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Sublethal Effects of Baculovirus in the Cabbage Moth, *Mamestra brassicae*

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Sublethal effects of pathogens such as baculoviruses, in particular vertical transmission to subsequent host generations, may play an important role in their ecology and population dynamics and could also be of relevance in their use as pest control agents. The effects of a range of sublethal concentrations of a nuclear polyhedrosis virus (NPV) were investigated in fourth and fifth instar larvae of the cabbage moth, *Mamestra brassicae*. Survivors of the NPV inoculation exhibited an extended developmental time in both the larval and the pupal phase compared with control larvae. There was a general trend toward increasing developmental time with increasing viral concentration. Pupal weight, sex ratio, fecundity, and egg viability were not significantly different between insects subjected to viral challenge and control groups. A low level of NPV mortality (0.55%) was recorded in the progeny of adults which had developed from larvae subject to viral challenge. Viral death in progeny larvae occurred predominantly during the second instar. Vertical transmission, although occurring at low levels, may be vital for the long-term persistence of the virus, particularly in a mobile pest species such as *M. brassicae*, which occupies ephemeral habitats.

KEY WORDS: **developmental time; sex ratio; fecundity; vertical transmission; transstadial transmission; nuclear polyhedrosis virus; sublethal infection; *Mamestra brassicae*.**

INTRODUCTION

Nonlethal viral infections in insects and vertical transmission of the pathogen to subsequent generations of the host have been greatly neglected in favor of more obvious lethal infections. Nevertheless, such infections may be important both in terms of the dynamics of insect–pathogen interactions (e.g., Anderson and May, 1981; Myers, 1988) and in relation to the design of biological pest control systems where sublethal effects might enhance long-term suppression of the pest population (e.g., de Moed *et al.*, 1990). Baculoviruses are among the most intensively studied of insect pathogens and data are already available on sublethal effects in some species (reviewed in Sait, 1992). However, no consistent pattern has emerged as to the effects of sublethal infection of either nuclear polyhedrosis viruses (NPVs) or granulosis viruses (GVs). Host responses range from none at all (Perelle and Harper, 1986; Murray *et al.*, 1991) to alteration of developmental time (Vail and Hall, 1969; Patil *et al.*, 1989; Sait *et al.*, in press), altered sex ratio (Melamed-Madjar and Raccah, 1979; Santiago-Alvarez and Vargas-Osuna, 1986), reduced fecundity (Geier and Oswald, 1977; Melamed-Madjar and Raccah, 1979; Young and Yearian, 1982; Shapiro and Robertson, 1987; Patil *et al.*, 1989; Sait *et al.*, in press), reduced egg viability (Melamed-Madjar and Raccah, 1979; Santiago-Alvarez and Vargas-Osuna, 1988; Vargas-Osuna and Santiago-Alvarez, 1988; Patil *et al.*, 1989; Sait *et al.*, in press), and vertical transmission of viral infection to progeny (Neelgund and Mathad, 1978; Melamed-Madjar and Raccah, 1979; Young and Yearian, 1982; Shapiro and Robertson, 1987; Smits and Vlak, 1988; Fuxa and Richter, 1991).

In this article the sublethal effects of NPV infection on *Mamestra brassicae* (L.) (Lepidoptera: Noctuidae), a common pest of *Brassica* crops throughout Europe, were examined in terms of developmental time, pupal weight, fecundity and egg viability, and whether males and females differed in their susceptibility to virus infection. Vertical transmission of the virus to the next host generation was also quantified.

