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TEMPERATURE AND CROWDING EFFECTS ON VIRUS MANIFESTATION IN *NEODIPRION SERTIFER* (HYMENOPTERA: DIPRIONIDAE) LARVAE

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ABSTRACT

Temperature and (or) crowding (larval density) functioned as stressors in the induction of symptoms associated with the nucleopolyhedrosis virus of the European pine sawfly, *Neodiprion sertifer*. Subsamples of larvae maintained at 30 and 35°C, with three levels of larval density each (20, 60, and 100/shoot) which had died under these conditions, revealed the presence of polyhedral inclusion bodies under microscopic examination. In contrast, larvae maintained at 25°C with the same three larval density levels experienced no symptoms of virus infection or mortality. The latter was consistent with field observations when temperatures during larval development ranged from 14°C to 27°C and larval densities were in the same general range.

The vertical transmission of sawfly nucleopolyhedrosis virus (NPV) between generations generally falls into two categories: environmental persistence and persistence in the host development sequence (Cunningham and Entwistle 1981). Environmental persistence has been well documented from foliage and soil samples; however, different explanations exist as to how the virus is maintained and spread by the adult sawflies. According to Cunningham and Entwistle (1981) there is no evidence that sawfly virus is transmitted from adults to progeny either within the egg or on it, even though Bird (1961) suggested ovarial transmission for both the European spruce sawfly, *Gilpinia hercyniae* (Hartig), and the European pine sawfly, *Neodiprion sertifer* (Geoffroy). It is established that infected adults are capable of contaminating the foliage with virus which may later be consumed by developing larvae. Bird (1961) did not favor the latent virus infection theory as a transmission possibility. Latent virus infection has been demonstrated in several lepidopteran species, manifesting itself in spontaneous outbreaks under various stressors including temperature and crowding (Steinhaus 1958). With coniferous sawflies, the only documented case of a spontaneous epizootic in North America was with the European spruce sawfly (Bird and Elgee 1957). The causative factors of this outbreak are unknown, though it is generally accepted that the virus was introduced from Europe through the release of contaminated exotic parasitoids. This paper provides some evidence for the possibility of latent viral infection in *N. sertifer* and the roles of temperature and crowding in disease expression.

MATERIALS AND METHODS

Second-instar larvae of *N. sertifer* were selected from a "virus-free" population in the Kettle Moraine State Forest in southeastern Wisconsin. Red pine shoots, with foliage from the previous year, were cut at the base and inserted in waxed, 1-pt containers filled with

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Table 1. Percent mortality ($\bar{x} \pm SE$) of *N. sertifer* larvae from nucleopolyhedrosis virus at three levels and three temperature regimes.[a]

	Density (larvae/shoot)		
Temperature (°C)	20	60	100
25	0%	0%	0%
30	77 ± 1%	84 ± 7%	95 ± 5%
35	100%	100%	100%

[a]At each temperature/density combination there were two replicates for a total of 18 units.

Table 2. Analysis of variance of virus-induced mortality in *N. sertifer* larvae.

Effect	*F* ratio	*P*
Density	$F(2,9) = 4.036$	$0.01 < P < 0.05$
Temperature	$F(2,9) = 1271$	$P < 0.005$
Temperature and density	$F(4,9) = 4.036$	$0.01 < P < 0.05$

water (400 ml). Larvae were transferred to the shoots at rates of 20, 60, and 100/shoot. These densities were comparable to those observed in the field on similar units of foliage. Six replicates were provided at each density. Each unit was sealed with a glass lantern globe and covered with cheesecloth for ventilation. The units were placed in controlled chambers at 25 ± 1°C, 30 ± 1°C, and 35 ± 1°C, RH at 50–60%, and a 16:8 photoperiod. The latter provided two replicates for each density and temperature combination or a total of six units in each chamber.

Each unit was monitored for 21 days with fresh foliage added when necessary. Dead larvae were removed at three-day intervals and stored singly in disposable test tubes at 0°C. From these larvae, three were picked at random from each replicate at each temperature-density combination. Each larva was macerated in a sterile glass tissue homogenizer and examined for polyhedral inclusion bodies (PIB) with brightfield microscopy at 600X. The number of PIB/larva was counted using a Levy chamber. Larvae in the field were monitored concurrently for symptoms of NPV infections.

Data were analysed using a multifactor analysis of variance. This allowed testing of the response (percent mortality), and PIB accumulation, as a function of temperature, density (crowding), or a combination of the two.

RESULTS AND DISCUSSION

Mortality due to NPV at the three levels of temperature and density (Table 1) shows that there was no mortality due to NPV at 25°C. This was consistent with the absence of any symptoms of NPV infection of larvae in the field under daily average temperatures which ranged from 13.9 to 27.0°C. during the same time period. At 30°C, the virus manifested itself in a progressive differential mortality directly related to the increase in larval densities. And lastly, there was a small temperature-density interaction that appeared to affect the response. To quantify the latter, a multifactor analysis of variance (Table 2) showed that the density and temperature interaction on the response was significant at $p < 0.05$. However, if the significance level was set at $p = 0.01$, it would appear that temperature alone was the significant factor in the induction of mortality by a latent virus. *Neodiprion sertifer* larvae are colonial feeders and one would expect that crowding (larval density) would have less effect than some of the other stressors.

Table 3. Average number of polyhedral inclusion bodies (PIB) (log_{10} units) observed in *N. sertifer* larvae at various densities or temperature conditions.

Larval density	PIB/larva[a] $\bar{x} \pm SD$	Temp. °C	PIB/larva[b] $\bar{x} \pm SD$
20	4.74 ± 0.61 (n = 12)A	30	4.87 ± 0.54 (n = 18)
60	4.80 ± 0.46 (n = 12)A	35	4.31 ± 0.33 (n = 18)
100	4.23 ± 0.28 (n = 12)B		

[a]Means followed by same letter not significantly different, and, those by different letters significantly different at $P = 0.05$ (df = 33) by Least Significant Difference Test.
[b]Means significantly different $P < 0.001$, df = 28 (*t*-test with unequal variances).

To analyse the accumulation of PIB in NPV-killed larvae, three larvae/replicate were subsampled at random from each temperature (30°C and 35°C) and density combination. Each of the 36 larvae was checked microscopically for the number of PIB present. Using log_{10} values to normalize the data, analysis of variance showed that temperature-density interaction on the accumulation of virus in the larvae was not statistically significant ($F(2,30) = 2.87$, $p > 0.05$). However, either density or temperature was significant in larval virus accumulation ($F(2,30) = 9.21$, $p < 0.01$; $F(1,30) = 15.43$, $p < 0.01$, respectively). Examination of the individual means (Table 3) showed that more virus was accumulated in the larvae at the lower densities (20 and 60 larvae/colony) and temperature (30°C) than at the higher density (100 larvae/colony) and temperature (35°C). These results suggest that temperature or density may limit the net accumulation of virus in the larvae.

Given the conditions of this experiment where the most stringent care was exercised in maintaining sterility in all objects and instruments used, and the choice of larvae from an area with no known history of NPV, it is possible to conclude that *N. sertifer* does harbor a latent virus. The conditions under which it manifests itself in the laboratory are harsh, particularly with regard to high temperatures. Thus, it is unlikely that epizootics caused by latent virus will be observed in the current range of this species where temperatures above 30°C are infrequent. It is possible, however, that latent virus could limit the southern range of *N. sertifer* where temperatures may average above 30°C.

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