

Artificially-Induced Nucleopolyhedrosis Virus Epizootic in Populations of *Neodiprion sertifer* (Hymenoptera: Diprionidae)¹

M. A. MOHAMED, H. C. COPPEL, AND J. D. PODGWAITE²

Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706

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ABSTRACT An artificially-induced epizootic was created in European pine sawfly (*N. sertifer*) populations at density levels of 5, 7, and 10 colonies per tree when plots were treated with 3.5×10^8 polyhedral inclusion bodies (PIB) per 0.04 ha. The progress of the epizootic was followed daily. The onset of mortality was at 13 days posttreatment, with a peak mortality period between 14 and 16 days across all density situations. During this period, ca. 70% of the total larval mortality was recorded. Of all the larval instars that died, the 4th experienced the greatest percentage of virus-induced mortality (ca. 58%). The average number of PIB accumulated per larva was inversely related to the density situations. That is, larvae in a low-density situation (five colonies per tree) accumulated more virus than those in a moderate-density situation (seven colonies per tree), and similarly between the moderate- and high-density situations (10 colonies per tree). This resulted in an inverse relationship between the average number of PIB produced per plot and colony density per tree: 2.92×10^9 , 9.00×10^8 , and 3.3×10^8 , in low-, moderate-, and high-density situations, respectively.

Laboratory and field studies by Bird (1953), and Bird and Whalen (1953) demonstrated the efficacy of the nucleopolyhedrosis virus (NPV) of the European sawfly, *Neodiprion sertifer* (Geoffroy), and initiated the basic methods for its utilization in suppressing populations of this insect. Their investigations in Canada and subsequent studies in the United States served as a basis for virus usage wherever *N. sertifer* occurred in large numbers. Aspects of artificially-induced epizootics, however, require further research to maximize the efficiency of virus usage and to establish criteria for clearance for general use. The course of an artificially-induced epizootic can be understood as a function of both time and the instars affected. This not only enables identification of parameters such as time of onset, peak, and decline phases of an epizootic but also the instar most affected. Further, some appreciation can be gained by the amount of virus liberated with respect to that initially used. This should provide some idea of whether or not the load of virus is magnified in the environment.

Materials and Methods

In 1979, nine 0.04-ha plots of even-aged (2.7 to 3.6 m high) red pine, *Pinus resinosa* Ait., harboring high densities of the European pine sawfly, were established in a 36.42-ha plantation in southeastern Wisconsin's Kettle Moraine Forest. Sawfly population had been monitored annually since 1972 for signs of NPV infection with negative results. Six of the nine virus-free plots were subdivided into three density subgroups of low (5 larval colonies per tree), moderate (7 larval colonies per tree), and high (10 larval colonies per tree), populations. These densities were established either by removing or adding egg-bearing terminals to five tagged trees randomly selected in each plot. These densities corresponded closely to those observed in the natural infestation.

When 50% of the larvae were in the 2nd instar, each of two 0.04-ha plots per density situation was treated

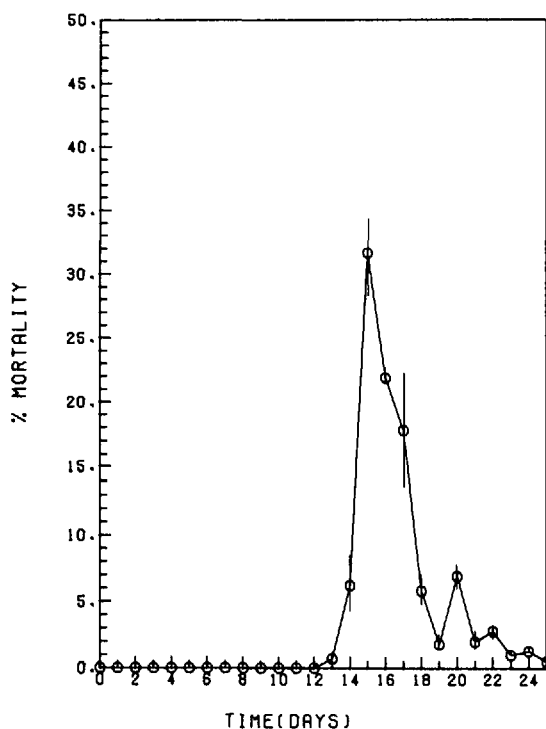


FIG. 1. Percent mortality (average of two replicates with SE indicated by vertical line) as a function of time in a low-density situation (five colonies per tree).

with 3.5×10^8 polyhedral inclusion bodies (PIB), using a mist blower. The formulation used per liter in the treatment consisted of 47 ml of Chevron Spray Sticker, 60 g of IMC-90-001 Shade, 4.6×10^8 PIB, and 953 ml of water. The remaining three plots served as controls. The average number (with SE) of larvae per colony in each replicate-density situation was estimated as: ($\bar{x} = 34.7 \pm 3.3$, $n = 25$, $\bar{x} = 47.5 \pm 5.4$, $n = 25$); ($\bar{x} = 40.5 \pm 3.6$, $n = 35$, $\bar{x} = 41.8 \pm 3.9$, $n = 35$);

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²Northeastern Forest Exp. Stn., Hamden, Conn.

