Ecology, 76(2), 1995, pp. 392–401

TRANSMISSION DYNAMICS OF A VIRUS IN A STAGE-STRUCTURED INSECT POPULATION[1]

D. GOULSON,[2] R. S. HAILS,[3] T. WILLIAMS, M. L. HIRST, S. D. VASCONCELOS, B. M. GREEN, T. M. CARTY, AND J. S. CORY

Ecology and Biocontrol Section, NERC Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford, OX1 3SR, UK

Abstract. Despite the blossoming interest in host–microparasite epidemiology, and in use of viruses in the biological control of insect pests, few empirical studies have attempted to quantify transmission and mortality rates under field conditions. We report a laboratory and field study in which the transmission parameter (ν) and mortality rate (α) due to nuclear polyhedrosis virus (NPV) are measured in different larval instars of the cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae). Laboratory studies of food consumption and virus susceptibility were used to produce crude estimates of relative transmission rates in successive instars. Increases in the rate of feeding outstrip increases in virus resistance with instar, so we predict that transmission rates should increase in older larvae (assuming rate of intake of virus to be proportional to rate of feeding). This prediction was tested in a field experiment in which a constant initial density of susceptible and infected (moribund) larvae were reared together on cabbage plants for 2–8 d. Estimates of the linear transmission parameter (ν) did not differ between instars and gave a mean value of 2.16×10^{-12} for all instars and time points. Causes for the discrepancy between predictions based on laboratory data and field measurements are discussed. Differences were found between instars in the time from infection to death (τ) (equivalent to $1/\alpha$, where α is the rate of mortality due to viral infection). Second-instar larvae died more rapidly once infected than third instars, which, in turn, died more rapidly than fourth instars. The effect of host population stage structure on patterns of viral infection can be pronounced and if recognized, may significantly increase the accuracy and predictive value of models of host pathogen systems.

Key words: baculovirus; instar; Lepidoptera; Mamestra brassicae; *mortality rate; nuclear polyhedrosis virus; pathogen; stage; susceptibility; transmission.*

INTRODUCTION

Theoretical ecologists, stimulated by the seminal works of Anderson and May (1979, 1981), have recently extended the scope of epidemiology beyond its traditional focus on human diseases to encompass the full breadth of animal–pathogen systems (e.g., Anderson et al. 1981, Murray et al. 1986, Dwyer et al. 1990). In particular, work has proliferated on the application of epidemiological models to insect–virus population dynamics (for example, Hochberg 1989, 1991, Hochberg et al. 1990, Onstad and Carruthers 1990, Begon et al. 1992, Dwyer 1994). Despite the ease with which insect populations can be manipulated and virus infections diagnosed, few empirical studies have examined the assumptions made in theoretical models or attempted to estimate their parameters so that quantitative predictions can be made (but see Dwyer 1991, Dwyer and Elkinton 1993). Determination of virus transmission rates has obvious implications for the use of viruses for insect biocontrol (Otvos et al. 1987) and may be influential in aiding our understanding of host population outbreaks and viral epizootics (Anderson and May 1981, Entwistle et al. 1983, Fuxa and Tanada 1987, Young and Yearian 1987).

Anderson and May (1981) type models assume, for the sake of simplicity, that host susceptibility to lethal infection does not vary with age. For insect–virus systems this is clearly not true. For example, lepidopteran baculoviruses are only able to produce patent infections in the larval stages of their hosts (patent infections invariably result in death). Successful predictions in baculovirus systems have been made by restricting models to one larval cohort (Dwyer and Elkinton 1993) but even this approach assumes that transmission rates do not differ according to instar. Transmission rates in the field are determined by a combination of several processes, principally the susceptibility of larvae to infection, and the consumption rate of foliage (and hence of the pathogen). Susceptibility of larvae to baculovirus infection is usually measured in the laboratory, using a range of known doses of virus administered individually to larvae over a short time period (<24 h), to derive the median dose that produces 50% mortality (the LD_{50}) (for example, Evans 1981). Larval susceptibility to viral disease decreases greatly with age: for example, the LD_{50} for the velvetbean caterpillar, *Anticarsia gemmatalis*, increases from ≈100 vi-

[1] Manuscript received 19 January 1994; revised 13 July 1994; accepted 17 July 1994.

[2] Present address: Department of Biology, University of Southampton, Southampton, UK.

[3] Address correspondence to this author.

rus occlusion bodies (OBs) for first-instar larvae to 4100 for fifth instars (Boucias et al. 1980). Comparable age-related susceptibility has been described in numerous studies (for example, Stairs 1965, Magnoler 1975, Boucias and Nordin 1977, Evans 1981, 1983). Measurement of susceptibility by LD_{50} in the laboratory does not take into account the greater food consumption of older larvae, which may increase the number of virus occlusion bodies (OBs) ingested in the field (e.g., Smits et al. 1987). In Lepidoptera, transmission normally occurs following the death of an infected larva, which releases many viral OBs onto the foodplant foliage where they may subsequently be eaten by healthy larvae. Hence Payne et al. (1981) suggested that, in species in which susceptibility in the laboratory decreases in proportion to size, decreasing susceptibility and increasing consumption with age may compensate each other, so that in the field, transmission rates do not differ according to instar. However, in species that show a disproportionate decrease in laboratory susceptibility with size, for example, *Pieris brassicae* and *Mamestra brassicae* (Evans 1981), consumption would be insufficient to compensate, so that later instars may become less prone to infection. The two published field studies that have investigated the relationship between age and virus transmission do not agree with either of these hypotheses, or with each other, as to which instars are most prone to infection, and both infer that differences in larval behavior underlie observed differences in transmission rates (Webb and Shelton 1990, Dwyer 1991).

