Sublethal Nucleopolyhedrovirus Infection Effects on Female Pupal Weight, Egg Mass Size, and Vertical Transmission in Gypsy Moth (Lepidoptera: Lymantriidae)

JUDITH H. MYERS,[1] RAKSHA MALAKAR,[2] AND JENNIFER S. CORY[3]

Environ. Entomol. 29(6): 1268–1272 (2000)

ABSTRACT Gypsy moth females that survived inoculation with *Lymantria dispar* (L.) nucleopolyhedrovirus (LdNPV) as fifth instars were smaller as pupae and laid fewer eggs as adults. Treatment with both wild type virus containing the *egt* gene and a genetically manipulated, *egt-* virus, lacking this gene, reduced pupal mass to a similar degree. Sublethal infection with wild type virus reduced the masses of surviving pupae at 20, 25, and 28°C. A relationship between virus dose (5,000, 50,000 and 500,000 occlusion bodies per larva), mass of pupae, and egg mass size only occurred in one experiment in which larvae were reared at 25°C and inoculated 5 d after molt to the fifth instar. Vertical transmission of overt infection occurred in two of 13 egg masses (15%) produced by females inoculated with virus as larvae. The five larvae infected with virus were ≈0.5% of the larvae tested. Whether sublethal effects of LdNPV infection occur in field populations of gypsy moth remains to be tested.

KEY WORDS *Lymantria dispar*, nucleopolyhedrovirus, vertical transmission, fecundity, population cycles, *egt-*

OUTBREAKS OF POPULATIONS of some forest Lepidoptera are associated with epizootics of nucleopolyhedrovirus (NPV) (Myers 1988). These double-stranded DNA viruses infect many species of Lepidoptera and are spread by the ingestion of leaf material contaminated by viral occlusion bodies (OBs) released with the death of infected host larvae. An aspect of the dynamics of disease and host populations that is not understood is how virus is maintained when hosts are rare. One possibility is that sublethally infected larvae are able to complete development and carry the disease to future generations either mostly by external contamination (Murray and Elkinton 1989, Murray and Elkinton 1990), as a low level, persistent infection (Kukan and Myers 1997) or in an inactive, latent form (Hughes et al. 1993). A number of studies have shown that pupae that have survived exposure to NPV as larvae are smaller than untreated controls and the moths emerging from these pupae have reduced fecundity (Rothman and Myers 1996). Similarly, several studies have demonstrated that overt infection can be transmitted from parent to progeny (Kukan 1999, Kukan and Myers 1999), although the source of this infection is not clear.

Whether sublethal effects and vertical transmission occur in gypsy moth, *Lymantria dispar* (L.), has been controversial. Shapiro and Robertson (1987) found persistence of NPV in adults and pupae of individuals surviving inoculation with virus as second instars. They also found that moths that survived exposure to virus at levels that killed 80% of the infected larvae had reduced fecundity, and vertically transmitted virus to offspring at a relatively high level (4.7–11.5%). However, Murray and Elkinton (1989) found that adults surviving virus challenge only transmitted virus to progeny at very low levels (<2.0%) and found no difference in pupal size or fecundity for individuals surviving exposure to virus as fourth instars (Murray et al. 1991).

Sublethal infection could influence the fecundity of moths by reducing the amount of fat and other nutrients available for egg or sperm production or through some associated cost of resistance mechanisms either when initially fighting off infection or in maintaining it at a nonlethal level (Myers and Kukan 1995). However, sublethal infection could also influence fecundity through an interaction with the hormonal balance of the individual during development. Infection of gypsy moth with NPV slows larval growth and inhibits larval molting and pupation (Burand and Park 1992). Most insect baculoviruses contain a gene (*egt*) that encodes the enzyme ecdysteroid UDP-glucosyl transferase which catalyzes the sugar conjugation of ecdysteroids (O'Reilly and Miller 1990). Fifth-instar gypsy moth infected with NPV do not show the increase in ecdysteroid titer before pupation that occurs in control caterpillars (Park et al. 1993). Additionally, female gypsy moth larvae infected with an *egt-* strain of LdNPV gained less weight than those

[1] Department Zoology, Centre for Biodiversity Research and Faculty of Agricultural Sciences, University of British Columbia, Vancouver, BC, V6T 1Z4, Canada.
[2] Department of Entomology, 235 Insectary Building, Cornell University, Ithaca, NY 14853.
[3] Ecology and Biocontrol Group, NERC, Institute of Virology and Environmental Microbiology, Mansfield Road, Oxford, UK 0X1 3SR.

