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Transmission dynamics of the multicapsid nucleopolyhedrovirus SeMNPV in *Spodoptera exigua* populations in greenhouse chrysanthemum

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The multicapsid nucleopolyhedrovirus, SeMNPV, is naturally maintained in *Spodoptera exigua* populations by vertical transmission from females to eggs and by horizontal transmission from cadavers of caterpillars to other caterpillars. Laboratory and greenhouse experiments were conducted to quantify transmission rates and provide baseline data to assess the potential of this virus to maintain itself naturally in pest populations after application in biocontrol programs. As to vertical transmission, 18% of the first instar larvae originating from egg batches produced by infected moths contracted SeMNPV, while 34% of the egg batches gave rise to one or more infected first instar larvae. As only few large egg batches were virus-free, 70% of all uninfescted larvae hatched in egg batches that contained one or more infected larvae. Substantial horizontal transmisson was observed under greenhouse conditions. Presence of 0, 1 or 10 infected first instar larvae in a batch of 100 first instar larvae resulted in survival to the fifth instar of 41, 30 and 20%, respectively. However, the effect on crop injury was marginal. The observed high transmission rates favour the maintenance of SeMNPV in *S. exigua* populations in greenhouses.

Keywords: *Spodoptera exigua* multicapsid nucleopolyhedrovirus, baculovirus, vertical transmission, horizontal transmission, biological control

Baculoviruses are pathogens that cause epidemics in insect populations. They are used as biological agents to control pest insect populations such as beet armyworm (*Spodoptera exigua*), gypsy moth (*Lymantria dispar*) and tobacco hornworm (*Heliothis virescens*) (Moscardi, 1999). Baculovirus populations are maintained in insect populations by transmission of the virus from infected to susceptible hosts. There are two major transmission routes: vertical and horizontal. Vertical transmission is the direct transfer of virus from parents to their own offspring, whereas horizontal transmission is the infection of susceptible individuals by ingestion of virus from the environment.

Vertical transmission has been studied in several virus-insect systems (Smits & Vlak, 1988a; Hamm & Young, 1974; Young, 1990; Fuxa & Richter, 1992). In these studies, vertical transmission was quantified by the proportion of eggs – laid by sublethally infected moths – that hatched into infected larvae. However, this characterization of vertical transmission is incomplete. It is epidemiologically significant to know how the infected individuals are distributed over egg batches (Anderson & May, 1981). Larvae that die of a vertically transmitted baculovirus infection spill newly produced virus on the foliage, hence new infections can occur when this is ingested by uninfected larvae. The number of encounters between vertically infected and uninfected hosts is greater when infected individuals are evenly distributed over all egg batches than when all infected individuals are deposited within a single egg batch. To understand baculovirus epidemics in insect populations, quantitative information of both vertical and horizontal transmission dynamics (including the distribution of infected larvae over egg batches) is needed.

Aim of this study is to quantify the vertical transmission rate of SeMNPV by studying the distribution of infected larvae over egg batches of sublethally infected *S. exigua* moths. In addition, the rate of the subsequent horizontal transmission is determined in populations *S. exigua* larvae in a greenhouse chrysanthemum crop.

MATERIAL AND METHODS

Insects and virus

Laboratory colonies of *S. exigua* were maintained as described by Smits *et al*. (1986). Larvae were reared from surface sterilized eggs and incubated at 25°C, 70-80% relative humidity and a 16 h photoperiod. The SeMNPV US isolate was used (Hunter & Hall, 1968) and was propagated in fourth instar *S. exigua* larvae via surface contamination of semi-synthetic diet (Smits & Vlak, 1988b). The virus was purified by grinding deceased larvae, filtering through a double layer of cheese-cloth and two centrifugation steps. The polyhedra were resuspended and stored in a glycerol/water (1:1) solution in the dark at 4°C.

