17).

No general closed-form solutions exist for equations 1 and 2 (12). However, numerical integration can be useful when parameter values are specified. If the right-hand side of equations 1 and 2 are represented by h_1 and h_2, respectively, then y_1 and y_2 at time τ (i.e., $y_{1,\tau}$ and $y_{2,\tau}$) can be written statistically as:

$$y_{1,\tau} = \int_0^{\tau} (h_1\, dt) + \xi_{1,\tau} \quad (3)$$

$$y_{2,\tau} = \int_0^{\tau} (h_2\, dt) + \xi_{2,\tau} \quad (4)$$

in which $\xi_{1,\tau}$ and $\xi_{2,\tau}$ are the error terms, or equivalently, the difference between the observed and expected disease incidence (assuming that equations 1 and 2 are correct) at the τ-th time during an epidemic (23). Integration in equations 3 and 4 was from the time of first symptomed plant ($t=0$) to the τ-th time ($t=\tau$).

We used a nonlinear regression procedure linked to a numerical integrator to fit equations 3 and 4 simultaneously to the tobacco virus disease progression data (14,15). In this BMDP procedure (AR), equations 1 and 2 were numerically integrated using a Runge-Kutta 5-th order algorithm. Initial level of disease incidence ($y_{i,0}$) was specified as being equal to the first nonzero incidence observed in the fields. Initial disease could have been specified as an unknown parameter, but that would have given four parameters per equation to estimate, which we felt were too many given the size of the data sets.

Combined virus disease progress data were analyzed with the integrated logistic model, which can be written statistically as:

$$y_\tau = K/(1+\exp(-(B+rt))) + \xi_\tau \quad (5)$$

in which y_τ is the proportion of plants infected by TEV or TVMV at the τ-th time; ξ_τ is the τ-th error term; r is the rate parameter; K is a parameter representing maximum disease incidence; and B is a parameter equal to $\ln(y_0/(K-y_0))$, with y_0 equal to the initial disease level. Parameters and associated statistics were estimated with BMDPAR (14).

Estimation of parameters with nonlinear models is an iterative procedure that starts with initial estimates, supplied by the investigator, and then iteratively modifies these estimates until convergence is achieved, i.e., the residual sum of square is minimized (1). Parameter boundary conditions (i.e., limits) can be specified to prevent testing unrealistic or meaningless parameter values. Lower limits for the rate parameters and the competition coefficients generally were zero; upper limits for these parameters were set at 3. Lower limits for the maximum disease level parameters were set at the final incidence + 0.0001 and upper limits at 1.0. If one of the parameters failed to converge at a value between its limits, at least two different initial estimates were attempted. After convergence, statistics such as the mean square error (MSE) and the standard deviations and covariances of estimated parameters were calculated. Standard deviations are measures of the precision of estimated parameters, i.e., how well they are determined by the regression procedure, given a certain data set. As with all nonlinear models, these statistics are correct

only asymptotically (1,8). Tests based on these statistics, therefore, are only approximate.

Appropriateness of all nonlinear models was appraised with the above-mentioned statistics, residual plots, and the coefficient of determination adjusted for degrees of freedom (R^2), which is often expressed as the proportion of variance accounted for. The adjusted R^2, although lower than the unadjusted coefficient, allows easy comparison of models with different numbers of parameters. For instance, a direct comparison of the goodness of fit of the pair of Lotka-Volterra equations (six parameters) with the logistic equation (three parameters) can be made with the adjusted R^2.

Relevant comparisons of estimated parameters (e.g., r_1 versus r_2) were made with Student's t tests by pooling their standard deviations (8). If a parameter converged to its upper or lower limit, then that estimate was treated as a constant in the t test. Standard deviations of derived parameters (e.g., r_1K_1) were calculated using asymptotic formulae (6). To control the overall significance level of multiple comparisons, t tests were limited to comparisons of estimated parameters within a field or between fields of a single pair. Tests were conducted at $P = 0.05$.

