Environmental Persistence of the Nucleopolyhedrosis Virus of the Gypsy Moth, *Lymantria dispar*[1,2,3]

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ABSTRACT

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A bioassay technique was used to estimate the concentrations of infectious gypsy moth nucleopolyhedrosis virus (NPV) that occur naturally in leaf, bark, litter, and soil samples taken from woodland plots in Connecticut and Pennsylvania. These concentrations were then compared to those in samples taken sequentially after treatment of these plots with NPV.

Results indicated that NPV is a natural component of the host's habitat, persisting in high concentrations in soil, litter, and on bark for at least one year after natural epizootics. Activity of NPV in spray deposits on foliage and bark was measurable for only 3–15 days after NPV treatment, whereas activity of NPV liberated from larval cadavers onto bark was measurable in high concentrations through May of the year following treatment.

The application of NPV at the rate of 2.5×10^{12} polyhedral inclusion bodies (PIB)/ha to an area already containing high concentrations of naturally occurring NPV did not cause an increase in the environmental NPV load. Although the application of NPV at the rate of 5×10^{12} PIB/ha to plots containing low natural concentrations of NPV resulted in measurable increases in the NPV load in these plots, these increases were not, overall, significantly higher than those occurring in a control plot that was not treated with NPV.

It is well documented that nucleopolyhedrosis viruses (NPV's) of insects naturally accumulate and persist in the habitats of their hosts (Jaques 1975). Although there is no evidence to indicate that these viruses are harmful to man or beneficial wildlife, there is concern that the deliberate introduction of these viruses into the environment for control of pest insects may increase the natural virus load and concurrently the risk of exposure of nontarget species.

The NPV of the gypsy moth, *Lymantria dispar* L., has been developed by the USDA for use as a biological control agent. This virus has been shown to persist for extended periods on the eggs of the insect and on environmental surfaces (Doane 1969, 1975). However, there have been no reported attempts to quantify the virus in the host's habitat. The following is a report on the length of time, the locations in which, and in what quantities this NPV persists after natural epizootics and how introduction of the virus may alter the environmental load.

Methods and Materials

NPV persistence was studied in woodland plots established in mixed hardwood stands in the Natchaug State Forest, Eastford, CT and in the Bald Eagle State Forest, Mifflinburg, PA.

Connecticut

Two 0.4-ha plots were established in a stand with a moderately dense gypsy moth population (725 egg masses/ha) known to have suffered a relatively high incidence of NPV disease in the preceding generation. One plot was treated with a formulation containing per liter: Cargill Insecticide Base® (CIB), 244 ml; Chevron Spray Sticker®, 23 ml; IMC 90-001®, 87.8 g; H_2O, 733 ml; and 1.3×10^{11} purified (Breillatt et al. 1972) polyhedral inclusion bodies (PIB) of gypsy moth NPV. The formulation was applied by mist blower at the rate of 2.5×10^{12} PIB and 18.7 liters/ha. The other plot was not treated and served as a control.

Four circular sampling areas, 30 cm diam, were established within each plot before treatment. These were located on the plot diagonals, 2 m from each corner, and were used for collecting soil and litter samples. In addition, one red oak, *Quercus rubra* L., and one red maple *Acer rubrum* L., was selected within each plot. Red maple, although not a primary gypsy moth host, was selected to determine if NPV persistence was different on a relatively smooth-barked species. Beneath each of these trees, and not more than 60 cm from its base, one sampling area was established where there was maximal runoff from the tree, and where NPV would most likely accumulate after rainfall.

Litter and the soil immediately below were collected with a cylindrical tool that sampled a 10-cm^2 area. Soil was taken to a depth of 2 cm for a final volume of 20 cm^3. Litter samples varied in volume depending on the amount of leaf cover.

Foliage samples, consisting of 2 terminal leaves from a branch, were taken from the midcrown of each tree.

Bark samples were taken from 2 locations on each tree: (1) around a point on the bole 2 m from the base and (2) around a point on the bole 30 cm from the base. Samples were 10 cm^2 and taken from that side of the tree determined to receive the least amount of direct sunlight.

Each sample was placed in a sterile plastic bag and stored at 4°C until it was prepared for bioassay.

Pennsylvania

The persistence of NPV was studied in conjunction with field experiments on the efficacy and safety of aerial application of the laboratory-produced virus material (Wollam et al. 1978). Three 14-ha plots, all supporting

[1] Lepidoptera: Lymantriidae.

[2] Mention of a proprietary product in this paper does not constitute an endorsement by the USDA.

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dense gypsy moth populations, were monitored. Plot 3 (2263 egg masses/ha) was treated twice (6 days apart) with a formulation containing per liter: CIB, 123 ml; Chevron Spray Sticker, 23 ml; IMC 90-001, 58.6 g; H_2O, 854 ml; and 1.3×10^{11} purified PIB. Plot 15 (2184 egg masses/ha) was similarly treated with a formulation containing per liter: Sandoz Virus Adjuvant 16-B® (SVA), 500 ml; H_2O, 500 ml; and 1.3×10^{11} purified PIB. Each application for both formulations was at the rate of 2.5×10^{12} PIB and 18.7 liters/ha. Plot 14 (1907 egg masses/ha) was untreated and served as a control.

Ten 0.1-ha subplots were established for efficacy assessment in each 14-ha plot. Persistence sampling areas, established as in Connecticut, were located in subplots 1, 3, 5, 6, 8, and 10. One tree, either chestnut oak, *Quercus prinus* L., white oak, *Quercus alba*, L., or red maple, was selected in subplots 1, 3, 8, and 10 of each plot. Only upper and lower bole bark samples were collected from these trees. Soil and litter samples were taken in all 6 subplots. The sampling procedure was the same as that used in Connecticut.