0046-225X/00/1268–1272$02.00/0

infected with the wild type virus, whereas, no difference in weight gain was found in males (Slavicek et al. 1999). More details of the interactions between the viral *egt* gene and ecdysteroid titers are necessary to understand the potential impacts of sublethal infection on the physiology of the host. It is possible that sublethal infection by NPV with the *egt* gene could have different impacts on surviving hosts than infection by an NPV without this gene. The existence of a strain of LdNPV lacking the *egt* gene provides an excellent tool for investigating the association of ecdysteroid titer and the potential sublethal effects of virus.

Rearing temperature is another factor that might influence the rate of development of larvae and possibly also that of the virus. We hypothesized that sublethal influences might have more opportunity for expression in inoculated larvae reared at a lower temperature, 20°C, than those reared at a warmer temperature, 28°C. Similarly the dose of virus might influence the opportunity for the expression of sublethal effects on surviving individuals. We expect that fewer individuals will survive exposure to higher viral doses, but that those that do will be more likely to demonstrate the effects of sublethal infection.

In the following study we investigated the influences of the presence and absence of the *egt* gene in the virus used for inoculation, viral dose, time of infection after molt to the fifth instar, and rearing temperature on the expression of sublethal viral effects indicated by pupal mass (a surrogate for fecundity) and egg mass size. In one experiment we reared larvae from egg masses of adults surviving inoculation with virus as larvae to determine if vertical transmission of virus occurred.

Materials and Methods

Gypsy moth egg masses and cups containing artificial diet (Bell et al. 1981) were obtained from the Methods Development Center, USDA (Otis, MA). Egg masses were surface sterilized by soaking in 10% formaldehyde for 1 h and washing under running tap water for 2 h. The hatched larvae were reared at 28°C in groups of 20 through the fourth instar on artificial diet in 150-ml cups. Slippage of the head capsule of larvae occurs just before the molt to fifth instar, and at that time larvae were placed in individual 50-ml diet cups where they were maintained until the start of the experiment. The sexes were distinguished by size of the larvae, and only results on females are considered here although treated males were kept for mating. Twenty-four hours before larvae were inoculated with virus or treated with distilled water as controls, they were placed in empty 50-ml cups. This period of starvation improves the rate of consumption of the 1.0-mm^3 diet cubes on which the virus or distilled water was placed.

Considerable mold contamination of diet cups occurred in the experiments in 1994. We are uncertain if the contamination came with the food, the caterpillars, or was associated with the rearing chambers. Experience had shown that caterpillars in cups with mold did not grow well and therefore, larvae in cups with mold were discarded without opening the cup lids.

LdNPV was provided by J. P. Burand (University of Massachusetts, Amherst). The concentration of occlusion bodies was determined by counting in a hemocytometer. In the first experiments, serial dilutions were made with distilled water to obtain concentrations of 1×10^6, 1×10^7, and 1×10^8 OBs/ml. Five microliters of NPV solution (dose of 5,000, 50,000, or 500,000 OBs per larva) were placed on a 1.0-mm^3 piece of diet and fed to larvae that had been in empty cups for ≈24 h. After 2–3 h, larvae that had eaten the whole diet cube were transferred to new 50-ml diet cups with sufficient diet to complete development. The few larvae that did not finish the diet cubes were discarded. Controls were treated in the same way but 5 μl of distilled water replaced the virus dose.

Three experiments were carried out, two in 1994 and one in 1995. In the first experiment, two types of virus were used, the G2 wildtype strain of LdNPV and the *egt-*, genetically modified strain, which lacks the *egt* gene (Slavicek et al. 1999). The viral dose was 5,000 OBs per larva and 82 female larvae were exposed to wild type virus, 83 to *egt-* and 82 were controls. Viral inoculation was carried out 5 d after the molt to fifth instar, and larvae were reared at 28°C. In another trial, 77 female larvae were inoculated with 5,000 OBs wild type virus and 79 larvae with *egt-* virus 5 d after molt to the fifth instar and reared at 25°C.

