ODC 411–145.7 x 18.77 *Lymantria dispar*

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Evaluation of Aerially-Applied Nuclear Polyhedrosis Virus for Suppression of the Gypsy Moth, *Lymantria dispar L.*

FOREST SERVICE RESEARCH PAPER NE-396
1978

FOREST SERVICE, U.S. DEPARTMENT OF AGRICULTURE
NORTHEASTERN FOREST EXPERIMENT STATION
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MANUSCRIPT RECEIVED FOR PUBLICATION 5 OCTOBER 1977.

ABSTRACT

Single and double applications of the nuclear polyhedrosis virus of *Lymantria dispar* L. were evaluated for suppression of gypsy moth infestations in Pennsylvania. The virus material, purified by isopynic centrifugation, was applied by air at the rate of 2.47 x 10^{12} polyhedral inclusion bodies per hectare. Two formulations of the virus were evaluated: one consisted of 1.12 kg of IMC-90001®, 0.88 liter of Chevron spray-sticker®, 4.67 liters of CIB®, and 13.14 liters of water/ha; the other was 9.34 liters of Sandoz virus adjuvant® and 9.34 liters of water/ha.[1] Application of the virus provided foliage protection, population reduction, and increased the number of virus-infected larvae. No consistent differences were observed between the two formulations, and the second application did not enhance treatment effects. In the following year, larval emergence from egg masses was significantly lower in the virus-sprayed areas than in the untreated population.

[1]The use of trade, firm, or corporation names is for the convenience and information of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service or Pennsylvania State University of any product or service to the exclusion of others that may be suitable.

INTRODUCTION

THE NUCLEAR polyhedrosis virus (NPV) of *Lymantria dispar* L. is a naturally occurring disease agent that induces epizootics in gypsy moth populations as their densities increase (Campbell 1963a). In laboratory investigations the virus was found to be most pathogenic to early instar larvae (Doane 1967; Magnoler 1974a). Determinations of the relative virulence of purified and nonpurified NPV preparations have produced conflicting results (Magnoler 1968a; Rollinson and Lewis 1973; Yendol and Hamlen 1973) and tests with different geographic isolates of the virus have shown the Hamden (Connecticut, USA) strain to be one of the most virulent (Magnoler 1970; Rollinson and Lewis 1973; Vasiljevic and Injac 1973).

Preliminary field studies utilizing ground (Magnoler 1967, 1968a, b, 1974b; Rollinson et al. 1965) and air (Yendol et al. 1977) applications have indicated that NPV is effective in causing larval mortality, protecting foliage, and reducing egg mass densities.

MATERIALS AND METHODS

The experimental site was in a gypsy-moth-infested area in Centre, Union, Clinton and Lycoming Counties, Pennsylvania. Fifteen treatment plots, 14.2 ha (379.0 x 379.0 m) in size, were established. The dominant tree species in the area were oaks, *Quercus* spp., 10 to 18 m in height. Ten 0.01 ha subplots (11.3 m diam.) were established in each plot for data collections.

A randomized complete block design was used to evaluate three replications of four treatments and a control. Gypsy moth population density was used as the criterion for the block design.

The virus material used was laboratory-produced gypsy moth NPV, Hamden isolate, purified by isopynic centrifugation in a 'K' rotor at the Atomic Energy Commission facility, Oak Ridge, Tenn. This virus preparation was applied by air at the rate of 2.47×10^{12} polyhedral inclusion bodies (PIB) per hectare per treatment.

Two commercial adjuvant-extending materials were evaluated with the virus. One formulation (the CIB formulation) consisted, for each hectare, of 1.12 kg of IMC-90001®, 0.88 liter of Chevron® spray sticker, 4.67 liters of CIB molasses material, and 13.14 liters of water. The other formulation (the SVA formulation) tested was Sandoz virus adjuvant® at the rate of 9.34 liters of adjuvant plus 9.34 liters of water per hectare. One and two applications of the virus with each adjuvant system were evaluated.

The initial spray date was determined by two factors: when at least 50 percent of the gypsy moth larvae were in the second instar and when at least 50 percent leaf expansion had occurred on the white oaks, *Q. alba* L. The first application was made either May 26, 27, or 28, 1975, and the second on June 2, 1975, to all plots designated for a second application. All spraying was conducted between 1530 and 1930 hours. A 450 hp Grumman AgCat®, equipped with a standard boom and six Beecomist® spinning nozzles (Model 275) with 80- to 100-micrometer perforated metal sleeve assemblies was used to apply the spray at 18.7 liters of finished spray per hectare.

