BIOLOGICAL CONTROL **12,** 119–126 (1998)
ARTICLE NO. BC980616

Influence of Larval Age on the Lethal and Sublethal Effects of the Nucleopolyhedrovirus of *Trichoplusia ni* in the Cabbage Looper

Maynard L. Milks,[1] Igor Burnstyn, and Judith H. Myers

Department of Zoology, University of British Columbia, Vancouver, British Columbia V6T 1W4, Canada
E-mail: milksm@em.agr.ca

Received July 14, 1997; accepted January 29, 1998

The nucleopolyhedrovirus of *Trichoplusia ni* (TnSNPV) shows promise as a bioinsecticide against cabbage loopers (*T. ni*). We examined the effects of larval age at time of treatment and of the dose of virus ingested on the lethal and sublethal effects of TnSNPV on *T. ni*. The survival and LD_{50} of cabbage loopers increased with the age of larvae at the time of treatment. The sublethal effects of TnSNPV included prolonged development and reduced pupal weight, egg production, and hatching success of eggs. The sublethal effects of TnSNPV did not vary among the three doses of virus used but were a function of larval age. The sublethal effects of TnSNPV were greatest when caterpillars were treated with virus at 92 and 144 h of age (third and fourth instar, respectively). Individuals that were treated at 192 h (fifth instar) and survived to adulthood did not appear to be impaired. These findings suggest that TnSNPV should be applied when cabbage loopers are in the early stages of development to maximize the bioinsecticidal activity of the virus. © 1998 Academic Press

***Key Words: Trichoplusia ni;* baculovirus; age dependence; dose independence; lethal effects; sublethal effects; bioinsecticide.**

INTRODUCTION

Nucleopolyhedroviruses (NPVs) are naturally occurring entomopathogens that have considerable potential for being developed as bioinsecticides, particularly against pest lepidopterans (Huber, 1986). NPVs can affect the demography of their hosts in two ways: they can have lethal or sublethal effects. Even when they are lethal, however, susceptibility to NPVs decreases with larval age at infection (e.g., Ignoffo, 1966; Boucias and Nordin, 1977; Burgerjon *et al.*, 1981; Smits and Vlak, 1988). Caterpillars succumbing to nucleopolyhedrosis are easily recognized (see Evans and Entwistle, 1987), and NPV epizootics have frequently been associated with collapsing populations of Lepidoptera (e.g., Steinhaus, 1949; Bird and Burk, 1961; Doane, 1976; Myers, 1988; Elkinton and Liebhold, 1990). The age structure of a population may play an important role in the development and magnitude of NPV epizootics (Webb and Shelton, 1990; Sait *et al.*, 1994a).

The sublethal effects of NPVs are more subtle and controversial, but they may nevertheless be of great importance in understanding the population dynamics of the host (e.g., Falcon, 1971; Perelle and Harper, 1986; Myers, 1988; Rothman and Myers, 1996). Rothman and Myers (1996) reviewed studies on sublethal infections and came to four conclusions. (1) Individuals surviving NPV infections during the larval stage are frequently impaired relative to controls. (2) The debilitating effects include prolonged development, lower pupal weight, and reduced reproductive capacity (egg production and hatching success of eggs). (3) The inclusion of debilitating effects caused by sublethal infections of NPVs could reduce the growth of populations by an additional 22% over that of NPV-induced mortality alone. (4) The magnitude of debilitating effects is generally greater when larvae are infected as late instars (fourth–fifth), but appears to be independent of the dose of virus that larvae ingest.

Three major drawbacks associated with the use of NPVs as bioinsecticides are that (1) they are expensive to produce and (2 and 3) the proportion and rate at which individuals die are frequently lower than with chemical insecticides. By determining which stage of the pest is most susceptible to NPVs, it may be possible to ascertain when the virus should be applied to maximize crop protection while minimizing the amount of virus required. Precise timing of applications could make NPV insecticides more cost effective. In addition, demonstrating that individuals that survive NPV infections are impaired may further aid in making NPVs marketable as bioinsecticides.

[1] Present Address: Agriculture and Agri-Food Canada, Pacific Agriculture Research Station, 4200 Highway 97, Summerland, BC Canada, V0H 1Z0.

1049-9644/98 $25.00

The cabbage looper (*Trichoplusia ni* Hübner) is found worldwide in subtropical regions (Mitchell and Chalfant, 1984). They are highly destructive agricultural pests that attack 160 species, varieties, or cultivars of plants (Sutherland and Greene, 1984). The single-embedded nucleopolyhedrovirus of *T. ni* (TnSNPV) shows a great deal of promise as a bioinsecticide against cabbage loopers (e.g., Jaques, 1972, 1977; Entwistle, 1983). The purpose of this study is to examine the effects of the age at which caterpillars are treated and of the dose of virus ingested on the lethal and sublethal effects of TnSNPV. Specifically, (1) are young larvae more susceptible to TnSNPV than older ones, (2) are individuals surviving infection with TnSNPV impaired relative to control insects, and (3) are these debilitating effects a function of the age of the larvae at time of treatment, dose of virus, or both?