RESULTS

Lotka-Volterra equations and individual virus diseases. The Lotka-Volterra competition equations provided excellent fits to the observed virus epidemic data (Figs. 1–3). Data for two fields in 1983 could not be fit by equations 3 and 4 because there were very few nonzero incidence values for each virus (Fig. 3). For the remaining fields, adjusted coefficients of determination were always greater than 0.90; 75% were greater than 0.97 (Table 1). All MSE values were less than 0.002. Residual plots (1) generally exhibited a random scatter of points. Parameter estimates varied among fields, among years, and with insecticide treatment.

Rate parameters (r_i) were estimated with the greatest precision. Except for field B-I in 1983, all r_i parameter estimates exceeded their asymptotic standard deviations, indicating that one can place a high degree of confidence in the estimates. The nonlinear regression procedure almost always resulted in the same r_i estimates no matter what initial estimates were used for a given epidemic. The r_i ranged from 0.1 to 0.3 per day except for one field.

The estimated rate of increase for TVMV-infected plants (r_2) was greater than that for TEV (r_1) in five epidemics (Table 1). Three of these fields were in 1985. However, $r_2 < r_1$ in four epidemics, none of which were in 1985. In five epidemics, r_2 exceeded r_1, although not significantly.

The K_i parameters were estimated less precisely than r_i. When an estimated K_i was close to the final disease level, its standard deviation was very small (e.g., K_2 for A-I in 1985; Table 1). When disease incidence for either virus leveled off at the end of an epidemic, the appropriate K_i converged to its lower boundary, which approximately equaled the maximum disease level

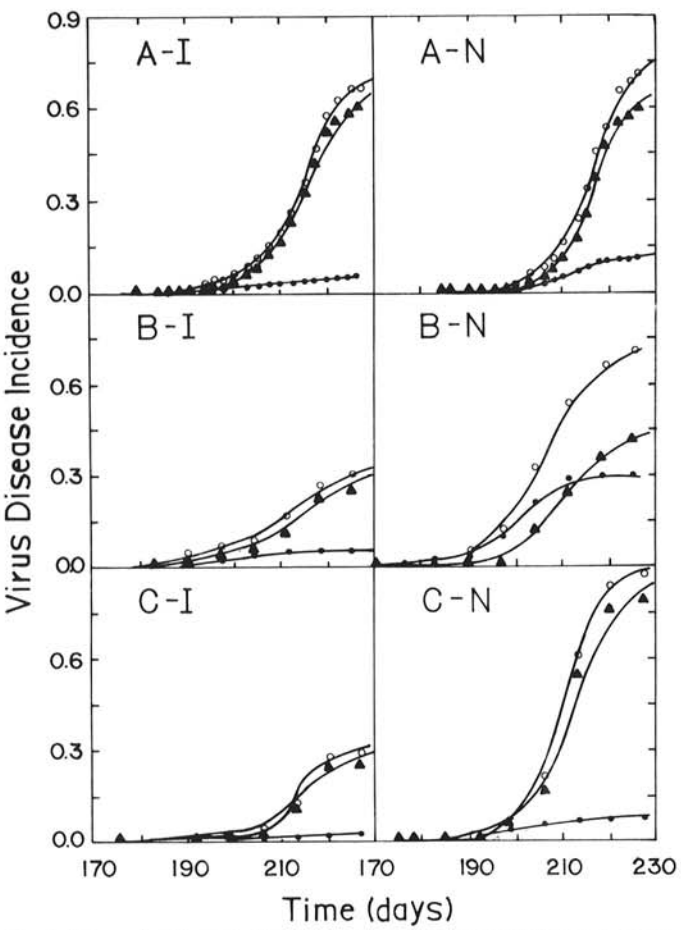

Fig. 1. Disease incidence due to tobacco etch virus (● = TEV), tobacco vein mottling virus (▲ = TVMV), and both viruses together (o) in 1985, together with predicted incidence (solid lines) based on the Lotka-Volterra (Eqs. 3 and 4) and logistic (Eq. 5) models. A, B, and C refer to the three tobacco field pairs; I and N indicate whether the field was treated or not treated with insecticides.

