Pea Enation Mosaic Virus Transmission by the Pea Aphid: A Multiphase Model of Virus Transmission

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ABSTRACT

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The median latent period (LP_{50}) for the transmission of a virus by an insect vector can be estimated by a log-probit analysis of serial transmission data. This approach assumes acquisition at a given instant during the acquisition access period (AAP), rather than distributed over the total AAP. A model is proposed that uses data from a two-plant readout system. The inoculation access period on the first plant is varied, and on the final plant, it is long enough to have a high probability of including any latent period. The data obtained can be used to estimate the acquisition rate, a minimum latent period, the LP_{50}, and (where applicable) other variable phases of the transmission process (eg, the time of inoculation, if such does not occur rapidly). The method is evaluated by using data on the transmission of the pea enation mosaic virus to and from sweet pea, *Lathyrus odoratus*, by the pea aphid, *Acyrthosiphon pisum*. The results suggest existence of a minimum latent period and that the LP_{50} is dosage sensitive.

The transmission of vectorborne viral plant pathogens is a process with several distinct phases, the nature of which depends upon the vector and the pathogen involved. For example, in the transmission of pea enation mosaic virus (PEMV) by the pea aphid, *Acyrthosiphon pisum* (Harris), three phases can be identified: an acquisition phase in which the aphid ingests virus as it feeds; a latent (or incubation) phase in which the virus transfers from the gut to the salivary gland of the aphid (it is not known whether the virus reproduces during this phase); and an inoculation and retention phase, in which the aphid may inoculate healthy plants on which it feeds as long as the virus is present in the saliva.

Various aspects of PEMV transmission by the pea aphid have been studied in depth by a series of authors, beginning with the early work of Osborn (10) (see Sylvester [14] for a comprehensive list of references to this and similar viruses), and elementary statistical distributions have been used to estimate the parameters of the transmission process. In particular, the latent period (phase) of transmission has been modeled by a log-normal distribution and its median (LP_{50}) taken as an appropriate characteristic of this period (13).

Sylvester (13) obtained experimental data on the latent period by allowing *A. pisum* larvae (average age, 6 hr) to feed on virus source plants (bur clover, *Medicago hispida*, or sweet pea, *Lathyrus odoratus*) for a 12-hr (20 and 30 C) or 24-hr (10 C) acquisition access period (AAP) after which each larva was transferred to a series of disease-free test plants at 12-hr (20 and 30 C) or 24-hr (10 C) intervals (inoculation access period [IAP]) for a minimum of six and a maximum of 10 consecutive transfers. A larva was assumed to have acquired PEMV only if one of the test plants developed symptoms of infection by PEMV. The cumulative percentage of first transmissions was calculated at the end of each IAP for all larvae that acquired virus. The LP_{50} was estimated by using a log-probit analysis (ie, fitting a log-normal distribution) of the data. It also was assumed that whenever acquisition took place, it did so halfway through the AAP, ignoring the fact that acquisition itself can be modeled by a probability density function, which we will denote by $p_a(\cdot)$. In fact, the exponential probability density function

$$p_a(\tau) = \alpha e^{-\alpha\tau} \qquad \tau \geqslant 0 \qquad (1)$$

provides a reasonable fit to the acquisition process, in which the parameter α is interpreted as the acquisition rate (ie, the rate at which individual aphids within a group become infective—see Fig. 2), and τ represents time.

In order to separate the latent phase from the acquisition phase, a model is required that accounts for the fact that vector-virus transmission consists of several different processes. In particular, we will consider a model of the transmission process that has three distinct phases: an acquisition phase, a latent period phase, and an inoculation phase. Additional phases, if relevant, may be included by using analogous arguments.

Model. Consider a vector that is allowed to feed on a virus source for s units of time (ie, the AAP is the time interval $[0,s]$) and then on a disease-free test plant until time t (ie, the IAP is the time interval $[s,t]$). If we choose we may, after a time t, transfer the vector to a second disease-free test plant on which the vector is allowed to feed until we are confident that almost all (eg, 99.9%) of the infective vectors will have transmitted the pathogen to the second test plants. The latter set of test plants can then be used to obtain the number of vectors that acquired virus. Acquisition information also can be obtained directly, but less reliably, from the first set of test plants. If a test plant in the first set develops symptoms of infection, then and only then do we assume that the corresponding vector has transmitted the virus. For a vector to transmit the virus we assume, only in a phenomenological sense, that acquisition must have been completed at some point σ on $[0,s]$, that the latent period must have been completed at some point τ on $(\sigma,t]$, and that the inoculation must have taken place at some point ω on the interval $[\max(s,\tau),t]$ (see Fig. 1).

