Persistence in Soil and on Foliage of Nucleopolyhedrosis Virus of the European Pine Sawfly, *Neodiprion sertifer* (Hymenoptera: Diprionidae)[1]

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ABSTRACT

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Six plots of pine trees harboring high densities of *N. sertifer* larvae were sprayed with the nucleopolyhedrosis virus (NPV) of this species. Half of these plots were resprayed in the second year of the study. Polyhedral inclusion bodies (PIB) were recovered in all plots from soil and foliage sampled at fixed time intervals within a 21-month period from the initial date of spraying. The concentration of PIB from both soil and foliage samples in the second year were generally lower in plots treated once than in those treated twice. NPV-induced larval mortality in plots sprayed once was 100% in the first season and 8% in the second. Laboratory bioassays of soil samples at intervals within the 21-month period resulted in 11 to 80% NPV-induced larval mortality. This study suggests that the NPV of *N. sertifer* can persist and retain some activity for at least 21 months under field conditions.

The inclusion viruses of lepidopterous species have been shown to persist in the environment for long periods (Bailey 1973, Jaques 1975). Bird and Burk (1961) observed the recurrence of epizootics over several consecutive years after European spruce sawfly, *Diprion hercyniae* (Hartig), populations were initially sprayed with its nucleopolyhedrosis virus (NPV). Bird (1961) also noted that the NPV of the European pine sawfly, *Neodiprion sertifer* (Geoffroy), persisted on trees in pine plantations. However, such observations did not casually relate mortality to NPV persistence, nor was the amount and longevity of NPV in the field determined.

Materials and Methods

In May 1979, nine 0.04-ha plots of red pine, *Pinus resinosa* Ait., were established in a 36.42-ha plantation in Wisconsin's Kettle Moraine Forest. Sawfly populations within these plots occurred at high densities since 1972 but showed no evidence of virus infection. The nine plots were subdivided into three groups as follows: two groups (consisting of three plots each) were sprayed with a formulation of *N. sertifer* NPV, whereas the third group served as a control. Each liter of NPV formulation, sprayed with a mist blower at 3.5×10^9 polyhedral inclusion bodies (PIB)/0.4 ha, included 47 ml of Chevron Spray Sticker, 60 g of IMC-90-001 Shade, 4.6×10^8 PIB, and 953 ml of water. Three of the sprayed plots were resprayed with a similar formulation of *N. sertifer* NPV in May 1980.

Soil and foliage were sampled in all plots at fixed time intervals within a 21-month period after treatment. The sampled soil consisted of a cylindrical plug (ca. 9 cm^3) of topsoil taken at the base of each of five tagged trees per plot. A handful of needles was taken also from ranches midway in the crown of each tagged tree. All samples were stored at 0°C in plastic bags.

Virus was recovered from 11 samples per group of plots, for a total of 33 samples within the 21-month period, by a procedure modified from Evans et al. (1980). Ten grams of each soil sample was weighed and suspended in a 2:1 (vol/wt) buffered solution (pH 7.0) of 0.1% sodium dodecyl sulfate. The suspension was shaken for 12 h on a rotary shaker and then allowed to settle for 1 h. The aqueous layer was pipetted off. This pro-

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Table 1.—Mortality of *N. sertifer* larvae from laboratory bioassays of aqueous soil suspensions sampled from 23 May 1979 (day 0) to 635 days posttreatment due to nucleopolyhedrosis virus (NPV)

Days after application	Mortality (% ± SE) of test larvae caused by NPV[a]		
	Nontreated plots	Plots treated 1 year	Plots treated 2 years
0	0.0	0.0	0.0
30	16.0 ± 0.5	60.4 ± 31.0	53.2 ± 18.1
60	12.4 ± 1.9	31.0 ± 8.6	50.0 ± 3.7
270	0.0	48.0 ± 7.8	42.4 ± 5.5
365	0.0	19.9 ± 13.6	54.0 ± 5.7
379	5.5 ± 1.4	34.0 ± 25.5	22.6 ± 2.3
395	9.1 ± 1.5	23.2 ± 1.9	80.4 ± 22.9
425	11.0 ± 0.0	33.0 ± 11.3	31.5 ± 12.0
625	0.0	11.0 ± 2.8	20.0 ± 11.3

[a]Percentages calculated as follows:{[number of NPV-killed larvae]/[(number of NPV-killed larvae + number of living prepupae) − (number that died from undetermined causes)]} × 100.

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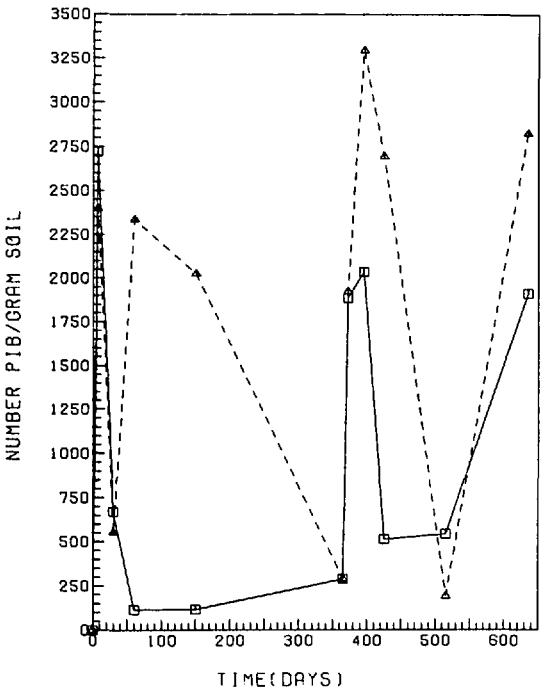

FIG. 1.—PIB recovery from soil versus time (solid and broken lines indicate plots sprayed with NPV only in the first year, and in 2 consecutive years, respectively).