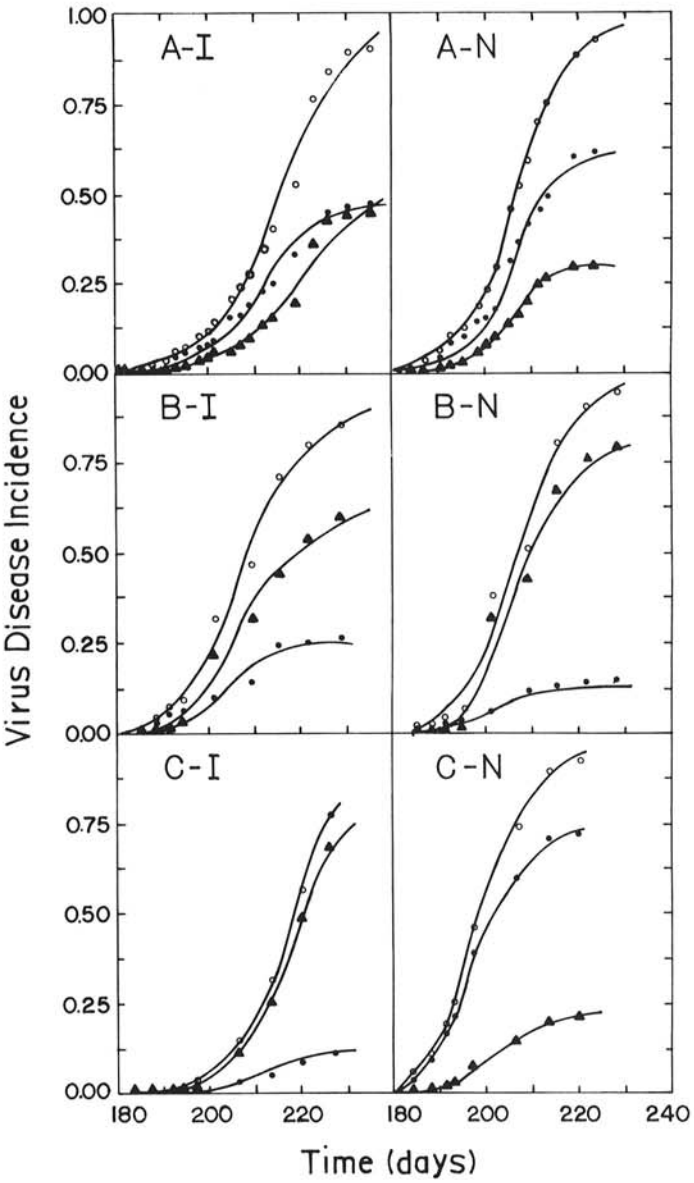

Fig. 2. Disease incidence due to tobacco etch virus (● = TEV), tobacco vein mottling virus (▲ = TVMV), and both viruses together (o) in 1984, together with predicted incidence (solid lines) based on the Lotka-Volterra (Eqs. 3 and 4) and logistic (Eq. 5) models. A, B, and C refer to the three tobacco field pairs; I and N indicate whether the field was treated or not treated with insecticides.

observed. This was especially prevalent in 1984 (e.g., K_2 for A-N and K_1 for B-I). In these cases, no standard deviation can be calculated. Also observed only for 1984, the estimated K_i for some epidemics reached the upper boundary of 1.0 (e.g., K_2 for C-I). Alternate initial estimates for K_i did not change this convergence to the upper limit.

Maximum disease incidence for TVMV (K_2), generally, was greater than that of TEV (K_1). In seven fields the difference was significant, and the trend obvious in another three. Only twice was K_1 significantly larger than K_2, although the trend was seen in another two fields. The 3 yr differed in the overall level of the estimated K_i. The lowest values of K_i were in 1983. The highest maximum for either virus in 1983 was 0.25 (K_2 in B-I). Some K_i were less than 0.05. The highest K_i estimates were in 1984, in which three fields had $K_2 = 1$ and one field with $K_1 = 1$. As expected, when one virus had a maximum of 1, the other virus had a much lower maximum. Even the lowest K_1s in 1984, however, were higher than the largest K_1 in 1983. The last year (1985) was characterized by intermediate estimates for K_1 and K_2. Neither parameter estimate reached its boundary conditions in any field.