Since $p_a(t)$ is the probability density function for acquisition, it follows that

$$\int_0^t p_a(\tau)\,d\tau = \text{the probability that acquisition is completed within } t \text{ units of time during the AAP.} \qquad (2)$$

Similarly we can define

$$\int_0^t p_l(\tau)\,d\tau = \text{the probability that the latent period is completed within } t \text{ units of time after acquisition has occurred.} \qquad (3)$$

and

$$\int_0^t p_i(\tau)\,d\tau = \text{the probability that inoculation is completed within } t \text{ units of time after completion of the latent period.} \qquad (4)$$

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Note that our argument is phenomenological in the sense that a vector may acquire dosages of the virus that could be too low either for transmission to occur or for the production of visible symptoms in the plant. In either case, the observer is unaware that the vector has acquired the virus. Our results remain consistent as long as the definition of transmission (which unfortunately may be observer-dependent) is based on the occurrence of visible symptoms in the test plant.

Let

$$P(s,t) = \text{probability that transmission occurs by time } t, \text{ given that the AAP is } [0,s] \text{ and that the IAP is } [s,t]. \quad (5)$$

Then it follows from the concatenation of the three distinct phases indicated in Fig. 1 that $P(s,t)$ is the convolution (multiplication of distributions) of the three distributions defined in equations 2 to 4 over the appropriate time intervals, ie,

$$P(s,t) = {}_0\int^s p_a(\sigma)\, {}_\sigma\int^t p_l(\tau-\sigma)\, {}_\kappa\int^t p_i(\omega-\kappa)\,d\omega\,d\tau\,d\sigma \quad (6)$$

in which

$$\kappa = \max(s,\tau). \quad (7)$$

As in previous papers (2,3,6,16,17) we will assume that inoculation occurs instantaneously, ie,

$${}_0\int^t p_i(\tau)\,d\tau = 1 \quad \text{for all} \quad t > 0. \quad (8)$$

This assumption is acceptable if inoculation occurs rapidly (of the order of several minutes) in comparison with the completion of the latent period (of the order of tens of hours). The available data support this assumption (1,4,5,7–9,11,12,15,16).

Assuming a distribution of the form given in equations 1 and 8, $P(s,t)$ in equation 6 reduces to

$$P(s,t) = \alpha\, {}_0\int^s e^{-\alpha\sigma}\, {}_\sigma\int^t p_l(\tau-\sigma)\,d\tau\,d\sigma. \quad (9)$$

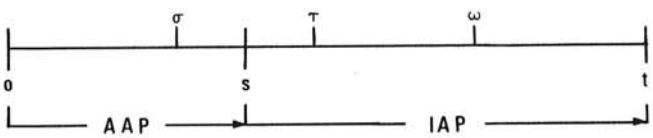

Fig. 1. Events and phases that occur if transmission of PEMV takes place on $[s,t]$. Acquisition occurs at $\sigma \in [0,s]$, completion of the latent period occurs at $\tau \in [\sigma, t]$, and inoculation occurs at $\omega \in [\max(s,\tau),t]$.

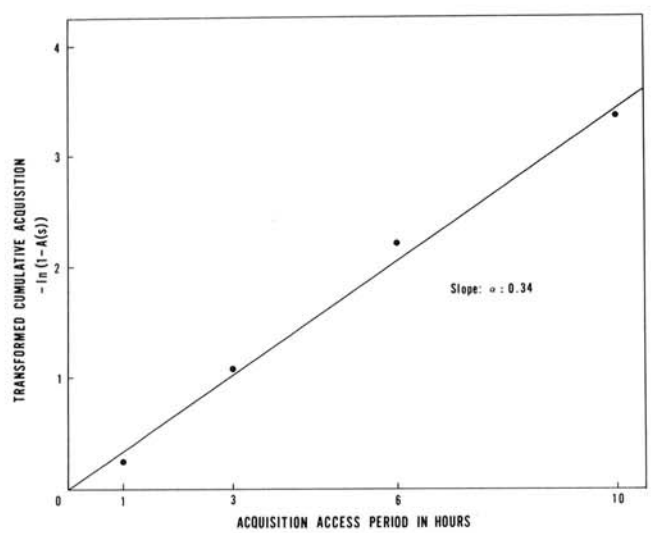

Fig. 2. Linear least squares fit of the relationship between the proportion of aphids acquiring virus and the length of the acquisition access period.

