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THE ROLES OF DENSITY, STAGE, AND PATCHINESS IN THE TRANSMISSION OF AN INSECT VIRUS[1]

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Abstract. Although the importance of insect viruses in the population dynamics of their hosts is widely acknowledged, ecologists are still relatively ignorant of the factors determining the rate of transmission of insect viruses in the field. I performed a series of field experiments in which I investigated the transmission dynamics of the nuclear polyhedrosis virus (NPV) of Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae), in northern Idaho, USA. In these experiments, I reared healthy and infected larvae together on seedling Douglas-fir (*Pseudotsuga menziesii*), and used the number of healthy larvae that became infected as a measure of transmission. I explored the influences of density, stage structure, and spatial structure on transmission by manipulating the density and stage distribution of healthy and infected hosts, and the spatial distribution of infected hosts. The experiments indicate that transmission is strongly affected by the densities of both healthy and infected hosts, but the effect depends on the instar of each. Late instars are both more infectious and more likely to become infected than are early instars, so that the NPV is more likely to spread in populations of late-instar tussock moth larvae. I also found that transmission is affected by the spatial distribution of infected hosts, and this effect also depends on the instar of healthy hosts. That is, transmission to healthy early instars decreases with increasing patchiness of infected hosts, but transmission to healthy late instars is essentially unaffected by patchiness. I discuss how these results can be interpreted in terms of behavioral differences among instars, and relate the results to the mathematical theory of disease and the use of viruses in biological pest control.

Key words: biological control; disease transmission; Douglas-fir tussock moth; epizootiology; mathematical epidemiology; nuclear polyhedrosis virus; susceptibility.

INTRODUCTION

Mathematical models have a long and successful history in human epidemiology, and represent one of the earliest applications of mathematics in population biology (Ross 1911, Bailey 1975). Recently, theoretical ecologists, led by Anderson and May (1979), have adapted these models to a variety of animal host–pathogen systems (Anderson and May 1981, Anderson et al. 1981, Levin and Pimentel 1981, Murray et al. 1986, Dwyer et al. 1990). Although models of different systems vary in their details, virtually all such models incorporate the assumption that transmission is linearly proportional to the density of healthy hosts times the density of infected hosts (but see Liu et al. 1987). This is written as

$$\text{transmission} \propto SI, \qquad (1)$$

where I is the density of infected hosts and S is the density of healthy hosts.

This assumption has rarely been tested quantitatively in the field (Anderson and May 1981), largely because experimental manipulations are difficult with most animal host–pathogen systems. However, insects and their associated viruses provide experimentally convenient systems for several reasons: (1) their densities can be easily reduced or supplemented for experimental purposes, (2) insects that are infected by viruses rarely recover, and (3) transmission is often direct (Evans and Allaway 1983, Evans and Entwistle 1987). Moreover, the transmission of insect viruses is especially interesting in light of the important role that viruses play in the dynamics of insect populations (Kaya and Anderson 1976, Harkrider and Hall 1978, Myers 1981, Fuxa 1982, 1983, Kalmakoff and Crawford 1982, Carter et al. 1983, Entwistle et al. 1983, Murdoch et al. 1985, Fleming et al. 1986). Also, knowledge of the determinants of virus transmission rates is believed to be one of the keys to understanding and predicting epizootics (outbreaks of disease) in insects (Stairs 1966, Zelazny 1977, Anderson and May 1981, Entwistle et al. 1983, Andreadis 1987, Fuxa and Tanada 1987, Young and Yearian 1987*a*), as well to using viruses effectively as biological insecticides (Thompson and Steinhaus 1950, Bird and Burk 1961, Stairs 1965, Klein and Podoler 1978, McLeod et al. 1982, Mohamed et al. 1983, Podgwaite et al. 1984, Fuxa 1987, Otvos et al. 1987*a*, *b*,).

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In this paper, I explore the adequacy of Eq. 1 in describing the transmission of the nuclear polyhedrosis virus (NPV) of Douglas-fir tussock moth (DFTM), *Orgyia pseudotsugata* McDunnough (Lepidoptera: Lymantriidae). It is certainly the case that host density is important in epizootics of virus disease in insect populations; in particular, disease epizootics are generally more likely to occur in dense host populations (Carter et al. 1983, Fleming et al. 1986, Woods and Elkinton 1987). Similarly, epizootics are also more likely to occur when the density of virus particles is high (Jaques 1974, Crawford and Kalmakoff 1977, Podgwaite et al. 1979, Entwistle et al. 1983, Fuxa and Geaghan 1983). This is also true for the particular case of the NPV of DFTM. Mason and Thompson (1971) found that, in NPV epizootics in DFTM populations of different densities, percent infection increased with host density. Millstein's (1988) data suggest that epizootic intensity increases dramatically with the density of both susceptible and infected hosts. However, rather than testing simply whether density has an effect on transmission, I tested the particular hypothesis embodied by Eq. 1; that is, *that transmission is linearly proportional to the densities of both host and pathogen.* Eq. 1 also embodies the implicit assumption that density is the most important determinant of transmission; the effects of age (or stage) structure, spatial structure, and other factors are assumed to be relatively unimportant. To test how much biological reality is lost with this simplification, I quantified the effects of stage structure and spatial structure upon the transmission of the NPV of DFTM. The stage structure of insect populations may be important in the transmission of virus diseases for two reasons. First, resistance typically increases by several orders of magnitude between first and last larval instars (adults usually are not susceptible), where resistance is measured as the number or dosage of virus particles that it takes to kill an individual host in a laboratory bioassay (Magnoler 1975, Boucias and Nordin 1977, Whitlock 1977, Evans 1981, Watanabe 1987, M. E. Martignoni, *personal communication*). This phenomenon is sometimes interpreted to mean that healthy late instars in the field are less likely to become infected with the disease (Entwistle et al. 1983, Tanada 1985, Watanabe 1987). Second, infectiousness, as measured by the number of virus particles produced by an infected host, increases with instar (Thompson and Scott 1979, Kaupp 1983, Teakle and Byrne 1989). This has likewise been interpreted to mean that infected late instars in the field are more likely to transmit the disease.

In contrast to the attention given to stage structure, the importance of spatial structure in the transmission of insect viruses has been virtually ignored (but see Entwistle et al. 1983). This is in spite of the fact that spatial structure and host movement rates are predicted to be important in the rate of spread of other diseases (Murray et al. 1986). Moreover, spatial structure should be especially important in the transmission of insect viruses because of the low mobility of many insect hosts, including larval DFTM.

In this paper, I present the results of three field experiments designed to test the influence of these factors upon the transmission of NPV. To begin with, I explicitly tested the assumption that transmission is linearly proportional to the density of healthy hosts times the density of infected hosts. I also tested whether the increase in infectiousness and decline in susceptibility of later instars in the laboratory (M. E. Martignoni, *personal communication*) translate into a higher infectiousness and a reduced risk of infection of later instars in the field. Finally, I determined whether the dispersion of infected larvae (clumped vs. uniform) influences the spread of NPV in small experimental populations of DFTM, and whether the effect of dispersion is in turn affected by the instar of healthy hosts.

Methods and Materials

Douglas-fir tussock moth and its nuclear polyhedrosis virus

DFTM is a defoliator of a variety of economically imporant trees including *Pseudotsuga menziesii* and *Abies grandis.* It ranges from British Columbia, Idaho, and Montana to California and Arizona. Males go through five larval instars, whereas females go through six (Brookes et al. 1978). Since females are flightless, mating and oviposition take place on the female's cocoon, and essentially all long-distance dispersal takes place when first-instar larvae balloon from the egg mass (Mitchell 1979). In many locations throughout its range, DFTM undergoes periodic outbreaks in which its density increases by at least four orders of magnitude; often, such outbreaks are terminated by epizootics of the NPV disease (Brookes et al. 1978). The NPV consists of particles of double-stranded DNA packed inside a polyhedron-shaped protein matrix. This matrix, termed a polyhedral inclusion body or PIB, enables the virus to survive for long periods outside the host, and is large enough (5–10 μm) to allow disease diagnosis under the light microscope (Evans and Entwistle 1987).

General methods

The experiments that I describe here were all modifications of the same basic protocol. For each experiment, I reared healthy tussock moth larvae on 2–3 yr old Douglas-fir together with infected larvae, and observed the fraction of the healthy larvae that became infected. All the experimental trees had been reared in western Washington in an area having no history of either DFTM or its NPV (Weyerhaeuser nursery in Rochester, Washington). Such trees have proved to be free of virus, as no larva feeding on them has ever become infected.

The larval densities that I used in the experiments ranged from 25 to 70 individuals/m^2 of foliage, depending on the experiment, which is within the range

of outbreak densities observed in the field (≈20–200 larvae/m^2 of foliage; Mason and Thompson 1971, Mason 1981, Otvos et al. 1987*a*). The number of initially infected larvae that I used per healthy larva ranged from 0.20 (5/25) to 0.38 (5/13). This is similar to the incidence of infection observed during natural virus epizootics, in which the fraction of infected larvae ranges from 0.1 to 0.4 (Mason and Thompson 1971).

In 1986 many of the larvae wandered away from the experimental trees. To prevent this from happening, in 1987, 1988, and 1989, I planted the trees in wading pools of ≈4 m^2 in area that were one-third filled with soil. In order to keep the larvae from crawling out of the pools, I coated the inner wall of each wading pool with a strip of Tanglefoot, an extremely sticky resin. Larvae that approached the Tanglefoot were repulsed and did not attempt to cross it. In 1986 there were six trees per experimental unit, while in 1987, 1988, and 1989 there were two trees per experimental unit. For all but the density experiment, treatments were grouped in blocks. Blocks were arranged within the site according to my subjective assessment of the amount of sunlight to which they were exposed. The number of blocks varied between experiments, as described under the subsections for each experiment.