The second experiment investigated the effect of viral dose and rearing temperature. Larvae were starved for 24 h and inoculated with 5,000, 50,000, or 500,000 OBs per larva of the wildtype LdNPV, 5 d after the molt to fifth instar. The control caterpillars were starved for 24 h and then fed the diet cubes inoculated with distilled water. Numbers of individuals in groups reared at 20 or 28°C, respectively, were control = 25, 25; 5,000 = 50, 60; 50,000 = 32, 33; 500,000 = 35, 36. Larvae were reared individually in 50-ml diet cups at 20 or 28°C, and checked every day until death or pupation. The cause of death of larvae was determined by observing smears with a light microscope. Pupae were weighed one day after pupation and kept at 25°C until the adults emerged. Adults were placed in brown paper lunch bags in groups of approximately three males and three females, from the same treatment group, for mating and oviposition. Egg masses were weighed and maintained at room temperature for one month, then chilled at 4°C for 206 d.

To determine if vertical transmission of NPV occurred, after chilling, 50% of the egg masses were disinfected with 10% formaldehyde and 50% were not. All of the egg masses were allowed to hatch at 25°C. Individual egg masses were placed in 50-ml plastic cups with a piece of wet paper towel. Larvae hatched from 21 egg masses and were collected and reared in groups of 1–10 individuals per 150-ml cup on artificial diet at 25°C. Dead caterpillars were smeared to determine if they were infected with virus.

Table 1. Mean, SE, and N of mass (g) of female pupae and of number of days from inoculation with NPV to pupation for gypsy moth larvae reared at 20 or 28°C, (ANOVA weight, $F = 5.74$; df = 7, 79; $P < 0.01$)

Dose (OBs)	20°C					28°C				
	Mean (gr)	SE	N	Mean (d)	SE	Mean (gr)	SE	N	Mean (d)	SE
5,000	1.46	0.07	13	9.4	0.2	1.40	0.06	15	6.6	0.2
50,000	1.28	0.06	16	10.6	0.2	1.39	0.09	7	6.6	0.3
500,000	1.40	0.08	9	10.2	0.3	1.22	0.06	14	6.7	0.3
Control	1.55	0.09	7	9.1	0.3	1.86	0.09	7	6.0	0.3

Tukey test showed that all means were significantly different from 28°C controls, (ANOVA development time, $F = 53.4$; df = 7, 79; $P < 0.01$). Tukey test showed that the development time of all groups reared at 20°C were significantly longer than those at 28°C but controls were not different from experimental groups.

The same viral doses were used for the third experiment as in the second (5,000; 50,000; and 500,000 OBs per larva), but the larvae were inoculated 1 d after molting to the fifth instar. Fifty female larvae were inoculated per viral dose and reared at 25°C until they died or pupated. The pupae were weighed a day after pupation, and were maintained at 25°C until they became adults. A pair of adults from the same treatment group were allowed to mate in a brown paper bag. The egg masses were collected and weighed, but were not stored for further development. A total of 30 egg masses was used for data analysis.

Statistical Analyses. Analysis of variance (ANOVA) and *t*-tests were performed using SYSTAT (SYSTAT 1992).

Results

The Effect of the *egt* Gene on Sublethal Effects. The masses of female pupae in the first experiment were significantly different when the three groups were compared with ANOVA ($F = 5.38$; df = 2, 55; $P = 0.01$). Pupal weights of those infected with the wild type virus (mean = 1.58 g, SE = 0.09, $n = 14$) were significantly smaller than the control pupae (mean = 1.91 g, SE = 0.06, $n = 30$) but not those infected with the *egt-* virus (mean = 1.69 g, SE = 0.09, $n = 14$) based on Tukey test. Mortality in treatment groups was 83% and of controls was 70%. A mold associated with the food cups increased the mortality of larvae. No control caterpillars died of virus. In a second trial comparing just virus treated larvae, pupal masses of gypsy moth females treated with wild type and *egt-* virus did not differ ([$t = 0.26$, df = 62, $P = 0.80$] [mean wild virus treated = 1.25 g, SE = 0.06, $n = 30$ and mean *egt-* treated = 1.27 g, SE = 0.06, $n = 24$]).