Sample Preparation

Individual leaf and litter samples were placed in a 500-ml Waring blender with 40 ml of sterile distilled water containing 50 ppm Triton-10-X (WT). The sample was blended for 15–20 sec until thoroughly homogenized. The homogenate was dispensed into a 260-ml capacity wide-mouthed bottle and the blender was rinsed with 10 ml WT. After addition of the rinse, the bottle was shaken vigorously 5 times and then left undisturbed for 5 min. One ml of the suspension was pipetted from just below the surface (top 2 mm) and used for bioassay.

Bark samples were treated as above, except that large (10-cm^2) pieces were broken with a hammer (while still in the plastic bags) before blending.

Soil samples were not blended, but rather placed directly into 160 ml capacity bottles with 50 ml WT. The samples were then shaken and treated as above.

Bioassay Procedure

Each sample suspension was bioassayed against 5 replicates of 10 newly molted 2nd-stage laboratory stock gypsy moth larvae reared in sterile plastic petri dishes (15×100 mm). Three 343-mm^3 artificial diet blocks (ODell and Rollinson 1966) were placed on the inside top of each dish and 0.03 ml of the test suspension was evenly applied over the 5 exposed surfaces of each block. The treated blocks were left undisturbed for one h to allow absorption of the test material. Larvae were then allowed to free feed on the treated diet for 48 h after which the treated diet was removed and replaced with untreated diet. Fifty control larvae, fed diet blocks treated with WT, were used for each 10 tests. Other groups of 50 larvae were fed suspensions prepared from sterile soil, bark, and litter to measure any direct effects of these materials on the insect. All larvae were reared for 16 days at 25°C and 16-h photoperiod. Larvae were checked daily for mortality, and deaths from NPV were confirmed microscopically.

Standard Bioassay

Numbers of infectious PIB in soil, litter, and bark samples were estimated from data obtained from the bioassay of steam sterilized samples that were treated with known amounts of purified PIB. Dosages were prepared from a stock suspension of purified PIB in WT and ranged from 1×10^5 to 2×10^7 PIB. Dosages in this range caused virus mortalities from 0 to 100% when administered to 2nd-stage gypsy moth larvae.

Soil and litter samples were prepared by first incorporating the appropriate NPV dose in 10 ml WT and then mixing this with 20 cm^3 of the material. The mixture was allowed to stand for 24 h to allow NPV to be absorbed by soil and litter particles. Samples were then bioassayed as above. Bark samples were treated in a similar manner except that the appropriate dose was allowed 24 h to dry on a 10-cm^2 piece of bark.

Dose-mortality data from the bioassay of these standard preparations were analysed by a logit chi-square analysis program (LOCSAN) (Paschke et al. 1968).

Results

Pennsylvania

Results of standard bioassays of sterilized bark, litter, and soil treated with known quantities of gypsy moth NPV are shown in Table 1. Equations derived from these data were used to estimate NPV levels over time within various strata in the Pennsylvania field plots.

Results of bioassays of bark, including percent mortality and extrapolated PIB values, are shown in Fig. 1 and 2. NPV activity at a height of 2 m on trees sampled in the NPV treated plots increased slightly one day after the 1st virus application but decreased to pretreatment activity 3 days after spray. NPV activity increased to slightly higher levels after the 2nd application either because of better coverage or the added effect of larvae dying and liberating PIB as a result of the 1st application. NPV activity increased dramatically between 14 and 22 days after the 1st application. This was most likely due to the PIB liberated from larvae dying from the result of NPV treatment as well as those dying from a natural epizootic that was coincident in the study area.

PIB concentrations on bark approximated $5 \times 10^6/cm^2$ through Oct. 1975, but with the onset of winter weather, decreased in Jan. 1976. NPV activity on bark nearly one yr after treatment (May 1976) indicated 16- and 6-fold increases in PIB concentrations in 2 treated plots, but only a 2-fold increase in the control plot.

NPV activity on bark samples at a height of 30 cm was similar to that obtained for upper tree bark, but indicated that there was only a 6- and 2-fold increase in PIB concentration in the 2 NPV-treated plots versus a 35-fold increase in the control plot one yr after spray.

NPV activity in litter did not increase appreciably until between 14 and 22 days after spray (Fig. 3). This was more likely due to PIB accumulating from dead larvae than to spray residue. Again, NPV activity in samples taken nearly one yr after spray indicated more of a PIB increase in the control plot (17-fold) than in the 2 NPV-treated plots (9- and 11-fold).

NPV activity in soil (Fig. 4), as in litter, did not increase until between 14 and 22 days after NPV application and was consistently lower than that in litter until the May 1976 sample. PIB concentrations at this time

Table 1.—Percent mortality and lethal dose values determined from bioassays of sterile bark, litter, and soil samples treated with various doses of gypsy moth NPV.

Sample	% mortality	Lethal dose[a]	95% fiducial limits	
			Upper	Lower
Bark[b]	10	4.44×10^{4}	6.91×10^{4}	2.55×10^{4}
	25	3.65×10^{5}	4.67×10^{5}	2.71×10^{5}
	50	3.00×10^{6}	3.57×10^{6}	2.53×10^{6}
	75	2.46×10^{7}	3.55×10^{7}	1.83×10^{7}
	90	2.02×10^{8}	3.80×10^{8}	1.22×10^{8}
Litter[c]	10	1.46×10^{5}	1.85×10^{5}	1.11×10^{5}
	25	4.99×10^{5}	5.82×10^{5}	4.20×10^{5}
	50	1.70×10^{6}	1.91×10^{6}	1.51×10^{6}
	75	5.82×10^{6}	6.88×10^{6}	5.02×10^{6}
	90	1.99×10^{7}	2.59×10^{7}	1.58×10^{7}
Soil[d]	10	1.94×10^{5}	2.39×10^{5}	1.52×10^{5}
	25	6.08×10^{5}	6.99×10^{5}	5.20×10^{5}
	50	1.91×10^{6}	2.13×10^{6}	1.71×10^{6}
	75	5.99×10^{6}	6.99×10^{6}	5.21×10^{6}
	90	1.88×10^{7}	2.39×10^{7}	1.52×10^{7}

[a] Lethal dose value in number of polyhedral inclusion bodies.
[b,c,d] Based on, respectively, 6, 5, and 4 replicates of 50 larvae/dose.