Because of the differences between the estimates of K_1 and K_2, differences in the estimated absolute rates r_1K_1 and r_2K_2 did not always agree with differences in r_1 and r_2. In some cases r_2 was greater than r_1 but r_2K_2 equaled r_1K_1 (e.g., B-N in 1985). In other cases, $r_2 = r_1$, yet $r_2K_2 > r_1K_1$ (e.g., C-I in 1984). Low estimated K_i in 1983 resulted in lower estimates of r_iK_i than in the other years, even though r_i was not necessarily lower. In general, large differences in the estimated r_i were obscured if the diseases were approaching very different K_i. Likewise, no differences in the r_i actually could indicate large differences in r_1K_1 or r_2K_2.

Competition coefficents (a_{ij}) were estimated with the least precision. Thirty-seven percent of the estimated a_{ij} reached a boundary of 0 (Table 1). Another 35% were nearly zero, thus indicating the general lack of virus interaction. Setting the lower a_{ij} boundary at -1, instead of 0, for the epidemics that converged to 0 always resulted in estimates that, although negative, were close to and not significantly different from 0 (data not shown).

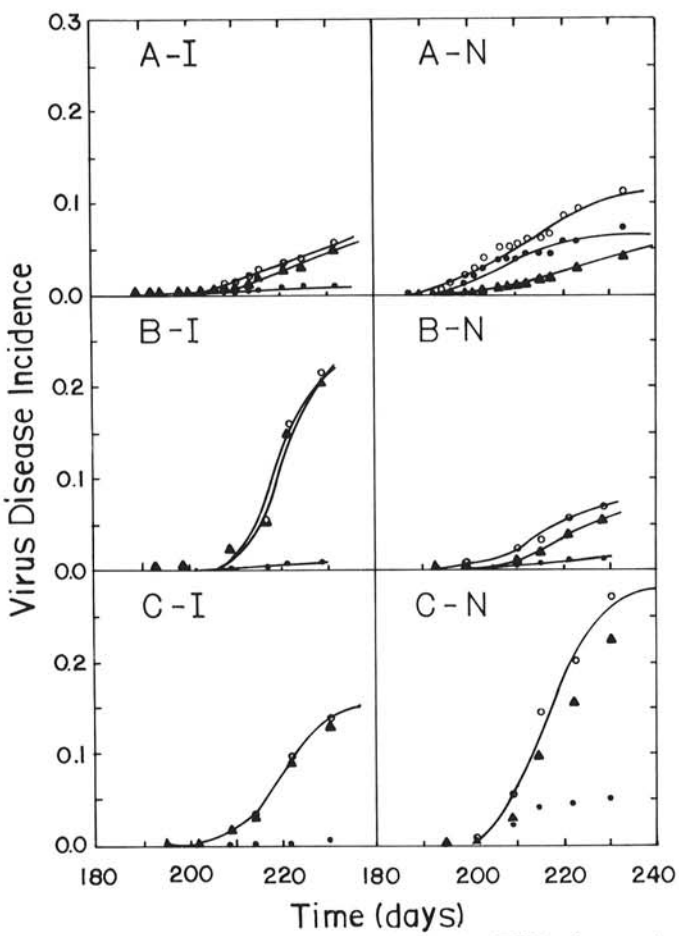

Fig. 3. Disease incidence due to tobacco etch virus (● = TEV), tobacco vein mottling virus (▲ = TVMV), and both viruses together (o) in 1983, together with predicted incidence (solid lines) based on the Lotka-Volterra (Eqs. 3 and 4) and logistic (Eq. 5) models. A, B, and C refer to the three tobacco field pairs; I and N indicate whether the field was treated or not treated with insecticides. It was not possible to fit equations 3 and 4 to the data in the C fields due to the low incidence.