The Effect of Temperature and LdNPV Dose. In the experiment 2 the mass of the female pupae did not differ with the temperature at which larvae were reared following inoculation (Table 1). The means of female pupal mass of all of the experimental groups were significantly lighter than that of the control group reared at 28°C but not of the control group reared at 20°C. The latter were light as compared with the controls in experiment 1 as well as the controls reared at 28°C. The time to pupation was significantly longer for larvae reared at 20°C compared with those reared at 28°C. Control pupae tended to develop more rapidly than those exposed to virus however this difference was not significant (Table 1). Survival to pupation was not obviously related to rearing temperature or viral dose, and was ≈25% for most groups with the 500,000 OBs, 28°C group having the highest survival at 39%.

Egg masses of females reared at 20°C were significantly lighter than those of females reared at 28°C ($t = 2.5$, df = 28, $P = 0.02$) (Table 2). Because the sizes of the egg masses of females exposed to virus at the different doses were not significantly different, the egg masses of the treated groups were combined and compared with controls for the two rearing temperatures. Egg masses from virus treated groups were significantly lighter than those from uninfected control insects (ANOVA, $F = 48.6$; df = 3, 26; $P > 0.01$). The Tukey test showed significant variation between the virus treated group reared at 20°C and the 28°C control group. Of 1,036 larvae from a total of 21 egg masses (13 from females inoculated with virus and eight controls) that were reared, only five larvae died of virus; one out of two hatching from one egg mass and four out of 62 from another. Neither of these egg masses had been surface sterilized and both were the offspring of females treated with 50,000 OBs.

In experiment 3, pupal mass varied significantly with increasing virus dose (Table 3) ($F = 9.9$; df = 3, 56; $P > 0.01$). Control pupae were significantly heavier than each of the experimental groups based on the Tukey test. The sizes of the egg masses produced by

Table 2. Mean mass (g), SE, number of gypsy moth egg masses of female moths infected as larvae with different doses of LdNPV and uninfected controls

Temp, °C	Dose (OBs)	Mean (g)	SE	N	Temp, °C	Dose (OBs)	Mean (g)	SE	N
20	5,000	—			28	5,000	0.335	0.060	2
	50,000	0.258	0.038	5		50,000	0.355	0.043	4
	500,000	0.283	0.049	3		500,000	0.322	0.035	6
	Mean	0.268	0.040	8		Mean	0.335	0.030	12
	Control	0.340	0.049	3		Control	0.427	0.035	6

Table 3. Mean mass, SE, and number of female gypsy moth pupae and egg masses of individuals surviving inoculation with LdNPV and controls. Rearing temperature 25°C

Dose (OBs)	Pupae (g)			Egg masses (g)		
	Mean (g)	SE	N	Mean (g)	SE	N
5000	1.812	0.092	16	0.377	0.041	4
50,000	1.737	0.107	14	0.301	0.027	9
500,000	1.394	0.156	7	0.267	0.018	7
Control	2.183	0.073	23	0.399	0.026	10

females also varied significantly ($F = 5.3$; df = 3, 26; $P > 0.01$) (Table 3) with egg masses of treated insects again being lighter than those of the controls.

Both exposure of gypsy moth larvae to virus 1 d after molting to the fifth instar (experiment 2) and 5 d after molting (experiment 3) reduced pupal mass and egg mass size of individuals inoculated with virus.

Discussion

The exposure of fifth-instar gypsy moth larvae to virus was associated with reduced female pupal mass and reduced size of egg masses of surviving individuals. In the experiment in which larvae were inoculated 1 d after molting to the fifth instar, the reduction in both pupal and egg mass sizes increased with the viral dose. A dosage effect was not apparent in the experiment in which larvae were inoculated 5 d after molting. The time for expression of differences associated with virus dose (≈6 d) may have been too short in this experiment. In experiments with cabbage loopers, *Trichoplusia ni* (Hübner), sublethal effects were associated with age at inoculation but not dose (Milks et al. 1998).

Prolonging the larval period by rearing at cooler temperatures did not have an apparent impact on the expression of sublethal effects as shown by pupal size, but egg masses of those reared at the cooler temperature were smaller. The temperature related growth curves of gypsy moth larvae and LdNPV are not known, but prolonging the time to pupation may have accentuated sublethal effects on fecundity. Survival of larvae reared at the two temperatures were similar.