A number of assumptions are implicit in the theoretical and empirical studies above, not least, that the probability of consuming infective virus is the same for all susceptible larvae. However, virus distribution in the field is likely to be highly contagious, in the vicinity of hosts that have succumbed to infection and released infective occlusion bodies. Such spatial heterogeneity in virus distribution may be highly influential in affecting transmission rates to different host instars, especially if those instars show differences in their feeding behavior or mobility. Hence the rate at which different instars become infected in the field (the transmission rate) may not be predictable from laboratory studies.

We aimed to investigate the outcome of age-related variation in food intake and susceptibility to infection in terms of differences in transmission rate between instars (the rate at which previously uninfected larvae pick up lethal infections). Consumption rates and virus susceptibility were quantified for each instar in the laboratory, and used to derive approximate predictions as to the relative rates of infection of each instar in the field. These predictions were then tested, within a single cycle of infection. Specifically, the transmission rate, the linear transmission parameter (ν), and the time from infection to death (τ) of larvae of different instars were measured in the field within experimental plots infested with a constant initial density of susceptible larvae. Inoculum was provided in the form of moribund infected larvae. Model assumptions of a constant density of inoculum ("free-living" infective stages) were also evaluated during the period of the trial.

M. brassicae (L.), the cabbage moth, provided a convenient subject as both the insect and its food plant, cabbage, are easily cultured, and because its multiply enveloped nuclear polyhedrosis virus (*Mb*NPV) (Baculoviridae) has been extensively studied in terms of its biological activity (Evans et al. 1981, Evans 1981, 1983), host range (Doyle et al. 1990), and biochemical characteristics (Brown et al. 1981, Possee and Kelly 1988). The dynamics of this system is also of relevance to biological control, for *M. brassicae* is an important pest of *Brassica* crops, while the virus is commercially used as a means of controlling outbreaks.

Methods

Insect material was obtained from a culture of *M. brassicae* maintained at the NERC Institute of Virology and Environmental Microbiology, Oxford, originally established in 1977 from females caught in Oxford at light. The virus strain was originally obtained from A. Gröner (Biologische Bundesanstalt, Darmstadt, Germany) in 1976 and originated from an epizootic in an insect culture in Darmstadt in 1973.

Food consumption in the laboratory

Estimates of larval feeding rates in terms of leaf area ingested were obtained for second, third and fourth instars by containing 50 newly moulted larvae individually in ventilated plastic pots (4 cm diameter × 3.5 cm high) each with a 17 mm diameter leaf disc of cabbage. Larvae were allowed to feed for 24 h in darkness at 24°C, and the leaf discs then removed for measurement. As larvae of all instars tend to graze on the surface of the leaf without penetrating through it, leaf area measurement devices proved to be unsuitable. Leaf areas grazed were estimated to within 1 mm^2 by eye with the leaf discs placed on fine-lined graph paper: areas grazed on the top and bottom of the leaf were summed, and a mean value calculated for each instar.

Estimation of LD_{50} values for each instar

LD_{50} values for second, third, and fourth instars were calculated by inoculating larvae with a range of virus doses, and assessing virus-induced mortality. For each instar six doses were used, with 50 larvae per dose, and with each dose replicated 3 times. Larvae were individually inoculated with 1 μL of virus solution on a small plug of artificial diet (sufficiently small to be consumed within 24 h) and subsequently reared at 24°C until pupation or death. Doses were chosen to span the values of LD_{50} for each instar published by Evans (1981).

TABLE 1. Number of replicate plots of each larval instar destructively sampled at each sampling time.

	Day			
	2	4	6	8
Virus plots	4	4	4	3
Control plots	5	3	3	4
Inoculum density monitoring plots	5	3	3	4

Measurement of transmission and mortality rates in the field

The field experiment was carried out on a freshly ploughed and harrowed arable field at the University of Oxford field station, Wytham UK, during July 1993. Sixteen cabbages (var. Spitfire 240) were planted in a four by four grid inside each of 90 1-m^2 plots, themselves arranged 2 m apart in a grid. Plots were constructed with 1.2 m wooden stakes at each corner. A barrier of clear polythene 30 cm high was stapled around each plot, and buried 10 cm into the soil, to minimize ingress of predators and egress of larvae. A polythene roof was stapled across the top of each plot immediately prior to introduction of virus inoculum, to prevent rainfall altering the inoculum density during the trial. Birds were excluded by netting. The cabbages were allowed to establish for 4 wk before the introduction of larvae.

The experiment was designed to examine the transmission rate within each experimental plot during a single cycle of infection, and to compare transmission rates in different instars. Plots were stocked with equal densities of healthy larvae; a concurrent trial demonstrated that proportionate mortality was linearly related to host density in this system, at least within the range of densities used (including that in the present study) (S. D. Vasconcelos, *unpublished data*).

All virus-treated plots were exposed to equal quantities of inoculum, so that only larval instar differed between plots. A subset of plots was then destructively sampled at successive time points. All larvae were removed and reared to pupation in the laboratory to assess the proportion infected. The design can be summarized as follows: 45 plots were assigned to each of the two treatments (inoculum vs. no inoculum). Within each treatment 15 plots were assigned to each of the three larval instars used. The 15 plots for each treatment/instar combination were divided between four sampling times, in which three to five plots (Table 1) were searched destructively for all larvae present.

