Efficacy of the *Neodiprion sertifer* (Hymenoptera: Diprionidae) Nucleopolyhedrosis Virus (Baculovirus) Product, Neochek-S

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ABSTRACT *Neodiprion sertifer* (Geoffroy) larval populations were treated with high and low doses of a nucleopolyhedrosis virus (NPV) product, Neochek-S. Larval population reduction due to Neochek-S was well over 90% in all sprayed plots 28 days after application, whereas overall protection of *Pinus resinosa* (Ait.) foliage was 94.0 ± 1.6%. The difference between doses was insignificant with respect to population reduction or foliage protection. A dose rate of 2.5×10^9 polyhedral inclusion bodies of *N. sertifer* NPV/ha, by ground application, provided acceptable control in a plantation infested with moderate to dense populations of the insect.

THE EUROPEAN PINE SAWFLY, *Neodiprion sertifer* (Geoffroy), is a pest of most species of two-needled pines (Lyons 1964). It is particularly serious in Scotch pine, *Pinus sylvestris* (L.), and red pine, *P. resinosa* (Ait.), plantations that are managed for Christmas tree production in the northeastern United States, because disfigurement of trees severely affects their marketability. This sawfly also causes significant growth loss in Scotch pine (Wilson 1966), but the resultant loss in timber production, in this and other species, remains undefined (Lyons 1964).

N. sertifer populations are reduced by a natural nucleopolyhedrosis virus (NPV) (Baculovirus) disease first reported by Escherich (1913) and later described in detail by Bird and Whalen (1953). The NPV was purposely introduced into Canada from Sweden in 1949 and has subsequently proven to be an extremely effective biological control agent against *N. sertifer* populations in southern-Ontario scotch pine plantations (Bird 1953) and red pine plantations in the United States (Dowden and Girth 1953).

The USDA Forest Service has registered an *N. sertifer* NPV product, Neochek-S, with the U.S. Environmental Protection Agency as an alternative to the use of chemical pesticides for *N. sertifer* control in the United States. Here we report efficacy information on this product.

Materials and Methods

Study Area and Design. In April 1981, three blocks were established within a 14-ha red pine plantation located 6.5 km east of La Grange, Walworth County, Wis. (Fig. 1). Blocks were selected on the basis of similar tree size and condition, and *N. sertifer* egg density. Three 0.04-ha plots, separated by at least 10 m, were established within each block and randomly assigned one of three treatments. The high-dose Neochek-S treatment was 12.5×10^9 polyhedral inclusion bodies (PIB) per ha, whereas 2.5×10^9 PIB per ha was applied in the low-dose treatment. The third treatment, no spray, served as the control.

Virus Source. Neochek-S was produced by infecting field-collected fourth-stage *N. sertifer* larvae with NPV by the method of Rollinson et al. (1970). Larval cadavers were harvested, frozen at −20°C, lyophilized, and micropulverized to yield a fine admixture of insect parts (99.95%) and *N. sertifer* PIB (0.05%) which was stored dry at −20°C. The Neochek-S (lot no. 206) used in this study contained 11.6×10^9 PIB per g. Microbiological quality control tests revealed a standard bacterial plate count of 2.00×10^6 per g and were negative for fecal coliform and pathogenic bacteria. The product elicited no mortality when injected intraperitoneally into mice at 0.4-ha-equivalent doses (Podgwaite and Bruen 1978).

Formulation and Application. The appropriate amount of Neochek-S for a 0.04-ha treatment was triturated in a tissue homogenizer with 5 ml of nonchlorinated water. The resulting suspension was added to a 22.7-liter container in which 240 ml of Acrylocoat spreader sticker, 30 ml of red food coloring, and 7.3 liters of nonchlorinated water had been premixed. The formulation was further mixed by vigorous agitation and then added to the tank of a Solo model-410 backpack mistblower, where it was again mixed before application.

A single application was sprayed at the rate of 187.0 liters/ha when *N. sertifer* larvae were in the first and second stages. Trees within the plots were 0.6 to 3 m tall; their spacing allowed complete coverage of each tree. Even coverage of the entire

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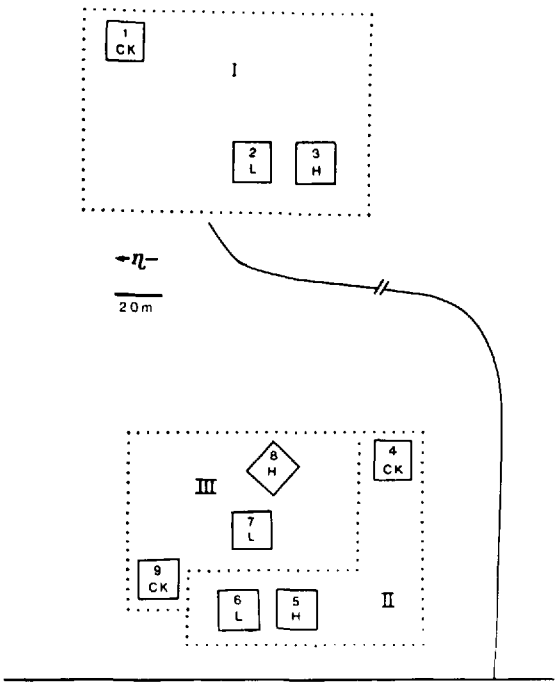