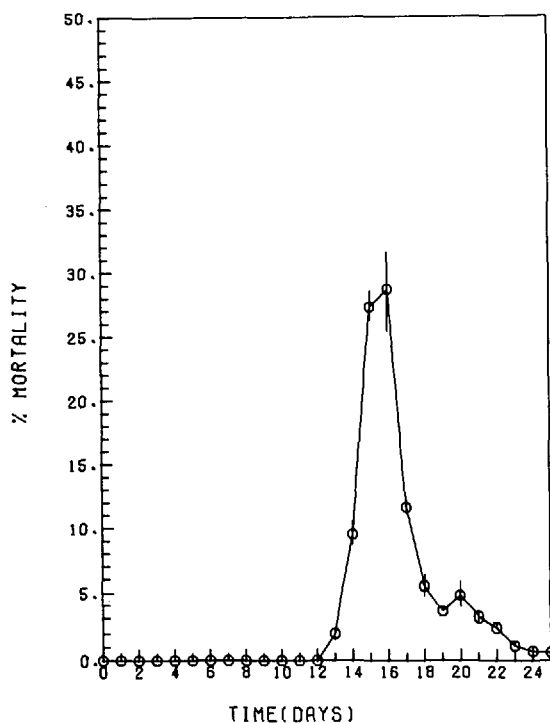


FIG. 2. Percent mortality (average of two replicates with SE indicated by vertical line) as a function of time in a medium-density situation (seven colonies per tree).

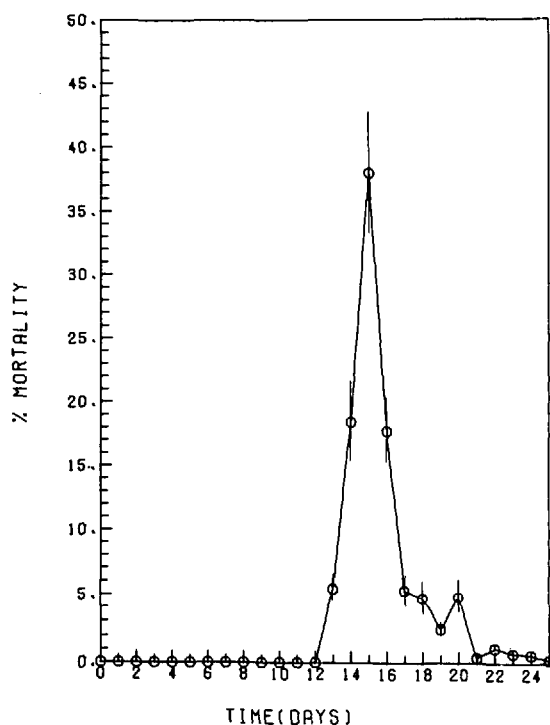


FIG. 3. Percent mortality (average of two replicates with SE indicated by vertical line) as a function of time in a high-density situation (10 colonies per tree).

and, ($\bar{x} = 38.9 \pm 2.8$, $n = 50$, $\bar{x} = 33.4 \pm 2.3$, $n = 50$), for low-, moderate-, and high-density situations, respectively, where n is the number of larval colonies.

The five tagged trees in each plot were monitored daily for 25 days, providing information needed to assess larval mortality over the time period of the epizootic. When available, one dead larva was subsampled at random per colony per tree per day from all plots and stored singly in a disposable test tube at 0°C. From these samples, 25 larval cadavers were selected at random from each replicate plot, for a total of 150 larvae. These were used for head capsule measurements to determine the larval instar and to provide a measure of the mortality experienced by each instar.

An additional 20 larval cadavers per replicate plot were subsampled at random for a total of 120 larvae. These larvae were macerated in sterile glass tissue homogenizers and recovered in 1 ml of water. The number of PIB was counted at various dilutions with a Levy chamber and brightfield microscopy at 600×. The data obtained from this procedure enabled us to estimate the net amount of PIB entering *N. sertifer's* habitat.

Three control plots were monitored during the same time period as were the six treated plots. Each contained five tagged trees with half the total number of larval colonies ($n = 110$) used in the treated plots. This allowed a comparison of larvae in treated plots with those in nontreated plots with respect to the presence or absence of symptoms of NPV infection.