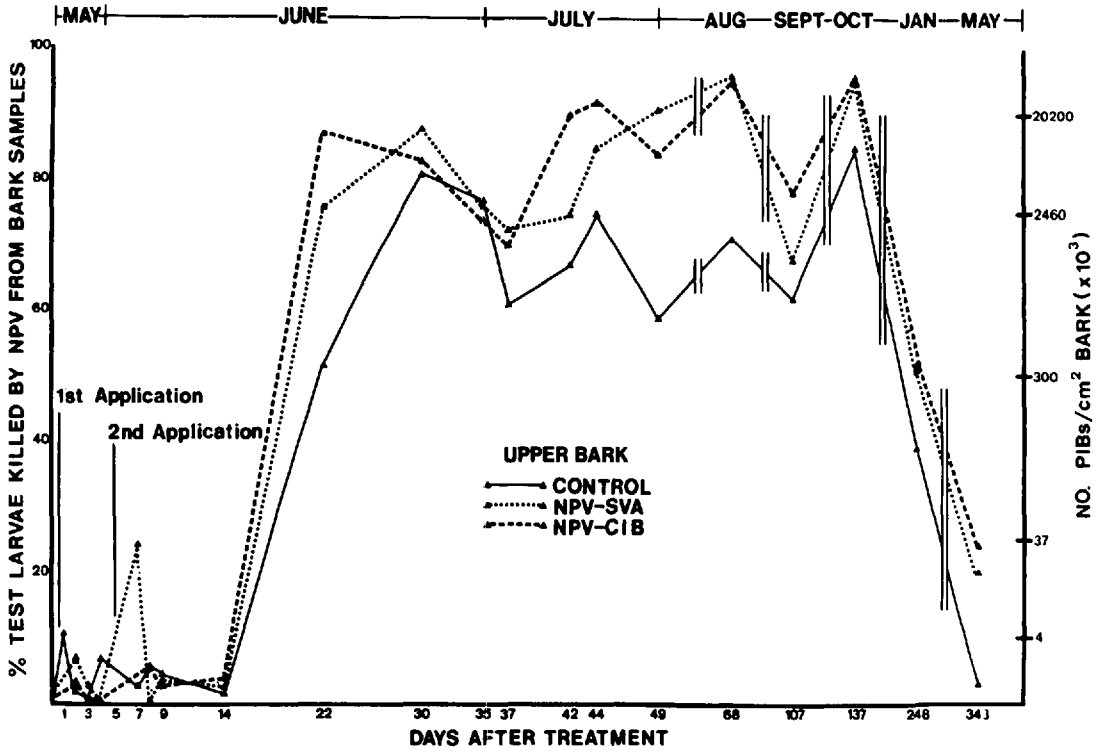

FIG. 1.—NPV activity and estimated PIB concentrations on tree bark (2 m) in 2 NPV treated plots and a control plot in Pa. Each point based on the bioassay of 4 samples.

represented an 18-fold increase over pretreatment concentration in each of the NPV-treated plots as compared to a 10-fold increase in the control plot.

Connecticut

Since the Connecticut plots were not sampled as intensively as those in Pennsylvania, extrapolated PIB levels per unit of bark, litter, or soil were not routinely determined. Relative NPV activity in samples was recorded as the percent mortality among 2nd-stage gypsy moth larvae with which they were bioassayed.

Bioassays of leaf samples indicated no NPV activity on red maple foliage in the treated plot 15 days after spray (Fig. 5). NPV activity on red oak foliage was negligible by 8 days after spray, but increased on day 15 because of PIB liberated from dead insects. There was no NPV activity on foliage in the control plot except in those samples taken 36 days after spray. This activity was due to natural NPV mortality and subsequent liberation of PIB onto foliage in the control plot.