The larvae that I used in all of the experiments were initially reared from eggs on commercial diet (Bio-Serv) according to the procedure of Robertson (1985). These larvae were from a laboratory population originating from eggs collected in 1986 near the site of my field experiments (Potlatch, Idaho). Laboratory studies on other insects have shown that, when larvae are intentionally infected while being reared on experimentally manipulated artificial diet, the composition of the diet can affect the fraction of larvae that ultimately die (Vail et al. 1968, Keating and Yendol 1987). It is not known, however, what effect a brief initial period of feeding on artificial diet has on subsequent susceptibility while feeding on natural hosts. Thus I do not know whether initially feeding DFTM larvae artificial diet altered my results from what would be observed in a completely natural situation. However, because my protocol was the same for all experimental treatments, my rearing procedure should not bias the results in any way.

Because of delays associated with rearing larvae in the laboratory, the experiments took place later in the summer (mid July–early September) than DFTM larvae naturally occur in the field (late May–mid July). By mid–late July in northern Idaho, predaceous vespid wasps (*Vespula pensylvanicus* and *Vespula maculatus*) are much more numerous than they are in May and June. In order to avoid the unnatural effects of high vespid populations, all the wading pools were enclosed in 122 × 122 × 122 cm wasp exclusion cages made of spun-bonded polyester (Reemay) supported by 152.4 cm high bamboo stakes.

I infected larvae for the experiments by feeding them artificial diet (without formalin, which inhibits infection; see Vail et al. 1968) onto which 0.5 mL of a homogenate of infected larvae in distilled H_2O had been pipetted. Since only dead larvae are infectious, it was important to use a fatal dose of the virus to infect larvae. Accordingly, I used a homogenate of 40 infected fourth-instar cadavers in 100 mL of distilled H_2O, which invariably produces 100% mortality. The virus that I used is the multicapsid morphotype (OPS-MM, Martignoni et al. 1980), originally obtained from Jeffrey Millstein (Washington State University, Pullman, Washington, USA). These laboratory-infected larvae are hereafter referred to as primary infecteds (Young and Yearian 1987*a*). The primary infecteds were marked with a fluorescent powder, so that they could be distinguished from any new infections, which are hereafter referred to as secondary infected larvae.

After the healthy larvae and the primary infecteds were placed on the trees, I censused the experiments 2–3 times per week and collected dead larvae every day or every other day. Except where noted, experiments ran until all larvae had died or pupated. Dead larvae that were not dried out were autopsied for cause of death. Smears were stained using Buffalo Black (or Naphthalene Black, Entwistle et al. 1983) and examined under the light microscope at 400× for the presence of PIBs.

In order to allow for the possibility of infections due to virus from extraneous sources, such as wind-blown dust from forest duff, I set up the experiments in a second-growth Douglas-fir and grand fir forest near Princeton, Idaho (University of Idaho Experimental Forest) that has had a history of DFTM outbreaks. This had the additional advantage that the experiments were performed under conditions that closely approximate the natural environment of DFTM larvae.

To assay for infections due to extraneous sources, and to test for accidental infection of healthy larvae before the start of the experiments, I set up a control for each experiment. These controls were exactly the same as the experimental treatments except that they had no primary infecteds. For the purposes of experimental design, the controls were assumed to be an additional treatment of infectious larvae. For example, for the experiment in which I examined the influence of stage structure on transmission, I used three treatments of infected larvae, i.e., third instars, fifth instars, and no infected larvae. The no infected larvae treatment thus corresponds to the controls for this experiment. In the interests of clarity, however, I do not refer to the controls when describing the experimental design. Moreover, I do not include the controls as a treatment in the statistical analysis, because not a single infected larva appeared in the 1986, 1987, and 1988 controls. However, in 1989 a few infections did appear in the controls, although at a significantly lower rate than in the experimental treatments (see *Results: Testing the effect of spatial dispersion on NPV transmission*). The appearance of infections in the 1989 controls could have been a result of contamination from par-

ticles blown up from the forest duff, or a result of accidental infection in the laboratory. One procedure that I altered in 1989 that could have produced laboratory contamination was that I did not surface sterilize the eggs (a change that I made to maximize hatching rate). In the *Results* I discuss only the 1989 controls, even though I had controls for each experiment.

In short, all the experiments were designed to measure a single cycle of transmission. That is, all the initially infected larvae (primary infecteds) either died shortly after being placed on the trees, or shortly before, and all subsequent infections (secondary infecteds) were removed shortly after they died. As a result, the free-living population of virus on the trees did not increase after the 1st d of each experiment, and all transmission was a result of contact between healthy larvae and virus from the primary infecteds.

Testing the effect of density on NPV transmission

The assumption that transmission is linearly proportional to the density of both healthy and infected insects, as embodied by Eq. 1, was first adapted to insect diseases by Anderson and May (1981). The complete model is

$$\frac{dS}{dt} = r(S + I) - bS - \nu SP \tag{2}$$

$$\frac{dI}{dt} = \nu SP - (\alpha + b)I \tag{3}$$

$$\frac{dP}{dt} = \lambda I - [\mu + \nu(S + I)]P, \tag{4}$$

where S is the density of healthy hosts, I is the density of infected hosts, P is the density of the pathogen outside of any hosts (the "free-living" pathogen population), r is the reproductive rate of the host, b is the nondisease mortality rate of the host, ν is the transmission coefficient, α is the disease-induced mortality rate of the host, λ is the rate of production of pathogen particles by infected hosts, μ is the decay rate of the pathogen, and t is time.

Note that, for the transmission term in Eq. 3, I has been replaced by P. This is because healthy larvae become infected when they ingest the free-living pathogen, not when they encounter infected (but not yet infectious) larvae. The relevant density is thus P. However, if infected larvae produce roughly similar numbers of pathogen particles (an assumption that I tested separately), manipulations of the number of primary infected larvae are equivalent to manipulations of the density of the pathogen. To test whether transmission increases linearly with the densities of both infected and healthy larvae, I thus manipulated the densities of primary infected (I) and healthy (S) larvae, measured the number of new (secondary) infections produced $\left(\frac{dI}{dt}\right)$, and assessed whether the resulting constant of proportionality was independent of density. If transmission is determined primarily by density, and in particular if transmission is accurately described by Eq. 1, then estimates of this proportionality constant should be consistent across densities and instars. However, Eq. 1 is too simple a model to be useful in analyzing the resulting data; most notably, it does not take into account loss of susceptibles due to pupation or nondisease mortality. On the other hand, although Eqs. 2–4 allow for this and other realistic features, they do not permit straightforward estimation of the transmission parameter ν (which corresponds to the proportionality between transmission and $I \times S$ in Eq. 1). I therefore modified Eqs. 2–4 to more closely model the conditions of my experiments. Because of the way the experiments were set up, these modifications allow me to simplify Eqs. 2–4 so that I can easily estimate ν without sacrificing biological detail. First of all, during my experiment there was no DFTM reproduction. Secondly, Anderson and May (1981) assume that the survival time of infected hosts is exponentially distributed, with mean $1/\alpha$. However, laboratory observations indicate that the distribution of survival times of infected larvae is decidedly not exponential (Fig. 1). Instead, the data indicate that there is a substantial incubation time before death, a process which I approximated by assuming a fixed incubation time. Although this time lag is probably not significant over the long time scales with which Anderson and May (1981) are concerned, on the time scale of my experiments it was important. I thus modified Eqs. 2–4 by dividing the infectious class into classes based on time since infection. Third, I assume that the decay of virus particles is negligible on the time scale of my experiments. Since the experiments lasted no longer than 40 d, this is probably a reasonable assumption (Olofsson 1988). Also, any new (secondary) infections, those resulting from contact with the primary infected larvae, were removed within 24 h of when they died. This was done to ensure that the pool of free-living virus in the environment (P) did not increase above and beyond what I put in at the beginning of the experiment. Further, I assume that the amount of virus eaten by healthy and infected hosts is negligible, because the amount of virus produced by an infected individual is very large relative to the amount of virus on the foliage consumed by larvae during an experiment.

Mathematically, the lack of change in the free-living pathogen population corresponds to setting

$$\frac{dP}{dt} = 0, \tag{5}$$

so that $P = P_0$. The additional modifications lead to the following equations:

$$\frac{dS}{dt} = -(b + \nu P_0)S \quad (6)$$

$$I(0, t) = \nu P_0 S \quad (7)$$

$$I(\tau, t) = I(0, t - \tau)e^{-b\tau} \quad (8)$$

$$\frac{dR}{dt} = I(T, t), \quad (9)$$

where S, I, b, ν, and t are as before, τ is time since infection, T is the incubation time between infection and death, and R is the number of secondary infections. $I(\tau, t)$, however, is no longer the number of infected larvae. Instead $I(\tau, t)$ is the number of larvae that enter the τ-th time-since-infection class per day at time t. $I(0, t)$ is thus the number of larvae becoming infected per day at time t.

It is straightforward to show that

$$R(t) = \frac{\nu P_0 S_0}{b + \nu P_0} e^{-bT}[1 - e^{-\nu P_0(t-T)}] \quad (10)$$

for $t > T$. Eq. 10 can be used to estimate the transmission parameter ν given the initial densities of susceptible larvae and virus particles (S_0 and P_0), the number of secondary infections $R(t)$, the incubation time T, and the nondisease mortality rate b. (Although b represents loss of larvae due to both nondisease mortality and pupation, I will refer to it as the nondisease or natural mortality rate because very few larvae ever survived to pupate.) An important feature of Eq. 10 is that, if the fraction of larvae in a replicate that became infected, $R(t)/S_0$ is much more than ½, the resulting transmission parameter ν is very sensitive to the natural mortality rate. In order to produce a meaningful estimate of ν, it is thus necessary to estimate the natural mortality rate quite accurately.