Treatments were evaluated on the basis of pre- and posttreatment egg mass numbers, larvae and pupae situated on or under burlap bands, larval and pupal collections, defoliation estimates, spray deposit, and bioassay.

The number of gypsy moth egg masses was determined by a method similar to that of Connola et al. (1966). Prespray egg mass densities ranged from 919 to 4920 egg masses per hectare. Evaluation of treatments was based on three population density levels: low—919 to 1344 egg masses per hectare, intermediate—1670 to 2282 egg masses per hectare, and high—3053 to 4920 egg masses per hectare. Numbers of egg masses were determined in November 1975, after the leaves had fallen.

Burlap bands were used to obtain data on relative densities of gypsy moth larvae and pupae, and for estimating the mortality from the virus in treated and untreated populations. Fifty oaks in each plot were banded as described by Yendol et al. (1973). All larvae and pupae un-

der or on the burlap or within 7.62 cm below the band were counted. Estimates of larval density and of the incidence of virus in larvae and pupae were obtained on June 12, 13, and 18. Numbers of viable pupae and sex ratios were determined on July 29.

Progress of the virus disease in each plot was monitored by collecting gypsy moth larvae: 100 were collected from each plot before it was sprayed and at weekly intervals thereafter. These larvae were returned to the laboratory and maintained in groups of 10 on washed oak foliage at 21 to 26°C with a variable photoperiod for 8 to 9 days. Dead larvae were examined to confirm the presence of NPV.

Defoliation in each subplot was usually estimated in 10 percent increments at the cessation of larval feeding.

White oil-sensitive spray cards were used to confirm treatment coverage in each plot. Cards were positioned on 0.3-m wire holders at ground level under an opening in the forest canopy in or near each subplot. They were collected immediately after spraying.

The residual activity of each virus formulation was evaluated from foliage samples from virus-treated and untreated plots. Three samples were collected from each treatment. Sequential sampling of foliage was initiated when it was sprayed and continued 1, 2, 3, 5, 7, 9, 11 and 13 days after spraying of the single-application plots. Plots that were sprayed twice were sampled when sprayed and 1, 2, 3, 5, and 7 days after each application. Untreated areas were sampled each time foliage was collected in treated areas. Samples were taken at midcrown at cardinal directions selected on a random basis.

Egg masses collected near the experimental site were prepared according to a method described by Yendol et al. (1973). Upon eclosion, larvae were transferred to artificial diet (ODell and Rollinson 1966) and allowed to feed until needed for the bioassay. Residual virus was bioassayed using second-instar gypsy moth larvae (4-6 mg). Leaf material from each sample area was placed in five 16 oz. containers. Ten larvae were placed in each container and maintained at 21 to 26°C. Dead larvae were removed daily and examined for NPV.

A second-year evaluation of treatment effects on the subsequent generations was begun in April 1976. Larvae collected, larval and pupal populations under burlap bands, egg masses collected, and estimated defoliation were the parameters evaluated.

Unpaired t-tests using unpooled variances were used to analyze the data from burlap-band determinations and defoliation. This technique was employed because Bartlett's tests indicated heterogenous variances among the plots. Treatments at each density were analyzed separately because of highly significant ($p < 0.01$) interactions determined by the analysis of variance.

RESULTS AND DISCUSSION

Burlap-band determinations

Examination of burlap bands on June 12 and 13 indicated that some of the virus treatment plots had significantly fewer live larvae than the controls (Table 1). The percentage of NPV-killed

Table 1.—Mean numbers of gypsy moth larvae recovered (per cm of burlap) after application of NPV to plots with different population densities

Treatment	Recovered June 12, 13			Recovered June 18		
	Low density	Intermediate density	High density	Low density	Intermediate density	High density
Control	0.121a b	1.375a	1.393a	0.272a	3.570a	6.433a
SVA, 1 application	0.048a	0.259b	1.154a b	0.122b	0.215b	2.656c
SVA, 2 applications	0.175b	2.609a	1.280a b	0.320a	1.278c	2.656c
CIB, 1 application	0.083a b	0.048c	0.934b	0.061c	0.048b	1.570d
CIB, 2 applications	0.100b	0.301b	0.647c	0.052c	0.437e	0.305b

Means based on 50 burlaped oaks per plot. Means in the same column not followed by the same letter are significantly different at the 1% level (unpaired t-test using unpooled variances).