Virus inoculum was provided in the form of infected larvae. Newly moulted second-instar larvae were each fed a lethal dose of ≈6400 NPV OBs on a plug of artificial diet over a 24-h period, and reared on in groups of 32 at 24°C. Five days after infection the larvae were placed in the field at a density of 2 per plant (32 larvae per plot). Eighty infected larvae were retained to establish the time of mortality. Control plots received no inoculum. Healthy larvae were placed out 24 h after the inoculum larvae, at a density of 9 per plant (144 larvae per plot), using either second-, third-, or fourth-instar larvae (neonates are very sensitive to handling, while larvae older than fourth instars may have pupated within the duration of the experiment). Only larvae that had recently moulted were used, so that within instars, larvae were of approximately equal age and size.

Destructive sampling was carried out at 2, 4, 6, and 8 d after introducing the uninfected larvae. By the 6th d most larvae had moulted to the following instar. Cabbages were cut at soil level and dissected in the field to recover all larvae within each plot. In addition, the soil surface and any self-seeded weeds within the plot were searched by hand. Control plots were searched before inoculated plots to reduce the risk of cross contamination. Larvae remaining within a plot were removed to the laboratory and reared individually on artificial diet at ≈24°C until death or pupation. They were examined at 48-h intervals and the time of death recorded. Where the cause of death was unclear it was further investigated by smearing, staining with Giemsa, and examination under the light microscope for viral OBs. None of the introduced (uninfected) larvae died of virus infection in the field during the 8 d of the trial, i.e., no secondary viral infections occurred and all transmission was a result of the primary inoculum.

Monitoring of inoculum density in the field by simplified bioassay

Fifteen additional plots were used to examine changes in virus inoculum levels during the period of the trial. These plots were set up simultaneously with the main trial, and were allocated random positions within the grid of plots. They were inoculated with infected larvae in an identical manner to, and at the same time as, the other virus-treated plots, but were not infested with healthy larvae at the beginning of the experiment. The 15 plots were divided between the four sampling time points (Table 1). Twenty four hours prior to each sampling point, 144 second-instar larvae were introduced to each plot, which were subsequently sampled destructively, and the larvae reared through to pupation in the laboratory as above. The proportion of larvae that acquired virus infections during this 24-h period was used as an indicator of changes in inoculum density.

Statistical analysis

LD_{50} values for each instar were calculated from the regression equation of the logit of proportionate virus-induced mortality against the log of the virus dose. We have assumed that the mean dose received by larvae of each instar will be directly proportional to the leaf area eaten: predicted relative rates of mortality are thus derived from the mean leaf area eaten for each instar

divided by the LD_{50}. Errors for the estimates for each instar were calculated by randomization methods.

Proportionate mortality in larvae from field plots was analyzed using GLIM (McCullagh and Nelder 1989), and was initially estimated with binomial errors; this error structure was substantiated during the analysis. If the degree of overdispersion was within acceptable limits (when Pearson's χ^2 divided by the residual degrees of freedom is <3), then a dispersion parameter was calculated and used to adjust the scale parameter. Where this procedure was found to be inappropriate (when data were overdispersed), an arcsine transformation with normal errors was used as an alternative.

To estimate the rate of transmission of the virus in each field plot, we followed a protocol recently suggested in the literature (Dwyer and Elkinton 1993). This involves a reduced, within-season version of an existing model (from Anderson and May 1981). Estimates of the rate of transmission consider only transmission events resulting in lethal infections, and assume that the rate of transmission is linearly related to the density of both the pathogen and susceptible hosts. The rate of change of susceptible host density due to virus infection is given by

$$\frac{dS}{dt} = -\nu PS,$$

the rate of change of infected host density is

$$\frac{dI}{dt} = \nu PS - \nu P_{(t-\tau)}S_{(t-\tau)},$$

and the rate of change of virus density in the environment is

$$\frac{dP}{dt} = \Lambda\nu P_{(t-\tau)}S_{(t-\tau)} - \mu P,$$

where S = density of susceptible hosts (number per plot), I = density of infected hosts (number per plot), P = density of pathogen in the environment (occlusion bodies per plot), ν = transmission parameter (square metres per occlusion body per day), τ = time between infection and death of the host (days), Λ = number of pathogen particles produced by an infected larva (occlusion bodies per larva), μ = decay rate of pathogen in the environment (per day), t = time (days).

This model makes a number of assumptions; for example, both the death rate of infected and susceptible larvae due to factors other than disease (referred to as b in Anderson and May 1981) is assumed to be negligible compared to the disease-induced death rate. We also assume that ν is constant over time (although one may expect it to vary according to the weather, particularly temperature, which presumably influences larval behavior). This model is then reduced further by considering a time span less than τ, i.e., the dynamics of an initial infection before any larvae have died and released additional inoculum into the field. Over the course of the experiment, we can also assume that $dP/dt = 0$, i.e., the virus is not lost from the plant substrate in significant quantities (as will be shown later). The model thus reduces to:

$$\frac{dS}{dt} = -\nu P_0 S,$$

$$\frac{dI}{dt} = \nu P_0 S.$$

Integrating we obtain

$$\nu = \frac{-1}{tP_0}\ln\left[1 - \frac{I_t}{S_0}\right],$$

where t is the number of days from the initiation of the experiment. Thus, each time point can give us a separate estimate of ν. P_0 was estimated from the density of inoculum larvae released per plot and the mean number of OBs produced per larvae. Inoculum larvae died as third instars, which release 6.21×10^8 OBs per larva (Evans et al. 1981), giving a value for P_0 of 2.0×10^{10} OBs/m^2. S_0 is the density of susceptible hosts at $t = 0$. If S_0 is taken to be the original number of hosts released into a plot, then we assume that the larvae that were not retrieved at later time points were not infected. Alternatively, if S_0 is taken as the number of hosts retrieved at a particular time point, then the assumption is that susceptible and infected larvae have been lost in equal proportions in the intervening period. We adopted the latter assumption when calculating ν, although adopting the former assumption does not alter the relative values of ν.