Eq. 10 is based on the assumption that the nondisease mortality rate b is constant. In 1987 and 1988, however, there was a sharp increase in natural mortality partway through the experiment (see *Results*), apparently because of increased ambient temperatures, and there was a similar but less profound increase in 1986. To describe this increase, I relaxed the assumption that the natural mortality rate b is constant, and estimated b from the data using a form of piecewise regression. That is, from the natural mortality data for each replicate, I estimated two different natural mortality rates, a low rate for the early part of the experiment, a high rate for the latter part of the experiment, and the changeover point between the two. To do this, I used Hudson's (1966) method, which involves successively dividing the data into two subsets for each sampling date, t. For the first, earlier, subset, I used linear regression to fit the equation

$$-\ln\left(\frac{N_t}{N_0}\right) = b_1 t \quad (11)$$

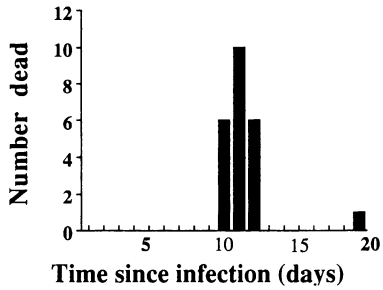

FIG. 1. Time until death of Douglas-fir tussock moth larvae infected with nuclear polyhedrosis virus in the laboratory and incubated at 20°C (n = 23).

to the pupation and nondisease mortality data for each replicate. Here, t is time, b_1 is the natural mortality rate, N_0 is the number of larvae in the replicate that died of nondisease causes or pupated, and N_t is the number of those larvae that survived to time t. (The motivation for this equation is that it is the solution to $\frac{dN}{dt} = -b_1 N$.)

To the second, later, subset, I again used linear regression to fit the equation

$$-\ln\left(\frac{N_t}{N_0}\right) = b_2 t + c, \quad (12)$$

where b_2 is the natural mortality rate after the time t^* (at which the nondisease mortality rate changes), and c is a constant. Without any a priori expectation for the time at which the natural mortality rate changes, it is necessary to estimate the changeover point t^*. This is essentially equivalent to choosing a particular pair of values b_1, b_2. t^* is thus chosen by minimizing the sum of the residual sum of squares of each least squares fitting, that is, by minimizing the sum of the residuals for b_1 and the residuals for (b_2, c).

In short, Hudson's method allows for simultaneous estimation of b_1, b_2, and t^*. The result is that the slope of the best fit line changes at t^*.

The solutions for $R(t)$ when the natural mortality rate changes partway through the experiment are given in the Appendix. The important point is that $R(t)$ can be described by the following function:

$$R(t) = f(\nu, S_0, P_0, b_1, b_2, t^*, T). \quad (13)$$

I can use this equation to estimate the transmission coefficient, ν, from experimental data, because S_0, $R(t)$, P_0, b_1, b_2, t^*, and T are all known constants. Since for this experiment I assume that each infected larva produces the same number of polyhedra, regardless of instar, $P_0 = \Lambda I_0$, where I_0 is the number of primary infected larvae in each replicate and Λ is the number of polyhedra per cadaver. For convenience, I used a value of $\Lambda = 10^7$, based on Thompson and Scott (1979),

TABLE 1. Densities of larvae at the beginning of each density experiment, parameter estimates used in estimating the virus transmission coefficient, ν, and 10^9 times estimates of ν. Parameters are: S_0: number of healthy larvae at the beginning of the experiment; I_0: number of primary infected larvae at the beginning of the experiment; b_1: nondisease mortality rate in early part of experiment (before time t^*); b_2: nondisease mortality rate in late part of experiment (after time t^*); t^*: time at which nondisease mortality rate changes; T: incubation time of disease; $10^9\nu$: transmission coefficient multiplied by 10^9.

Year	S_0	I_0	b_1	b_2	t^*	T	ν
			1986†				
1	133/3	76/3	0.071	0.237	17.74	14	0.28
2	78/3	5/3	0.039	0.176	17.66	14	0.01
			1987‡				
1	53	10	0	0.203	14.57	15	2.90
2	47	10	0	0.107	10.10	15	6.82
3	57	10	0.009	0.243	19.43	15	1.23
4	48	10	0	0.154	14.08	15	1.62
5	53	10	0	0.179	13.85	15	4.28
6	24	10	0	0	···	15	2.39
			1988				
1	29	5	0.012	0.138	13.81	11	0.28
2	30	5	0.006	0.179	24.21	11	0.20
3	27	5	0.003	0.192	15.18	11	2.27
4	31	2	0.029	0.208	17.19	11	0.24
5	16	5	0.057	0.166	24.97	11	0.29
6	17	2	0.012	0.132	23.33	11	0.85
7	17	2	0.020	0.440	35.77	11	0.07

† In 1986, I used six trees instead of three, so values of S_0 and I_0 are divided by 3.

‡ Nondisease mortality in replicate 6 was negligible.

but since Λ is constant, for the purposes of this experiment its actual value is unimportant. The remaining parameters can be derived from the data. The estimation techniques for b_1, b_2, and t^* are described above. Since the incubation time T is sensitive to temperature, laboratory estimates of T are not necessarily reliable. Accordingly, for the value of T for each replicate, I used the time until the first infection appeared in any replicate in that year (except for 1986, all replicates in a given year were begun at the same time).

Using the above information, ν is the only unknown in Eq. 13 and can thus be estimated for each experimental replicate using a nonlinear fitting procedure (Seber 1977). I did this by using a computer to find, for each replicate, the value of ν which minimized the sum of the squared differences between the value of $R(t)$ calculated from the model and the successive values of $R(t)$ for the replicate.

In summary, I conducted this experiment by manipulating the relative densities of susceptible and infected hosts, estimating the transmission parameter ν, and determining whether the resulting value of ν is consistent across densities. I performed this experiment in 1986, 1987, and 1988; the densities for the replicates in each year are shown in Table 1, in terms of the number of larvae per two trees. Since two trees (each $\approx$1 m tall) comprise $\approx$0.75–1.0 m^2 of foliage, the densities that I used ranged from $\approx$25–70 larvae/m^2, depending on experiment and tree size. These densities were chosen to be roughly in the range of outbreak densities, yet low enough so that larvae did not consume all of the foliage on the trees.

The major difference between years was that in 1986 and 1988, I used a combination of late third- and early fourth-instar larvae for both primary infected and healthy larvae, but in 1987 I used only late fifth instars. Other differences were that in 1986 I used six trees instead of two, and in 1988 not all larvae had died or pupated by 15 September. To avoid losing the remaining 29 larvae to frost, I removed all larvae and reared them in the laboratory until they had died or pupated. (This difference leads to a slight modification of the equations in the Appendix.)

Testing whether larval instar influences infectiousness and risk of infection

In order to investigate the effects of larval instar upon transmission, I performed an experiment in which I varied the instars of both the infected and healthy larvae. Specifically, I used two instars of primary infecteds, early (third) and late (fifth), and two instars of healthy larvae, early (third) and late (fifth). I used these particular instars because thirds are the earliest instar that can be easily manipulated and fifths are the last instar in males. The four experimental treatments were thus (1) third-instar infecteds with third-instar healthy, (2) fifth-instar infecteds with third-instar healthy, (3) third-instar infecteds with fifth-instar healthy, and (4) fifth-instar infecteds with fifth-instar healthy. Each treatment was replicated four times for a total of 16 wading pool units. Since it takes $\approx$10 d for infected larvae to die, the primary infecteds were fed the virus 9 d before the experiment began. The experiment was begun on 16 July 1988 by distributing 25 healthy and 5 infected larvae uniformly over the two trees in each wading pool. These densities are again roughly equivalent to natural outbreak densities of DFTM (Mason and Thompson 1971, Mason 1981, Shepherd et al. 1984, Otvos et al. 1987*a*). Since third-instar larvae take longer to pupate than do fifth instars, I removed all living larvae when the first fifth instar pupated 13 d after the start of the experiment, which ensured that all larvae were exposed to the virus for the same length of time. These removed larvae were subsequently reared on artificial diet until they either died or pupated. Dead larvae were autopsied as previously described.

It should be emphasized that, although third and fifth instars do occasionally occur together in the field (Shepherd et al. 1984), I did not design this experiment to mimic field conditions exactly. Instead, my intent was to assess the relative infectiousness and susceptibility of larvae in different instars, and this necessitated a manipulative experiment. For example, if I had simply exposed healthy fifth instars to infected fifths, and healthy thirds to infected thirds, and then observed lower transmission among the fifths, I would not know whether to attribute this difference to lower risk of

infection or lower infectiousness of fifth instars. This experiment was thus designed to examine how infectiousness and risk of infection combine to determine overall transmission. To analyze the data from this experiment, I applied a log-linear G test for multiway tables (Sokal and Rohlf 1981) to the fraction of healthy larvae that became infected.

Testing the effect of spatial dispersion on NPV transmission

To test for an effect of the spatial distribution of the virus on transmission, I manipulated the spatial dispersion of the virus by placing the primary infecteds on the trees after they had died, so that they would not move. I contrasted a patchy dispersion of cadavers with a uniform dispersion of cadavers. For the uniform treatment, I distributed infected cadavers as uniformly as possible over the trees, with no branch having more than one cadaver. For the patchy treatment, I placed infected cadavers as close together as possible, usually touching, on a single branch.

As in the previous experiment, in which I tested for an effect of instar, my manipulations of virus spatial dispersion were not necessarily designed to mimic natural conditions during an epizootic. Instead, I intentionally used what may well have been an artificial dispersion of infected larvae; that is, I chose extreme spatial dispersions in order to make as clear as possible the relationship between cause (spatial dispersion) and effect (transmission). In any event, there are no published data giving the spatial dispersion of infected larvae.