In the past, it always has been assumed that the distribution of the latent period is unbounded above but bounded below with zero as its greatest lower bound (g.l.b.) The log-normal distribution is a typical example and is commonly used. We may, however, generalize the form of $p_l(t)$ by assuming that its g.l.b. is an unknown parameter ρ, which will be estimated along with the other parameters of the distribution $p_l(t)$ using a suitable estimation process (nonlinear regression). If ρ turns out to be substantially larger than zero (ie, of the same order of magnitude as the LP_{50} (median of $p_l(t)$) then this will strongly suggest the existence of a minimum latent period.

A log-normal distribution with a g.l.b. has the form

$$\begin{aligned} p_l(t) &= 0 \qquad t < \rho \\ p_l(t) &= \exp\left\{-1/2[\ln(t-\rho)-\mu]^2/\lambda^2\right\}/(t-\rho)\lambda\sqrt{2\pi} \\ &\qquad t \geqslant \rho \end{aligned} \quad (10)$$

in which $\mu > 0$ and $\lambda > 0$ are, respectively, the mean and the variance of the distribution. In this case, equation 9 can be written as

$$P(s,t) = \alpha\, {}_0\int^s e^{-\alpha\sigma} F(\ln(t-\rho-\sigma-\mu)/\lambda)\,d\sigma \quad (11)$$

in which F is the cumulative distribution function of the standard normal distribution, ie,

$$F(z) = \text{Prob}\left\{Z \leqslant z \mid Z \sim N(0,1)\right\} \quad (12)$$

Since this function, equation 12, cannot be expressed in a closed form, the integral of equation 11 must be evaluated numerically in which case F must be separately evaluated either by direct integration of the normal distribution or by interpolating tabulated values which are readily available.

Another family of distributions unbounded above, but possessing a g.l.b., ρ, is the shifted gamma probability distribution

$$\begin{aligned} p_l(t) &= 0 \qquad t < \rho \\ p_l(t) &= \beta^\gamma (t-\rho)^{\gamma-1} e^{-\beta(t-\rho)}/(\gamma-1)! \\ &\qquad t \geqslant \rho \end{aligned} \quad (13)$$

in which $\beta > 0$ and $\gamma \geqslant 1$ are parameters of the distribution. This distribution has the advantage that equation 9 can be integrated whenever γ is a positive integer, and for $\gamma = 2$ it has a similar shape to the log-normal distribution. Integrating equation 9 for the case $\gamma = 2$ in equation 13, we obtain

$$\begin{aligned} P(s,t) = {} & (1-e^{-\alpha\hat{s}}) - \alpha(1+\beta(t-\rho))e^{-\beta(t-\rho)}(1-e^{-(\alpha-\beta)\hat{s}})/(\alpha-\beta) \\ & + \alpha\beta e^{-\beta(t-\rho)}(1-(1+(\alpha-\beta)\hat{s})e^{-(\alpha-\beta)\hat{s}})/(\alpha-\beta)^2 \end{aligned} \quad (14)$$

in which

$$\hat{s} = \min(s, t-\rho).$$

MATERIALS AND METHODS

The virus source was a strain of PEMV originally collected in Berkeley and maintained in sweet pea by insect inoculation. Test plants were 1-wk-old sweet pea seedlings planted in 5 × 5-cm-square plastic pots. Vectors were pea aphids derived from a clonal line maintained at Berkeley. During the AAP and IAP, the test plants were kept in growth chambers at 19.6 ± 1.06 C and constant light of 8,600 to 11,000 lux at plant level. All test plants used for the first IAP were preconditioned for temperature equilibrium by placing them in the growth chambers for several hours prior to use.