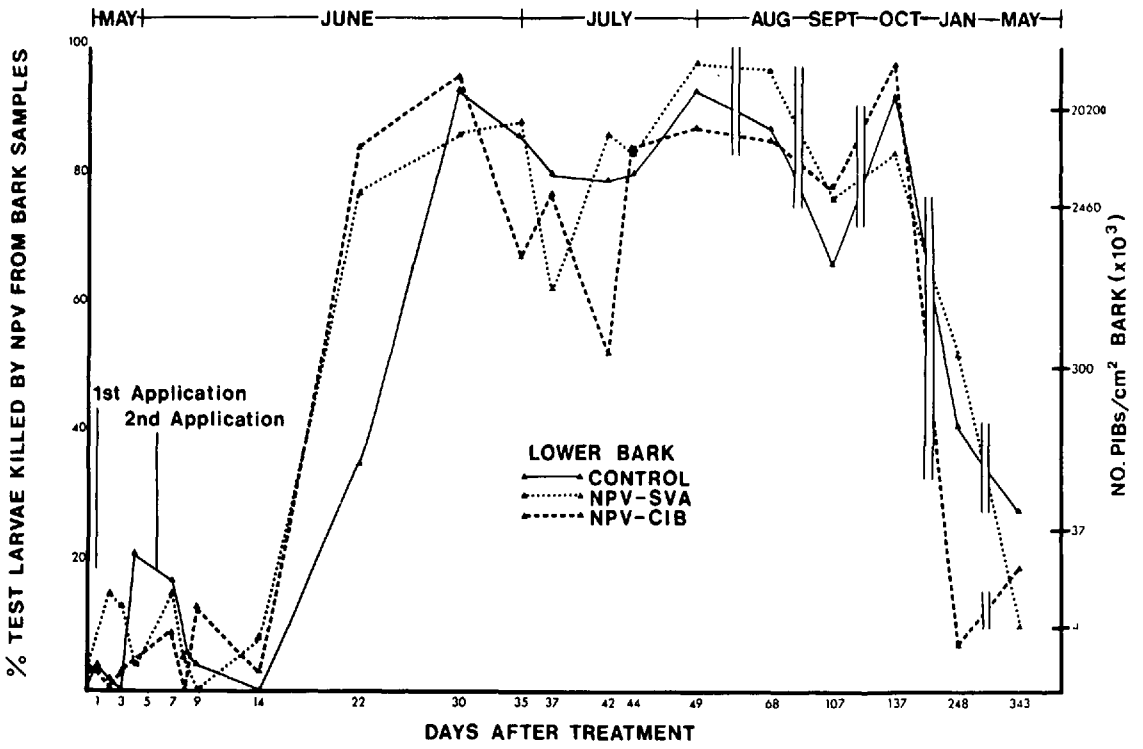

FIG. 2.—NPV activity and estimated PIB concentrations on tree bark (30 cm) in 2 NPV treated plots and a control plot in Pa. Each point based on the bioassay of 4 samples.

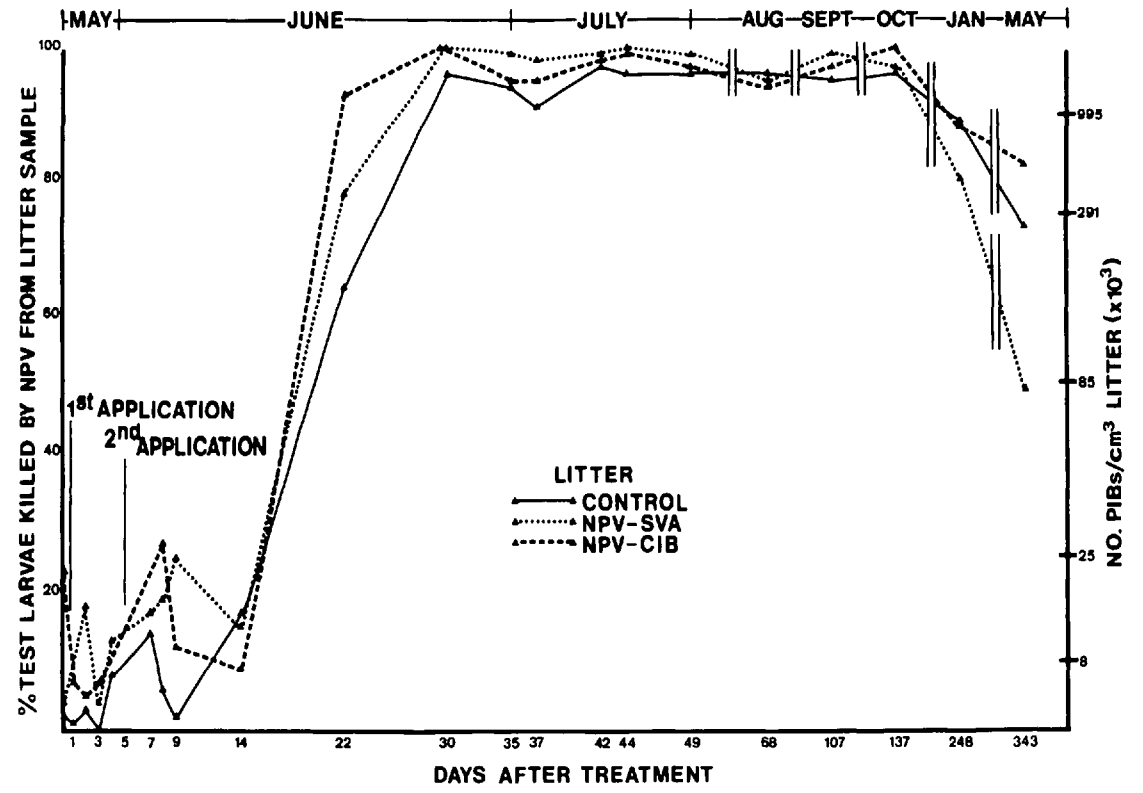

FIG. 3.—NPV activity and estimated PIB concentrations in litter in 2 NPV treated plots and a control plot in Pa. Each point based on the bioassay of 6 samples.