MATERIALS AND METHODS

The *M. brassicae* culture used in this study was initiated from the offspring of female moths trapped at light

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near Winchester, Hampshire, in 1991, and since reared continuously in captivity on artificial diet (Hunter *et al.*, 1984). The culture has been shown to be free of latent infection of *M. brassicae* NPV by comparison with a laboratory culture of *M. brassicae* carrying a latent infection, using the polymerase chain reaction (PCR) to detect viral DNA (Hughes *et al.*, 1993). Larvae were reared for the first four instars in virus-free conditions in groups of approximately 100, in ventilated clear plastic boxes (16 × 28 × 10 cm) lined with tissue paper, and stored in the dark at 24°C. The constant larval rearing density avoided any possible effect of density on resistance to disease, which has been found in both *M. brassicae* (D.G., unpublished data) and *Mythimna separata* (Kunimi and Yamada, 1990). Experimental larvae were removed within 24 h of molting so that they were of standard age (±12 h). Only the first batch of larvae to reach the appropriate instar from each box were used so that all larvae were of equal developmental stage. Sublethal effects were examined in larvae challenged with virus during their fourth and fifth instars (there are six instars in total).

Infection with M. brassicae NPV

The *M. brassicae* NPV (MbNPV) was obtained from A. Gröner (BBA, Darmstadt, Germany) in 1976 and originated from an epizootic in a culture of *M. brassicae* in Darmstadt in 1973. It has since become known as the Oxford isolate and has been extensively studied in terms of biological activity (Evans, 1981, 1983), host range (Doyle *et al.*, 1990), and biochemical characteristics (Brown *et al.*, 1981; Possee and Kelly, 1988).

Individual fourth and fifth instar larvae were weighed and then administered virus by the diet plug method (Doyle *et al.*, 1990). Small plugs of diet were inoculated with 1 μl of virus suspension and the larvae left to feed for 24 h. All larvae consumed the diet plug within this time. They were subsequently transferred individually to clean diet and reared at 24°C in the dark until pupation. Three concentrations of virus were used, 1.0×10^3, 10^4, and 10^5 polyhedral occlusion bodies (POBs) per larva and a control (deionized water). Each larva was examined daily, and the time to pupation, pupal sex, and weight, and duration of the pupal phase were recorded. Fifty larvae were used per concentration. Experiments were replicated four times (800 larvae in total) for fifth instars and twice for fourth instar larvae (400 larvae in total). Viral deaths were diagnosed from the characteristic white appearance and subsequent lysis of the cadaver. Cadavers which did not exhibit these symptoms were smeared and stained with Giemsa, and examined under a light microscope (×1000) for the presence of POBs.

Adults from the first replicate only were mated to examine differences in fecundity according to treatment. On emerging, adults were paired with another survivor from their treatment group and placed in plastic containers (10 cm in diameter × 6 cm high) lined with tissue paper. Each pair was provided with a pad of tissue soaked in 10% honey solution which was renewed every 2 days. Containers were stored at 24°C with an 18:6 h light:dark cycle. The total number of eggs laid and the number that subsequently hatched were recorded. Pairs which failed to mate were discarded. In total there were 71 successful pairings from larvae treated as fourth instars and 59 from larvae treated as fifth instars. The first 100 larvae to hatch from each pairing (or less if fewer were available) were reared individually to record viral deaths in the progeny larvae. Where the cause of death was unclear, the larvae were smeared onto a microscope slide, stained with Giemsa and examined under the light microscope for POBs.

To confirm that offspring deaths were caused by MbNPV, virus from five progeny deaths was multiplied by feeding to fourth instar *M. brassicae* larvae (selected at random from parents infected during the fifth instar). Virus was purified from the larvae and viral DNA was extracted using phenol–chloroform and subjected to restriction enzyme analysis (*Hin*dIII and *Eco*RI) for comparison with the original inoculum stock (protocol in Doyle *et al.*, 1990).

Statistical Analysis

Analysis of variance was used to examine sublethal effects on survivors of viral challenge. Fecundity was analyzed by pair, rather than by individual, as all moths were paired with moths which had received the same treatment. Hence differences between virus effects on male sperm viability and female egg production were not distinguished. Pairs which produced no eggs, and thus presumably had not mated, were excluded from the analysis. Approximation to normality in the error structure was confirmed by examination of residuals generated by the program GLIM (Generalised Linear Interactive Modelling) (McCullagh and Nelder, 1989).