Because I suspected that the effect of dispersion might depend upon both overall virus density and the instar of healthy hosts, I performed two variations on this experiment in two different years. In 1988, I crossed the two dispersion treatments with two densities of infected cadavers (high = 10 individuals, low = 5 individuals) for a total of four treatments (patchy-high, patchy-low, uniform-high, and uniform-low). In this version, 35 healthy larvae were placed on each set of two trees, as uniformly as possible. All larvae, both healthy and infected, were early-fourth or late-third instars. Each treatment was replicated four times for a total of 16 wading pool units. Larvae were placed on the trees on 10 August 1988; by 15 September, not all larvae had died or pupated. To avoid losing the remaining 74 larvae to frost, I removed all larvae and reared them in the laboratory until they died or pupated.

In 1989, I crossed the two dispersion treatments with two instars of healthy larvae, third and fifth, again for a total of four treatments (patchy-third, patchy-fifth, uniform-third, and uniform-fifth). In this version, 13 healthy larvae and 5 primary infected larvae were placed on each set of two trees. All primary infected larvae were third instars. Each treatment was replicated three times for a total of 12 wading pool units, plus six controls with no primary infected larvae.

Larvae were placed on the trees on 2 July 1989. Since third-instar larvae take longer to pupate than do fifth instars, I removed all living larvae 11 d after the start of the experiment (2 d after the first fifth instar pupated), which again ensured that all larvae were exposed for roughly the same length of time. To analyze the data from this experiment, I again applied a G test for multiway tables to the fraction of healthy larvae that became infected.

Results

The effect of density on NPV transmission

To calculate the transmission parameter ν, I first estimated the natural mortality rate for each replicate. These data, with the associated best fit lines, are given in Fig. 2.

The data showing the time course of transmission in each replicate are shown in Fig. 3, with the corresponding fit of the model. The calculated values of ν for each replicate are given in Table 1 along with the parameters used in the calculations. Since for this experiment the amount of replication of densities within years was low, it was difficult to compare values of the transmission parameter, ν, among densities within a single year. Consequently, to test for consistency of ν, I compared the estimates of ν between years.

Note that the values of ν are more consistent within years than among years. This can be seen more clearly in Fig. 4, in which the value of ν is plotted for each replicate in each year. The mean value of ν was higher in 1987 than in 1986 or 1988 (Table 2). To test whether this difference is statistically significant, I used a bootstrap procedure (Efron and Tibshirani 1986) to estimate the 95% confidence intervals for the estimates of ν for 1987 and for 1988 pooled with 1986. The bootstrap is a method of estimating the frequency distribution of any statistic. For example, for 1987, for which there were 6 values of ν, the procedure consisted of randomly sampling with replacement 6 times from the set of 6 values, calculating a mean for each resulting set of 6, and repeating this procedure 1000 times. The resulting distribution of means can be used to approximate the actual distribution of ν for the 1987 data. The procedure for the combined 1986 + 1988 data was the same except that, since there were 9 values of ν, I sampled 9 times instead of 6.

The value of ν was significantly higher for 1987 than for 1986 and 1988 (Table 2; $P < .05$). In other words, the value of ν was not consistent among years in my experiments. This inconsistency is probably due to the fact that I used later instar larvae in 1987 than in 1986 or 1988, as the next experiment demonstrates.

Testing whether larval instar influences infectiousness and risk of infection

The results from the instar experiment indicate that there was a significant interaction between instar of primary infected, instar of healthy larvae, and exper-

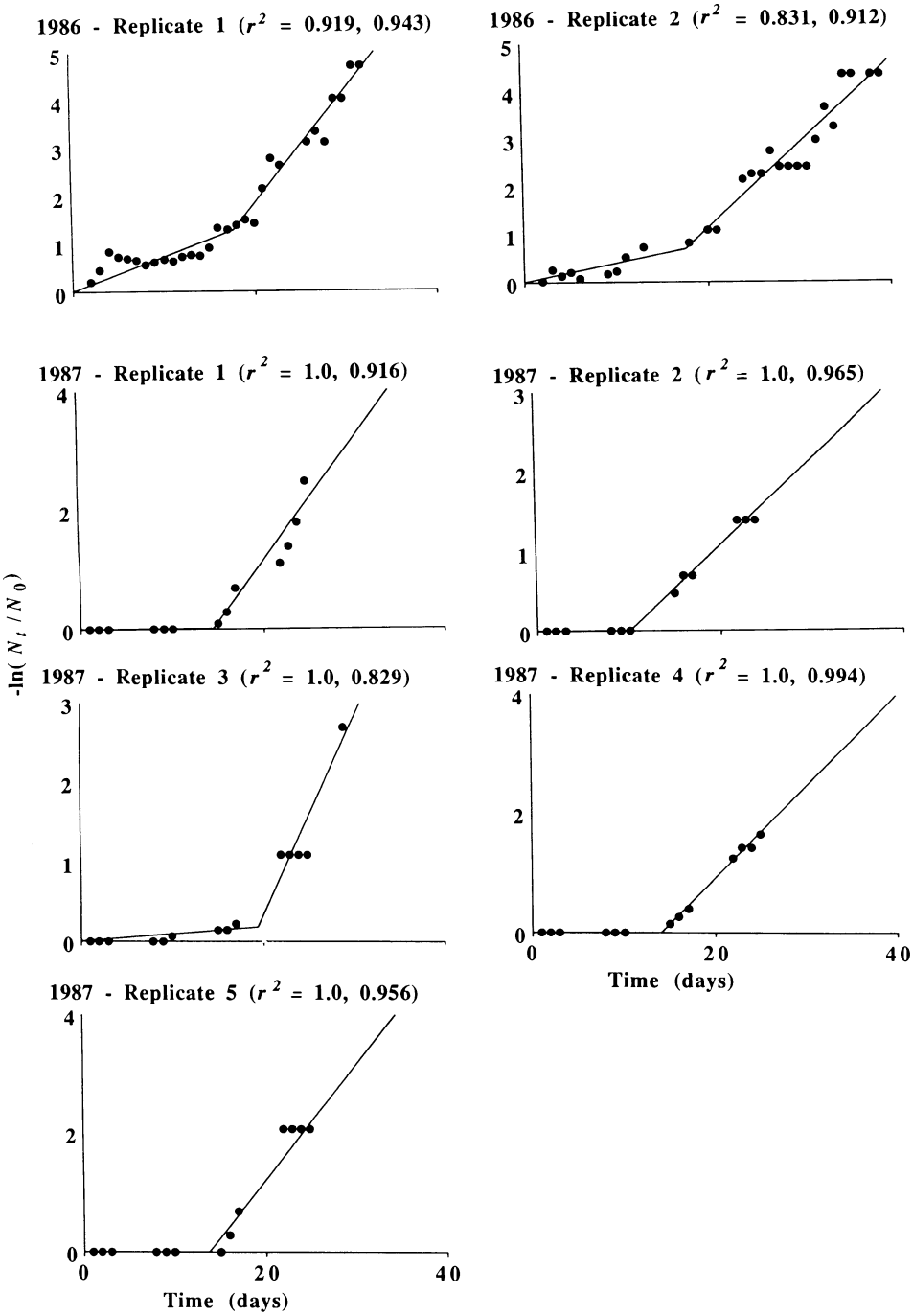

FIG. 2. Time series of nondisease termination of the larval stage (mortality plus pupation) for each replicate of density experiment. Vertical axis is $-\ln(N_t/N_0)$, a measure of mortality. Lines indicate least-squares best-fit using the method of Hudson (1966). The coefficient of determination is given for each fitted line segment. The point of change in slope is t^*.

imental block (G = 11.50, df = 3, P = .009; Fig. 5) with respect to the fraction of healthy larvae that became infected. This can be interpreted as either a significantly higher risk of infection of fifth instars depending upon the instar of the primary infecteds, or as a significantly higher infectiousness of fifth instars depending on the instar of the healthy larvae. In spite of these complicating interactions, two overall effects were evident in the data (Fig. 5): (1) fifth instars were more likely to become infected than third instars, and (2) fifth instars were on average more likely to transmit than third instars. It is important to note that these