Results

Predicting relative transmission rates using laboratory data

The area of food plant surface ingested by larvae increased greatly in successive instars (9.0 ± 0.4, 33.0 ± 2.4, and 241.7 ± 10.7 mm^2/24 h, means ± 1 SE for second, third and fourth instars, respectively) ($F_{2,135}$ = 389, $P < 0.001$) (Fig. 1). The LD_{50} value for second-instar larvae was 4036 OBs per larva (95% CL 1767–9219), for third instars 7274 (6167–8578), and for fourth-instar larvae 22 925 (15 033–34 544). The predicted relative rates of transmission, calculated from the ratio of leaf area grazed to LD_{50}, indicated that mortality in fourth instars should be higher than third- and second-instar larvae (Table 2).

Inoculum levels during the field experiment

In total, 990 second-instar larvae were recovered from the 15 plots used to study inoculum persistence (45.8% recovery). The proportion of viral deaths did not vary significantly during the period of the experiment ($F_{3,11}$ = 1.16), indicating that there were no appreciable changes in the level of inoculum in the field during the experimental period. The proportion of

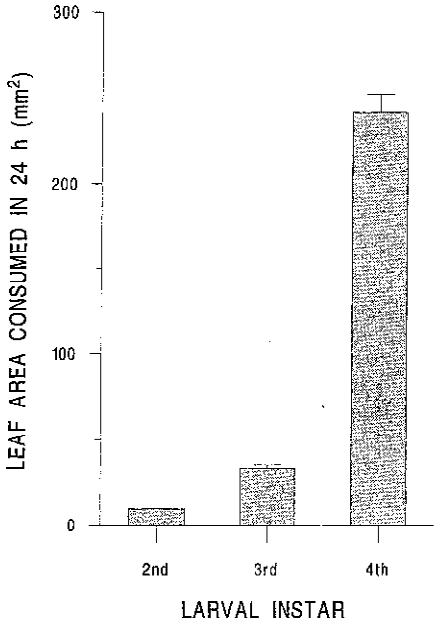

FIG. 1. Differences in leaf area grazed (mm^2) in 24 h between *M. brassicae* larval instars 2–4 (± 1 SE). $N = 50$ for each instar.

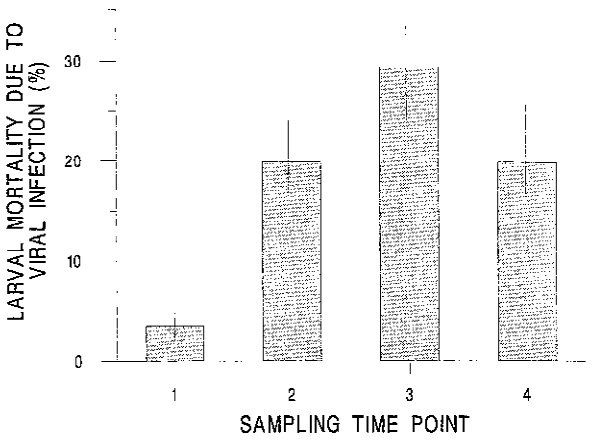

FIG. 2. Mortality (%) of second-instar *M. brassicae* larvae exposed to inoculated plots for 24 h prior to sampling, for each of the four sampling time points (± 1 SE). Mortality provided a measure of changes in density of the inoculum during the period of the trial. $N = 328$, 203, 236 and 223 for time points 1, 2, 3, and 4, respectively.

deaths at the beginning of the experiment was lower than at subsequent time points, although this trend was not significant (Fig. 2). The low mortality may be due to inoculum cadavers having not lysed by the first sampling point.

Transmission rates in the field

In total we recovered 8712 larvae from 90 plots (excluding those plots used to study inoculum persistence): 4330 from control plots and 4382 from plots inoculated with virus (66.8 and 67.6% recovery, respectively). These were reared on to determine whether they were lethally infected with virus (the possibility that a proportion of the survivors may have carried covert infections was not addressed). Only 12 virus deaths occurred in control plots (0.28%), confirming that the background level of virus in the environment was low, and that experimental procedures were rigorous enough to minimize cross contamination. Of the larvae retrieved from inoculated plots, 846 (19.3%) subsequently died due to viral infection. Predictably, there was an increase in the proportion of infected larvae in samples taken at later time points ($\chi^2 = 120.1$, df = 3, $P < 0.001$), for these larvae had been exposed to virus for longer periods (Table 2). The only exception to this trend was the incidence of viral mortality in fourth instar larvae collected at the last (fourth) time point, which was unusually low, albeit based on a reduced sample size ($n = 95$) (Table 2).

In contrast to the predicted increase in mortality in successive instars based on feeding behavior and LD_{50}s, the proportion of larvae infected did not differ significantly among instars ($\chi^2 = 2.58$, df = 2) (Table 2). The mortality for all time points combined was 21.6, 17.7, and 19.0% for instars 2, 3, and 4, respectively. Accordingly, estimates of the transmission parameter,

TABLE 2. Percentage viral mortality observed in each larval instar according to sampling time, and predicted relative rates of infection of larvae. Predictions were calculated from laboratory estimates of the rate of leaf consumption and susceptibility to virus (LD_{50} of each instar) with the assumption that transmission depends on their ratio (consumption/LD_{50}).