Vertical transmission

Two-hundred fifth instar larvae were allowed to ingest SeMNPV surface contaminated semi-synthetic medium with a blue stained $1x10^3$ polyhedra/ mm^2 solution, whereas a control group was reared in absence of virus. The larvae were reared at 25°C without light until pupation. Pupae were collected and transferred to an oviposition cylinder. The cylinder contained vermiculite and two layers of paper on the wall for egg deposition. After four and six days a paper with egg batches was removed from the cylinder. Neither pupae nor egg batches were surface-decontaminated in order to allow potential vertical transmission through external virus. The egg batches were reared individually and the egg batch size, the number of hatched larvae and the number of virus-killed larvae were recorded after four days of incubation in a stove at 25°C without light. Egg batch size, hatching and the number of infected larvae were analyzed using the Wilcoxon rank-sum test with Genstat (Genstat 5 Committee, 1993).

Horizontal transmission

The experiment was conducted in a greenhouse from April 6 to May 16 1994 in Wageningen, The Netherlands. Temperatures ranged from 20-30°C in the daytime, and were kept at 20°C during the night. A total of 48 plots with chrysanthemum plants were constructed in the greenhouse. Each plot consisted of 54 plants, which were planted in eight rows at distances of 10 cm. At the start of the experiment the plants were 8 weeks old and 100 cm high. The plots were surrounded by sticky tape to prevent larvae from escaping.

The treatment factors of the experiment were two chrysanthemum cultivars, ‘Tiger’ and ‘Tigerrag’, and three density levels of primary infected larvae (PIL). The PIL density levels were: 0, 1 and 10 infected larvae per 100 first instar *S. exigua* larvae. The two chrysanthemum cultivars were assigned to the 48 plots in alternating design. The three PIL density levels were assigned to the plots in a completely randomised design.

Virus infection was obtained by allowing newly hatched larvae to ingest an LD_{100} of surface contaminated semi-synthetic medium ($1x10^3$ polyhedra/mm^2) with a blue stained virus solution. After 24 h, larvae were selected for the experiment by their blue colour. Primarily infected and uninfected larvae were released in the plots by placing petri-dishes with a total of 100 first instar larvae upside down on top of a plant in the middle of the plot, allowing the larvae to descend into the crop. The number of larvae not leaving the petri dish was noted. The experiment was terminated when fifth instar larvae were first observed in the crop (22 and 23 days after the release of the larvae). Destructive sampling was carried out by dividing the plants in three foliage strata with an equal number of leaves. All larvae in each stratum were collected and counted. The total number of virus-killed and recaptured larvae, their instar, and the number of larvae recaptured on tape were recorded per plot. Recaptured larvae were individually reared on semi-synthetic diet until the larvae had either pupated or died. The number of virus-killed larvae was calculated as the sum of recaptured virus-killed larvae in plots and larvae that died of SeMNPV infection during the incubation period. These larvae were assumed to be secondarily infected larvae since the primarily infected first instar larvae disintegrate rapidly and are unlikely to be found. Crop injury was recorded by counting the number of damaged leaves. Larval survival and virus-induced mortality were analysed by ANOVA with Genstat.