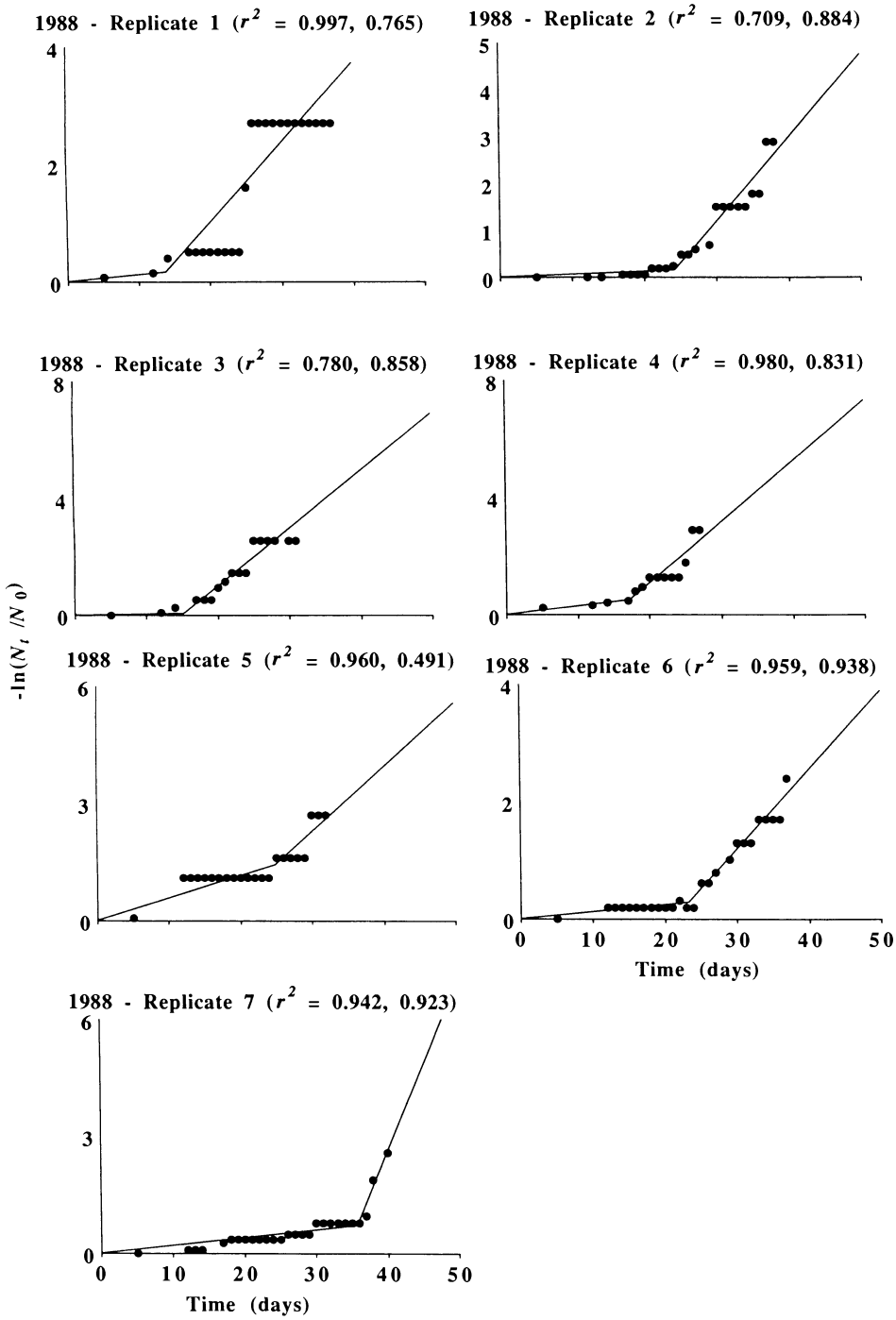

FIG. 2. Continued.

effects were not small; depending on the contrasts involved, the fraction infected varied by as much as a factor of 3.

Testing the effect of spatial dispersion on NPV transmission

The results of the 1988 spatial dispersion experiment (Fig. 6) show that there was a significant effect of spatial dispersion on the fraction of healthy larvae that became infected (G = 16.49, df = 6, P = .0113), although this effect depended upon the experimental block. Similarly, there was an effect of the density of primary infecteds (G = 20.00, df = 6, P = .0028), although this effect also depended on the experimental block. There was, however, no interaction between density and spatial dispersion (G = 2.21, df = 4, P = .697).

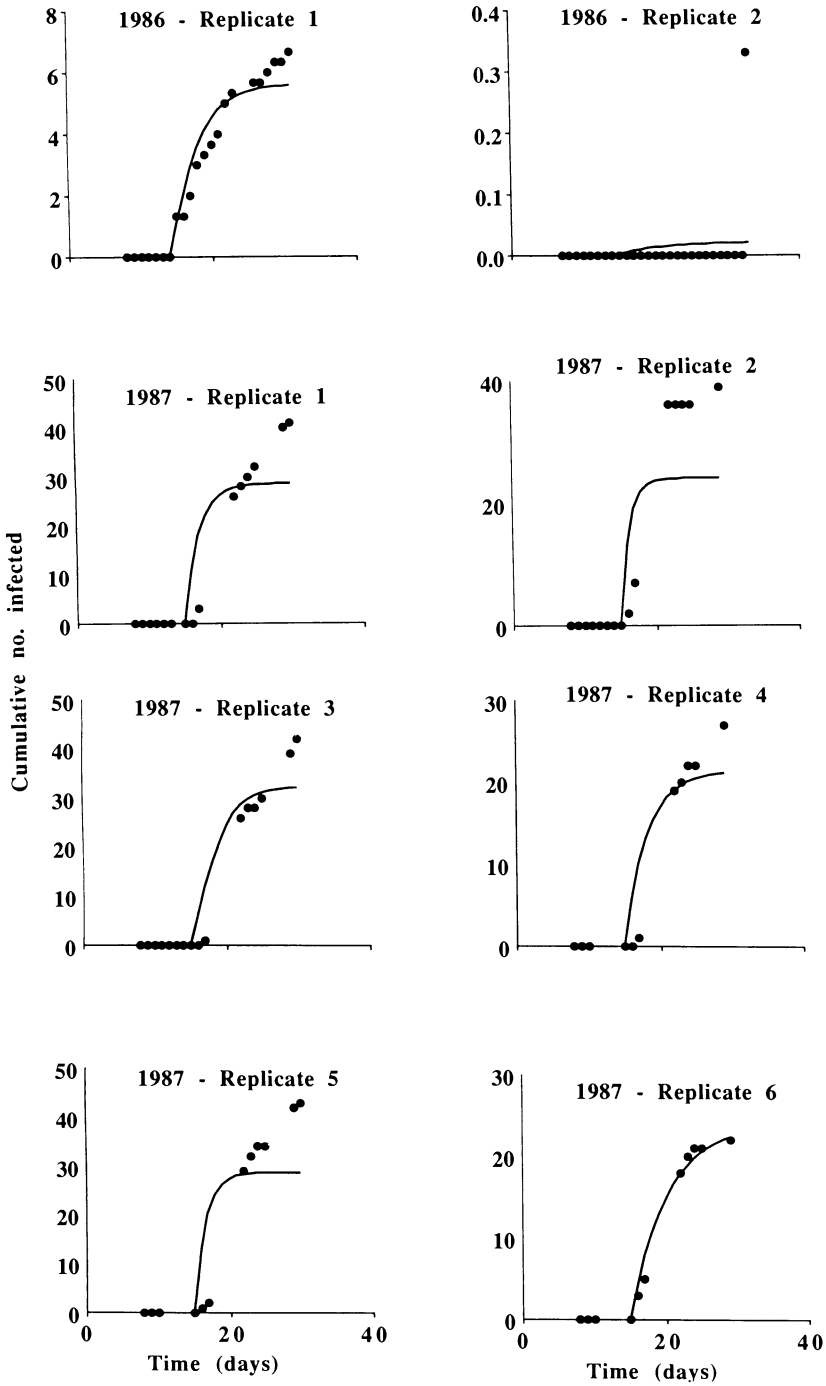

FIG. 3. Time series of disease mortality in each replicate of density experiment. Smooth curves indicate best fit of model (see Appendix) using parameters in Table 1.

The results of the 1989 spatial dispersion experiment (Fig. 7) were not as clear as the results of 1988. In particular, in 1989, although the fraction of third instars that became infected was higher in the uniform treatment, the trend was reversed in fifth instars. However, this interaction between instar and spatial dispersion was not statistically significant ($G = 3.88$, df $= 3$, $P = .2$). One result that was consistent between years was that the fraction of fifth instars that became infected was significantly higher than the fraction of third instars that became infected ($G = 22.08$, df $= 10$, $P = .015$).

Two caveats, however, apply to the 1989 spatial dispersion experiment. First of all, in contrast to earlier

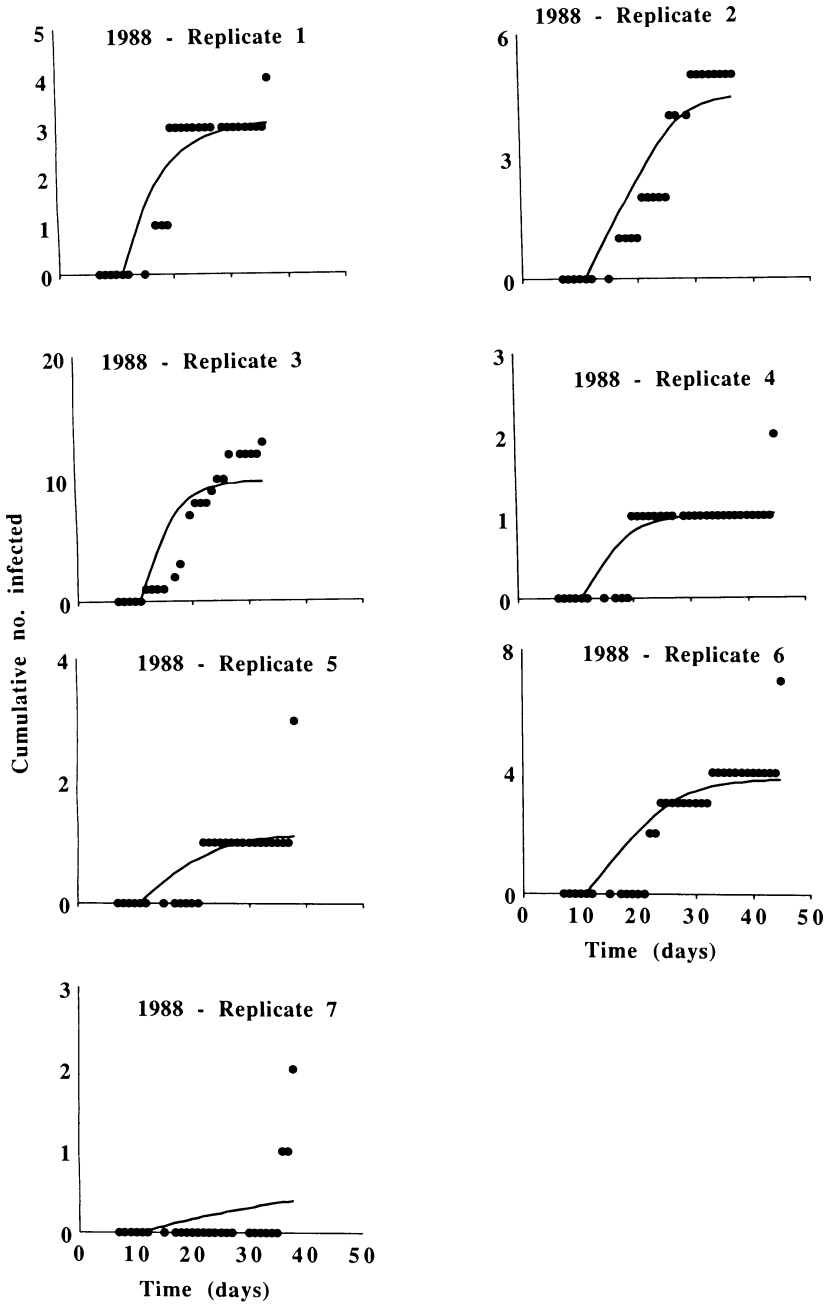

FIG. 3. Continued.

experiments, six infections occurred among control insects. Three of these (one fifth instar and two third instars) occurred within 10 d of the start of the experiment. Since this is less than the typical incubation time of the disease in third instars in the field or in the laboratory, these infections were undoubtedly due to accidental contamination in the laboratory. The remaining three infections were all in fifth instars, in which the disease often has a very long incubation time. In short, all the infections among control insects were probably due to contamination in the laboratory rather than to extraneous virus in the field. Because of the infections in my controls, I did not count any infections that occurred in the experimental treatments within 10 d of the start of the experiment. Secondly, in one cage of the fifth instar/patchy virus treatment, ants killed 10 larvae, which I then had to exclude from the analysis.