	Instar 2				Instar 3				Instar 4			
	Mortality				Mortality				Mortality			
Day	%	Lower 95% CL	Upper 95% CL	*n*	%	Lower 95% CL	Upper 95% CL	*n*	%	Lower 95% CL	Upper 95% CL	*n*
2	2.3	1.0	4.2	342	2.7	1.2	4.7	485	5.3	3.2	8.0	434
4	24.7	19.7	30.1	389	17.0	12.7	21.8	411	18.1	13.7	23.0	414
6	28.0	22.8	33.6	396	23.5	18.6	28.8	492	40.8	35.0	46.8	296
8	31.5	25.6	37.7	288	31.5	25.6	37.7	340	16.8	12.2	21.9	95
Mean	21.6	12.8	31.2	1415	17.7	6.5	30.4	1728	19	7.4	32.1	1239
Predicted relative transmission rate (±95% CL)	1.0	0.6	1.5		1.9	1.5	2.3		4.5	3.5	7.1	

TABLE 3. Mean estimates for the infection transmission parameter ν for each instar and time point combination, calculated from observed incidence of virus infection at each time point. All numbers are 10^{-13} times the actual number.

	Instar 2		Instar 3		Instar 4	
Day	ν	SE	ν	SE	ν	SE
2	5.95	8.5	6.83	8.5	13.7	8.5
4	35.6	8.5	23.5	8.5	25.1	8.5
6	27.5	8.5	22.5	8.5	44.0	8.5
8	23.5	9.8	23.7	9.8	11.6	9.8

ν, did not differ significantly among instars ($F_{2,42} = 0.57$) (Table 3), with a mean for all instars and time points combined of 2.16×10^{-12}. The transmission paramcter did differ with time; the first time point produced estimates that were significantly lower than time points 2 and 3 ($t = 2.88$, df = 41, $P < 0.01$, and $t = 3.49$, df = 41, $P < 0.01$ for time points 2 and 3, respectively). This may be due to the inoculum larvae failing to release all OBs before the first time point (mortality was also lower for the first time point in larvae used to monitor the inoculum level). The transmission estimates for time point 4 are lower than earlier estimates, but not significantly so.

Time from infection to death (τ)

Monitoring of larvae once recovered from the field provided a survival curve for each instar at each time point. The exponential distribution was found to be an inadequate model for the data, as this assumes the hazard function (the instantaneous risk of death) is constant. The Weibull distribution, which allows the hazard function to vary with time, was found to be a significantly better description of the data; an improvement in the χ^2 value of 213.9 (df = 1).

Of the 846 virus deaths in larvae retrieved from virus-inoculated plots, the time to death varied from 9 to 22 d post release. Survivorship curves for the three instars indicated that second-instar larvae died more quickly once infected (Fig. 3), which was confirmed by Weibull analysis of the times to death (Table 4). Instars 3 and 4 differed significantly from instar 2 ($t = 2.44$, df = 35, $P < 0.05$ and $t = 2.90$, df = 35, $P < 0.01$, respectively) and from each other ($t = 2.60$, df = 35, $P < 0.01$). This is shown as the rate of mortality due to virus, α (the inverse of τ), in Fig. 4. The slower mortality of larvae sampled at later time points (Table 4) was presumably a compound effect of the average date of infection (larvae sampled later may have acquired their lethal dose at a later date), and higher temperatures in the laboratory speeding the development of infection in early samples.

DISCUSSION

Differences in food consumption rates and susceptibility to viral infection among different larval instars of *M. brassicae* in the laboratory lead to the prediction that the transmission rate of *Mb*NPV in the field should be higher in fourth-instar larvae compared to earlier instars. This prediction was tested in a field trial, based on plots of cabbages contaminated with a known inoculum density in the form of virus-infected *M. brassicae* cadavers. No significant differences were detected in the linear transmission parameter, ν, between instars, although a significant time effect was evident. The interval between infection and death, τ, increased with larval age, such that the rate of mortality due to virus, α (the inverse of τ), was significantly reduced in older instars.

Laboratory predictions based on food consumption and susceptibility to virus provide at best a crude estimate of relative transmission rates. At least two factors may influence relative transmission rates in the field. Firstly, heterogeneity in the distribution of virus combined with differences in behavior between instars (mobility, feeding sites, etc.), may affect the probability of virus acquisition. Secondly, extrapolation of laboratory data to the field assumes that environment and diet will not affect the relative magnitudes of feeding and susceptibility.

Spatial heterogeneity in virus distribution caused by the limited dissemination of virus released from infected corpses may favor transmission to those instars that are most mobile, i.e., those with the greatest probability of encountering the remains of an infected conspecific. In Lepidoptera generally, later instars tend to be more mobile, leading to the expectation that later instars may exhibit higher rates of transmission. Similarly, each instar may adopt particular patterns of feeding or movement that may bias the probability of consuming virus. Consequently, age-related differences in insect behavior may overtly alter actual virus transmission rates in the field. Changes in virus spatial distribution may explain the lower rates of infection (and hence lower estimates of ν) found in larvae sampled

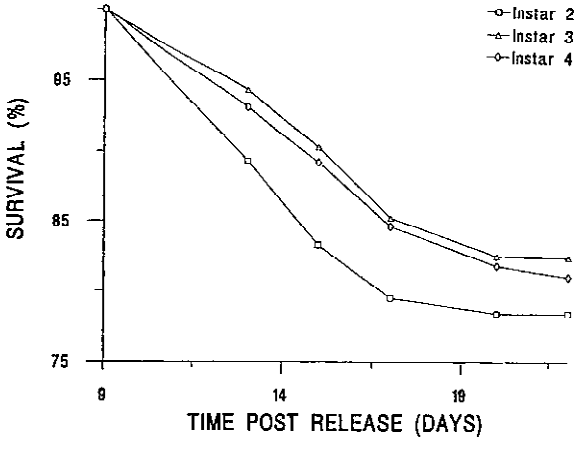

FIG. 3. Survivorship curves for the three larval instars, averaged across all time points (only viral mortality included).

