Response of Small Mammals to Aerial Applications of the Nucleopolyhedrosis Virus of the Gypsy Moth, *Lymantria dispar*[1,2,3]

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ABSTRACT

Environ. Entomol. 7: 676–684 (1978)

Resident populations of white-footed mice, *Peromyscus leucopus* Rafinesque, red-backed voles, *Clethrionomys gapperi* Vigers, opossums, *Didelphis marsupialis* L., chipmunks, *Tamias striatus* L., and raccoons, *Procyon lotor* L., were evaluated to detect any short term effects from aerial applications of the nucleopolyhedrosis virus (NPV) of the gypsy moth. NPV in 2 formulations was sprayed on woodland plots in central Pennsylvania at the rate of 2.5×10^{12} polyhedral inclusion bodies (PIB)/ha.

Comparisons of prespray and postspray censuses of white-footed mice and red-backed voles in control and treated plots revealed no changes in populations or body weight that could be attributed to NPV treatments.

Data from 47 caged and 250 free-living mammals showed no significant differences in organ and tissue weights, hematological values or necropsy and histopathological rankings between control and treated mammals when sample sizes were large and mean total weight between groups similar. It was concluded that aerial applications of NPV at 2.5×10^{12} PIB/ha caused no short term adverse effects to those mammals that either contacted NPV during it's application or subsequently fed on NPV infected gypsy moths or other NPV-contaminated food sources.

The nucleopolyhedrosis virus (NPV) of the gypsy moth, *Lymantria dispar* L. has been developed by the USDA as a biological control agent. Podgwaite et al.[6] have shown that gypsy moth NPV is a natural component of the host's habitat, persisting naturally at high levels in soil, litter and on bark following natural epizootics. However, before registration of this microbial insecticide, its safety following large scale application had to be assured. Of particular concern was the possible harmful effects of NPV sprayed on wild populations of small mammals, some of which are important predators of the gypsy moth (Smith and Lautenschlager, in press).

Laboratory studies have shown that gypsy moth NPV causes no harmful effects either when fed to rats,[7,8] dogs,[9] white-footed mice, *Peromyscus leucopus*, Rafinesque, and short-tailed shrews, *Blarina brevicauda* Say (Lautenschlager et al. 1977) or when applied to the skin of guinea pigs and the eyes and skin of rabbits.[7] However, there have been no reports on the effects of field application of NPV on resident populations of wild mammals. This paper presents the results of a study designed to determine the following in response to field applications of NPV: 1) demographic changes in free-living white-footed mice and red-backed voles; 2) pathologic conditions in free-living white-footed mice, red-backed voles, *Clethrionomys gapperi* Vigers, chipmunks, *Tamias striatus* L., and raccoons, *Procyon lotor* L.; and 3) pathologic conditions of caged young and adult opossums, *Didelphis marsupialis* L., and white-footed mice.

Materials and Methods

Study Area

The study area was located in the Bald Eagle State Forest (central mountain region of Pennsylvania), latitude, 41° 05′ N; longitude, 77°15′ W, 27.5 km west of Milton, PA. In the spring of 1975, fifteen 14-ha plots were established on the relatively xeric ridgetops. Nine of these plots, which ranged in elevation from 550–650 m, and which supported moderately dense to dense (500–5000 egg masses/ha) populations of gypsy moths, were used for this study.

Vegetation on these plots was analyzed by strata. In the tree stratum (above 3 m), both northern red oak, *Quercus rubra* L., and scarlet oak, *Quercus coccinea* Muenchh, were co-dominant with chestnut oak, *Quercus prinus* L. Red maple, *Acer rubrum* L., and white oak, *Quercus alba* L., were also common. The shrub stratum (½–3 m) was dominated by mountain laurel, *Kalmia latifolia* L., with abundant witch hazel, *Hamamelis virginiana* L., and red maple. The herb stratum (0–½ m) was dominated by mountain laurel and lowbush blueberry, *Vaccinium vacillans* Torr., while huckleberry, *Gaylussacia baccata* Wang., also was common.