MATERIALS AND METHODS

Experimental Protocol

A stock sample of TnSNPV was obtained from Dr. R. P. Jaques (Agriculture and Agri-Food Canada, Harrow, Ontario). The occlusion bodies (OBs) had been purified from infected *T. ni* as described in Potter *et al.* (1978). The OB concentration of the sample was determined using an improved Neubauer hemacytometer, and the sample was serially diluted in sterile distilled water (see Milks, 1997a).

The following experiment was conducted twice. Recently laid eggs (≤24 h old) were obtained from a colony of cabbage loopers kept in our laboratory. The colony had been established approximately 2 years earlier (ca. 25 generations of *T. ni*) by mixing individuals of the Hughes, Ignoffo, Jaques, Kaupp, and Keddie populations (see Milks, 1997a). The eggs were surface-sterilized using 0.1% sodium hypochlorite as described in Milks (1997a). After hatch, neonates were placed individually in 30-ml plastic cups containing high wheat germ diet (Jaques, 1967) and reared at 26 ± 1°C with a photoperiod of 16L:8D.

At 96, 144, or 192 h after hatch (third, fourth, or fifth instar, respectively), the larvae were transferred individually to a second cup containing only a plug of diet (thickness 2–3 mm, diameter 5 mm) treated with distilled water, as controls (the performance of cabbage loopers treated with distilled water or an extract of homogenized healthy *T. ni* larvae did not differ significantly; M. L. Milks, unpublished data), or with 2570, 12,900, or 25,700 TnSNPV OBs (10 μl of 2.57×10^5, 1.29×10^6, or 2.57×10^6 OBs/ml, respectively). Cohorts of 20 larvae were used as controls in each age group. Each TnSNPV dose was replicated four times. The number of larvae per replicate of each dose for each larval age is shown in parentheses: 96 h group: 2570 (20), 12,900 (25), and 25,700 (35) OBs; 144 h group: 2570 (15, but 10 in Trial 2), 12,900 (18), and 25,700 (20) OBs; 192 h group: 2570 (8), 12,900 (8), and 25,700 (12) OBs. The number of individuals per replicate varied to ensure that there would be a sufficient number of surviving adults in each age × TnSNPV dose category. Larvae that consumed the plug within 36 h were returned to their original cups while those failing to do so were discarded. Young larvae eat slowly and thus this period of time (36 h) was selected to ensure that the caterpillars in each of the three age groups would have enough time to consume the plug. Larvae were checked daily, and mortality, pupal weight (on day of pupation), sex, and time of adult emergence were recorded. Diagnosis of viral mortality was based on gross symptoms. Overall, 96% of TnSNPV-inoculated larvae that died exhibited the typical signs of nucleopolyhedrosis (i.e., discoloration and liquefaction) (Evans and Entwistle, 1987).

Seven female and male moths from each age × TnSNPV dose were randomly selected and paired in 500-ml paper cups (i.e., one pair/cup; 7 cups × 3 larval ages × 4 TnSNPV treatments [3 virus doses + 1 control] = 84 cups). The inside of each cup was lined with a piece of paper toweling as oviposition substrate, and a 10% sucrose solution was provided for adult feeding. The first paper lining was collected 4 days after a pair was formed and every second day thereafter until the female died. The number of eggs per lining was counted and, in Trial 2, the hatching success of eggs was also estimated.

Statistics

The equation of the dosage–mortality curves and LD_{50}s (dose required to kill 50% of treated insects) with associated 95% CIs were computed for each age group of each trial using PROC PROBIT with a logit link (SAS Institute, 1990). Since the mortality of control individuals never exceeded 5%, the data used in the analyses were not corrected for control mortality. For each trial, the slope of the three dosage–mortality curves were compared as described by Collett (1991).

The possible sublethal effects of TnSNPV on the pupal weight, developmental time, number of eggs laid per pair, and hatching success of eggs (arcsine square root transformed) of cabbage loopers were examined using multifactor analyses of variance (ANOVA) (PROC GLM; SAS Institute, 1990). The independent variables were larval age at time of treatment (fixed effect), TnSNPV treatment (fixed effect), and trial (random effect; not used in the ANOVA of hatching success). Sex (fixed effect) was also considered an independent variable in the ANOVAs of pupal weight and developmental time.

The data of each dependent variable were analyzed in two steps. First, to determine if a dependent variable varied among the three TnSNPV doses, an ANOVA was

TABLE 1

Number of Larvae, Percentage of Survival in Relation to *Trichoplusia ni* Single-Embedded Nucleopolyhedrovirus (TnSNPV) Dose, LD_{50}s and 95% CIs, and Slope of Dosage–Mortality Curves

Age of larvae at infection (h)	n[a]	Percentage of survival per TnSNPV dose (OBs)				LD_{50}	95% Confidence intervals	Slope (SE)
		0 (Control)	2570	12,900	25,700			
Trial 1								
96	305	95	80	36	22	9,403	7,294–11,613	3.06 (0.39)
144	226	100	86	62	41	19,240	13,751–31,445	2.05 (0.43)
192	132	100	91	69	78	604,880	—[b]	0.73 (0.59)
Trial 2								
96	300	100	74	30	13	6,025	4,427–7,713	2.79 (0.34)
144	207	95	85	60	38	17,211	11,851–27,964	1.94 (0.48)
192	130	100	95	77	39	18,971	13,445–29,482	3.00 (0.74)

[a] Total number of larvae, including controls, that consumed the plug within 36 h.
[b] 95% CI could not be estimated by SAS Institute (1990).
* $P < 0.05$.

conducted without the control groups. The second ANOVA was conducted on all available data, including the controls. Data were pooled across replicates within a TnSNPV dose. Examination of residuals did not suggest that the assumptions of ANOVA were violated in any test.