TABLE 4. Weibull model estimates of mean time to death (days) of infected larvae, τ, (excluding nonvirus mortality) for second, third and fourth instars from each sampling time point.

	Instar 2			Instar 3			Instar 4		
Day	τ	Lower 95% CL	Upper 95% CL	τ	Lower 95% CL	Upper 95% CL	τ	Lower 95% CL	Upper 95% CL
2	12.8	12.5	13.0	13.8	13.6	14.0	14.4	14.2	14.6
4	13.5	14.4	14.8	14.6	14.4	14.8	15.3	15.1	15.1
6	14.8	17.8	16.2	16.0	15.8	16.2	16.8	16.5	17.0
8	15.9	15.7	16.1	17.2	16.9	17.4	18.0	17.8	18.2

at the first time point compared to subsequent samples. We suggest that inoculum cadavers may not all have fully lysed by the time that uninfected larvae were released, so reducing the availability of virus in the period prior to the first sampling point.

Little is known of the feeding behavior or mobility of *M. brassicae* larvae. In other Lepidoptera, larvae occupy different microhabitats on the plant according to age. For example, larvae of *Pieris rapae*, which also feed on cabbage, feed on the lower surface of outer leaves in the early instars, and move towards the heart of the cabbage in later instars (Samson and Geier 1983, Hoy and Shelton 1987). The present study provides limited evidence that larval behavior may differ with age in *M. brassicae*: the recapture rate declined markedly for fourth instars at the last sampling time point, suggesting increased dispersal from the experimental plots. The larvae that were recaptured exhibited a lower infection rate than at previous time points, posing the possibility that, at this age, infected larvae are more prone to dispersal, thus biasing the sample towards uninfected larvae. This is supported by an unreplicated study by Evans and Allaway (1983) who describe enhanced dispersal of infected third-instar *M. brassicae* larvae compared to uninfected controls. A rigorous empirical study of larval behavior in relation to infection is required.

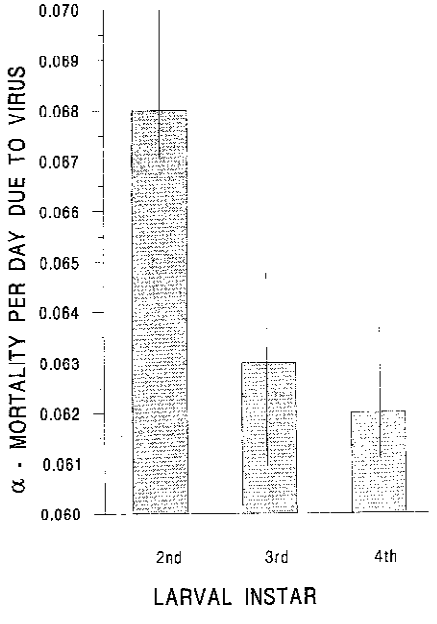

FIG. 4. Weibull model estimates of α (rate of virus-induced mortality of infected larvae) for second-, third-, and fourth-instar *M. brassicae* larvae, combining sampling time points ($\pm$ 1 SE). α is equivalent to $1/\tau$, where τ is the mean time from infection to death.

Both food quality (Keating et al. 1990, Hunter and Schultz 1993) and temperature (Ali and Young 1991) may influence susceptibility to baculovirus infection. Laboratory experiments were all carried out at 24°C, and LD_{50}s calculated for larvae feeding on artificial diet, which may produce different results to larvae exposed to virus under field conditions. Induction of plant defences occurs in proportion to damage, and has been shown to reduce susceptibility of larvae to baculovirus infection (Hunter and Schultz 1993). This process may reduce the relative susceptibility of late-instar larvae, compared to early instars, since late instars eat more.

Previous field studies on the importance of stage structure differ in their conclusions. Dwyer (1991) described an approximately fivefold increase in the rate of infection of fifth-instar Douglas-fir tussock moth (*Orgyia pseudotsugata*) when compared with third-instar larvae, using a primary inoculum of infected third-instar larvae. When inoculum was provided by infected fifth instars, however, there was no significant difference in the rate of infection of fifths compared to thirds. Webb and Shelton (1990) found rates of infection to be higher in mid-instar *Pieris rapae* than early or late instars (mortality 8, 72, and 44% for first, third, and fifth instars, respectively), when exposed to spray applications of granulosis virus. In contrast, we found no difference in field-measured rates of infection according to instar. We suggest that generalizations as to the role of instar on transmission are problematic, for the outcome of compensatory feeding rate and susceptibility interactions will depend on the behavior of susceptible and infected larvae, on food plant chemistry and architecture, and not least on spatial heterogeneity in the density of virus inocula.