Table 2.—Estimated incidence of virus-caused mortality (in percent) in gypsy moth populations of different densities treated with NPV

Treatment	Recovered June 12, 13 [a]			Recovered June 18 [b]		
	Low density	Intermediate density	High density	Low density	Intermediate density	High density
Control	0.00a	0.38a	4.53a	0.00a	6.49a	11.55a
SVA, 1 application	8.84a	4.26a b	5.80a	44.77b	34.87b c	48.54b
SVA, 2 applications	10.51a	8.70b	21.38b	48.50b	36.51b c	52.79b
CIB, 1 application	5.56a	26.93a b c	19.76b	49.82b	56.40b	21.49c
CIB, 2 applications	1.61a	21.01c	3.81a	41.49b	27.69c	14.23a c

Means in the same column not followed by the same letter are significantly different at the 1% level (unpaired t-tests using unpooled variances).
[a] Means based on 19-50 burlaped oaks/plot (number of observations/plot varied because some trees had no larvae).
[b] Means based on 28-50 burlaped oaks/plot (number of observations/plot varied because some trees had no larvae).

larvae also increased significantly, compared with the controls (Table 2). On June 18, estimated larval populations in virus-treated plots were significantly lower than in the controls, except in the low-density double-SVA treatment. The virus-treated plots, except the high-density double-CIB application, had significantly higher virus-caused mortality than the controls. This delayed appearance of virus mortality in a treated population was also reported by Rollinson et al. (1965) and Magnoler (1974b).

In general, population was reduced and virus incidence increased when NPV was applied. Plots that received a single application showed as much and sometimes more population reduction than those that received a double application. The one exception was in the high-density CIB plots. Estimated virus incidence also indicated that single applications were as effective as double applications. In the intermediate-density CIB plots, the single application produced significantly more NPV-killed larvae than the double application. The SVA plots were more consistent in virus incidence than the CIB plots.

Collections of larvae

In gypsy moth larvae collected before spraying, mortality from NPV ranged from 0 to 5 percent among the 15 treatment plots (Fig. 1). In the first larvae collected after spraying, virus incidence remained low in the untreated areas, while all treated plots showed substantial increases in the number of virus-killed larvae. Virus mortality in the untreated area with low population density remained low throughout the experiment, but virus mortality continued to increase in the other untreated populations to a high of 70.0 percent in the third postspray collection from the area of intermediate density. In the high-density untreated area, the percentage of virus-killed larvae increased from 12.1 to 76.0 between the first and second postspray collections. Virus mortality remained high in this area for the remainder of the experiment.

The percentage of larvae killed by the virus between the first and second postspray collections increased in all treatment plots, except the high-density plot treated twice with CIB. The plots treated twice with SVA had the highest percentage of virus mortality during the third and fourth postspray collections of all densities.

Sex ratio determinations

Examination of pupal populations under burlap bands on July 29 showed significant reductions in the number of pupae in all the virus-sprayed plots except those with high population density that were treated with CIB (Table 3). In some of the intermediate and all of the high-density plots there were significant reductions in the percentage of females, as determined from pupal collections (Table 4). Two of the low-density areas had significant increases in the numbers of female pupae. Campbell (1963b) indicated that in gypsy moth populations disease was strongly selective against females, especially among late instars and prepupae. The distortion of sex ratios observed in the intermediate and high density

Figure 1.—Incidence of virus-caused mortality in low, intermediate, and high density gypsy moth populations after aerial applications of NPV.