Pupal masses of larvae exposed to the wild type virus and the *egt-* virus were similar, although only those infected with wild type virus were significantly smaller than controls. Slavicek et al. (1999) found that 5 d after infection cumulative weight gain of fifth-instar, female, gypsy moth larvae declined for larvae infected with *egt-* virus but continued to increase for those infected with a virus strain with the *egt* gene. The growth curves of larvae infected with *egt-* virus were similar to those of controls, but the infected larvae lost weight after the controls had pupated. In these experiments the final weights of larvae infected with a wild type virus were similar to those of controls, but larvae infected with the *egt-* virus were significantly lighter. Eventually, all the infected larvae in these experiments died. Similarly, Wilson et al. (2000) found that *T. ni* larvae infected with *egt-* virus were smaller at the fourth instar than were those infected with virus with the *egt* gene. These results indicate differences in the growth curves of larvae infected with *egt-* and wild type viruses. There was no clear impact of the *egt* gene on the expression of sublethal effects in our experiments.

Vertical transmission occurred in larvae from two egg masses and these were not surface sterilized. It is possible that this infection was associated with external contamination of the egg mass, but it is unlikely that it was associated with the original inoculation of the larvae because the virus dose was put on a small piece of food, the larvae consumed all of the food and virus, and then larvae were placed individually into new cups. In the studies of Shapiro and Robertson (1987), contamination was a possible explanation for vertical transmission because larvae were reared on media containing viral OBs. In most studies in which vertical transmission has been studied, overt infection only occurs in 0.5–2% of larvae (Kukan 1999), and our results and those of Murray and Elkinton (1989) with gypsy moth are similar. Although the number of infected larvae is small, the expression of virus in two of 13 egg masses of females that had been inoculated with virus (15%) is more substantial. Transmission of the virus to other caterpillars in the family group could be influential in natural populations. The potential impact of sublethal infection and vertical transmission of NPV on population dynamics of forest Lepidoptera has been explored in several models (Regniere 1984, Beukema 1992, Myers 1993, Briggs and Godfray 1996, Boots and Norman 2000) and these indicate that sublethal infection and vertical transmission can promote cyclic dynamics under some conditions. Therefore, these results are of interest to the understanding of gypsy moth population dynamics.

Sublethal effects of the granulosis virus, PiGV, on both males and females of laboratory populations of the Indian meal moth, *Plodia interpunctella* (Hübner), have been detailed (Sait et al. 1994, 1998). In this situation, exposure to virus influences the size and fertility of moths of both sexes and both the overt and sublethal aspects of viral infection are thought to influence the population dynamics of these laboratory cultures. Nothing is known about virus effects in wild populations of grain moths.

Reduced fecundity is a characteristic of population declines in several species of forest caterpillars (Myers 1988) including the tent caterpillar *Malacosoma pluviale* (Myers and Kukan 1995). Reduced fecundity has also been shown to occur following experimental exposure of late instar tent caterpillar larvae to NPV (Rothman and Myers 1994, Rothman 1997). Egg masses of high density gypsy moths are generally

markedly smaller than those from low density populations (Campbell 1978, Myers et al. 1998) and although viral infection is usually a characteristic of outbreaks as well; whether the reduced fecundity of moths is associated with sublethal infection has not been shown. These experiments show that sublethal effects are possible in this species and deserve further investigation in field populations.

Acknowledgments

This work was carried out in the Department of Entomology of the University of Massachusetts, Amherst, in the laboratory of Joe Elkinton, and J.H.M. appreciates the hospitality, facilities, and many excellent discussions provided there. We thank John Burand (Departments of Entomology and Microbiology, University of Massachusetts) for providing LdNPV and USDA-APHIS Laboratory (Otis Air National Guard Base, MA) for providing gypsy moth eggs and cups of synthetic diet. Jeff Boetner kindly helped with the laboratory rearing. This research was partially supported by the Natural Science and Engineering Research Council of Canada.

References Cited

Bell, R., C. Owens, M. Shapiro, and J. Tardif. 1981. Mass rearing and virus production, pp. 599–606. *In* C. Doane and M. McManus [eds.], The gypsy moth: research toward integrated pest management. USDA, Washington, DC.

Beukema, S. J. 1992. Towards more realistic models of disease and insect population dynamics: virus-tent caterpillar interactions. M.S. thesis, University of British Columbia, Vancouver.

Boots, M., and R. Norman. 2000. Sublethal infection and the population dynamics of host-microparasite interactions. J. Anim. Ecol. 69: 517–524.

Briggs, C. J., and H.C.J. Godfray. 1996. The dynamics of insect-pathogen interactions in seasonal environments. Theor. Popul. Biol. 50: 149–177.