Fig. 1. Study block and plot configuration within the red pine plantation. L, Plots treated with a low NPV dose; H, plots treated with a high NPV dose; CK, controls.

0.04 ha was approached through timed walks paralleling tree rows. Spray deposit cards were placed in check plots to detect drift, but drops were not counted. All spraying was done on 6 May 1981, between 0900 and 1400 (CDT). Conditions were dry and bright; temperatures were 7 to 10°C, with northeasterly winds at 15 to 18 km/h.

Sampling and Evaluation. Before treatment, three isolated *N. sertifer* larval colonies were selected on each of 10 trees in each plot within blocks II and III (Fig. 1). In plots within block I, it was not always possible to find three isolated colonies in each tree, so extra trees were selected to provide 30 colonies per plot. Larval densities were determined by counting all larvae in each selected colony 1 day before treatment and at weekly intervals thereafter. The number of healthy, dead, and infected larvae was recorded on each occasion. Infection and mortality due to NPV was assessed on the basis of gross pathology. Larval enumeration continued until all surviving larvae had dropped to the ground to spin cocoons.

Defoliation was measured as centimeters of branch defoliated per 100 larvae. The initial larval counts were taken the day before spraying, and defoliated branch length was measured after all surviving larvae had dropped to the ground. One colony-bearing branch on each of 10 trees per plot was selected for measurement.

For the statistical analysis, the precision of the population reduction estimates for each tree was increased by the jacknife method applied to the block survival ratio (Miller 1964). Bias-adjusted tree values, for all measurement dates, were analyzed by multivariate analysis of variance (MANOVA) to compare population changes over time between plots. An analogous univariate procedure (ANOVA) was used to evaluate defoliation. To separate effects of spraying from natural changes and development in the larval population, net virus mortality effects and net foliage saved were determined by Abbott's formula (Abbott 1925).

Results and Discussion

Significant ($P < 0.001$) Neochek-S larval mortality was noted from the first postspray evaluation—9 days after NPV application—through the last evaluation at 34 days, by which time most surviving larvae had dropped to the ground. Fourteen days after treatment, all but a few colonies in all sprayed plots contained NPV-infected larvae. Also at this time, polyhedrosis was noted in several colonies in the control for block III (plot 9). This, in all likelihood, resulted from spray that was seen drifting into a corner of this plot during

Table 1. Number of live sawfly larvae per colony and defoliation estimates in NPV-treated and control plots, with SEs

Treatment	Block	Days after treatment						Defoliation
		0	9	14	21	27	34	
High dose	I	41.27 ± 4.51	42.00 ± 5.89	18.37 ± 3.27	2.00 ± 0.66	0.00 ± 0.00	0.00 ± 0.00	1.00 ± 0.37
	II	46.43 ± 4.56	22.60 ± 3.06	5.27 ± 2.16	2.17 ± 1.04	1.13 ± 0.79	0.17 ± 0.17	1.40 ± 0.83
	III	44.63 ± 3.66	33.63 ± 3.44	21.13 ± 2.83	4.90 ± 1.57	0.20 ± 0.17	0.37 ± 0.26	1.10 ± 0.53
Low dose	I	55.63 ± 3.39	50.03 ± 4.01	29.53 ± 2.79	3.43 ± 1.26	0.00 ± 0.00	0.00 ± 0.00	0.55 ± 0.28
	II	43.97 ± 3.26	21.03 ± 3.08	10.10 ± 2.90	2.63 ± 0.96	0.90 ± 0.59	0.00 ± 0.00	1.00 ± 0.52
	III	43.13 ± 3.06	33.73 ± 3.89	25.97 ± 3.74	7.80 ± 2.61	2.07 ± 1.12	0.10 ± 0.10	0.40 ± 0.22
Control	I	41.03 ± 3.57	43.13 ± 4.11	43.10 ± 4.64	41.33 ± 3.92	23.63 ± 3.90	0.10 ± 0.07	17.00 ± 3.45
	II	37.80 ± 2.42	36.20 ± 2.91	32.37 ± 2.52	23.70 ± 1.99	18.13 ± 1.85	0.93 ± 0.47	11.20 ± 1.84
	III	30.27 ± 2.13	27.17 ± 2.26	10.00 ± 2.03	7.97 ± 1.84	6.37 ± 1.69	0.90 ± 0.34	5.30 ± 2.98

Number of live larvae (mean ± SE) per colony, based on 30 colonies per plot. Defoliation refers to centimeters of branch defoliated per colony (mean ± SE) in 10 colonies per plot.