In spite of these caveats, the overall effects when examining the 1988 and 1989 experiments together are clear. That is, healthy early-instar larvae in these experiments were generally more likely to become in-

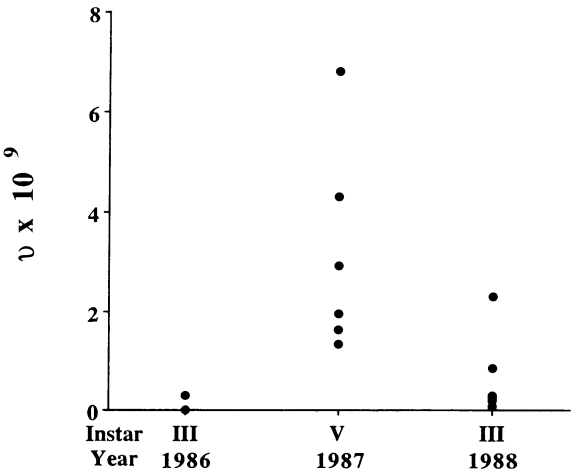

FIG. 4. Estimates of the virus transmission parameter ν, in Eqs. 6 and 7, for each replicate in density experiments in 1986, 1987, and 1988.

fected when exposed to uniformly distributed infected cadavers than when exposed to patchily distributed infected cadavers (Figs. 6 and 7), but the risk of infection of late instars was roughly the same regardless of the spatial dispersion of infected cadavers. Healthy larvae were also more likely to become infected when exposed to higher densities of infected cadavers, as is anticipated by Eq. 1. Finally, late instars were again in general more likely to become infected than were early instars.

DISCUSSION

My experiments show that host density, host instar, and virus spatial distribution all have significant effects on the rate of transmission of the virus in the field. More specifically, the results of the density experiments indicate that the proportionality between transmission and host density times pathogen density (PS) can vary markedly. In particular, it appears that transmission rates probably varied between 1986–1988 and 1987 as a result of differences in the instar of the larvae used in 1987 vs. 1986 and 1988. This interpretation is suggested by my experimental finding that healthy fifth instars exposed to infected fifth instars are more likely to become infected than are healthy third instars exposed to infected third instars. Moreover, this higher transmission rate among fifth instars was consistent across densities in the density experiment (from 25 to 55 healthy larvae per two trees) and years (1987, 1988, and 1989).

Since later instar larvae produce more virus particles per larva when infected (Thompson and Scott 1979), one would expect that the fraction of larvae infected by fifth instars would be higher than that infected by third instars. Fig. 5 shows that the fraction of third instars infected by fifth instars was indeed much higher than the fraction infected by third instars. The fraction of fifth instars infected by fifth instars, however, was slightly lower than the fraction infected by third instars. I suspect that this curous result was due to the fact that it took longer for fifth instars to die than it took for third instars to die, and the disease can only be transmitted after the larvae have died. As a result, fifth instars had less time to transmit the disease, which lowered the fraction that they infected.

Since the physiological resistance of DFTM larvae increases with age, one might further expect that, in field epizootics, the fraction of larvae that became infected would be consistently higher among healthy third instars than among healthy fifths. Fig. 3 shows that the opposite is true: the fraction of healthy larvae that became infected was consistently higher among fifth instars than among third instars. This greater risk of infection of late instars can be due to their higher feeding rate; for many caterpillars, including DFTM, feeding rate increases with body mass (and thus instar; Crawley 1983). I suggest, however, that the higher mobility of later instars may partly explain their higher risk of infection. Since late instars are much more mobile than are early instars (G. Dwyer, *personal observation*), late instars are presumably at greater risk of encountering an infectious dose of the virus. In other words, the greater resistance of late instars may be less important than their greater risk of contacting an infectious dose. Moreover, the number of PIBs in each infected cadaver is many times an infectious dose; if a healthy larva encounters the virus at all, it probably encounters many times an infectious dose. These speculations are supported by my results on the effects of the spatial dispersion of the virus. That is, the fact that when the virus is clumped, the risk of infection for early instars is lowered, but the risk of infection for late instars is the same, suggests that the higher risk of infection of late instars is at least partially due to their higher mobility. In particular, if the lower risk of infection of early instars was solely due to their lower feeding rate, then there would be no effect of clumping, since the total amount of virus (and thus the amount of virus-contaminated foliage) was the same in both patchy and uniform treatments in both 1988 and 1989.

The fact that the effects of instar and spatial dispersion in the 1988 experiments were significantly affected by the experimental block indicates that there was some effect of shading. Because I did not record the relative

TABLE 2. Means and bootstrapped 95% confidence intervals for the virus transmission coefficient ν (shown multiplied by 10^9), for 1987 and pooled 1988 and 1986 density experiments.

Year	Lower CL	Mean	Upper CL
1987	1.76	3.13	5.27
1988 + 1986	0.21	0.55	1.15

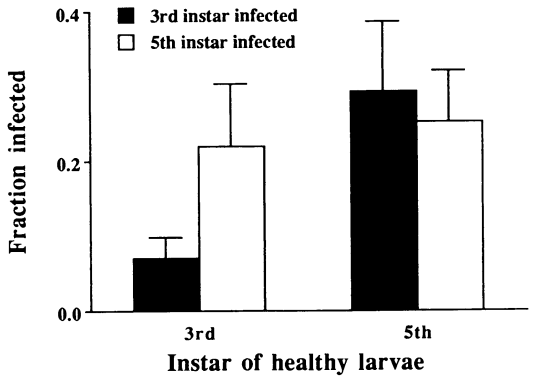

FIG. 5. Fraction of healthy larvae that became infected in the presence of infected third- or fifth-instar larvae (host stage structure experiment). Error bars indicate 1 SE.

shadiness of the different blocks, the direction of any such effects is unknown. The block effect may have more to do with temperature than with amount of light, as on the time scale of these experiments virus decay due to exposure to sunlight is probably minimal (Olofsson 1988). In so far as the block effects have to do with temperature, they may have affected disease incidence by increasing host movement rates, as DFTM movement is strongly affected by temperature (G. Dwyer, *personal observation*).

Regardless of the mechanism, the result that late instars are both more infectious and more likely to become infected indicates that, in epizootics in natural populations, infection rates should be highest among late instars. This is indeed true in naturally occurring virus epizootics in many Lepidoptera (and diprionid Hymenoptera [sawflies]) (Kaupp 1983, Tanada 1985, Woods and Elkinton 1987) including DFTM (Mason and Thompson 1971, Mason 1981), although often there is a peak of infection in the first instar due to the overwinter survival of virus on contaminated eggs (Murray and Elkinton 1989). This correspondence between the results of my experiments and observations of naturally occurring epizootics suggests that my results are not peculiar to my small experimental arenas. Apparently, the pattern of disease incidence with respect to stage structure is the same in epizootics at both the large scale of natural epizootics and the small scale of my experiments.

The possibility that transmission rates are a function of instar-specific movement rates, virus spatial distribution, and host and virus density has implications for the use of bioassays and mathematical models, as well as for the application of viruses in biological control. First of all, it is clear that bioassay results must be carefully interpreted. Even though bioassays have established that late-instar DFTM larvae are less susceptible than are early-instar DFTM larvae, in the field late instars are more likely to become infected than are early instars. This apparent contradiction between laboratory bioassays and field experiments could be due to any of a number of important facets of DFTM biology that are not present in a laboratory experiment; however, my experiments suggest that part of the problem lies in not knowing what the distribution of infectious doses is in the field. If larvae either encounter many times an infectious dose of the virus, in the form of an infected cadaver, or do not encounter the virus at all, then the average density of infectious doses may not be as important as their spatial distribution (Richter et al. 1987).

The dependence of the transmission coefficient for the NPV on the instar of DFTM suggests that models of this and similar systems would be more useful (for prediction, at least) if they included stage structure. It is not certain what effect such additional complexity would have on the dynamics of Anderson and May's (1981) model. Anderson and May's (1981) conclusion, that insect diseases having long-lived infectious stages will tend to drive long-term host population cycles, would probably be unchanged; that is, adding stage structure would probably promote such cycles (Brown 1984, Liu et al. 1987, Andreasen 1989). However, without stage structure, models of insect host–virus systems will be of little use in understanding the dynamics of epizootics over shorter time scales, as for example within a season. Fortunately, the basic mathematical framework developed by Anderson and May (1981) easily could be modified to accomodate this complexity; clearly, this deserves more attention.

The greater risk of infection among late instars has implications for the biological control of insect pests, including DFTM. Because early instars are more susceptible in the laboratory, it is sometimes assumed that, when NPVs are used as biological insecticides, maximum population reduction will be achieved by spraying early instars (Watanabe 1987, Young and Yearian 1987*b*). However, if late instars are more likely to become infected, then the opposite may be true.