RESULTS

Effect of Larval Age at Time of Treatment on the Lethal Effects of TnSNPV

The slopes of the dosage–mortality curves varied with the age of larvae at time of treatment in Trial 1 ($\chi^2 = 12.6$, $df = 2$, $P < 0.001$). However, the only pairs of slopes that were significantly different were those of the 96- and 192-h age groups ($\chi^2 = 11.8$, $df = 1$, $P < 0.001$). The slope of the three dosage–mortality curves did not differ in Trial 2 ($\chi^2 = 2.4$, $df = 2$, $P > 0.25$).

Survival within a given dose and LD_{50}s increased with the age of larvae at time of infection (Table 1). However, it was not possible to calculate a 95% CI for the LD_{50} of the 192-h group of Trial 1, and the 95% CIs of the 144- and 192-h groups of Trial 2 overlapped (Table 1).

Effect of Larval Age at Time of Treatment and Virus Dose on the Sublethal Effects of TnSNPV

The pupal weight, developmental time, number of eggs laid per pair, and hatching success of eggs did not vary across the three TnSNPV doses (all terms involving TnSNPV dose had $P > 0.10$ for all dependent variables). Hence, to maximize the power of ANOVA (Zar, 1987), subsequent analyses for each of the dependent variables were conducted with the doses pooled (TnSNPV treatment = 2 levels, control or infected).

Pupal weight. The pupal weight of cabbage loopers that survived to adulthood was influenced by three factors: first, the pupae of females were lighter than those of males (Table 2, Fig. 1). Second, the weight of

TABLE 2

Four-Way ANOVA of the Pupal Weight and Developmental Time of Individuals That Survived to Adulthood and of the Number of Eggs Laid per Pair, with Sex, (Pupal Weight and Developmental Time Only), *Trichoplusia ni* Nucleopolyhedrovirus (TnSNPV) Treatment (Control or Infected), Age of Larvae at Time of Infection, and Trial as Predictor Variables

Factor	Pupal weight		Developmental time		Eggs laid per pair	
	F (df)	P	F (df)	P	F (df)	P
Sex	40.2 (1)	<0.001	48.3 (1)	<0.001		
TnSNPV treatment	8.8 (1)	0.003	10.8 (1)	0.001	6.4 (1)	0.012
Age at infection	12.4 (2)	<0.001	12.9 (2)	<0.001		
Trial	6.8 (1)	0.009			35.2 (1)	<0.001
Age at infection × TnSNPV treatment	6.5 (2)	0.002	3.2 (2)	0.042	3.2 (2)	0.042
Age at infection × trial	10.9 (2)	<0.001	4.1 (2)	0.017		

Note. Only factors that were significant ($P < 0.05$) are given.