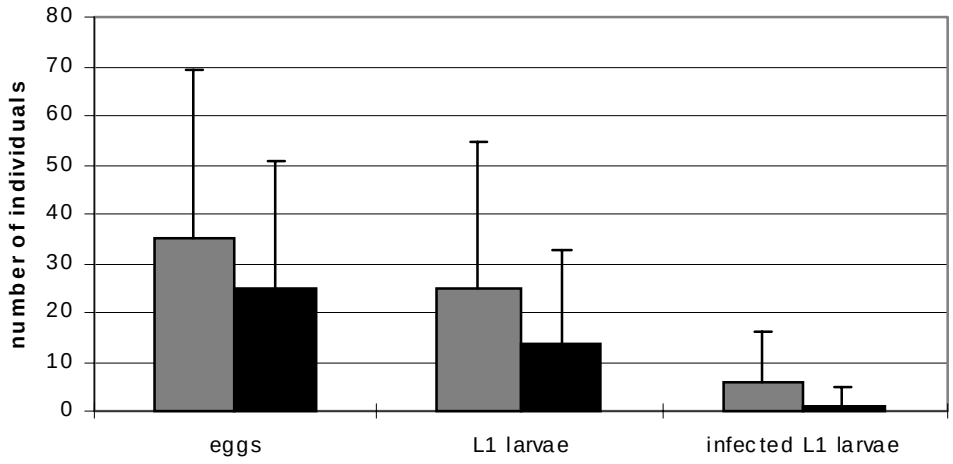

Figure 1. Number of eggs, number of hatched larvae, and number of infected L1 larvae in egg batches laid by sublethally infected *Spodoptera exigua* during the first four days after emergence (light bars) and during the consecutive two days (dark bars). Bars indicate mean and standard deviation

RESULTS

Vertical transmission

The ingestion of SeMNPV surface contaminated diet caused 67% larval mortality and 42 larvae developed into adults. The first paper layer in the oviposition cylinder was removed four days after the first adults had emerged and contained 52 egg batches. A second group of 54 egg batches was collected two days later, after which the moths rapidly died without producing any more eggs. The size of the egg batches ranged from 5 to 175 eggs per batch and the 106 batches added up to a total of 3179 eggs. After four days of incubation, 95 egg batches contained hatched larvae and 64% of the deposited eggs hatched. Thirty-two egg batches contained virus-killed larvae, which is 34% of the hatched egg batches. The total number of progeny that developed SeMNPV infection was 367, which is 18% of the total number of hatched larvae. In Fig. 1 the mean number are given of eggs, hatched larvae, and infected L1 larvae that hatched from egg batches of the first four days and the consecutive two days.

The first group contained a higher number of eggs, hatched larvae, and infected L1 larvae per egg batch than the second group, although these differences only were significant for the number of eggs and the number of infected larvae ($P < 0.05$). The cumulative frequency distributions of the number of eggs, the number of hatched larvae and the number of infected L1 larvae are presented in Fig. 2. The number of eggs and the number of hatched larvae were highly correlated ($R^2 = 0.939$, $P < 0.001$) (Fig. 3a). The relations between the number of infected L1 larvae and the egg batch size and the number of hatched larvae are presented in Figs. 3b and 3c. The number of infected L1 larvae per egg batch was positively correlated with the egg batch size ($R^2 = 0.631$, $P < 0.001$) and the number of hatched larvae ($R^2 = 0.684$, $P < 0.001$). As a consequence, 80% of the egg batches larger than 40 eggs contained infected larvae. Seventy percent of all uninfected larvae hatched in egg batches that contained one or more infected larvae.

Horizontal transmission

The survival and SeMNPV-induced mortality in populations of *S. exigua* larvae with 0, 1 and 10 primary infected larvae are shown in Table 1. Almost 80% of the recaptured larvae in the control plots had reached the fifth instar and the rest was still in the fourth instar. The chrysanthemum cultivar had no effect on the number of surviving ($P = 0.13$) and virus-killed larvae ($P = 0.38$). The PIL density levels significantly affected the number of *S. exigua* larvae that survived in the plots and the subsequent incubation period ($P < 0.001$). Survival of larvae of all three PIL density levels with 0, 1 and 10 SeMNPV-infected larvae differed significantly ($P < 0.05$) with 41, 30 and 20% survival. The number of virus-killed larvae of the two PIL density levels with 1 and 10 infected

larvae were significantly higher than the control, but the difference between these two density levels was not significant (P = 0.20). The proportion of damaged leaves was 29%, 25% and 22.5% for treatments with respectively 0, 1 and 10 infected larvae.