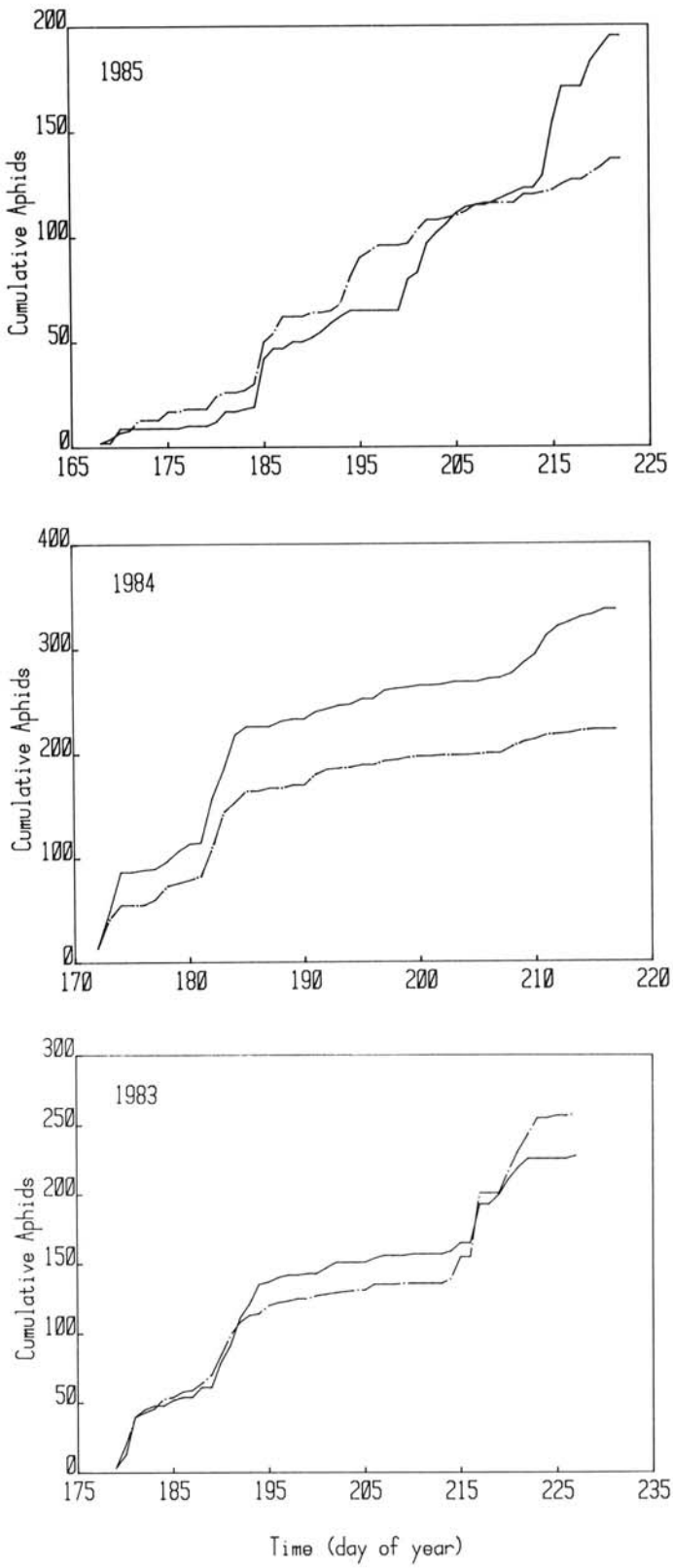

Fig. 4. Cumulative numbers of aphids collected in five horizontal ermine-lime traps placed in A-N (solid line) and A-I (line and dash) from 1983-85.

Logistic equation and combined virus disease. The logistic equation (Eq. 5) provided excellent fits to the combined virus disease incidence data (Figs. 1–3). Adjusted R^2 values were all greater than 0.97; MSE's were less than 0.003 (Table 1). Residual plots, generally, had a random pattern. All parameters were estimated very precisely, i.e., estimates were at least twice the value of their standard deviation.