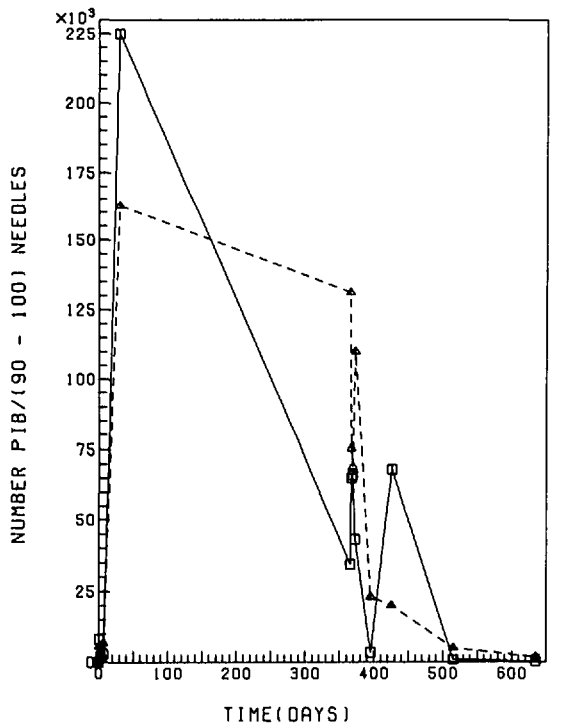

FIG. 2.—PIB recovery from foliage versus time (solid and broken lines indicate plots sprayed with NPV only in the first year, and in 2 successive years, respectively).

cedure was repeated, and the final solution was pooled. Particulate matter was removed by centrifugation at 180 × *g*, and the supernatant was again centrifuged at 2,000 × *g* for 20 min to sediment any polyhedra present. The pellet was resuspended in 1 ml of water and examined for PIB under bright field microscopy at 600×.

Soil was sampled nine times during the 21-month period from the base of an additional tree in each group of plots. This provided 27 samples which were bioassayed by spraying aqueous suspensions (10 g of soil per 50 ml of water) on bouquets of pine foliage with an atomizer. When the spray deposit had dried, 20 2nd-instar larvae of *N. sertifer* (obtained from Bassett, Wis., 50 km removed from the experimental plots) were transferred to the foliage. Each bouquet was enclosed in a lantern globe and kept at 25 ± 1°C, 40 to 50% relative humidity (RH), and a photoperiod of LD 16:8. Each bioassay was replicated. Fresh foliage was added after the sprayed foliage was eaten. Dead larvae were removed and stored at 0°C for subsequent microscopic examination.

Foliage (90 to 100 needles), sampled 13 times per group of plots during the test period, was cut to 1- to 2-cm lengths and immersed in a buffered solution of 0.1% sodium dodecyl sulfate. The extraction and counting of virus was done as previously described for soil samples.

To gain some idea of foliar persistence and activity of NPV in the field, 110 colonies of *N. sertifer* (15 to 105 larvae per colony) in each group of plots were monitored for 24 days in both years of the experiment. Dead larvae were recorded and collected daily and stored at 0°C. Twenty of these larvae per group of plots were subsampled at random for microscopic examination.

An estimate of the percent efficiency of PIB recovery from soil samples, using the phosphate-buffered sodium dodecyl sulfate solution, was calculated by incorporating known quantities of purified polyhedra (2.5×10^6 PIB/ml) in sterile soil (5 g). Reextraction and purification was done as previously described.

Results and Discussion

Bioassays of aqueous soil suspensions (Table 1) show that some viral activity is retained up to 21 months from the initial date of spraying. Respraying of the plots (in 2 consecutive years) increased the concentration of virus in the soil (Fig. 1), the values being generally greater than those observed from plots sprayed once. This build-up of virus was probably due to the accumulation of NPV-killed larvae in the soil during both years, as well as the action of rain in moving virus from the foliage. Our control soil bioassays showed low NPV-induced mortality, possibly related to the actions of predaceous organisms moving from treated to untreated plots.

Only 28% of the known concentration of PIB added to sterile soil could be recovered by using the sodium dodecyl sulfate solution. Given this efficiency, all concentrations of PIB extracted from sampled soils were corrected by a factor of 3.6.

Persistence studies of PIB on foliage provided data similar to that recorded from the soil. Inclusion bodies were recovered (Fig. 2) up to the 21st month from the

initial date of spraying. The numbers of PIB were very low—lower than those recorded several days after spraying or when compared with the resprayed plots (except at 425 days). The latter could possibly be due to the removal of larval smears or PIB by rain or snow. Field observations in the first year indicated 100% mortality in sprayed plots and 0% in the control plots. In the second year, the average larval mortality was 8.6% (9.1% of all colonies were infected) for the unsprayed plot in contrast to 100% in the resprayed plots, and 0% mortality in the controls. Microscopic examination of 20 dead larvae per plot revealed the presence of PIB, indicating that the field mortality observed was indeed virally induced.

PIB concentrations from soil and foliage samples in plots treated only the first year had generally comparable values to those recorded by Dubois (1976) at LC_{50} and LC_{90} of 1.55×10^3 and 4.06×10^5 PIB/ml, respectively. Our observations of mortality in the field and in our laboratory bioassays were lower than expected. This implies that the difference may have been caused by inactivated viral material. Jaques (1973) hypothesized that larval smears of *N. sertifer* could protect the virus from ultraviolet light and thus retain its activity. In this way, NPV on foliage would persist to act as foci for enzootics and lead to epizootics through horizontal transmission by biotic and abiotic factors.

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