DISCUSSION

Spodoptera exigua moths that were treated with SeMNPV as fifth instar larvae transmitted the virus to 18% to their progeny. Reported vertical transmission rates of SeMNPV through sublethally infected moths vary. Young (1990) and Goulson & Cory (1995) found no or minimal vertical transmission in *S. ornithogalli* and *Mamestra brassicae* whereas Young & Yearian (1982) and Smits & Vlak (1988a) found vertical transmission rates of 8 to 28% in *Pseudoplusia includens* and *S. exigua*.

Thirty-four percent of the hatched egg batches contained infected larvae. In general, these contaminated egg batches contained high numbers of eggs and were typically laid within four days after emergence of the adults. It is well established that the rate of vertical transmission of moths exposed to polyhedra declines in time (Hamm & Young, 1974; Elnagar *et al*., 1982). This suggests that the first egg batches deposited, which are generally larger, have a higher probability to be contaminated with polyhedra. In contrast, smaller egg batches, which are deposited later, may be almost free of polyhedra because the majority already has been deposited with previous egg batches.

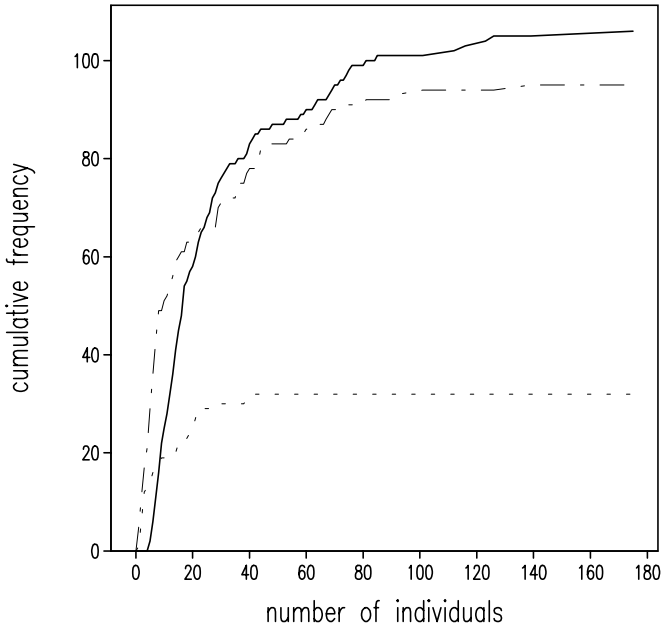

Figure 2. Cumulative frequency distributions of the number of eggs (solid line), number of hatched larvae (dashed line), and number of infected L1 larvae (dotted line) in egg batches laid by sublethally infected *Spodoptera exigua* after six days

Table 1. Mean and SEM of the fate of populations of 100 *Spodoptera exigua* larvae in greenhouse chrysanthemums with 0, 1 and 10 primary infected larvae

	Primary Infected Larvae (PIL) – treatment		
	0	1	10
survival	41.2 ± 2.7	29.9 ± 3.6	19.6 ± 2.9
primarily infected	0 ± 0	1 ± 0	10 ± 0
secondarily infected	0 ± 0	6.7 ± 1.4	9.1 ± 1.1
background mortality	52.2 ± 2.9	56.9 ± 3.0	56.4 ± 2.8
caught on tape	0.9 ± 0.3	1.1 ± 0.3	1.3 ± 0.3
unreleased	5.7 ± 3.1	4.4 ± 2.5	3.6 ± 2.5