Differences in the sex-ratio of surviving larvae (determined in the pupae) were assessed by comparison with controls and used to examine differential susceptibility of the sexes. Sex-ratios were analyzed using GLIM with binomial errors, as were differences in the proportion of second-generation larvae that succumbed to viral infection. This enabled partitioning of variation due to inoculum concentration and instar. In both analyses the degree of overdispersion was within acceptable limits (when Pearson's χ^2 divided by the residual degrees of freedom is <3), and a dispersion parameter was calculated and used to adjust the scale parameter.

RESULTS

Predictably, larval mortality increased with viral concentration ($\chi^2 = 264$, $df = 3$, $P < 0.01$, and $\chi^2 = 23.8$, $df = 3$, $P < 0.01$, fourth and fifth instars, respectively). This reduced the number of larvae surviving to pupation at higher concentrations. For the four concentration levels (control, 10^3, 10^4, and 10^5 POBs/larva) pupal sample sizes were 96, 73, 69, and 48 for those treated at the fourth instar and 191, 167, 118, and 68 for those treated at the fifth instar. Overall mortality levels were thus 4, 27, 31, and 52% for fourth instars and 4.5, 16.5, 41, and 66% for fifth instars. Deaths from causes other than virus were few (overall 4.2%) and were mainly failed pupations. These deaths were similarly distributed between control and infected treatments ($\chi^2 = 0.15$, $df = 3$, and $\chi^2 = 0.45$, $df = 3$, fourth and fifth instars, respectively).

Developmental Rate and Pupal Weight

In both fourth and fifth instars inoculation with MbNPV increased developmental time from inoculation to pupation compared with controls ($F = 3.84$, $df = 3252$, $P < 0.01$, and $F = 5.92$, $df = 3537$, $P < 0.01$ for fourth and fifth instars, respectively) (Fig. 1). The increases in developmental time were greater at higher concentrations. Pupal weight was significantly greater in females than in males: 0.50 ± 0.003 for females and 0.43 ± 0.002 for males (mean (g) ± SE, instars and virus treatments combined). However, pupal weight did not differ according to treatment ($F = 0.36$, $df = 2,252$ and $F = 0.12$, $df = 3537$, fourth and fifth instars, respectively) or according to the instar at which larvae were inoculated ($F = 0.71$, $df = 1796$).

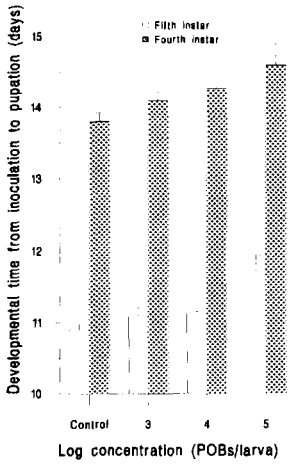

FIG. 1. Developmental time (±SE) from inoculation to pupation, according to concentration of a nuclear polyhedrosis virus of *Mamestra brassicae*. Larvae were inoculated in the fifth instar. POBs, polyhedral occlusion bodies.

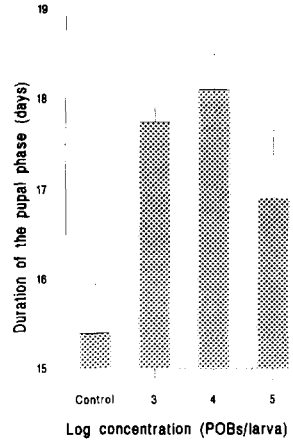

FIG. 2. Duration of the pupal phase (±SE) according to viral concentration of a nuclear polyhedrosis virus of *Mamestra brassicae*. The larvae were inoculated in the fifth instar. POBs, polyhedral occlusion bodies.