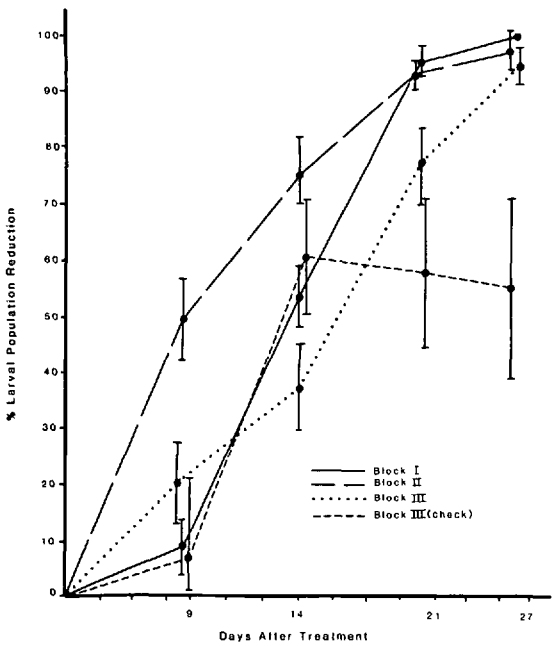

Fig. 2. *N. sertifer* larval population reduction in three treatment blocks; corrected for natural mortality (Abbott 1925).

treatment of plot 6. For that reason, plot 9 was eliminated as a control; because of similarities between blocks II and III, plot 4 was used as a control for sprayed plots in both blocks II and III.

The larval density per colony and the defoliation recorded during the evaluation period are given in Table 1. MANOVA showed there were no significant differences ($P > 0.5$) between the dose rates of 12.5×10^9 PIB per ha and 2.5×10^9 PIB/ha in any block. Plot 9 in block III, when compared with control plots for blocks I and II, showed significantly different ($P < 0.001$) population levels, providing further evidence that it had been inadvertently treated with virus.

The estimates of net (Neochek-S–induced) mortality are illustrated in Fig. 2. Twenty-one days after application, Neochek-S had accounted for a reduction in the sawfly population of > 90% on blocks I and II and a reduction of nearly 80% on block III. A week later, larval populations collapsed completely in block I sprayed plots, and reduction levels were well over 90% in the other sprayed plots. Neochek-S had a significantly ($P < 0.001$) earlier (days 9 and 14 after spray) mortality impact in block II than in block I or block III, though the difference from block I had eroded by day 21. This difference may have been due to better spray coverage, or a higher incidence of smaller, more susceptible larvae in the block.

Defoliation and foliage protection estimates on NPV-treated and control plots are shown in Table 2. Again, using the control plot in block II as a control for both blocks II and III, analysis of variance revealed no detectable difference in defoliation between the two NPV doses, but did show that all NPV treatments significantly ($P < 0.001$) reduced defoliation when compared with the control plots. Foliage protection was highest on block I (96.2 ± 1.2%) and was 94.0 ± 1.6% overall.

Neochek-S compares most favorably with other *N. sertifer* NPV preparations, both in population reduction and foliage protection (Dowden and Girth 1953, Cunningham et al. 1975, Entwistle et al. 1980). Furthermore, previous evidence suggests that doses of 5×10^9 to 9×10^9 PIB per ha were needed to afford adequate control and foliage protection (Cunningham and Entwistle 1981). Our results with Neochek-S at 2.5×10^9 PIB per ha were most encouraging. This dose level, by ground application, provided acceptable control (no disfigured trees) in moderate to dense sawfly populations in plantations where complete coverage can be assured through an application rate of 187 liters/ha.

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Table 2. Defoliation and foliage protection estimates for sprayed Neochek-S and control plots

Treatment	Block			
	I	II	III	All
High dose	2.0 ± 0.6	2.7 ± 1.9	3.0 ± 1.4	2.6 ± 1.4
Low dose	1.1 ± 0.5	2.1 ± 1.1	1.1 ± 0.5	1.4 ± 0.8
Control	40.2 ± 5.9	26.4 ± 4.1	18.6 ± 9.4[a]	33.3 ± 3.6
Protection (%)	96.2 ± 1.2	90.0 ± 4.6	92.2 ± 3.2	94.0 ± 1.6

Defoliation refers to centimeters of branch defoliated per 100 larvae (mean ± SE).
[a] NPV drift into this plot verified.

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