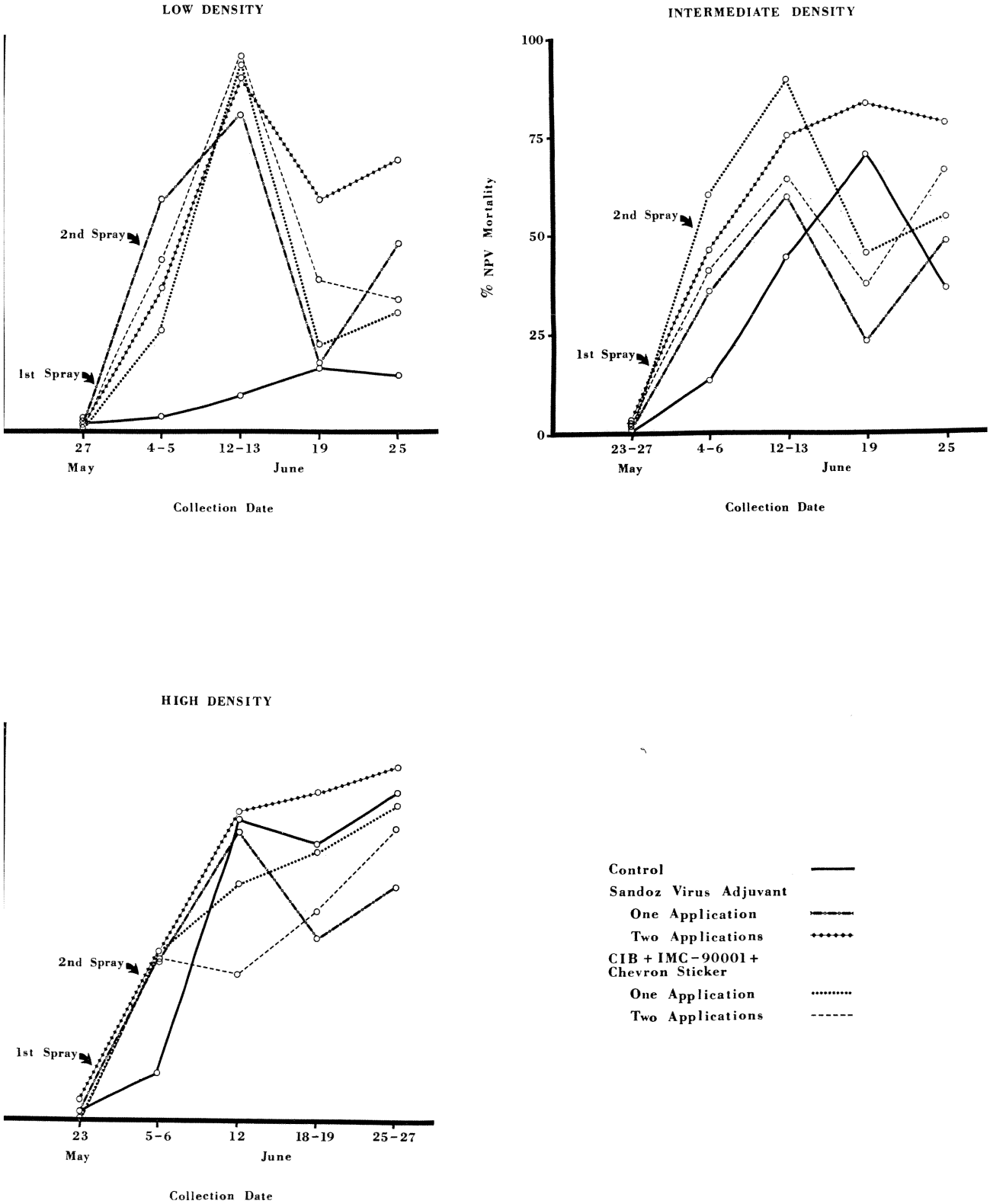

Table 3.—Mean numbers of gypsy moth pupae (per cm of burlap) recovered after applications of NPV

Treatment	Population density		
	Low	Intermediate	High
Control	2.968a	7.190a	4.315a
SVA, 1 application	0.436b c	2.543b	1.049b
SVA, 2 applications	0.731b	2.562b	2.040c
CIB, 1 application	0.369b c	1.231c	4.140a
CIB, 2 applications	0.311c	1.255c	3.766a

Means based on 50 burlaped oaks/plot. Means in the same column not followed by the same letter are significantly different at the 1% level (unpaired t-test using unpooled variances).

Table 4.—Percentage of females among gypsy moth pupae in NPV-treated and control plots

Treatment	Population density		
	Low	Intermediate	High
Control	77.0*	24.0*	25.0*
SVA, 1 application	51.5	44.0	34.0*
SVA, 2 applications	45.0	14.0*	24.0*
CIB, 1 application	63.0*	37.0*	36.0*
CIB, 2 applications	51.0	56.0	27.0*

Based on a sample of 98 to 100 pupae per plot.
*Significantly different from a 1:1 ratio at the 5% level (X^2 with Yates' correction for continuity).