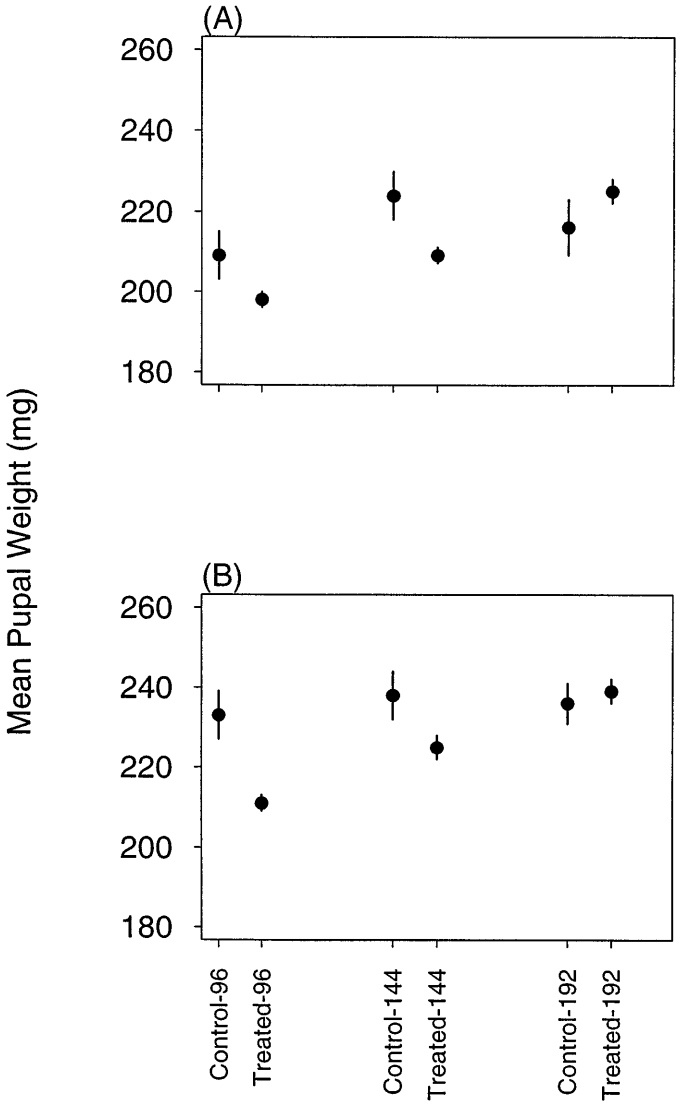

FIG. 1. Mean (±1 SE) pupal weight (mg) of (A) females and (B) males surviving to adulthood in relation to TnSNPV treatment and the age in hours at which larvae were treated. Control-96, individuals mock infected at 96 h of the larval stage. Infected-96, pooled category denoting all individuals that were treated with virus (i.e., 2570, 12,900, or 25,700 OBs of TnSNPV) at 96 h of age.

pupae varied between trials in some age groups (Table 2). Male and female pupae in the 144-h group of Trial 1 were heavier than those of Trial 2 (Table 3). Third, the pupal weight of survivors was a function of the age of the larvae when treated (Table 2, Fig. 1). The pupae of caterpillars treated at 96 and 144 h of age were smaller than their control counterparts (Table 3, Fig. 1).

Developmental time. The developmental time of cabbage loopers was influenced by three factors: first, female moths emerged before males (Table 2, Fig. 2). Second, developmental time varied between trials in some age groups (Table 2). Individuals in the 192-h group emerged sooner in Trial 2 than in Trial 1 (Table 3). Third, the effect of TnSNPV infection on developmental time was a function of the age at which the larvae were treated (Table 2, Fig. 2). The developmental time of infected individuals was longer than that of controls in the 96 h and close to significance ($F = 3.3$, $df = 1$, $P = 0.069$) in the 144-h group (Table 3, Fig. 2).

Eggs laid per pair. The number of eggs laid per pair was influenced by two factors: first, pairs produced more eggs in Trial 1 than in Trial 2 (Table 2). Second, the effect of TnSNPV infection was a function of the age at which larvae were treated (Table 2, Fig. 3). Pairs that had been treated at 96 h of the larval stage produced fewer eggs than controls (Table 3, Fig. 3).

Hatching success of eggs. The main effect of TnSNPV treatment was significant in the ANOVA of hatching success ($F = 3.9$, $df = 1$, $P = 0.050$), and pairs treated at 96 and 144 h hatched approximately 25% fewer eggs than controls (Fig. 4).

DISCUSSION

Effect of Larval Age at Time of Treatment on the Lethal Effects of TnSNPV

Overall, there was little evidence that the slope of dosage–mortality curves varied with the age of caterpillars at time of treatment. This suggests that increasing doses of TnSNPV led to a similar increase in mortality in all three age groups. The effect of larval age at time of treatment on the slope of dosage–mortality curves appears to vary across insect–baculovirus system. For example, age at treatment did not affect the slope of dosage–mortality curves in *Hyphantria cunea* Drury–GV/NPV (Boucias and Nordin, 1977), *Heliothis armigera* (Hübner)–NPV (Teakle *et al.*, 1985), *H. punctigera* Wallengren–NPV (Teakle *et al.*, 1986), or *Plodia interpunctella* (Hübner)–GV (Sait *et al.*, 1994b). However, the slopes of dosage–mortality curves varied and got smaller (shallower) with larval age at time of treatment in *Laspeyresia pomonella* (L.)–GV (Sheppard and Stairs, 1977), *Mamestra configurata* Walk.–NPV (Bucher and Turnock, 1983), *M. brassicae* (L.)–NPV (Evans, 1983), and *Spodoptera exigua* (Hübner)–NPV (Smits and Vlak, 1988).

The survival and LD_{50} of cabbage loopers infected with TnSNPV increased with the age at which caterpillars were treated (Table 1). Decline in susceptibility to baculoviruses with larval age at time of treatment has been reported in many other species (see Hochberg, 1991).

Effect of Larval Age at Time of Treatment and Virus Dose on the Sublethal Effects of TnSNPV

Cabbage loopers that survived infection with TnSNPV had longer developmental time, had reduced pupal weight, produced fewer eggs, and hatched fewer eggs

TABLE 3

Three-Way ANOVAs of the Pupal Weight, Developmental Time, and Number of Eggs Laid per Pair for Each of the Three Larval Ages at Infection

Age of larvae at infection (h)	Factor	Pupal weight		Developmental time		Eggs laid per pair	
		F	*P*	*F*	*P*	*F*	*P*
96	Sex	11.3	<0.001	15.3	<0.001		
	TnSNPV treatment	15.7	<0.001	11.8	<0.001	12	0.001
	Trial					7.2	0.010
144	Sex	9.9	0.002	14.9	<0.001		
	TnSNPV treatment	8.2	0.005				
	Trial	26.1	<0.001			20.6	<0.001
192	Sex	16.4	<0.001	25.0	<0.001		
	TnSNPV treatment						
	Trial			14.3	<0.001	9.9	0.003

Note. The predictor variables were sex (pupal weight and developmental time only), *Trichoplusia ni* single-embedded nucleopolyhedrovirus (TnSNPV) treatment (control or infected), and trial. Only factors that were significant ($P < 0.05$) are given. Degrees of freedom = 1 for all factors.

than controls. Sublethal baculovirus infections have been shown to adversely affect some or all of these life history traits in several other species (see Rothman and Myers, 1996).