The duration of the pupal phase differed according to treatment in the fifth instar ($F = 3.60$, $df = 3537$, $P < 0.05$), although there was no clear response to increasing concentration (Fig. 2). Larvae given low and medium concentrations (10^3 and 10^4 POBs/larva) took longer to develop than controls (pairwise comparison, $t = 2.62$, $df = 336$, $P < 0.01$, low concentration versus controls; $t = 2.79$, $df = 287$, $P < 0.01$, medium concentration versus controls). However, at the highest concentration developmental time was shorter than at low and medium concentrations and was not significantly higher than controls ($t = 1.08$, $df = 237$). There was no significant difference in the duration of the pupal phase in larvae inoculated in the fourth instar.

Sex Ratio of Survivors

The numbers of males and females surviving inoculation with NPV compared with controls are shown in Table 1. Although there was a consistent trend toward an increasing proportion of females with increasing concentration, suggesting that males may be more susceptible to MbNPV than females, in neither instar was this relationship significant ($\chi^2 = 1.56$, $df = 3$ and $\chi^2 = 5.59$, $df = 3$ for instars four and five, respectively).

As weight affects susceptibility to NPV, larval weights were compared according to sex: these calculations were based on control larvae only, as differential mortality according to sex and weight may bias comparisons using inoculated larvae. Both the fourth and fifth instars males were lighter than females, although the relationship was not significant ($t = 0.72$, $df = 96$ and $t = 1.6$, $df = 192$, respectively).

Adult Fecundity

Adult fecundity per pair was variable within all treatment groups, the number of eggs laid ranging from

4 to 863 (267 ± 13.4, mean ± SE). If larval inoculation had an effect on adult fecundity, it was obscured by this within-treatment variation, for no differences were apparent in the number of eggs produced compared with controls ($F = 0.66$, $df = 3, 68$ and $F = 1.29$, $df = 3, 56$ for larvae inoculated as fourth and fifth instars, respectively). Similarly, the number of fertile eggs produced did not differ according to treatment ($F = 0.79$, $df = 3, 68$ and $F = 0.65$, $df = 3, 56$ for fourth and fifth instars, respectively).

Vertical Transmission

In total 3528 larvae were reared from 75 pairings. Of these 1021 were controls (21 pairs). There was no viral mortality in control larvae. There were 14 viral deaths among larvae reared from adults which developed from virus-treated larvae (0.55% mortality among offspring of inoculated larvae) (Table 2). Virus from five deaths was subjected to restriction enzyme analysis and found to be indistinguishable from the original inoculum (data not shown). Mortality of offspring from larvae inoculated as fifth instars was higher than that from larvae inoculated as fourth instars, although the difference was not significant ($\chi^2 = 2.28$, $df = 1$). However, the mortality of offspring from parents which developed from virus-treated larvae was significantly higher than that of controls ($\chi^2 = 8.185$, $df = 3$, $P < 0.05$). The absence of viral deaths in controls is therefore unlikely to be due to chance, suggesting that inoculation of larvae that eventually became parents was responsible for the observed mortality in their offspring (rather than laboratory contamination during rearing). Thirteen of the 14 larvae which died due to viral infection died as second instars, the remaining larva as a third instar.

TABLE 1

Numbers of Male and Female *M. brassicae* Surviving Inoculation with a Range of Concentrations of Nuclear Polyhedrosis Virus (NPV)

Treatment (POBs/larva)	Total number of each sex and percentage of males					
	Fourth instar inoculated			Fifth instar inoculated		
	Male	Female	%	Male	Female	%
Control	32	20	61.5	106	63	62.7
10^3	38	31	55.1	95	71	57.2
10^4	36	20	54.5	59	59	50.0
10^5	22	23	48.9	34	33	50.7

Note. Sex was determined in the pupal stage. Deformed or dead pupae were excluded from analysis. Replicates are combined. POBs, polyhedral occlusion bodies.

TABLE 2

Nuclear Polyhedrosis Viral (NPV) Mortality in Second Generation *Mamestra brassicae* Larvae (the Offspring of Larvae Inoculated with Virus).