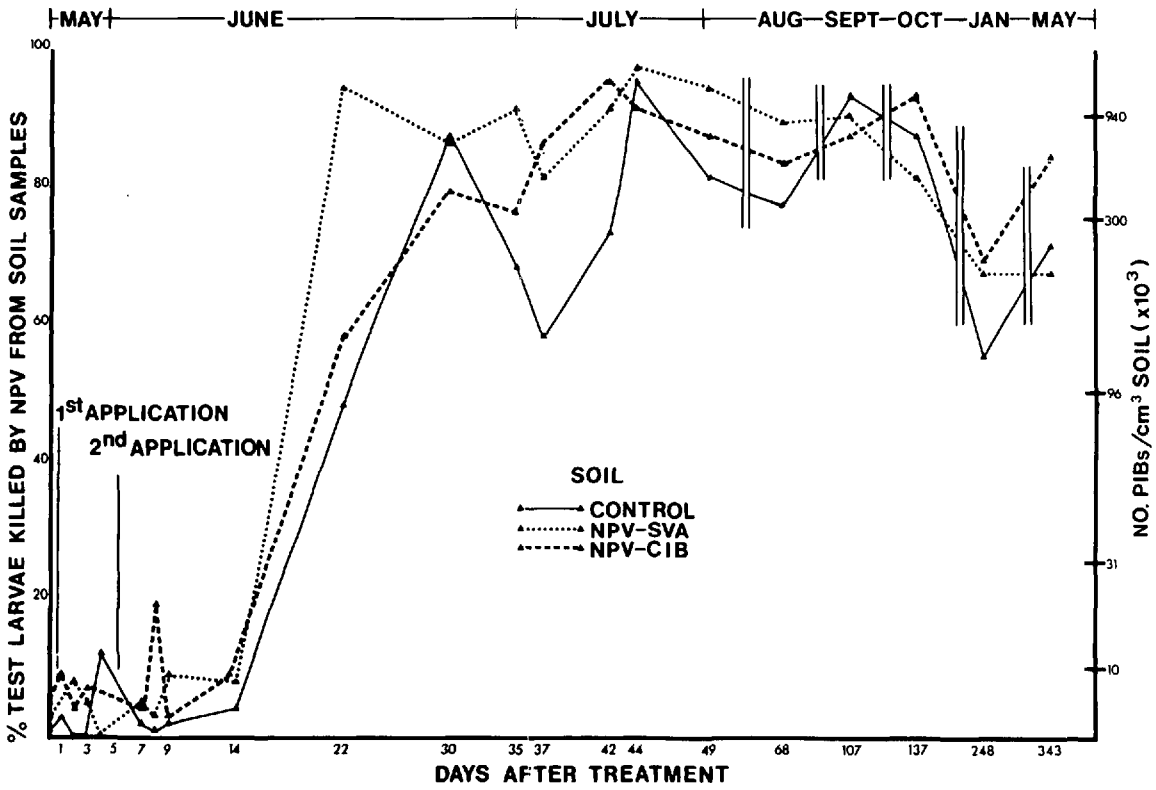

FIG. 4.—NPV activity and estimated PIB concentrations in soil in 2 NPV treated plots and a control plot in Pa. Each point based on the bioassay of 6 samples.

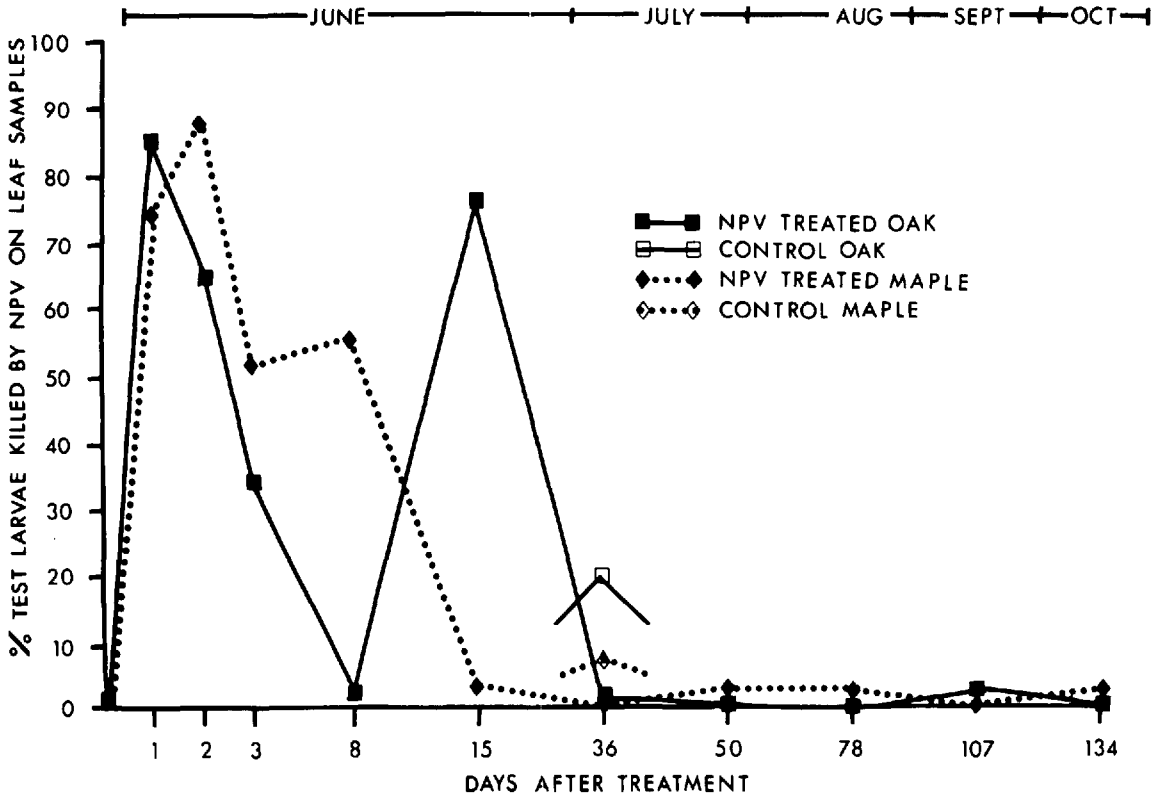

FIG. 5.—NPV activity on foliage in an NPV treated and in a control plot in Conn. Mortality data corrected according to Abbott (1925).