These debilitating effects of TnSNPV did not appear to be dose-dependent, a result which is consistent with several other studies (see Rothman and Myers, 1996). However, the age of cabbage looper larvae at the time of treatment did have an influence, and sublethal effects were greatest when caterpillars were treated at third and fourth instar. Cabbage loopers may only partially fit the pattern of increased occurrence of sublethal effects in fourth–fifth instars (see Rothman and Myers, 1996) because *T. ni* have a short larval stage (11–13 days at 26°C). The duration of the fifth instar of cabbage loopers is only 3 days, and pupation may disrupt viral replication (Stairs, 1965; Evans, 1983; Murray *et al.,* 1991). Hence, the virus may have little time to have an impact when cabbage loopers are infected at fifth instar.

Pupal weight and egg production per female are correlated in many species of insects, including *T. ni.* Despite this, the magnitude of debilitating effects on fecundity was greater than on pupal weight. Females that were infected at 96 h and that survived to adulthood laid 40% fewer eggs but, on average, weighed only 6–10% less than control females (see Figs. 1 and 2). This suggests that differences in egg production per pair cannot be entirely explained by reduction in the pupal weight of females. Rothman and Myers (1994) made a similar finding in *Malacosoma californicum pluviale* (Dyar) and proposed that the additional reduction in egg production may have occurred because the virus affected the reproductive tissues of females. Alternatively, part of the reduced fecundity of infected pairs of cabbage loopers may be attributable to males. In *Spodoptera littoralis* and *P. interpunctella,* uninfected females that mated to males that were sublethally infected with a baculovirus had a lower reproductive success (egg production and/or egg viability) than those mated to uninfected males (Santiago-Alvarez and Vargas Osuna, 1988; Sait *et al.,* 1994a).

Ignoffo (1964) and Vail and Hall (1969) did not observe any sublethal effects when first or second instars of *T. ni* were treated with TnSNPV. Several reasons may be given to explain this discrepancy. First, the occurrence of sublethal effects is not consistent in other species, either (Rothman and Myers, 1996). Goulson and Cory (1996) outlined several methodological and biological reasons to explain this inconsistent pattern. Second, in *T. ni,* sublethal effects may occur only when larvae are infected at third or fourth instar. First and second instars may be so susceptible to viral infection that the only ones that survive are those that escape infection because of mechanical (pipeting) errors. In this case, no difference would be expected between control and putatively treated individuals. Conversely, individuals that are sublethally infected at fifth instar may suffer few debilitating effects because the virus may have little time to adversely affect them (see above). Third, variation among studies may also be influenced by genetic resistance to baculoviruses (e.g., Aratake, 1973; Reichelderfer and Benton, 1974; Briese, 1982). More resistant strains may survive better and have a greater probability of demonstrating sublethal effects. The cabbage loopers used in this study came from five different populations (see Materials and Methods) and may have been more genetically diverse than those used by Ignoffo (1964) and Vail and Hall (1969).

Several mechanisms have been proposed to explain how sublethal doses of virus might adversely affect the fitness of surviving individuals (see Sait *et al.,* 1994a). One of them suggests that energy used for growth is diverted to inactivate and eliminate the pathogen. In

the early stage of an infection, caterpillars may be able to fight off the virus by shedding infected midgut cells into the lumen of the gut (Keddie *et al.*, 1989). In the later stages of infection, infected cells may be encapsulated by hemocytes and destroyed in the epidermis of the tracheae of the midgut (Stairs, 1964; Begon *et al.*, 1993; Washburn *et al.*, 1996). However, it is not known if either of these two "immune" responses are sufficiently costly to generate the sublethal effects of TnSNPV that were observed in this study.

Rothman and Myers (1996) argued that sublethal effects could only be confirmed by observing occlusion bodies or viral nucleic acids in the survivors. If, however, sublethal effects are the result of the mechanism outlined in the previous paragraph, then sublethally infected individuals may be impaired without occlusion bodies or viral nucleic acids being detectable.