The estimated r parameters ranged from 0.1 to 0.3 per day, except for field B-I in 1983. Year did not have a substantial overall effect on r estimates. The maximum virus disease levels ranged from a low of 0.07 in 1983 to 1.0 in 1984. In general, 1983 had the lowest estimated K's and 1984 the highest. In 1984, two of the K's reached their boundary of 1.0, and all K's were greater than or equal to ~ 0.90. The estimated K's in 1985 were somewhat less than in 1984 with all values less than 0.90. Differences in the K's resulted in substantial changes in the rK's. For instance, r for C-I and C-N in 1985 were virtually identical, but rK for C-N was about three times larger than C-I.

Insecticide effects and aphid counts. Treating plants with insecticide reduced the rate of disease increase or maximum disease level in some fields but not others (Table 1). In 1985, r_2 and r_2K_2 (TVMV) were reduced by insecticide treatment in all field pairs, but r_1 and r_1K_1 (TEV) were not. K_2 was reduced in the B and C fields but not in A. K_1 was not significantly affected by insecticide treatment. The r for the combined virus epidemics was not reduced in 1985, but K was reduced in the B and C fields, reflecting the reduction in K_2. This reduction of K resulted in reductions of rK in the same two fields. The lack of insecticide effect on epidemics caused by TEV likely interfered in detecting a reduction of r with the combined virus epidemics.

In 1984, r_1, K_1, and r_1K_1 estimates were reduced with insecticides in two of the three fields; r_2 was reduced in two, but r_2K_2 was reduced in only the B field. r_2, K_2, and r_2K_2 increased with insecticide treatments in the C field. With the combined virus data, r and rK were reduced in three and two fields, respectively, by insecticide applications. Although K was significantly higher in two of the insecticide treated fields than the controls, differences were only minor (0.02 and 0.05).

In the low disease year of 1983, only r_1 and r_1K_1 were reduced in one field. With combined virus data, rK was reduced twice but also increased once by insecticide treatment. K_1 and K_2 were not significantly affected by insecticide applications.

Aphid colonization virtually was eliminated by insecticide treatment in the A fields. Daily observation of plants indicated few or no aphid colonies. As expected, however, landings of transitory aphids in both fields were substantial each year (Fig. 4). Only in 1984 was there a substantial difference in total aphids trapped between the two A fields. Interestingly, r_1, r_2, K_1, r_1K_1, r, and rK were higher in the 1984 control field than in the insecticide-treated field. Such large differences in epidemic characteristics were not

TABLE 1. Estimated parameters and associated statistics from fitting the Lotka-Volterra equations to the progression data of tobacco etch and tobacco vein mottling and the logistic equation to the combined virus disease data