Table 5.—Percentage defoliation in plots with different population densities, after treatment with gypsy moth NPV

Treatment	Oaks			All species		
	Low density	Intermediate density	High density	Low density	Intermediate density	High density
Control	45.0a	95.0a	95.0a	39.0a	80.0a	88.0a
SVA, 1 application	9.0b	42.0b	27.0b	5.0b	31.0b c	16.0b
SVA, 2 applications	22.0b	81.0c	89.0c*	16.0b*	55.0b	78.0c
CIB, 1 application	7.0b	6.0d	72.0c*	5.0b	5.0d	60.0c*
CIB, 2 applications	8.0b	35.0b	84.0c*	5.0b	27.0c d	76.0c*

Means based on defoliation in ten 0.01 ha subplots/plot. Means in the same column not followed by the same letter are significantly different at the 1% level.
*Significantly different from the control at the 5% level (unpaired t-test using unpooled variances.).

untreated areas may indicate that natural virus was affecting these populations late in the season.

Defoliation estimates

There was significantly less defoliation of oaks and total defoliation in all treatment plots than in the control plots (Table 5). Plots that received a single application had as much foliage protection, or more, than those that received two applications. At the low density, no difference was observed between the formulations. However, in the intermediate-density plots a single application of the CIB formulation gave the best foliage protection to oaks. In the high density areas, a single application of SVA gave the best foliage protection.

Spray deposit cards

Spray deposits were recovered from all treatment areas. The CIB formulation had an average coverage of 10.18 droplets per square centimeter. SVA deposits had a mean of 33.01 droplets/cm^2, 3 times as much coverage. This explains, in part, the better performance of SVA.

A difference was also observed between the number median diameters (NMD) of the two formulations. The NMD was 90 μm for the SVA formulation and 140 μm for the CIB formulation. The mass median diameters (MMD) were similar for the two formulations: 320 μm for the SVA formulation and 310 μm for the CIB formulation.

Spray residue bioassay

Virus-caused mortality ranged from 63.3 to 76.0 percent when larvae were fed foliage collected during the first 24 h after the first spray application (Fig. 2). Foliage collected during the initial day after the second application produced 59.3 to 66.6 percent mortality among test larvae. Over the course of the experiment, virus-caused mortality in larvae fed foliage from unsprayed areas ranged from 4.0 to 13.3 percent.

The residual activity of the virus dissipated more rapidly after the second application than after the first application. Rainfall undoubtedly contributed; after the first spray application, there was no appreciable precipitation for 3 or 4 days—until June 1, when 7.9 mm was recorded. More than 19 mm of rainfall was recorded on June 5, 2 days after the second spray application.

After the initial reduction in virus-caused mortality in the first 2 or 3 days after spraying, the number of test larvae that died from virus remained fairly constant. Although the infectivity of the virus dissipated rapidly, the remaining activity still caused significantly more mortality than occurred among larvae fed on untreated foliage. There was an unexplained increase in virus-caused mortality among larvae that were fed foliage collected on June 3 from the area treated with a single application of SVA.

Final egg mass determination

In the low-density control area, the number of egg masses increased substantially while in the virus-treated areas it changed only slightly from prespray estimates (Table 6). Significant differences in egg mass numbers were observed between the untreated plots and the virus-

Figure 2.—Dissipation of infectivity of gypsy moth NPV applied by air to the experimental area.

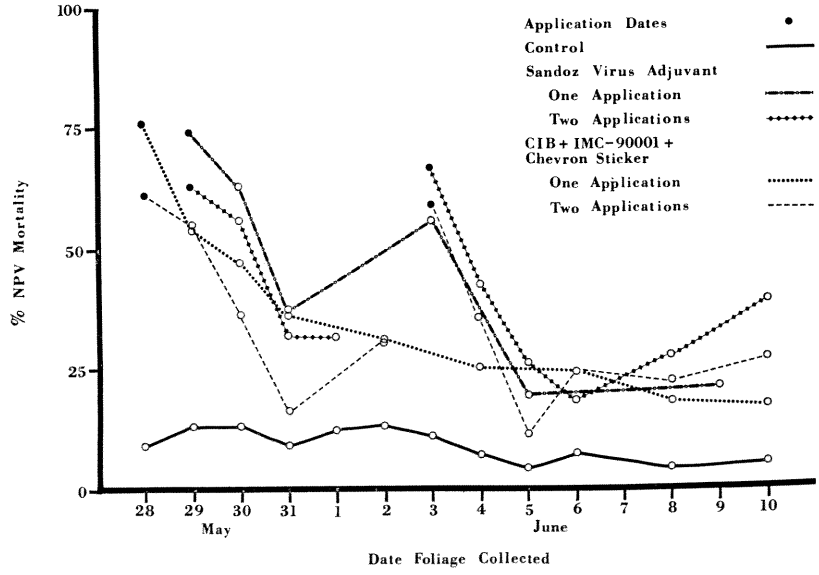

Table 6.—Estimated numbers of gypsy moth egg masses per hectare and differences (in percent) between pre- and posttreatment estimates