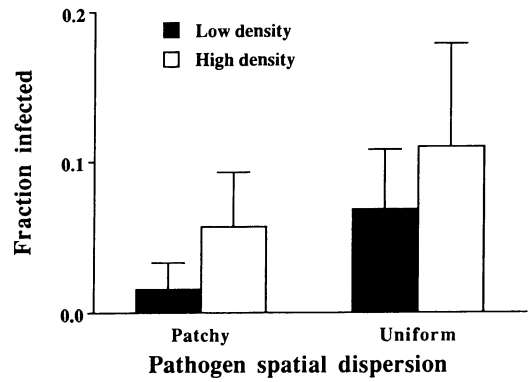

FIG. 6. Fraction of healthy larvae that became infected in the presence of infected cadavers with two different densities and distributions (pathogen spatial dispersion experiment, 1988). Error bars indicate 1 SE.

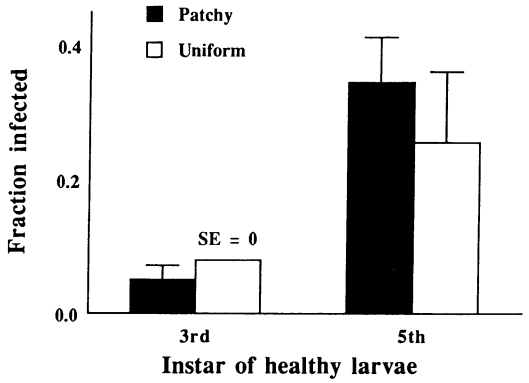

FIG. 7. Fraction of healthy third- or fifth-instar larvae that became infected in the presence of infected cadavers with two different distributions (host stage structure times pathogen spatial dispersion experiment, 1989). Error bars indicate 1 SE.

Although it is often perilous to extrapolate from small-scale experiments to large-scale population control, published reports of the use of the NPV of DFTM as a biological insecticide indicate that such an extrapolation may be justified. In two different DFTM–NPV spraying programs, although the virus was applied in the first instar, infection was highest in the last or second-to-last instar (Shepherd et al. 1984, Otvos et al. 1987*a*). Late instars may thus be at greater risk of infection at both large and small spatial scales.

Larval movement rates also may be relevant to biological control programs. Otvos et al. (1987*b*) observed NPV epizootics as much as 500 m away from sprayed plots, and therefore suggest that spraying alternate swaths of forest may be as effective as blanket spraying. Since they believe that this movement of the epizootic out of the sprayed zone may be due in part to the movement of infected larvae, a better understanding of the influence of larval movement on the rate of spread of the disease may lead to cheaper and more effective biological control, not just of DFTM but of many insect pests.

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LITERATURE CITED

Anderson, R. M., and R. M. May. 1979. Population biology of infectious diseases. Nature **280**:361–462.

Anderson, R. M., and R. M. May. 1981. The population dynamics of microparasites and their invertebrate hosts. Philosophical Transactions of the Royal Society, B, Biological Sciences **291**:451–524.

Anderson, R. M., H. C. Jackson, R. M. May, and A. M. Smith. 1981. Population dynamics of fox rabies in Europe. Nature **289**:765–771.

Andreadis, T. G. 1987. Transmission. Pages 159–176 *in* J. R. Fuxa and Y. Tanada, editors. Epizootiology of insect diseases. Wiley, New York, New York, USA.

Andreasen, V. 1989. Disease regulation of age-structured host populations. Theoretical Population Biology **36**:214–239.

Bailey, N. T. J. 1975. The mathematical theory of infectious disease and its application. Second edition. Griffin, London, England.

Bird, F. T., and J. M. Burk. 1961. Artificially disseminated virus as a factor controlling the European spruce sawfly, *Diprion hercyniae* (Htg.) in the absence of introduced parasites. Canadian Entomologist **93**:228–238.

Boucias, D. G., and G. L. Nordin. 1977. Interinstar susceptibility of the fall webworm, *Hyphantria cunea,* to its nucleopolyhedrosis viruses. Journal of Invertebrate Pathology **30**:68–75.

Brookes, M. H., R. W. Stark, and R. W. Campbell, editors. 1978. The Douglas-fir tussock moth: a synthesis. United States Department of Agriculture Technical Bulletin **1585**.

Brown, G. C. 1984. Stability in an insect-pathogen model incorporating age-dependent immunity and seasonal host reproduction. Journal of Mathematical Biology **46**:139–153.

Carter, J. B., E. I. Green, and A. J. Kirkham. 1983. A *Tipula paludosa* population with a high incidence of two pathogens. Journal of Invertebrate Pathology **42**:312–318.

Crawford, A. M., and J. Kalmakoff. 1977. A host–virus interaction in a pasture habitat. Journal of Invertebrate Pathology **29**:81–87.

Crawley, M. J. 1983. Herbivory: the dynamics of animal–plant interactions. University of California Press, Riverside, California, USA.

Dwyer, G., S. A. Levin, and L. Buttel. 1990. A simulation model of the population dynamics and evolution of myxomatosis. Ecological Monographs **60**:423–447.

Efron, B., and R. Tibshirani. 1986. Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. Statistical Science **1**:54–77.

Entwistle, P. F., P. H. W. Adams, H. F. Evans, and C. F. Rivers. 1983. Epizootiology of a nuclear polyhedrosis virus (Baculoviridae) in European spruce sawfly (*Gilpinia hercyniae*): spread of disease from small epicentres in comparison with spread of baculovirus diseases in other hosts. Journal of Applied Ecology **20**:473–487.

Evans, H. F. 1981. Quantitative assessment of the relationships between dosage and response of the nuclear polyhedrosis virus of *Mamestra brassicae.* Journal of Invertebrate Pathology **37**:101–109.

Evans, H. F., and G. P. Allaway. 1983. Dynamics of baculovirus growth and dispersal in *Mamestra brassicae* L. (Lepidoptera: Noctuidae) larval populations introduced into small cabbage plots. Applied and Environmental Microbiology **45**:493–501.

Evans, H. F., and P. F. Entwistle. 1987. Viruses. Pages 257–322 *in* J. R. Fuxa and Y. Tanada, editors. Epizootiology of insect diseases. Wiley, New York, New York, USA.

Fleming, S. B., J. Kalmakoff, R. D. Archibald, and K. M. Stewart. 1986. Density-dependent virus mortality in populations of *Wiseana* (Lepidoptera: Hepialidae). Journal of Invertebrate Pathology **48**:193–198.

Fuxa, J. R. 1982. Prevalence of viral infections in populations of fall armyworm, *Spodoptera frugiperda,* in southeastern Louisiana. Environmental Entomology **11**:239–242.

———. 1983. Multiple-regression analysis of factors affecting prevalence of nuclear polyhedrosis virus in *Spodoptera*

frugiperda (Lepidoptera: Noctuidae) populations. Environmental Entomology **12**:311–316.

———. 1987. Ecological considerations for the use of entomopathogens in IPM. Annual Review of Entomology **32**: 225–251.

Fuxa, J. R., and J. P. Geaghan. 1983. Multiple-regression analysis of factors affecting prevalence of nuclear polyhedrosis virus in *Spodoptera frugiperda* (Lepidoptera: Noctuidae). Environmental Entomology **12**:311–316.

Fuxa, J. R., and Y. Tanada. 1987. Epidemiological concepts applied to insect epizootiology. Pages 3–22 *in* J. R. Fuxa and Y. Tanada, editors. Epizootiology of insect diseases. Wiley, New York, New York, USA.

Harkrider, J. R., and I. M. Hall. 1978. The dynamics of an entomopoxvirus in a field population of larval midges of the *Chironomus decorus* complex. Environmental Entomology 7:858–862.

Hudson, D. J. 1966. Fitting segmented curves whose join points have to be estimated. Journal of the American Statistical Association **61**:1097–1129.

Jaques, R. P. 1974. Occurrence and accumulation of viruses of *Trichoplusia ni* in treated field plots. Journal of Invertebrate Pathology **23**:140–152.

Kalmakoff, J., and A. M. Crawford. 1982. Enzootic virus control of *Wiseana* spp. in the pasture environment. Pages 435–438 *in* E. Kurstak, editor. Microbial and viral pesticides. Dekker, New York, New York, USA.

Kaya, H. K., and J. F. Anderson. 1976. Biotic mortality factors in dark tussock moth populations in Connecticut. Environmental Entomology **5**:1141–1145.

Kaupp, W. J. 1983. Estimation of nucelar polyhedrosis virus produced in field populations of the European sawfly, *Neodiprion sertifer* (Geoff.) (Hymenoptera: Diprionidae). Canadian Journal of Zoology **61**:1857–1861.

Keating, S. T., and W. G. Yendol. 1987. Influence of selected host plants on gypsy moth (Lepidoptera: Lymantriidae) larval mortality caused by a baculovirus. Environmental Entomology **16**:459–462.

Klein, M., and H. Podoler. 1978. Studies on the application of a nuclear polyhedrosis virus to control populations of the Egyptian cottonworm, *Spodoptera littoralis*. Journal of Invertebrate Pathology **32**:244–248.

Levin, S. A., and D. Pimentel. 1981. Selection for intermediate rates of increase in parasite-host systems. American Naturalist **117**:308–315.

Liu, W., S. A. Levin, and Y. Iwasa. 1987. Influence of nonlinear incidence rates upon the behavior of SIRS epidemiological models. Journal of Mathematical Biology **23**: 187–204.

Magnoler, A. 1975. Bioassay of nucleopolyhedrosis virus against larval instars of *Malacosoma neustria*. Journal of Invertebrate Pathology **25**:343–348.