Table 1. Percent mortality for each instar-density situation^a

Density	$\bar{x} (\pm SE)$ % Mortality of instar:		
	3	4	5
Low	0	68 ± 17.0	32 ± 17.0
Moderate	20 ± 5.7	60 ± 11.3	20 ± 17.0
High	30 ± 4.1	46 ± 25.5	24 ± 11.3

^aAverages computed on the basis of 25 larvae randomly sampled from each replicate plot-density situation.

Results and Discussion

All plots treated with NPV showed 100% larval mortality. Visual observation of all colonies revealed the characteristic symptoms of NPV infection such as sluggishness, nonresponsiveness to any stimuli, and finally, hanging by the abdominal tip from the needle when dead. Larvae from control plots showed no such symptoms, nor was any mortality observed. Further, larval tissue smears from each treated plot (20 randomly sampled larvae per plot) examined microscopically revealed the presence of polyhedra which were similar to known purified samples, without exception. These findings indicated that all field mortality could be attributed to the NPV.

To assess mortality as a function of time, the number of dead larvae per day per colony was summed and compared with the total number of larvae started within

Table 2. Mean number of PIB per larva and estimates of PIB produced in each density situation, respectively^a

Colony density/tree	$\bar{x} \pm SD$		
	PIB/larva ^b	Estimated PIB produced ^b	(PIB produced)/(PIB used initially)
5	$3.02 (\pm 1.5) \times 10^6$ ^a	$2.92 (\pm 0.92) \times 10^6$ ^a	8.35 ± 2.62
7	$5.95 (\pm 1.67) \times 10^6$ ^b	$9.00 (\pm 1.41) \times 10^6$ ^b	2.6 ± 0.42
10	$1.85 (\pm 0.5) \times 10^6$ ^c	$3.30 (\pm 0.57) \times 10^6$ ^c	0.95 ± 0.21

^aMeans computed from two replicates per plot, each replicate consisting of 20 randomly sampled larvae at each density situation. Each plot of 0.04 ha sprayed with 3.5×10^8 PIB initially.

^bMeans with different letters significantly different, $P \leq 0.05$, 3 df, by least significant difference test. Log₁₀ values used in all statistical analyses.

each plot. The average of the two replicate plots in each density situation was then plotted against time (Fig. 1 to 3). From these plots several conclusions can be drawn. First, the onset of mortality in the epizootic was at 13 days and the termination at 25 days posttreatment across all densities. Second, within this time period, a peak mortality period was recorded at day 15 for density situations of 5 and 10 colonies per tree, and day 16 for seven colonies per tree. And last, the greatest proportion of mortality occurred within a 3-day period (days 14 to 16) accounting for 74.1, 67.6, and 71.4% of the larvae in the three density situations, respectively.

The percent mortality experienced by each instar is shown in Table 1. Two factor analysis of variance with interaction resulted in nonsignificant F values ($P > 0.05$) in the mean percent mortality either for the effect of different densities or the combination of density and larval instars. However, mean percent mortality was significantly different between instars, $F(2, 9) = 12.84$, $P < 0.05$. Further comparison of the percent mortality in each instar by the least significant difference test showed the following: mean percent (with SE) mortality of the 4th instar (58 ± 7.2 , $n = 6$) was significantly different from that of the 3rd and 5th instars (16.7 ± 6.2 and 25.3 ± 5.3 , $n = 6$, respectively) at $P < 0.05$, $df = 15$. Thus, it seems that the epizootic exerts most of its effect in the 4th-instar larvae.

Comparison of the mean number of polyhedra per larva in the three density situations (Table 2) showed that larvae in a low-density situation accumulated more virus than those in moderate density situation, and similarly, between moderate- and high-density situations. A similar result was obtained for average net PIB produced in the three density situations, with the ratio of PIB produced to that used initially, being highest in the low-density situation. The latter values were obtained by multiplying the total number of larvae per plot times

the average number of PIB per larva in each density situation. One explanation of this trend is that it may be due to crowding, which is often associated with latent virus expression (Steinhaus 1958). Larval resistance due to crowding could be reduced in high-density situations, making them susceptible to lower virus accumulation. Alternatively, under crowded conditions, the growth rate of larvae is slower in high-density situations than in low-density situations. This results in smaller cadavers which in turn contain lower numbers of PIB per larva.

An artificial epizootic in *N. sertifer* population has several well-defined features. There are definite time periods for both the onset and the peak of mortality; there is an instar most affected by the virus; and finally, virus accumulation in its habitat seems to be inversely related to colony density per tree.

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