Prespray NPV activity on bark from red oaks in both the treated and the control plot was significantly higher than that on red maple bark taken from both plots (Fig. 6 and 7). NPV activity on treated red oak bark sampled at both 30 cm and 2 m remained relatively high over the sampling period, whereas activity on treated red maple declined to below prespray levels one yr after spray. Activity on the control oak dropped below prespray activity by the May 1976 sampling date. Little or no NPV was detected on the control red maple during the study.

Based on the NPV activity in 6 samples, the prespray PIB concentration in litter in the treated plot averaged 4.00×10^5 PIB/cm^3 (Fig. 8). Nearly one year later, this concentration had decreased to 1.06×10^5 PIB/cm^3, a decrease of 74% of the prespray level. Prespray concentrations in the control plot averaged 1.65×10^5 PIB/cm^3. This concentration decreased to 4.50×10^4 PIB/cm^3 one yr later, a reduction of 73%.

None of the postspray soil samples taken from the treated plot displayed NPV activities significantly higher than those obtained from the prespray samples (Fig. 9). Avg prespray PIB concentration in the treated plot was 1.55×10^5 PIB/cm^3. This dropped to 6.75×10^4 PIB/cm^3 one yr later, a reduction of 56%. Over the same period, PIB concentrations in the control plot decreased from a prespray avg of 5.00×10^4 PIB/cm^3 to 2.15×10^4 PIB/cm^3, a 57% reduction.

Discussion

Both the Pennsylvania and Connecticut studies indicated that the nucleopolyhedrosis virus of the gypsy moth is a naturally occurring constituent of the habitat of its host and that it persists in high concentrations for at least one yr, and probably longer, after natural NPV epizootics.

It is apparent that NPV contained in exudates from larval cadavers is more resistant to deactivation than NPV in spray formulations. Data from the Pennsylvania plots indicated that NPV in spray deposits on bark retained measurable activity above prespray levels for only 3 days after treatment. This was most likely due to the inactivation of the virus by solar radiation or to its removal from bark due to heavy rains. As a result of treated larvae dying and the subsequent liberation of PIB from cadavers, NPV accumulated more rapidly on bark in the treated plots than in the control plot. However, following a natural epizootic, NPV activity on control bark rose to approximate NPV activity on treated bark. In fact, one yr after spray, NPV activity was higher on lower tree bole bark in the control plot than in either of the treated plots.

NPV activity in samples from Connecticut plots indicated that the virus accumulated and persisted at higher levels on red oak bark than on red maple bark. The physical nature of the red oak bark probably accounts for this

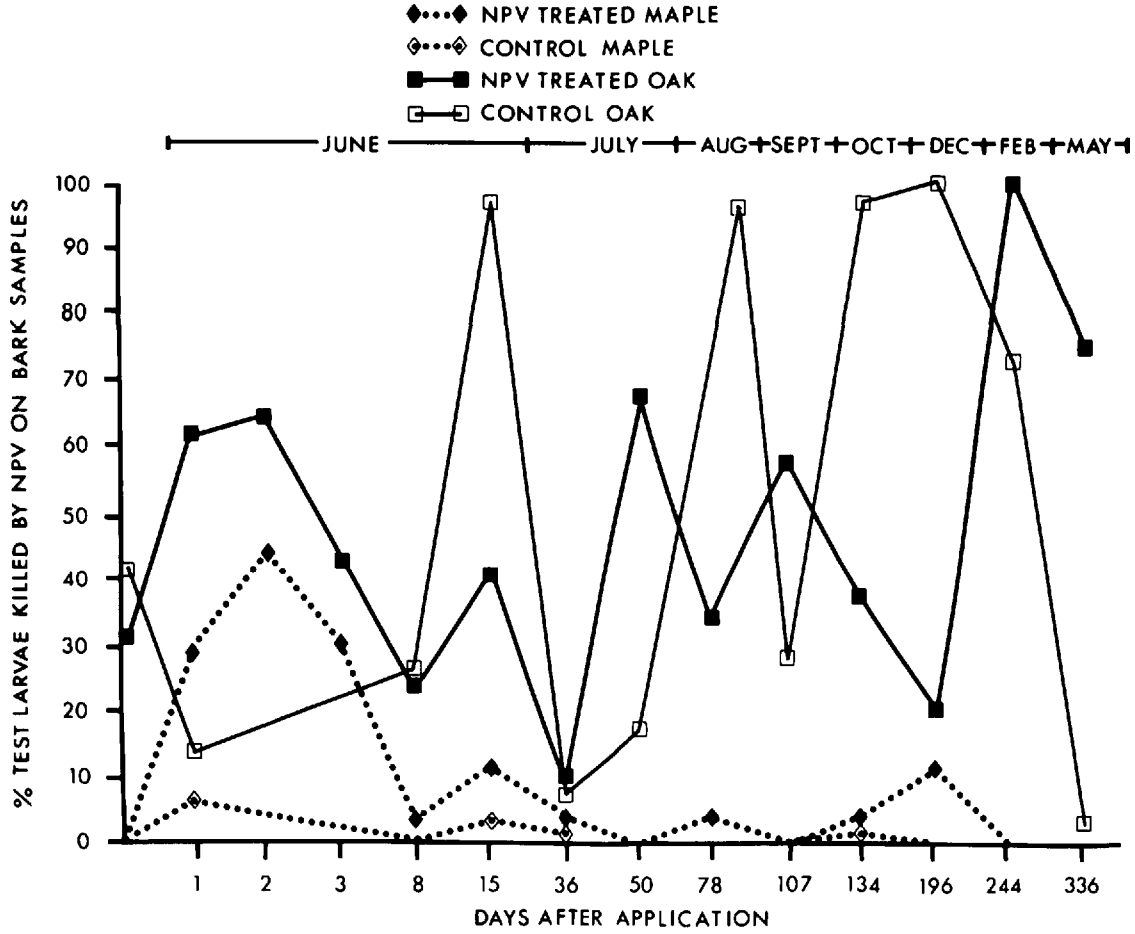

FIG. 6.—NPV activity on tree bark (2m) in an NPV treated and in a control plot in Conn. Mortality data corrected according to Abbott (1925).