Burand, J., and E. Park. 1992. Effect of nuclear polyhedrosis virus infection on the development and pupation of gypsy moth larvae. J. Invertebr. Pathol. 60: 171–175.

Campbell, R. 1978. Some effects of gypsy moth density on rate of developoment, pupation time, and fecundity. Ann. Entomol. Soc. Am. 71: 442–448.

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

Kukan, B, 1999. Vertical transmission of nuclear polyhedrosis virsus. J. Invertebr. Pathol. 74: 103–111.

Kukan, B., and J. Myers. 1997. Prevalence and persistence of nuclear polyhedrosis virus in fluctuating populations of forest tent caterpillars (Lepidoptera: Lasiocampidae) in the area of Prince George, British Columbia. Environ. Entomol. 26: 882–887.

Kukan, B., and J. Myers. 1999. Dynamics of viral disease and population fluctuations in western tent caterpillars (Lepidoptera: Lasiocampidae) in southwestern British Columbia, Canada. Environ. Entomol. 28: 44–52.

Milks, M. L., I. Burnstyn, and J. H. Myers. 1998. Influence of larval age on the lethal and sublethal effects of the nucleopolyhedrovirus of *Trichoplusia ni* the cabbage looper. Biol. Control 12: 119–126.

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

Murray, K., and J. Elkinton. 1990. Transmission of nuclear polyhedrosis virus to gypsy moth (Lepidoptera: Lymantriidae) eggs via contaminated substrates. Environ. Entomol. 19: 662–665.

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

Myers, J. H. 1988. Can a general hypothesis explain population cycles of forest Lepidoptera. Adv. Ecol. Res. 18: 179–284.

Myers, J. H. 1993. Population outbreaks in forest Lepidoptera. Am. Sci. 81: 240–251.

Myers, J. H., G. Boettner, and J. Elkinton. 1998. Maternal effects in gypsy moth: only sex ratio varies with population density. Ecology 75: 305–314.

Myers, J. H., and B. Kukan. 1995. Changes in the fecundity of tent caterpillars: a correlated character of disease resistance or sublethal effect of disease? Oecologia 103: 475–480.

O'Reilly, D., and L. Miller. 1990. Regulation of expression of a baculovirus ecdysteroid UDP-glusyl transferase. Science 245: 1110–1112.

Park, E., J. P. Burand, and C.-M. Yin. 1993. The effect of baculovirus infection on ecdysteroid titer in gypsy moth larvae. J. Insect Physiol. 39: 791–796.

Regniere, J. 1984. Vertical transmission of diseases and population dynamics of insects with discrete generations: a model. J. Theor. Biol. 107: 287–301.

Rothman, L. 1997. Immediate and delayed effects of a viral pathogen and density on tent caterpillar performance. Ecology 78: 1481–1493.

Rothman, L., and J. Myers. 1994. Nuclear polyhedrosis virus treatment effect on reproductive potential of western tent caterpillar (Lepidoptera: Lasiocampidae). Environ. Entomol. 23: 864–869.

Rothman, L., and J. Myers. 1996. Debilitating effects of viral diseases on host Lepidoptera. J. Invertebr. Pathol. 67: 1–10.

Sait, S. M., M. Begon, and D. J. Thompson. 1994. Long-term population dynamics of the Indian meal moth *Plodia interpunctella* and its granulosis virus. J. Anim. Ecol 63: 861–870.

Sait, S. M., M.J.G. Gage, and P. A. Cook. 1998. Effects of a fertility-reducing baculovirus on sperm numbers and sizes in the Indian meal moth, *Plodia interpunctella.* Funct. Ecology 12: 56–62.

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

Slavicek, J. M., H.J.R. Popham, and C. I. Riegel. 1999. Deletion of the *Lymantria dispar* multicapsid nucleopolyhedrovirus ecdysteroid UDP-Glucosyl transferase gene enhances viral killing speed in the last instar of the gypsy moth. Biol. Control 16: 91–103.

SYSTAT. 1992. Statistics. SYSTAT, Evanston, IL.

Wilson, K., D. R. O'Reilly, R. S. Hails, and J. S. Cory. 2000. Age-related effects of the *Autographa californica* MNPV *egt* gene in the cabbage looper (*Trichoplusia ni* Hübner) (Lepidoptera: Noctuidae). Biol. Control (in press).

Received for publication 10 February 2000; accepted 22 August 2000.