Treatment (POBs/larva)	Number of larval deaths due to NPV (*n*)
	Fourth Instar
Control	0 (599)
10^3	1 (531)
10^4	3 (472)
10^5	0 (301)
	Fifth instar
Control	0 (422)
10^3	2 (545)
10^4	3 (391)
10^5	5 (267)

Note. All deaths are from different broods, except fifth instar at 10^5 polyhedral occusion bodies (POBs), in which three of the five deaths were from the same brood.

DISCUSSION

The only significant effect of virus inoculation on surviving larvae was an increase in the developmental time to pupation compared with controls and, in larvae inoculated as fifth instars, an increase in the duration of the pupal phase. Developmental time to pupation tended to increase with increasing concentration. However, pupal weight, pupal sex ratio, adult fecundity, and egg viability were unaffected by sublethal concentrations of *M. brassicae* NPV in the fourth and fifth instars. The likely cause of the observed increases in developmental time is the establishment of sublethal infections in at least some of the surviving larvae. However, the extent and duration of these infections remain to be determined. A low level of viral mortality (0.55%) was recorded in *M. brassicae* larvae which had been sublethally infected with NPV during either the fourth or fifth instar, suggesting that these infections (or perhaps the original inoculum) can persist through metamorphosis within the host.

The effects of sublethal infection on developmental time have not been thoroughly investigated in many insect–baculovirus systems. An increase in larval and pupal developmental time after baculovirus infection appears to be a more common response than a decrease (Mardan and Harein, 1984; Patil *et al.*, 1989), although both have been recorded (Sait *et al.*, in press). In a few systems there was no alteration in developmental time (Vargas-Osuna and Santiago-Alvarez, 1988; Young, 1990). The most detailed study of the effect of sublethal infection on developmental time is the recent work of Sait *et al.* (in press) who examined the interaction of

the flour moth, *Plodia interpunctella* and its GV in all instars. They found no consistent response to sublethal GV concentrations, recording both increases and decreases in developmental time, although the former was more common.

Effects on fecundity as a result of sublethal infections have been studied in more detail than development and there are numerous reports of reductions in oviposition rate or fecundity (Melamed-Madjar and Raccah, 1979; Young and Yearian, 1982; Patil *et al.*, 1989; Young, 1990; Sait *et al.*, in press) and egg viability (Melamed-Madjar and Raccah, 1979; Young and Yearian, 1982; Santiago-Alvarez and Vargas-Osuna, 1988; Vargas-Osuna and Santiago-Alvarez, 1988; Patil *et al.*, 1989; Sait *et al.*, in press). However, there are also several instances in which sublethal concentrations have had no effect on either, as in the current study (Perelle and Harper, 1986; Smits and Vlak, 1988; Murray *et al.*, 1991).

It is difficult to draw any general conclusions from the reported data on sublethal infections; different techniques have been used for dosing the larvae, the sample sizes and statistical rigor of the experimental designs varied considerably, different aged insects were used, and studies differed in the set of parameters measured. Additionally, in some studies, the untreated control insects have shown evidence of vertically transmitted baculovirus infection, albeit at a lower level than those which have been treated, but which might mean that both populations were in fact sublethally infected by virus, negating any valid comparisons. Sait *et al.* (in press) are particularly critical of techniques which have a long (24 h) dosing period as they suggest that the fitter individuals are more likely to be killed by the virus because they ingest the virus quickly, while the less fit individuals will ingest the virus more slowly, perhaps giving them a higher likelihood of survival. The technique of droplet dosing, whereby larvae imbibe viral suspension, allows for precise timing of inoculation. However, individual larvae differ widely in the volume which they take in (Smits and Vlak, 1988). As Sait *et al.* (in press) admit, fitter larvae may imbibe more, again biasing results toward survival of less fit larvae. The situation is further complicated as the type of food with which the virus concentration is administered may itself alter susceptibility (Keating *et al.*, 1990; Hunter and Schultz, 1993). Detailed examination of the pathology of sublethal infections may be necessary to provide an explanatory framework for the diversity of sublethal effects so far described. Larvae may fight off infection in its early stages by shedding infected midgut cells into the gut lumen (Keddie *et al.*, 1989), the cost of which may be manifest as a reduced development rate.