Ten-day-old apterous female aphids were confined by caging on healthy sweet pea seedlings and allowed to larviposit for 24 hr. They were then removed, the plants recaged, and the progeny allowed to develop until they reached an average age of 59 hr. The larvae were then collected by cutting off the seedlings and shaking them gently into 40 butyrate-acetate cages, distributing the number as evenly as possible. The cages were then sealed with Parafilm®

TABLE 1. Transmission ($P[s,t]$) and acquisition ($A[s]$) frequencies of pea enation mosaic virus by larval *Acyrthosiphon pisum* aphids, using sweet pea (*Lathyrus odoratus*) as the virus source during variable acquisition access periods (AAP)[a]

Length of the AAP											
s = 1 hr			s = 3 hr			s = 6 hr			s = 10 hr		
t	$P(1,t)$	$A(1)$	t	$P(3,t)$	$A(3)$	t	$P(6,t)$	$A(6)$	t	$P(10,t)$	$A(10)$
6.85	0/30[b]	10/30	6.70	0/30	20/30	7.00	0/30	27/30	11.40	1/30	20/30
12.00	0/30	9/30	11.80	1/30	23/30	11.60	0/30	26/30	12.20	2/30	29/30
16.00	0/30	7/30	15.85	1/30	23/30	15.70	1/30	30/30	16.10	1/30	30/30
23.65	3/30	5/30	23.50	9/30	19/30	23.35	20/30	28/30	23.20	12/30	29/30
27.45	1/30	6/30	27.30	11/30	18/30	27.15	14/30	28/30	26.00	18/30	30/30
31.45	3/30	7/30	31.30	10/30	16/30	31.15	23/30	27/30	31.00	24/30	27/30
36/45	5/49	8/47	36.30	21/47	32/47	36.15	35/46	40/46	36.00	42/48	46/48
...	...	...	48.40	11/24	12/24	...	...	...	...	...	...

[a]AAP of length (s) and inoculation access period (IAP) of length (t) were at 20 C and constant light of 8,600 to 11,000 lux.
[b]The numerator is the number of plants that exhibited symptoms; the denominator, the number tested.

and the insects left to fast for ~2 hr at ~27 C. At the end of the fasting period the Parafilm was removed and the cages were quickly inverted over 40 sweet pea plants infected with PEMV. When all cages were in place, they were immediately tapped with a pencil to knock the insects to the soil from where they could migrate to the plants and begin to feed.

After an AAP of 1 hr, 10 caged virus source plants were selected at random, the cages removed, the plants cut off, and the aphids shaken into a single cage to pool the sample. The aphids then were transferred as rapidly as possible, by using microaspirators, to a tray of 1-wk-old sweet pea seedling test plants, one insect per plant, and caged. Six trays of 30 plants and one tray of 50 plants were prepared. Each tray was put into a growth chamber as the insect transfers were complete, and the elapsed time noted.

At the end of each IAP of ~6.8, 12, 16, 23.6, 27.4, 31.4, and 36.4 hr 30 aphids were transferred to a second set of test plants for a final IAP of 4–6 days, a period sufficiently long to insure that most, if not all, of the infective insects would transmit the virus. Any missing or dead insects found at the time of the second transfer were replaced by using insects from the next tray in the sequence such that the ultimate source of substitute insects was the tray of 50 insects set up for that purpose.

The above procedure was repeated after the 3, 6, and 10-hr AAP (an extra tray of 30 was set up for the 3-hr trial). The actual IAPs were: ~6.7, 11.8, 15.8, 23.5, 27.3, 31.3, 36.3 (48.4 for the extra tray of 30); 7.0, 11.6, 15.7, 23.4, 27.2, 31.3, 36.2; and 11.4, 12.2, 16.1, 23.2, 26.0, 31.0, and 36.0 hr for the 3-, 6-, and 10-hr AAP trials, respectively.

After the insects were removed from the test plants, all plants were fumigated with nicotine and placed in a greenhouse for symptom development. Symptoms were recorded 2 wk after termination of the final IAP.

All calculations were carried out on a CDC 6400 and the regressions were performed by a combination of a simplex method (to locate a neighborhood of the least squares solution) and a variable metric method (which is efficient in a region close to the least squares solution).

RESULTS AND DISCUSSION

The data obtained are given in Table 1. Assuming that the acquisition probability distribution is exponential (see equation 1), the proportion of vectors A(s) that acquired the virus in an AAP of length s is given by

$$A(s) = \int_0^s \alpha e^{-\alpha t} dt = 1 - e^{-\alpha s} \quad (15)$$

from which it follows that

$$-\ln(1 - A(s)) = \alpha s \quad (16)$$

The parameter α can be estimated using a nonlinear least squares fit of equation 15 or a linear least squares fit of the transformed variable $-\ln(1-A(s))$ as depicted in equation 16. These values of α together with the values of α that satisfy equation 15 exactly for each separate AAP are given in Table 2 along with an estimate of the median acquisition period (AP_{50}). The linear least squares fit to the acquisition data is shown in Fig. 2. The AP_{50} estimates in Table 2 are all similar except for the case s = 1 hr, which may reflect the time taken for the aphids to migrate to the plant and begin to feed.