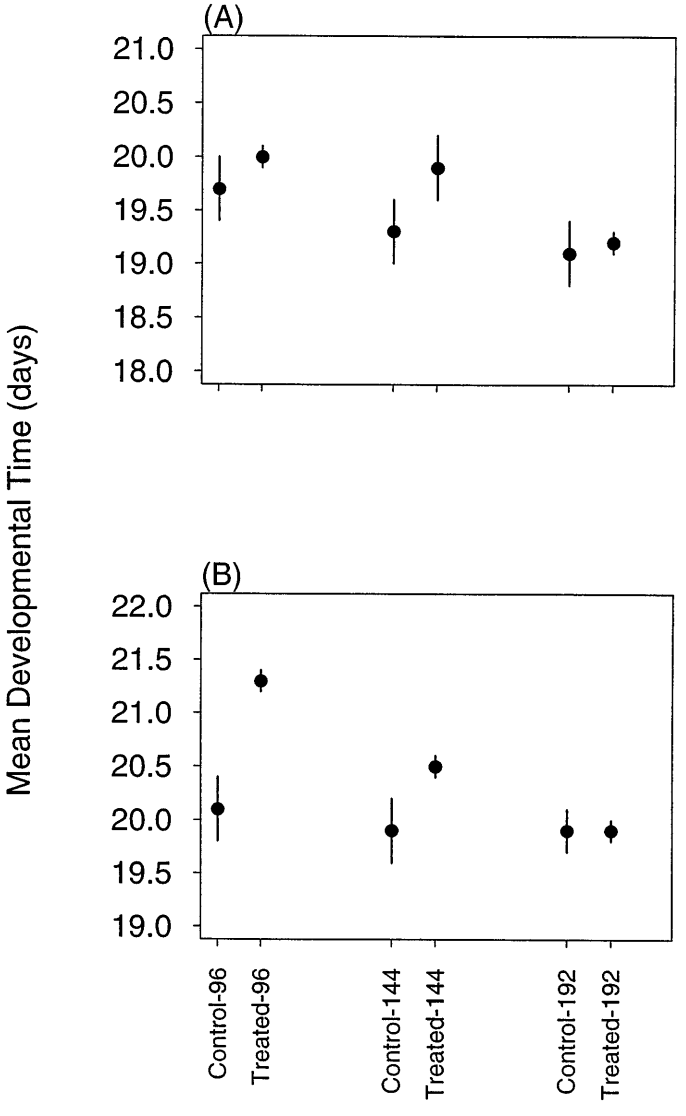

FIG. 2. Mean developmental time (days) of (A) females and (B) males surviving to adulthood in relation to TnSNPV treatment and the age in hours at which larvae were treated. Control-96, individuals mock infected at 96 h of the larval stage. Infected-96, pooled category denoting all individuals that were treated with virus (i.e., 2570, 12,900, or 25,700 OBs of TnSNPV) at 96 h of age.

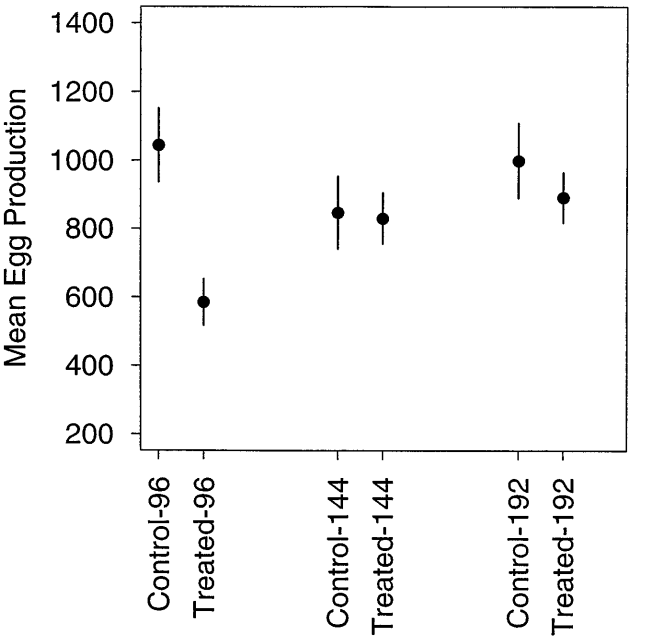

FIG. 3. Mean egg production per female in relation to TnSNPV treatment and the age in hours at which larvae were treated. Control-96, individuals mock infected at 96 h of the larval stage. Infected-96, pooled category denoting all individuals that were treated with virus (i.e., 2570, 12,900, or 25,700 OBs of TnSNPV) at 96 h of age.

Sait *et al.* (1994a) criticized the use of the diet contamination techniques when studying sublethal effects of baculoviruses. Their criticism rests on the assumption that individuals that feed and develop more slowly may ingest the virus over an extended period of time and have a higher probability of survival. Thus, the inoculation technique *per se* could select for smaller, slower developing individuals. Milks (1997b) examined this assumption and observed that the time to ingest the treated plug does not affect the survival of *T. ni*. Hence, the diet contamination technique used in this study is unlikely to have generated the sublethal effects of TnSNPV that were observed.

Implications of Findings for Biological Control

This study suggests that application of TnSNPV when *T. ni* are in the midstages of development may result in more efficient control of cabbage loopers because young larvae are more susceptible to the disease, and survivors may have impaired development and reproductive success. These sublethal effects could accentuate the bioinsecticidal properties of TnSNPV on cabbage loopers.