In addition to detrimental but nonlethal effects in inoculated insects, many studies have found secondary infections in the offspring of these insects. Vertical transmission of virus from one generation to the next may occur either via contamination of the egg surface with virus during oviposition (transovum) or via virus POBs or viral DNA within the eggs (transovarial), (e.g., Doane, 1969; Hamm and Young, 1974; Melamed-Madjar and Raccah, 1979; Young and Yearian, 1982; Fuxa *et al.*, 1992). As with other sublethal effects, the presence or absence of vertically transmitted virus appears to vary between host species and between viruses, and according to the host instar inoculated. For example, in a direct comparison, NPV isolated from *Spodoptera frugiperda* produced 14.3% mortality in offspring of infected *S. frugiperda* larvae, while under the same conditions no evidence could be found for vertical transmission of *Anticarsia gemmatalis* NPV in *A. gemmatalis* larvae (Fuxa and Richter, 1993). Even studies of the same host–virus system show disagreements. Murray *et al.* (1991) found that no sublethal effects or vertical transmission followed sublethal infections of *Lymantria dispar* NPV in fourth instar *L. dispar* larvae, while in contrast Shapiro and Robertson (1987) found both a reduction in egg mass weight and viral mortality (4.7 to 11.5%) in progeny larvae following sublethal infections in second instar larvae. This may be related to the different ages of insect or differences in virus or insect stocks, or to differences in methodology. Several authors have found evidence for vertical transmission in other systems including, *Mythimna separata*/NPV (Neelgund and Mathad, 1978), *Sesamia nonagroides*/GV (Melamed-Madjar and Raccah, 1979), *Spodoptera exigua*/NPV (Smits and Vlak, 1988) and *S. frugiperda*/NPV (Fuxa and Richter, 1991). The levels of mortality varied but reached up to 28% in *S. exigua* (Smits and Vlak, 1988). Fuxa and Richter (1991) were able to increase the rate of vertical transmission of *S. frugiperda* NPV by artificial selection on the virus, suggesting that the ability to transmit vertically is determined genetically.

Recently, a latent infection of MbNPV in *M. brassicae* has been described in which the viral DNA is passed from generation to generation without producing any symptoms of disease (Hughes *et al.*, 1993). In the artificially maintained host population studied by Hughes *et al.* (1993) expression of apparent infections was triggered by challenging larvae with another baculovirus. Environmental stress (high and low temperatures, starvation, crowding) were not effective in triggering apparent infections in this population (D.G., unpublished data). Given that MbNPV is known to be capable of latency for many successive host generations, it is possible that some survivors of viral challenge became so infected, and that offspring which did not exhibit symptoms of infection may have been carrying the virus. Only molecular studies (e.g., Hughes *et al.*, 1993; Williams, 1993) could clarify this possibility.

Vertical transmission may be of great biological significance to host and pathogen, even when occurring at low levels. Anderson and May (1981) model the implications of vertical transmission (model C) and predict that it reduces the threshold density of hosts necessary for persistence of the pathogen (for at low host densities horizontal transmission alone may be insufficient to maintain the virus population). Their model probably underestimates the importance of vertical transmission for it does not incorporate spatial structuring of the host population or periods when hosts are not available (as usually occurs in seasonal insects). One may predict that vertical transmission should be more common in virus–host systems in which the host usually occurs at low density. However, information as to the prevalence of vertical transmission and on host population biology is as yet too sparse to test this hypothesis. Vertical transmission enhances virus dispersal by enabling the virus to track movements of the adult host insect. This is likely to be particularly important in a mobile pest species such as *M. brassicae* and also in migratory species, in which successive larval generations are unlikely to occupy the same site (Fuxa and Richter, 1993). Clearly further empirical research is necessary to establish the occurrence and ecological importance of vertical transmission and other sublethal effects.