Treatment	Population density					
	Low		Intermediate		High	
	No. [a]	%	*No.*	%	*No.*	%
Control	7479 a	+713.8	563	− 70.5	148	−97.0
SVA, 1 application	1146 b	+ 14.8	4337	+107.0	1551	−62.4
SVA, 2 applications	968 b	− 16.3	731	− 68.0	99	−98.0
CIB, 1 application	1057 b	+ 13.8	1739	+ 4.1	3646	− 6.8
CIB,+ 2 applications	1314 b	− 2.2	2272	+ 0.5	4377	+43.4

Egg mass densities were estimated from counts made in ten 0.01=ha subplots per plot.
[a] Means in this column not followed by the same letter are significantly different at the 2% level (unpaired t-test using unpooled variances.

treated areas, but there was no differences among virus treatments. In the intermediate- and high-density untreated areas, the population collapsed naturally, as evidenced by the large reductions in the numbers of egg masses. The trend toward natural collapse of these gypsy moth populations was observed in larval collections as large increases in virus mortality, and in pupal collections as distorted sex ratios. The intermediate- and high-density plots that received a double application of SVA also had substantial reductions in egg masses, which appeared to be due to a natural virus epizootic. Therefore, we made no statistical analyses of the intermediate- and high-density plots.

Second year evaluation

Egg masses were collected from the low-density plots in April 1976. These plots were selected for further evaluation because there was no evidence that a natural virus epizootic had occurred in them. In some of the sprayed plots and both controls in the intermediate- and high-density areas populations had been drastically reduced by natural virus epizootics.

Egg mass size was significantly smaller in the plots that received a double application of SVA than in the control plots (Table 7). In the other virus-treated plots, the egg masses were not significantly different from those in the untreated area. Kaya et al. (1974) reported increases in the number of eggs per egg mass following *Bacillus thuringiensis* treatment, and Doane (1968) noted increases in egg mass size in gypsy moth populations treated with a chemical insecticide. It appears that treatment of gypsy moth populations with NPV does not increase egg mass size. Egg masses from all virus-sprayed plots produced significantly fewer larvae than those collected from the untreated plot.

Burlap-band estimates of larval populations made on June 18 and July 1, 1976, showed no significant differences between treated and untreated areas. But in the final determination, made on July 12, the treated plots had significantly more larvae and pupae than the unsprayed area. There was a significantly higher mortality from virus infection in the control area than in the treated plots on July 1. This indicates a natural virus epizootic in the control plots and may explain the reduced population noted on July 12.

Collections of larvae also showed more virus-caused mortality among larvae collected in the untreated areas than in the treated plots. In the final collection, NPV mortality in the plots that had had one and two applications of SVA and those that had had one and two applications of

Table 7.—Mean numbers of eggs and larvae per egg mass in the low-density plots the year after virus applications

Treatment	Eggs/mass	Larvae/mass
Control	274 a	168 a
SVA, 1 application	234 a	114 b
SVA, 2 applications	147 b	79 b
CIB, 1 application	264 a	99 b
CIB, 2 applications	260 a	90 b

Means based on 47-49 egg masses/plot. Means in the same column not followed by the same letter are significantly different at the 1% level (unpaired t-test using unpooled variances).

CIB were 86, 51, 84, and 88 percent, respectively. In the untreated area, virus mortality was 96 percent, a further indication of a developing virus epizootic.

Defoliation was estimated in July 1976 at 40 percent for all tree species in the untreated area. The plots treated with one and two applications of SVA had 14 and 7 percent defoliation, respectively; the plot that received a single application of CIB had 35 percent defoliation and the plot that received a double application had 23 percent defoliation.

Our results indicate that application of NPV to gypsy moth infestations below 4920 egg masses per hectare will provide foliage protection and population reduction while increasing virus-caused mortality, compared with untreated areas. This experiment suggests that virus applications do not improve the population quality of successive generations, as has been observed with *B. thuringiensis* and chemical insecticides; i.e., there was no increase in egg mass size, emergence from egg masses was reduced, and the virus infection was prolonged in the treated populations. Additional experiments are needed to further evaluate the field efficacy of the gypsy moth NPV, and to provide additional information on residual effects of the virus on the progeny of treated populations.

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