TABLE 2. Acquisition rates of pea enation mosaic virus by larval *Acyrothosiphon pisum*[a] aphids

Acquisition period (hr)	Estimation procedure	Acquisition rate	Median acquisition period (hr)[b]
1	Single point	0.26	2.7
3	Single point	0.35	2.0
6	Single point	0.37	1.9
10	Single point	0.34	2.0
Combined	Nonlinear least squares (see equation [15])	0.33	2.1
Combined	Linear least squares (see equation [16])	0.34	2.0

[a]Maintained at 20 C and constant light of 8,600 to 11,000 lux, based on the acquisition data in Table 1.
[b]The median acquisition period (AP_{50}) = $-1/\alpha \ln(1/2)$—see equation 16.

Parameters characterizing the latent period were obtained in several different ways from the data in Table 1. Specifically: a standard log-probit analysis was done in which the beginning of the transmission period was assumed to be halfway through the AAP; a nonlinear least squares fit of the transmission data to the distribution given by the gamma model (equation 14) was done for the case in which the value of α was a priori assigned before the nonlinear least squares regression on equation 14 according to the acquisition results listed in Table 2 (ie, the second set of test plant data was utilized); and, using the gamma model except that α was not assigned an a priori value, but was estimated as part of the nonlinear least squares regression on equation 14 (ie, the second set of test plant data was not utilized). The results are listed in Table 3.

The analysis based on the regression on equation 14 strongly suggests the existence of a minimum latent period of 10–14 hr. Furthermore, the results of the log-probit analysis and those of the preassigned regression on equation 14 suggest the possibility that the LP_{50} may decrease with dosage. By contrast, the three-parameter regression yields remarkably similar estimates for the LP_{50} and minimum LP for each individual acquisition period.

Similar results were obtained for a regression on the log-normal model where equation 11 was approximated numerically by using Gaussian Quadrature. For example, in the case of an acquisition period of 10 hr, the LP_{50} and minimum LP of 21.5 and 10.5 hr, respectively, were obtained. The regression on equation 11, however, was substantially more expensive to run than that on equation 14 although it should be kept in mind that equation 11 contains an extra parameter. For the data subset s = 10, the regression on the gamma distribution (equation 14) cost $0.43 while the regression on the log-normal distribution (equation 11)

TABLE 3. Results of regression analysis pertaining to the latent period (LP) process of pea enation mosaic virus being transmitted by larval *Acyrthosiphon pisum* aphids from and to sweet pea *Lathyrus odoratus*[a]

Acquisition period (hr) = (s)	Log-probit analysis median, LP (hr)	Regression on equation 14					
		Preassigned acquisition rate			Acquisition rate estimated from regression equation		
		Rate	Median LP (hr)	Minimum LP (hr)	Rate	Median LP (hr)	Minimum LP (hr)
1	36.2	0.26	36.3	7.8	0.10	20.7	13.9
3	25.4	0.35	27.2	5.1	0.20	19.8	12.3
6	20.9	0.37	20.2	11.0	0.23	18.0	12.4
10	17.7	0.34	21.4	13.8	0.26	20.7	14.2
All *s*	21.7	0.33	21.6	10.7	0.19	18.1	13.5

[a] Maintained at 20 C and constant light of 8,600–11,000 lux.

using a five-point Gaussian Quadrature formula cost $2.72. The accuracy of the five-point Gaussian Quadrature compared very favorably with a 15-point Gaussian Quadrature (0.014% difference for the LP_{50} and 0.82% difference for the minimum LP).

It is felt, however, that the data listed in Table 1 are insufficient to reliably estimate the latent period parameter of the transmission process, especially at the subsample level, ie, considering each individual acquisition period separately. The results obtained in Tables 2 and 3 do, however, demonstrate the application of the modeling approach proposed in this paper and indicate that the question of a dosage-sensitive latent period needs to be investigated more fully.

The method proposed relies on the computer implementation of suitable minimization algorithms to perform the nonlinear regression procedure. If such are not available, then a standard log-probit analysis (which assumes the latent period to begin at the midpoint of the AAP and makes no allowance for a minimum LP) will give a reasonable estimate for the LP_{50} (see Table 3).

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