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REFERENCES

Anderson, R. M., and May, R. M. 1981. The population dynamics of microparasites and their invertebrate hosts. *Philos. Trans. R. Soc. London B.* **291,** 451–524.

Boots, M., and Begon, M. 1993. Trade-offs with resistance to a granulosis virus in the Indian meal moth, examined by a laboratory evolution experiment. *Funct. Ecol.* **7,** 528–534.

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

de Moed, G. H., van der Werf, W., and Smits, P. H. 1990. Modelling the epizootiology of *Spodoptera exigua* nuclear polyhedrosis virus in a spatially distributed population of *Spodoptera exiqua* in greenhouse chrysanthemums. SROP/WPRS Bull. XIII/5 (1990), 135–141.

Doane, C. C. 1969. Trans-ovum transmission of nuclear-polyhedrosis virus in the gypsy moth and the inducement of virus susceptibility. *J. Invertebr. Pathol.* **14,** 199–210.

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

Evans, H. F. 1981. Quantitative assessment of the relationships between dosage and response of the nuclear polyhedrosis virus of *Mamestra brassicae*. *J. Invertebr. Pathol.* **37,** 101–109.

Evans, H. F. 1983. The influence of larval maturation on responses of *Mamestra brassicae* L. (Lepidoptera: Noctuidae) to nuclear polyhedrosis virus infection. *Arch. Virol.* **75,** 163–170.

Fuxa, J. R., and Richter, A. R. 1991. Selection for an increased rate of vertical transmission of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus. *Environ. Entomol.* **20,** 603–609.

Fuxa, J. R., and Richter, A. R. 1993. Lack of vertical transmission in *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) nuclear polyhedrosis virus, a pathogen not indigenous to Louisiana. *Environ. Entomol.* **22,** 425–431.

Fuxa, J. R., Weidner, E. H., and Richter, A. R. 1992. Polyhedra without virions in a vertically transmitted nuclear polyhedrosis virus. *J. Invertebr. Pathol.* **60,** 53–58.

Geier, P. W., and Oswald, L. T. 1977. The light-brown apple moth, *Epiphyas postvittana* (Walker) 1. Effects associated with contaminations by a nuclear polyhedrosis virus on the demographic performance of a laboratory strain. *Austr. J. Ecol.* **2,** 9–29.

Hamm, J. J., and Young, J. R. 1974. Mode of transmission of nuclear-polyhedrosis virus to progeny of adult *Heliothis zea*. *J. Invertebr. Pathol.* **24,** 70–81.

Hughes, D. S., Possee, R. D., and King, L. A. 1993. Activation and detection of a latent baculovirus resembling *Mamestra brassicae* nuclear polyhedrosis virus in *M. brassicae* insects. *Virology* **194,** 608–615.

Hunter, F. R., Crook, N. E., and Entwistle, P. F. 1984. Viruses as pathogens for the control of insects. *In* "Microbiological Methods for Environmental Biotechnology" (J. M. Grainger and J. M. Lynch, Eds.), pp. 323–347. Academic Press, London.

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

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

Keddie, B. A., Aponte, G. W., and Volkman, L. E. 1989. The pathway of infection of *Autographa californica* nuclear polyhedrosis virus in an insect host. *Science* **243,** 1728–1730.

Kunimi, Y., and Yamada, E. 1990. Relationship of larval phase and susceptibility of the armyworm, *Pseudaletia seperata* Walker (Lepidoptera: Noctuidae) to a nuclear polyhedrosis virus and a granulosis virus. *Appl. Entomol. Zool.* **25,** 289–297.

Mardan, A. H., and Harein, P. K. 1984. Susceptibility of malathion-resistant Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), to a granulosis virus. *Environ. Entomol.* **13,** 79–80.