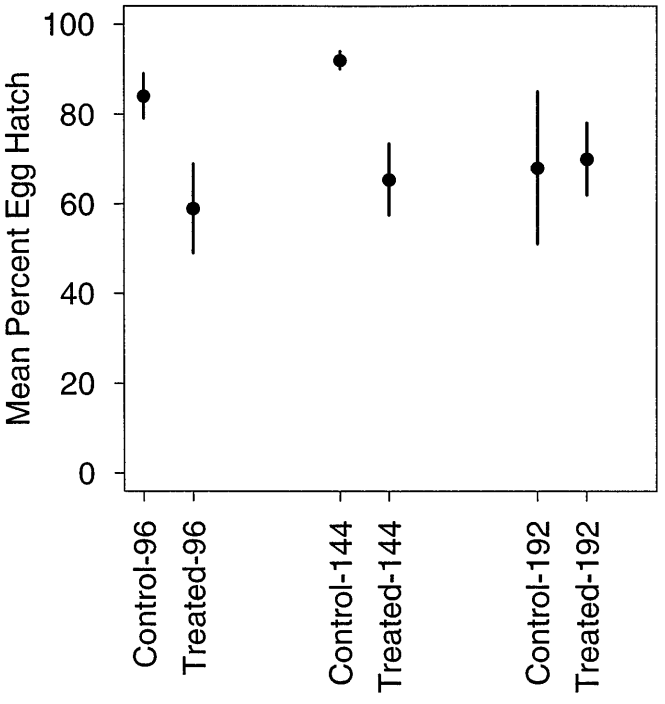

FIG. 4. Mean % egg hatch in relation to TnSNPV treatment and the age in hours at which larvae were treated. Control-96, individuals mock infected at 96 h of the larval stage. Infected-96, pooled category denoting all individuals that were treated with virus (i.e., 2570, 12,900, or 25,700 OBs of TnSNPV) at 96 h of age.

ACKNOWLEDGMENTS

This study would not have been possible without the outstanding laboratory assistance of Michelle Leptich and Thuy Nguyen. Martin Adamson, James Fuxa, Patrick Hughes, Murray Isman, Lorne Rothman, Geoff Scudder, Dolph Schluter, and David Theilmann commented on an earlier version of the manuscript. This study was supported by an operating grant to J.H.M. and a postgraduate scholarship to M.L.M. from the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

Aratake, Y. 1973. Strain differences of the silkworm, *Bombyx mori* L., in resistance to a nuclear polyhedrosis virus. *J. Seric. Sci. Jpn.* **42,** 230–238.

Begon, M., Haji Baud, K. B., Young, P., and Howells, R. E. 1993. The invasion and replication of a granulosis virus in the Indian meal moth, *Plodia interpunctella:* An electron microscope study. *J. Invertebr. Pathol.* **61,** 281–285.

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

Boucias, D. G., and Nordin, G. L. 1977. Interinstar susceptibility of the fall webworm, *Hyphantria cunea,* to its nucleopolyhedrosis and granulosis viruses. *J. Invertebr. Pathol.* **30,** 68–75.

Briese, D. T. 1982. Genetic basis for resistance to a granulosis virus in the potato moth, *Phthorimaea operculella. J. Invertebr. Pathol.* **39,** 215–218.

Bucher, G. E., and Turnock, W. J. 1983. Dosage responses of the larval instars of the bertha armyworm, *Mamestra configurata* (Lepidoptera: Noctuidae), to a native nuclear polyhedrosis virus. *Can. Entomol.* **115,** 341–349.

Burgerjon, A., Biache, G., Chaufaux, J., and Petre, Z. 1981. Sensibilité comparée, en fonction de leur âge, des chenilles de *Lymantria dispar, Mamestra brassicae* et *Spodoptera littoralis* aux virus de la polyhèdrose nucléaire. *Entomophaga* **26,** 47–58.

Collett, D. 1991. "Modeling Binary Data." Chapman & Hall, London.

Doane, C. C. 1976. Ecology of pathogens of the gypsy moth. *In* "Perspectives in Forest Entomology" (J. F. Anderson and H. K. Kaya, Eds.), pp. 285–293. Academic Press, New York.

Elkinton, J. S., and Liebhold, A. M. 1990. Population dynamics of gypsy moth in North America. *Annu. Rev. Entomol.* **35,** 571–596.

Entwistle, P. F. 1983. Viruses for insect pest control. *Span* **26,** 31–34.

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.

Evans, H. F., and Entwistle, P. F. 1987. Viral diseases. *In* "Epizootiology of Insect Diseases" (J. R. Fuxa and Y. Tanada, Eds.), pp. 257–322. Wiley, New York.

Falcon, L. A. 1971. Microbial control as a tool in integrated control programs. *In* "Biological Control" (C. B. Huffaker, Ed.), pp. 346–364. Plenum, New York.

Goulson, D., and Cory, J. S. 1996. Sublethal effects of baculovirus in the cabbage moth, *Mamestra brassicae. Biol. Control* **5,** 361–367.

Hochberg, M. 1991. Viruses as costs to gregarious feeding behaviour in the Lepidoptera. *Oikos* **61,** 291–296.

Huber, J. 1986. Use of baculoviruses as pesticides. *In* "The Biology of Baculoviruses" (R. R. Granados and B. A. Federici, Eds.), pp. 181–202. CRC Press, Boca Raton, FL.