Martignoni, M. E., P. J. Iwai, and G. F. Rohrmann. 1980. Serum neutralization of nucleopolyhedrosis viruses (*Baculovirus* subgroup A) pathogenic for *Orgyia pseudotsugata*. Journal of Invertebrate Pathology **36**:12–20.

Mason, R. R. 1981. Numerical analysis of the causes of population collapse in a severe outbreak of the Douglas-fir tussock moth. Annals of the Entomological Society of America **74**:51–57.

Mason, R. R., and C. G. Thompson. 1971. Collapse of an outbreak population of the Douglas-fir tussock moth *Hemerocampa pseudotsugata* (Lepidoptera: Lymantriidae). United States Department of Agriculture Forest Service Research Note **PNW-139**.

McLeod, P. J., S. Y. Young, and W. C. Yearian. 1982. Application of a baculovirus of *Pseudoplusia includens* to soybean: efficacy and seasonal persistence. Environmental Entomology **11**:412–416.

Millstein, J. A. 1988. The population dynamics of the Douglas-fir tussock moth: a study of multiple interactions. Dissertation. Washington State University, Pullman, Washington, USA.

Mitchell, R. G. 1979. Dispersal of early instars of the Douglas-fir tussock moth. Annals of the Entomological Society of America **72**:291–297.

Mohamed, M. A., H. C. Coppel, and J. D. Podgwaite. 1983. Artificially-induced nucleopolyhedrosis virus epizootic in populations of *Neodiprion sertifer* (Hymenoptera: Diprionidae). Environmental Entomology **12**:397–399.

Murdoch, W. W., J. Chesson, and P. L. Chesson. 1985. The theory and practice of biological control. American Naturalist **125**:344–366.

Murray, J. D., E. A. Stanley, and D. L. Brown. 1986. On the spatial spread of rabies among foxes. Proceedings of the Royal Society, London, Series **B**, **229**:111–150.

Murray, K. D., and J. Elkinton. 1989. Environmental contamination of egg masses as a major component of transgenerational transmission of gypsy moth nuclear polyhedrosis virus (LdMNPV). Journal of Invertebrate Pathology **53**:324–334.

Myers, J. H. 1981. Interactions between western tent caterpillars and wild rose: a test of some general plant herbivore hypotheses. Journal of Animal Ecology **50**:11–25.

Olofsson, E. 1988. Environmental persistence of the nuclear polyhedrosis virus of the European pine sawfly in relation to epizootics in Swedish Scots pine forests. Journal of Invertebrate Pathology **52**:119–129.

Otvos, I. S., J. C. Cunningham, and L. M. Friskie. 1987*a*. Aerial application of nuclear polyhedrosis virus against Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Lymantriidae). I. Impact in the year of application. Canadian Entomologist **119**:697–706.

Otvos, I. S., J. C. Cunningham, and R. I. Alfaro. 1987*b*. Aerial application of nuclear polyhedrosis virus against Douglas-fir tussock moth, *Orgyia pseudotsugata* (McDunnough) (Lepidoptera: Lymantriidae). II. Impact 1 and 2 years after application. Canadian Entomologist **119**:707–715.

Podgwaite, J. D., P. Rush, D. Hall, and G. S. Walton. 1984. Efficacy of the *Neodiprion sertifer* (Hymenoptera: Diprionidae) nuclear polyhedrosis virus (Baculoviridae) product, Neochek-S. Journal of Economic Entomology **77**:525.

Podgwaite, J. D., K. S. Shields, R. T. Zerillo, and G. S. Walton. 1979. Environmental persistence of the nucleopolyhedrosis virus of the gypsy moth *Lymantria dispar*. Journal of Environmental Entomology **8**:528–536.

Richter, A. J., J. R. Fuxa, and M. Abdel-Fattah. 1987. Effect of host plant on the susceptibility of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) to a nuclear polyhedrosis virus. Environmental Entomology **16**:1004–1006.

Robertson, J. 1985. *Orgyia pseudotsugata*. Pages 401–406 *in* P. Singh and R. F. Moore, editors. Handbook of insect rearing. Volume II. Elsevier, New York, New York, USA.

Ross, R. 1911. The prevention of malaria. Second edition. Murray, London, England.

Seber, G. A. F. 1977. Linear regression analysis. John Wiley & Sons, New York, New York, USA.

Shepherd, R. F., I. S. Otvos, R. J. Chorney, and J. C. Cunningham. 1984. Pest management of Douglas-fir tussock moth (Lepidoptera: Lymantriidae): prevention of an outbreak through early treatment with a nuclear polyhedrosis virus by ground and aerial applications Canadian Entomologist **116**:1533–1542.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman, New York, New York, USA.

Stairs, G. R. 1965. Artificial initiation of virus epizootics in forest tent caterpillar populations. Canadian Entomologist **97**:1059–1062.

———. 1966. Transmission of virus in tent caterpillar populations. Canadian Entomologist **98**:1100–1104.

Tanada, Y. 1985. A synopsis of studies on the synergistic

property of an insect baculovirus: a tribute to Edward A. Steinhaus. Journal of Invertebrate Pathology **45**:125–138.
Teakle, R. E., and V. S. Byrne. 1989. Nuclear polyhedrosis virus production in *Heliothis armigera* infected at different larval ages. Journal of Invertebrate Pathology **53**:21–24.
Thompson, C. G., and D. W. Scott. 1979. Production and persistence of the nuclear polyhedrosis virus of the Douglas-fir tussock moth, *Orgyia pseudotsugata* (Lepidoptera: Lymantriidae), in the forest ecosystem. Journal of Invertebrate Pathology **33**:57–65.
Thompson, C. G., and E. A. Steinhaus. 1950. Further tests using a polyhedral virus to control the alfalfa caterpillar. Hilgardia **19**:411.
Vail, P. V., T. J. Henneberry, A. N. Kishaba, and K. Y. Arakawa. 1968. Sodium hypochlorite and formalin as antiviral agents against nuclear polyhedrosis virus in larvae of the cabbage looper. Journal of Invertebrate Pathology **10**:84–93.
Watanabe, H. 1987. The host population. Pages 71–112 *in* J. R. Fuxa and Y. Tanada, editors. Epizootiology of insect diseases. Wiley, New York, New York, USA.
Whitlock, V. H. 1977. Effect of larval maturation on mortality induced by nuclear polyhedrosis and granulosis virus infections of *Heliothis armigera*. Journal of Invertebrate Pathology **30**:80–86.
Woods, S. A., and J. S. Elkinton. 1987. Bimodal patterns of mortality from nuclear polyhedrosis virus in gypsy moth (*Lymantria dispar*) populations. Journal of Invertebrate Pathology **50**:151–157.
Young, S. Y., and W. C. Yearian. 1987*a*. Intra- and intercolony transmission of a nuclear polyhedrosis virus of the loblolly pine sawfly *Neodiprion taedae linearis* Ross, on pine. Journal of Entomological Science **22**:29–34.
Young, S. Y., and W. C. Yearian. 1987*b*. Transmission of a nuclear polyhedrosis virus in *Anticarsia gemmatalis* (Hubner) (Lep.: Noctuidae) larval populations on caged soybean. Environmental Entomology **16**:691–698.
Zelazny, B. 1977. Transmission of a baculovirus in populations of *Oryctes rhinoceros*. Journal of Invertebrate Pathology **27**:221.

APPENDIX

In this appendix, I present the solutions to Eq. 9 in the case when the natural mortality rate varies with time. In all cases, incubation time of the disease, $T <$ time t. P_0 is the number of pathogen particles at the beginning of the experiment, and $R(t)$ is the cumulative number of secondary infections at time t.

First, if the incubation period is less than the time until the natural mortality rate changes, t^*, (i.e., $T < t^*$), then

$$R(t) = \nu P_0 S_0 \left\{ \frac{e^{-b_1 T}}{(b_1 + \nu P_0)} [1 - e^{-(b_1 + \nu P_0)(t - T)}] \right\}$$

for $t < t^*$

$$R(t) = \nu P_0 S_0 \left\{ \frac{e^{-b_1 T}}{(b_1 + \nu P_0)} [1 - e^{-(b_1 + \nu P_0)(t^* - T)}] + \frac{e^{\nu P_0 T + (b_2 - b_1)t^*}}{(b_2 + \nu P_0)} [e^{-(b_2 + \nu P_0)t^*} - e^{-(b_2 + \nu P_0)t}] \right\}$$

for $t^* < t < (t^* + T)$

$$R(t) = \nu P_0 S_0 \left\{ \frac{e^{-b_1 T}}{(b_1 + \nu P_0)} [1 - e^{-(b_1 + \nu P_0)(t^* - T)}] + \frac{e^{\nu P_0 T - (b_1 + \nu P_0)t^*}}{(b_2 + \nu P_0)} [1 - e^{-(b_2 + \nu P_0)T}] + \frac{e^{-b_2 T - (b_1 + \nu P_0)t^*}}{(b_2 + \nu P_0)} [1 - e^{-(b_1 + \nu P_0)(t - t^* - T)}] \right\}$$

for $(t^* + T) < t$.

If, instead, the incubation period is greater than the time until the natural mortality rate changes, so that $t^* < T$, then

$$R(t) = \nu P_0 S_0 \left\{ \frac{e^{-\nu P_0 T + (b_2 - b_1)t^*}}{(b_2 + \nu P_0)} [e^{-(b_2 + \nu P_0)T} - e^{-(b_2 + \nu P_0)t^*}] \right\}$$

for $t < (t^* + T)$

$$R(t) = \nu P_0 S_0 \left\{ \frac{e^{-b_2 T + (b_2 - b_1)t^*}}{(b_2 + \nu P_0)} [1 - e^{-(b_2 + \nu P_0)t^*}] + \frac{e^{-b_2 T - (b_1 + \nu P_0)t^*}}{(b_2 + \nu P_0)} [1 - e^{-(b_2 + \nu P_0)(t - t^* - T)}] \right\}$$

for $(t^* + T) < t$.