McCullagh, P., and Nelder, J. A. 1989. "Generalized Linear Models" Chapman and Hall, London.

Melamed-Madjar, V., and Raccah, B. 1979. The transstadial and vertical transmission of a granulosis virus from the corn borer *Sesamia nonagroides*. *J. Invertebr. Pathol.* **33,** 259–264.

Murray, K. D., Shields, K. S., Burand, J. P., and Elkinton, J. S. 1991. The effect of gypsy moth metamorphosis on the development of nuclear polyhedrosis infection. *J. Invertebr. Pathol.* **57,** 352–361.

Myers, J. H. 1988. Can a general hypothesis explain population cycles of forest Lepidoptera? *In* "Advances in Ecological Research," Vol. 18, pp. 179–242. Academic Press, London.

Neelgund, Y. F., and Mathad, S. B. 1978. Transmission of nuclear polyhedrosis virus in laboratory population of the armyworm, *Mythimna (Pseudaletia) separata*. *J. Invertebr. Pathol.* **31,** 143–147.

Patil, U. R., Savanurmath, C. J., Mathad, S. B., Aralaguppi, P. I., and Ingalhalli, S. S. 1989. Effects of nuclear polyhedrosis virus on the growth, development and reproduction in surviving generations of the armyworm *Mythimna (Pseudaletia) separata* (Walker). *J. Appl. Entomol.* **108,** 527–532.

Perelle, A. H., and Harper, J. D. 1986. An evaluation of the impact of sublethal dosages of nuclear polyhedrosis virus in larvae on pupae, adults and adult progeny of the fall armyworm, *Spodoptera frugiperda*. *J. Invertebr. Pathol.* **47,** 42–47.

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

Sait, S. M. 1992. "The Population Dynamics of an Insect–Virus Interaction." Ph.D. Thesis, Univ. of Liverpool.

Sait, S. M., Begon, M., and Thomson, D. J. The effects of a sublethal infection in the Indian meal moth, *Plodia interpunctella*. *J. Anim. Ecol.*, in press.

Santiago-Alvarez, C., and Vargas-Osuna, E. 1986. Differential mortality between male and female *Spodoptera littoralis* larvae infected with a baculovirus. *J. Invertebr. Pathol.* **47,** 374–376.

Santiago-Alvarez, C., and Vargas-Osuna, E. 1988. Reduction of reproductive capacity of *Spodoptera littoralis* males by a nuclear polyhedrosis virus (NPV). *J. Invertebr. Pathol.* **52,** 142–146.

Shapiro, M., and Robertson, J. L. 1987. Yield and activity of gypsy moth (Lepidoptera: Lymantriidae) nucleopolyhedrosis virus recovered from survivors of viral challenge. *J. Econ. Entomol.* **80,** 901–905.

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

Vail, P. V., and Hall, I. M. 1969. The influence of infections of nuclear polyhedrosis virus on adult cabbage loopers and their progeny. *J. Invertebr. Pathol.* **13,** 358–370.

Vargas-Osuna, E., and Santiago-Alvarez, C. 1988. Differential response of male and female *Spodoptera littoralis* (Boisduval) (Lep., Noctuidae) individuals to a nuclear polyhedrosis virus. *J. Appl. Entomol.* **105,** 374–378.

Williams, T. 1993. Covert iridovirus infection of blackfly larvae. *Proc. R. Soc. London B.* **251,** 225–230.

Young, S. Y. 1990. Effect of nuclear polyhedrosis virus infection in *Spodoptera ornithogalli* larvae on post-larval stages and dissemination by adults. *J. Invertebr. Pathol.* **55,** 69–75.

Young, S. Y., and Yearian, W. C. 1982. Nuclear polyhedrosis infection of *Pseudoplusia includens* (Lep.: Noctuidae) larvae: Effect on post larval stages and transmission. *Entomophaga* **27,** 61–66.