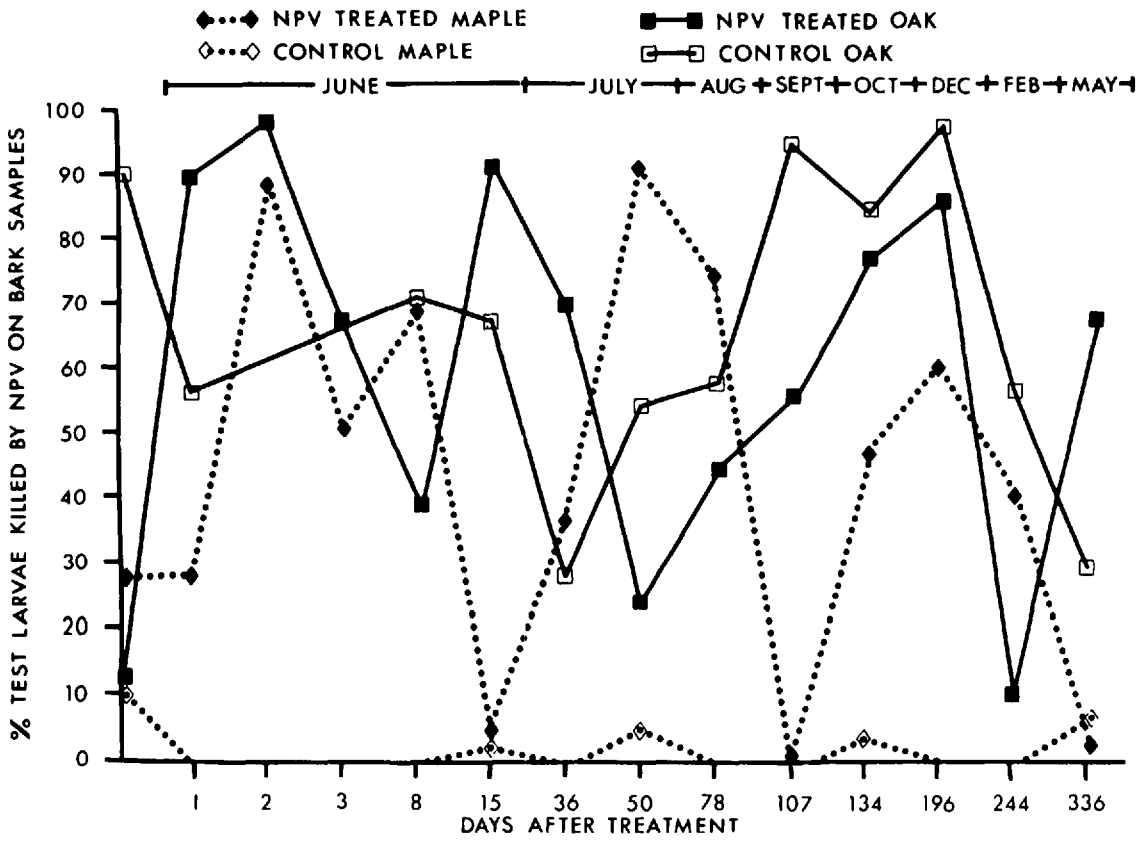

FIG. 7.—NPV activity on tree bark (30 cm) in an NPV treated and in a control plot in Conn. Mortality data corrected according to Abbott (1925).