The transmission parameter, ν, has, to our knowledge, been estimated in only two other systems. In *Orgyia pseudotsugata*, estimates from experiments repeated in three consecutive years ranged from 1×10^{-12}

to 6.8×10^{-9}, with a mean for 1987 of 3.13×10^{-9}, and for 1988 and 1989 combined of 5.5×10^{-10} (Dwyer 1991). Dwyer and Elkinton (1993) derived a mean estimate for ν of 1.45×10^{-12} in *Lymantria dispar*, similar to our mean estimate of 2.16×10^{-12}. However, comparison with either study is difficult, as estimation of ν depends on the area used. Dwyer (1991) used a unit area of two seedling Douglas-fir trees (*Pseudotsuga menziesii*) in a 4-m^2 cage, Dwyer and Elkinton (1993) measured the leaf area within mesh bags used to contain larvae on the foodplant (*Quercus rubra*), while our estimates are based on 1 m^2 of soil planted with 16 cabbages. Differences between studies may also arise due to differences in susceptibility of host species: the number of virus occlusion bodies required to produce an infection is inversely proportional to ν. For example, *Trichoplusia ni* is more susceptible to *Mb*NPV than is *M. brassicae* (Doyle et al. 1990), therefore, under otherwise identical conditions, the transmission parameter for *T. ni* should be higher.

Our estimates of the rate of mortality due to virus infection, α (0.062–0.068) are towards the lower end of those derived from published laboratory studies of the time from NPV infection to death. This is perhaps not surprising as most laboratory measurements are likely to have been made at higher temperatures than are found in the field in northern Europe. For example, α = 0.18–0.30 in *Spodoptera exigua* (Smits and Vlak 1988), 0.13–0.20 in *Pseudoplusia includens* (Ali and Young 1991), 0.11 in *Hyphantria cunea* (Nordin and Maddox 1972), 0.090 in *Lymantria dispar* (Doane 1967), and 0.053 in *Malacosoma americanum* (Smirnoff 1967). Field measurements have rarely been made due to the difficulties involved in finding dead larvae, which disintegrate rapidly. The published estimates give similar values to laboratory studies: 0.071 in *Lymantria dispar* (Woods and Elkinton 1987) and 0.067–0.091 in *Orgyia pseudotsugata* (Dwyer 1991). Previous studies that examined mortality rates of infected larvae in successive instars are in accordance with our finding that α decreases as larvae age (Boucias and Nordin 1977, Whitlock 1977, Smits and Vlak 1988, Ali and Young 1991, Sait 1992). This may simply be size related, i.e., more virus reproduction is necessary to kill a larger host. As α decreases in successive instars, so infected larvae will have longer to feed and grow before they succumb. Larval size at death is directly correlated with the number of virus particles released (Λ) (Evans et al. 1981).

This study suggests that incorporation of stage-related shifts in α (but not in ν) would increase the accuracy of invertebrate-pathogen models, at least for the *M. brassicae*/*Mb*NPV system. Adding host population stage structure to Anderson and May type models is thought to promote long-term host population cycles (Brown 1984, Liu et al. 1987, Andreason 1989), although these studies do not specifically consider variation in α between susceptible stages. Anderson and May type models are simplistic since they ignore many factors that are thought to influence insect-pathogen dynamics, including amongst others, host stage structure, foraging behavior, spatial distribution, food plant structure, quality and distribution, and a suite of abiotic variables. For example, it is probable that both transmission and virus-induced mortality vary according to temperature, since temperature influences both feeding rate and the time from infection to death. Similarly, rainfall may influence transmission by redistributing virus in the environment (washing virus downwards or conversely splashing virus from the soil onto plants). Transmission rate is therefore likely to vary both within and between seasons, which in turn may influence host and virus population densities. If abiotic conditions vary between parameter estimation and model testing, accurate prediction of population densities may be difficult. Nevertheless, Anderson and May type models have been used successfully to produce approximate predictions of within-season Lepidoptera–baculovirus dynamics (Dwyer and Elkinton 1993). Incorporation of further refinements, such as allowing ν and α to vary with host instar or environmental conditions, may increase model accuracy but at the cost of increased complexity. Only detailed empirical testing of model assumptions in field studies can identify which factors are most likely to significantly enhance the predictive value of invertebrate–pathogen models.

Acknowledgments

We are very grateful to David Sharpe for all his help in setting up the field site and the Wytham committee for allowing us to use the Oxford University Farm. We would like to thank Anne Miller, Andy Richards, and Joanna Sloley for assistance in field work. S.D. Vasconcelos was funded by a Brazilian Government studentship from CNPq. We are indebted to Greg Dwyer, Mark D. Hunter, and an anonymous referee for comments on an earlier draft.

Literature Cited

Ali, A., and S. Y. Young. 1991. Influence of larval age and temperature on effectiveness of a nuclear polyhedrosis virus in the soybean looper, *Pseudoplusia includens* (Lepidoptera: Noctuidae) on soybean. Biological Control **1**:334–338.

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.

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.

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

Begon, M., R. G. Bowers, N. Kadianakis, and D. E. Hodgkinson. 1992. Disease and community structure: the importance of host self-regulation in a host-host-pathogen model. American Naturalist **139**:1131–1150.

Boucias, D. G., D. W. Johnson, and G. E. Allen. 1980. Effects of host age, virus dosage, and temperature on the infectivity of a nucleopolyhedrosis virus against velvetbean caterpil-

lar, *Anticarsia gemmatalis*, larvae. Environmental Entomology **9**:59–61.

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.

Brown, D. A., H. F. Evans, C. J. Allen, and D. C. Kelly. 1981. Biological and biochemical investigations on five European isolates of *Mamestra brassicae* nuclear polyhedrosis virus. Archives of Virology **69**:209–217.