	Lotka-Volterra:[a]										Logistic:[b]				
Field[d]	r_1	r_2	K_1	K_2	a_{12}	a_{21}	MSE[c] ($\times 10^5$)	R^{2e}	r_1K_1	r_2K_2	r	K	MSE[c] ($\times 10^5$)	R^{2e}	rK
1985															
A-I	*0.176*	*0.216*	0.064	*0.682**	0.03	(0.00)	15.3	0.987	0.011	*0.147*	*0.216*	*0.727*	35.0	0.994	*0.157*
A-N	*0.196*	*0.243**[f]	*0.126*	*0.664**	0.02	(0.00)	10.3	0.997	*0.025*	*0.161**	*0.240*	*0.770*	27.6	0.996	*0.184*
B-I	*0.087*	*0.164**	0.415	*0.306*	1.81	(0.00)	8.9	0.978	0.036	*0.050*	*0.182*	*0.326*	7.6	0.995	*0.059*
B-N	*0.129*	*0.187**	*0.784*	*0.454**	*1.34*	(0.00)	13.2	0.994	*0.101*	*0.085**	*0.187*	*0.732**	13.0	0.998	*0.136**
C-I	*0.128*	*0.172*	0.035	*0.326*	0.07	(0.00)	18.6	0.922	0.004	*0.056*	*0.264*	*0.304*	20.0	0.987	*0.080*
C-N	*0.140*	*0.202**	0.204	*0.861**	0.17	(0.00)	51.1	0.979	0.029	*0.174**	*0.258*	*0.895**	35.5	0.998	*0.231**
1984															
A-I	*0.196*	*0.155**	(0.470)	*0.510*	0.05	(0.00)	54.7	0.979	*0.092*	*0.079**	*0.136*	(1.00)	75.3	0.993	*0.136*
A-N	*0.246**	*0.280**	(1.00) (*)	(0.300) (*)	*1.42*	0.04	62.0	0.981	*0.246**	*0.084**	*0.171**	*0.976**	14.6	0.999	*0.167**
B-I	*0.207*	*0.209*	(0.270)	(1.00) (*)	0.03	*1.56*	86.9	0.971	*0.056*	*0.209**	*0.150*	*0.898*	116.0	0.990	*0.135*
B-N	*0.223*	*0.261**	0.175	(1.00)*	0.06	*1.67**	172.0	0.971	0.039	*0.261**	*0.176**	*0.962*	288.0	0.982	*0.169*
C-I	*0.167*	*0.183*	(0.115)	(1.00) (*)	0.01	*1.43**	5.9	0.999	*0.019*	*0.183**	*0.155*	(1.00)	7.7	0.999	*0.155*
C-N	*0.200**	*0.159**	*0.722**	*0.232**	(0.00)	(0.00)*	8.7	0.999	*0.144**	*0.037**	*0.187**	*0.950**	16.0	0.999	*0.178**
1983															
A-I	*0.135*	*0.180**	*0.008*	(0.055)*	(0.00)	*1.43**	0.2	0.941	*0.001*	*0.010**	*0.138*	*0.071*	0.6	0.983	*0.010*
A-N	*0.235**	*0.182**	(0.071)	0.075	*0.32**	0.39	2.6	0.979	*0.017**	0.014	*0.130*	*0.121**	2.6	0.980	*0.016**
B-I	0.108	*0.220*	0.069	0.252	0.12	(0.00)	18.8	0.994	0.007	0.055	*0.388*	*0.223*	13.5	0.983	*0.086*
B-N	*0.225*	*0.155**	(0.015)	*0.076**	*0.07*	(0.00)*	0.2	0.906	*0.003*	*0.012**	*0.150**	*0.085**	0.3	0.995	*0.013**
C-I	...[g]	...	...	...	...	...	...	...	...	...	*0.206*	*0.147*	0.6	0.998	*0.030*
C-N	...	...	...	...	...	...	...	...	...	...	*0.186*	*0.284**	29.8	0.975	*0.053**

[a] Lotka-Volterra estimated parameters. r_i represents the rate parameter (in units per day) for tobacco etch virus (TEV; $i = 1$) or tobacco vein mottling virus (TVMV; $i = 2$); K_i represents the maximum disease incidence of each virus when the other is *not* present; and a_{ij} is a competition coefficient that represents the inhibitory effect of virus j on the increase of virus i. See equations 1 and 2 in text for a listing of the model.

[b] Logistic estimated parameters: r is a rate parameter (in units per day); and K represents the maximum disease incidence for the combined virus data. See equation 5 in text for a listing of the model.

[c] Mean square error for the agreement between observed and predicted disease incidence.

[d] I: Field treated with insecticides. N: Field not treatment with insecticide.

[e] Coefficient of determination adjusted for degrees of freedom for the agreement between observed and predicted disease incidence.

[f] Estimated parameters *followed* by an asterisk (*) indicate that the two viruses (1 and 2) are significantly different from each other ($P = 0.05$); estimates with an * *below* the value indicate that the corresponding parameters for the pair of fields (e.g., A-I and A-N) are significantly different from each other ($P = 0.05$); italicized values are greater than their asymptotic standard deviations. Values in parentheses converged to a boundary and are treated as constants.

[g] Insufficient nonzero data points to fit the Lotka-Volterra equations.

observed in the A fields during the other 2 yr in which aphid landings were similar.