Ignoffo, C. M. 1964. Bioassay technique and pathogenicity of a nuclear polyhedrosis virus of the cabbage looper, *Trichoplusia ni* (Hubner). *J. Invertebr. Pathol.* **6,** 237–245.

Ignoffo, C. M. 1966. Effects of age on mortality of *Heliothis zea* and *Heliothis virescens* larvae exposed to a nuclear-polyhedrosis virus. *J. Invertebr. Pathol.* **8,** 279–283.

Jaques, R. P. 1967. The persistence of a nuclear polyhedrosis virus in the habitat of the host insect, *Trichoplusia ni.* I. Polyhedra deposited on foliage. *Can. Entomol.* **99,** 785–796.

Jaques, R. P. 1972. Control of the cabbage looper and imported cabbageworm by viruses and bacteria. *J. Econ. Entomol.* **65,** 757–760.

Jaques, R. P. 1977. Field efficacy of viruses infectious to the cabbage looper and imported cabbageworm on late cabbage. *J. Econ. Entomol.* **70,** 111–118.

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.

Milks, M. L. 1997a. Comparative biology and susceptibility of cabbage looper (Lepidoptera: Noctuidae) lines to a nuclear polyhedrosis virus. *Environ. Entomol.* **26,** 839–848.

Milks, M. L. 1997b. Ingestion time does not influence the susceptibility of *Trichoplusia ni* to a nuclear polyhedrosis virus. *J. Invertebr. Pathol.* **70,** 165–166.

Mitchell, E. R., and Chalfant, R. B. 1984. Biology, behavior, and dispersal of adults. *In* "Suppression and Management of Cabbage Looper Populations" (P. D. Lingren and R. L. Greene, Eds.). *Tech. Bull. U. S. Dep. Agric.* **1684,** 10–20.

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 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,** 179–242.

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.

Potter, K. N., Jaques, R. P., and Faulkner, P. 1978. Modification of *Trichoplusia ni* nuclear polyhedrosis virus passaged *in vivo*. *Intervirology* **9,** 76–85.

Reichelderfer, C. F., and Benton, C. V. 1974. Some genetic aspects of the resistance of *Spodoptera frugiperda* to a nuclear polyhedrosis virus. *J. Invertebr. Pathol.* **23,** 378–382.

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

Rothman, L. D., and Myers, J. H. 1996. Debilitating effects of viral diseases on host lepidoptera. *J. Invertebr. Pathol.* **67,** 1–10.

SAS Institute. 1990. SAS user's guide: Statistics. SAS Inst., Cary, NC.

Sait, S. M., Begon, M., and Thompson, D. J. 1994a. The effects of a sublethal baculovirus infection in the Indian meal moth, *Plodia interpunctella*. *J. Anim. Ecol.* **63,** 541–550.

Sait, S. M., Begon, M., and Thompson, D. J. 1994b. The influence of larval age on the response of *Plodia interpunctella* to a granulosis virus. *J. Invertebr. Pathol.* **63,** 107–110.

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.

Sheppard, R. F., and Stairs, G. R. 1977. Dosage-mortality and time-mortality studies of a granulosis virus in a laboratory strain of the codling moth, *Laspeyresia pomonella*. *J. Invertebr. Pathol.* **29,** 216–221.

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

Stairs, G. R. 1964. Changes in the susceptibility of *Galleria mellonela* (Linnaeus) larvae to nuclear-polyhedrosis virus following blockage of the phagocytes with India ink. *J. Invertebr. Pathol.* **6,** 371–376.

Stairs, G. R. 1965. The effect of metamorphosis on nuclear polyhedrosis virus infecting certain lepidoptera. *Can. J. Microbiol.* **11,** 509–512.

Steinhaus, E. A. 1949. "Principles of Insect Pathology." McGraw–Hill, New York.

Sutherland, D. W. S., and Greene, G. L. 1984. Cultivated and wild host plants. *In* "Managing Cabbage Looper Populations" (P. D. Lindgren and G. L. Greene, Eds.). *Tech. Bull. U. S. Dep. Agric.* **1684,** 1–13.

Teakle, R. E., Jensen, J. M., and Giles, J. E. 1985. Susceptibility of *Heliothis armiger* to a commercial nuclear polyhedrosis virus. *J. Invertebr. Pathol.* **46,** 166–173.

Teakle, R. E., Jensen, J. M., and Giles, J. E. 1986. Age-related susceptibility of *Heliothis punctiger* to a commercial formulation of nuclear polyhedrosis virus. *J. Invertebr. Pathol.* **47,** 82–92.

Vail, P. V., and Hall, I. M. 1969. Susceptibility of the pupa of the cabbage looper, *Trichoplusia ni*, to nuclear polyhedrosis virus. *J. Invertebr. Pathol.* **14,** 227–236.

Washburn, J. O., Kirkpatrick, B. A., and Volkman, L. E. 1996. Insect protection against viruses. *Nature* **383,** 767.

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

Zar, J. 1987. "Biostatistical Analysis." Prentice Hall, Englewood Cliffs, NJ.