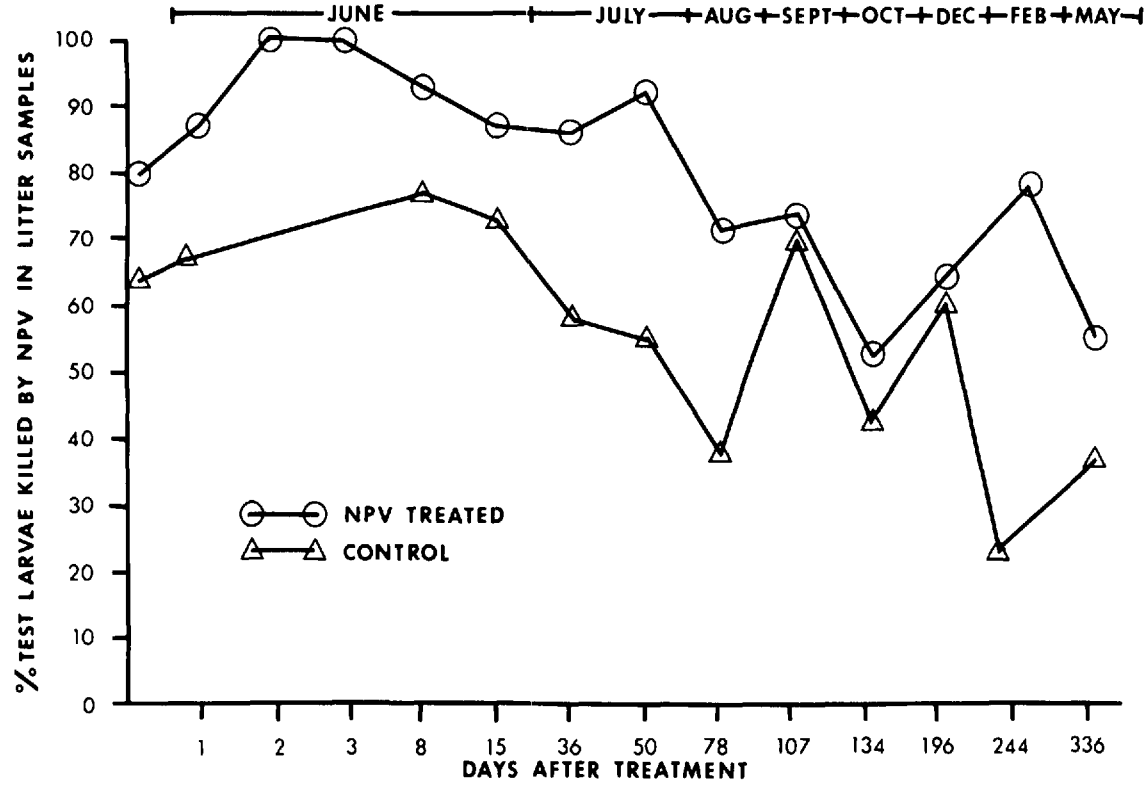

FIG. 8.—NPV activity in litter in an NPV treated and in a control plot in Conn. Each point based on the bioassay of 2 samples. Mortality data corrected according to Abbott (1925).

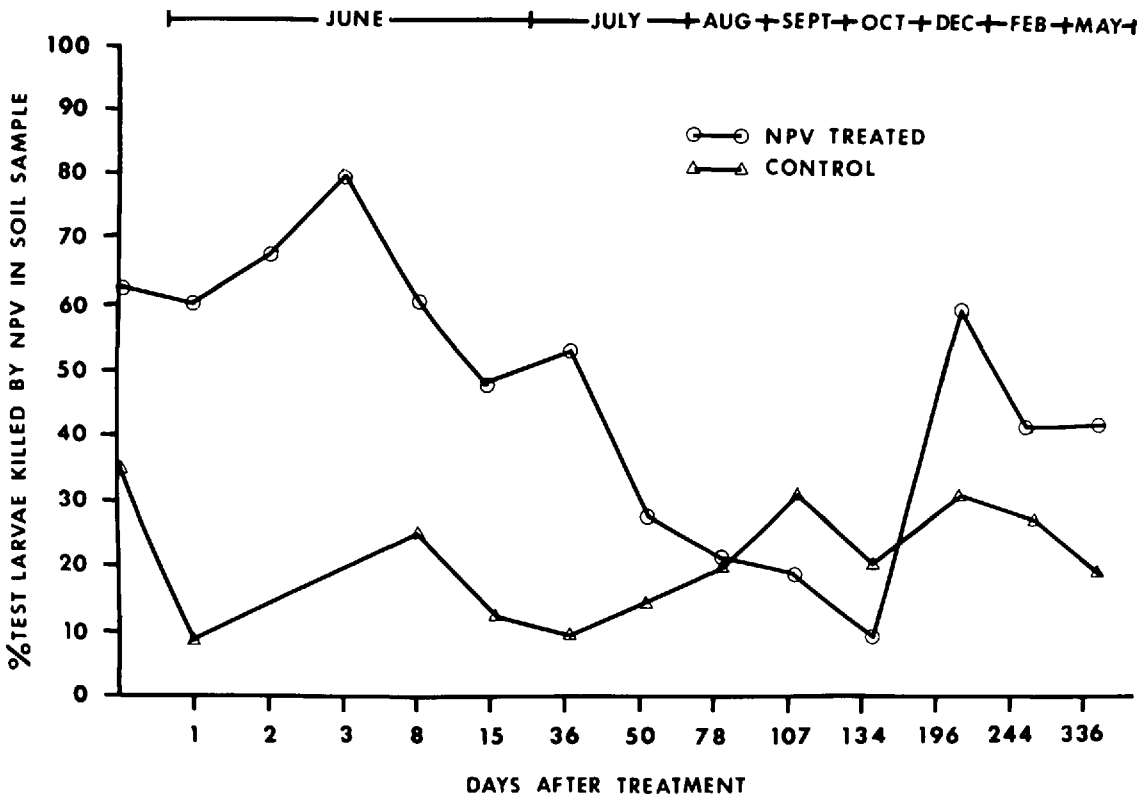

FIG. 9.—NPV activity in soil in an NPV treated and in a control plot in Conn. Each point based on the bioassay of 2 samples. Mortality data corrected according to Abbott (1925).

difference—its corrugated, rough surface affords the virus protection against adverse environmental factors, particularly solar radiation.

The extended persistence on bark is consistent with Clark's (1955) work on overwintering of tent caterpillar NPV and the work of Bird et al. (1972) on spruce budworm NPV. Whether or not the extended persistence of gypsy moth NPV on bark is important in the generation-to-generation transmission of the agent remains to be determined.

NPV persisted at the highest observed levels in litter and soil. This is in accordance with what is known of the accumulation and persistence of other NPV's (Jaques 1964, 1967, Hukuhara and Namura 1972, Thomas et al. 1972). Interestingly, the Connecticut data indicated that the addition of PIB at the rate of 2.5×10^{12} PIB/ha did not result in an increase in PIB concentrations in soil and litter over naturally occurring concentrations. In fact, in the treated plot there was a 75% decrease in PIB in litter and a 57% decrease in soil one yr after spray. Furthermore, data indicated that although there were increases in PIB over prespray concentrations in all strata in the Pennsylvania treatment plots, these increases were not markedly higher or lower than those observed in the control plot.

Thus the deliberate introduction of gypsy moth NPV at the stated levels does not appear to cause changes in the NPV load over those that would occur naturally. Therefore, any increase in the risk of exposure of nontarget species to NPV appears to be minimal.

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