DISCUSSION

Despite the many studies on the spread of virus diseases into or within experimental fields, "results obtained have seldom been analyzed in sufficient detail to obtain the maximum possible information on the various factors influencing the sequence and rates of spread observed" (20). We have been able to quantify with high precision (as measured by R^2 and MSE) combined virus disease progression with the logistic equation and individual virus disease progression with the Lotka-Volterra equations. These models were used successfully for years with low maximum virus incidence (K_1, K_2, and K) (1983) and very high incidence (1984). Rates at which the viruses approached their maximum (r_1, r_2, and r) were remarkably stable over these years. Multiplying these relative rates by maximum disease levels to achieve absolute rate parameters usually resulted in larger differences among fields or years. There was a slight tendency for r_2 or r_2K_2 to exceed r_1 or r_1K_1, respectively, although no differences or the reverse also were observed. Additionally, insecticide treatments often reduced these rates, but no differences or increases in rates also occurred.

The data on aphid counts in the A fields clearly indicated that TEV and TVMV increased in tobacco fields with little or no aphid colonization (A-I). Probing and feeding by transitory aphids were responsible for most of this disease increase. Large differences in aphids between fields resulted in large differences in disease progress (A-I and A-N in 1984). Moreover, slight differences in aphids resulted in only slight differences in disease progress (1983 and 1985). In a separate paper, disease incidence will be modeled as a function of numbers of individual aphid species.

The BMDPAR procedure was used successfully to fit the Lotka-Volterra equations to the individual virus progression data. The iterative nonlinear procedure always converged when there were five or more nonzero values for each virus. The main difficulty with the analysis was the high standard deviations that sometimes were calculated for estimates of a_{ij}. Reestimating parameters with new initial values usually did not change the resulting standard deviations, implying that these parameter estimates were imprecise and not different from zero. Using BMDPAR, it would be easy to incorporate additional unknown parameters such as initial disease incidence into the models. Also, it would be feasible to add a differential equation (with additional parameters) to describe the increase in incidence of plants infected by both viruses (y_{1+2}). This latter analysis depends on the feasibility of identifying plants that are doubly infected. Unfortunately, y_{1+2} could not be estimated with this tobacco potyvirus system. The major constraint to generalizing the Lotka-Volterra equations by adding parameters is the resulting need for disease observations at additional times to permit parameter estimation and model evaluation.

The ecological literature is rich in articles on the population dynamics of organisms that are competing for the same limited resources (11). Numerous papers deal with equilibrium conditions, i.e., population changes around K_1 and K_2 when dy_1/dt and dy_2/dt are near zero. Plant disease epidemics usually are not at equilibrium. In fact, few papers in epidemiology have dealt with equilibrium conditions (4,5,22). Because of the way virus diseases are measured with the tobacco system, dynamic fluctuations around the maximum (K_i) are not possible. Such dynamic fluctuations could be measured and studied with other pathosystems.

Rigorously defined, the Lotka-Volterra equations quantified the increases in symptomatic plants, or equivalently, the increases in plants first infected by each virus. There is no evidence for cross-protection between TEV and TVMV (Pirone, *unpublished*), and tobacco plants generally can be infected by both viruses. The two viruses thus were not competing for the same resource, the plant, at least at the population level. Therefore, one would expect the competition coefficients (a_{ij}) to equal zero. This was found for over 70% of the a_{ij}. Even some relatively large estimated competition coefficients had larger standard deviations, indicating the lack of interaction. Competition could occur when infection by virus i was so severe that the plant was removed from the epidemic. Then inoculation of a plant by virus j, which was previously infected by i, would not contribute to increase in y_j. This would be expected to happen when plants were infected early and for a long time. Interestingly, most of the large a_{ij} were for the 1984 epidemics when disease incidence was the highest of the 3 yr.

The low estimates of a_{ij} in this study suggest that the individual virus disease epidemics could have been described with separate logistic equations. Such a decision, however, could only be made after the a_{ij} were calculated. We did not believe that the additional regressions were necessary. Additionally, separate regressions would have neglected the fact that y_1 and y_2 were simultaneously estimated with potentially correlated error terms (ξ_1 and ξ_2). Such correlation would have resulted in biased parameter estimates (23).

The BMDPAR procedure has many applications to epidemiological modeling and analysis. One can estimate parameters of a single differential equation that does not have an analytic solution. Also, one can fit other sets of linked differential equations, such as the theoretical equations of Jeger (3), to disease progress data. Modeling results then can be evaluated using standard techniques (1, 7–9).

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