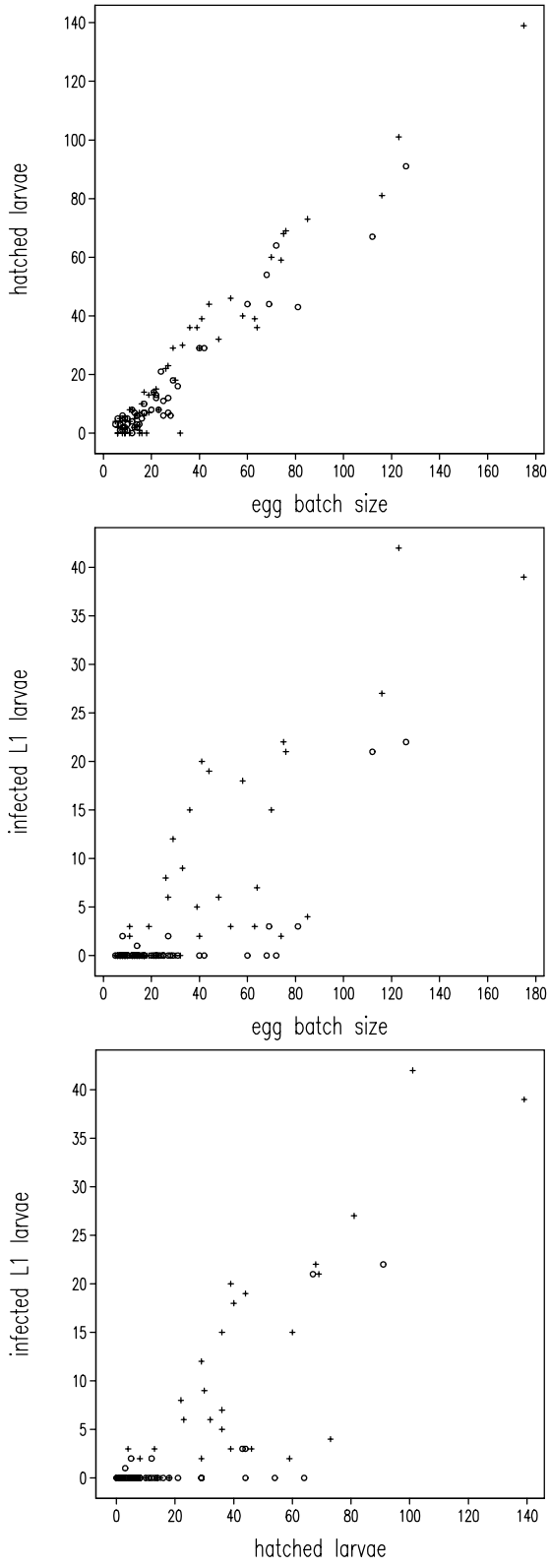

Figure 3. Relation between the egg batch size, the number of hatched larvae and the number of infected L1 larvae in egg batches deposited by sublethally infected *Spodoptera exigua*. Egg batches laid during the first four days are indicated as '+' and egg batches laid during the consecutive two days are indicated as 'o'

The number of *S. exigua* larvae recovered in the horizontal transmission experiment was much lower than the number released. Overall mortality in the control plots was almost 60% and are in accordance with observations of Smits *et al*. (1987) who found similar percentages of background mortality in greenhouse chrysanthemum. The survival of groups of 100 *S. exigua* larvae was significantly reduced by the release of 1 or 10 SeMNPV-infected larvae, indicating that horizontal transmission impacted survival. The number of surviving larvae was negatively correlated with the number of primary infected larvae released into the plots. On the other hand, the number of recaptured SeMNPV-killed larvae in plots with 1 and 10 initially infected larvae were not significantly different with average values of 7 and 9 larvae, respectively. These high horizontal transmission rates suggest that uninfected larvae may be attracted to virus-killed larvae, or that virus-killed larvae contaminate many leaves. Although larval survival was reduced at increasing PIL densities, crop injury was hardly reduced in plots with such PIL densities.

The results of this study indicate that sublethally infected *S. exigua* moths are efficient transmitters of SeMNPV to their progeny. Because the highest numbers of infected larvae tend to be deposited in early laid, large egg batches, the resulting horizontal transmission is likely to infect a substantial part of the larval population. Therefore, vertical transmission may play an important role in maintaining the baculovirus epidemic within the insect population. Comprehensive simulation models can be used to evaluate the consequences of these transmission dynamics at the population level (Van der Werf *et al*., 1991).

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