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

Doane, C. C. 1967. Bioassay of nuclear-polyhedrosis virus against larval instars of the gypsy moth. Journal of Invertebrate Pathology **9**:376–386.

Doyle, C. J., M. L. Hirst, J. S. Cory, and P. F. Entwistle. 1990. Risk assessment studies: detailed host range testing of wild-type cabbage moth, *Mamestra brassicae* (Lepidoptera: Noctuidae), nuclear polyhedrosis virus. Applied and Environmental Microbiology **56**:2704–2710.

Dwyer, G. 1991. The roles of density, stage, and patchiness in the transmission of an insect virus. Ecology **72**:559–574.

———. 1994. Density dependence and spatial structure in the dynamics of insect pathogens. American Naturalist **143**: 533–562.

Dwyer, G., and J. S. Elkinton. 1993. Using simple models to predict virus epizootics in gypsy moth populations. Journal of Animal Ecology **62**:1–11.

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.

Entwistle, P. F., P. H. W. Adams, H. F. Evans, and C. F. Rivers. 1983. Epizootiology of a nuclear polyhedrosis virus (Baculoviridae) in European sawfly (*Gilpinia hercyniae*): spread of disease from small epicentres in comparison with spread of baculovirus disease 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.

———. 1983. The influence of larval maturation on responses of *Mamestra brassicae* L. (Lepidoptera: Noctuidae) to nuclear polyhedrosis virus infection. Archives of Virology **75**:163–170.

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., C. J. Lomer, and D. C. Kelly. 1981. Growth of nuclear polyhedrosis virus in larvae of the cabbage moth, *Mamestra brassicae* L. Archives of Virology **70**:207–214.

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.

Hochberg, M. E. 1989. The potential role of pathogens in biological control. Nature **337**:262–265.

———. 1991. Non-linear transmission rates and the dynamics of infectious disease. Journal of Theoretical Biology **153**:301–321.

Hochberg, M. E., M. P. Hassell, and R. M. May. 1990. The dynamics of host-parasitoid-pathogen interactions. American Naturalist **135**:74–94.

Hoy, C. W., and A. M. Shelton. 1987. Feeding response of *Artogeia rapae* (Lepidoptera: Pieridae) and *Trichoplusia ni* (Lepidoptera: Noctuidae) to cabbage leaf age. Environmental Entomology **16**:680–682.

Hunter, M. D., and J. C. Schultz. 1993. Induced plant defences breached? Phytochemical induction protects an herbivore from disease. Oecologia **94**:195–203.

Keating, S. T., M. D. Hunter, and J. C. Schultz. 1990. Leaf phenolic inhibition of gypsy moth nuclear polyhedrosis virus. Journal of Chemical Ecology **16**:1445–1457.

Liu, W., S. A. Levin, and Y. Iwasa. 1987. Influence of nonlinear incidence rates upon the behaviour 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.

McCullagh, P., and J. A. Nelder. 1989. Generalized linear models. Chapman and Hall, London, England.

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.

Nordin, G. L., and J. V. Maddox. 1972. Effects of simultaneous virus and microsporidian infection on larvae of *Hyphantria cunea*. Journal of Invertebrate Pathology **20**: 66–69.

Onstad, D. W., and R. I. Carruthers. 1990. Epizootiological models of insect diseases. Annual Review of Entomology **35**:399–419.

Otvos, I. S., J. C. Cunningham, and L. M. Friskie. 1987. 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.

Payne, C. C., G. M. Tatchell, and C. F. Williams. 1981. The comparative susceptibilities of *Pieris brassicae* and *P. rapae* to a granulosis virus from *P. brassicae*. Journal of Invertebrate Pathology **38**:273–280.

Possee, R. D., and D. C. Kelly. 1988. Physical maps and comparative DNA hybridisation of *Mamestra brassicae* and *Panolis flammea* nuclear polyhedrosis virus genomes. Journal of General Virology **69**:1285–1298.

Sait, S. M. 1992. The population dynamics of an insect-virus interaction. Dissertation. University of Liverpool, Liverpool, England.

Samson, P. R., and P. W. Geier. 1983. Induction of crop damage by the cabbage white butterfly, *Pieris rapae* (Lepidoptera: Pieridae), on cabbage. Protection Ecology **5**:199–233.

Smirnoff, W. A. 1967. Effect of gamma radiation on the larvae and the nuclear-polyhedrosis virus of the Eastern tent caterpillar, *Malacosoma americanum*. Journal of Invertebrate Pathology **9**:264–266.

Smits, P. H., M. C. van Velden, M. van de Vrie, and J. M. Vlak. 1987. Feeding and dispersion of *Spodoptera exigua* larvae and its relevance for control with a nuclear polyhedrosis virus. Entomologia Experimentalis et Applicata **43**:67–72.

Smits, P. H., and J. M. Vlak. 1988. Biological activity of *Spodoptera exigua* nuclear polyhedrosis virus against *S. exigua* larvae. Journal of Invertebrate Pathology **51**:107–114.

Stairs, G. R. 1965. Quantitative differences in susceptibility to nuclear-polyhedrosis virus among larval instars of the forest test caterpillar, *Malacosoma disstria* (Hübner). Journal of Invertebrate Pathology **7**:427–429.

Webb, S. E., and A. M. Shelton. 1990. Effect of age structure on the outcome of viral epizootics in field populations of imported cabbageworm (Lepidoptera: Pieridae). Environmental Entomology **19**:111–116.

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, H. 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. 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.