The mammals most frequently live-trapped in the study area were white-footed mice, red-backed voles, chipmunks, raccoons, opossums, and shrews, both the short-tailed shrew and the masked shrew, *Sorex cinereus* Kerr.

NPV Application

Three of the 9 study plots received 2 aerial applications of a molasses-base formulation (CIB); each liter contained: Cargill Insecticide Base®, 123 ml; Chevron Spray Sticker®, 23 ml; IMC 90–001®, 58.6 g; water, 854 ml and 1.3×10^{11} purified (Breillatt et al. 1972) polyhedral inclusion bodies (PIB) of gypsy moth NPV. Three other plots were similarly

[1] Lepidoptera: Lymantriidae.

[2] The use of trade, firm, or corporation names in this paper is for the information and convenience of the reader. Such use does not constitute an endorsement or approval by the USDA or the Forest Service of any product or service to the exclusion of others that may be suitable. Received for publication Feb. 22, 1978.

[3] The work reported was funded in part by a USDA sponsored program entitled "The Expanded Gypsy Moth Research and Development Program."

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[6] Podgwaite, J. D., K. S. Shields, R. T. Zerillo and R. B. Bruen. Environmental persistence of the nucleopolyhedrosis virus of the gypsy moth, *Lymantria dispar* L. (Unpublished report); Forest Insect and Disease Laboratory, Hamden, CT 06514.

[7] Litton Bionetics, Inc. 1975a. Acute toxicity tests: Nucleopolyhedrosis virus (NPV) of the gypsy moth. Final report submitted to: Forest Insect and Disease Laboratory, Hamden, CT 06514.

[8] Litton Bionetics, Inc. 1975b. 2-year carcinogenicity study in rats: Nucleopolyhedrosis virus (NPV) of the gypsy moth. Final report submitted to: Forest Insect and Disease Laboratory, Hamden, CT 06514.

[9] Litton Bionetics, Inc. 1975c. Subacute toxicity tests. Nucleopolyhedrosis virus (NPV) of the gypsy moth. Final report submitted to: Forest Insect and Disease Laboratory, Hamden, CT 06514.

Table 1.—Amount of NPV found[a] in alimentary tracts of 4 species of mammals from the study area.

Species	NPV sprayed			Control		
	No.	% of N with NPV	$\bar{x}\pm$SE of NPV $\times 10^4$	No.	% of N with NPV	$\bar{x}\pm$SE of NPV $\times 10^4$
Peromyscus	41	93	13.30± 5.36[b]	28	54	0.64±0.33
Clethronmys	20	85	8.99± 6.26	15	93	6.37±3.61
Tamias	5	100	35.29±29.66	2	50	0.04±0.04
Procyon	5	60	0.15± 0.10	4	50	0.01±0.01

[a] Determined by bioassay.
[b] Significantly different ($P < 0.05\%$).

treated, but with a different formulation (SVA); each liter contained: Sandoz Virus Adjuvant 16-B®, 500 ml; water, 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 liter/ha. The plots were sprayed on May 28, 1975, and on June 2, 1975, from a 450-hp Grumman AgCat® equipped with 6 Beecomist® nozzles. Three untreated plots served as controls.

Mammal Census

To determine which plots would be censused, we established a transect trap line, consisting of 30 traps spaced 15 m on center in each of the 9 study plots. Each trap on each transect line was a 7.5×7.5×30-cm Sherman® live trap. Live traps were used for censusing small mammals in this study since the establishment of pitfall traps on the study area was impractical because of the rocky substrate. Traps were baited with rolled oats mixed in peanut butter and supplied with cotton for nesting material. Preliminary trapping (before treatment) indicated that 3 plots had similar numbers of mice, voles, and chipmunks. Therefore, near the center of each of these 3 plots, we established a permanent 9×9 grid—81 live traps—one/grid point, spaced at 13.7-m intervals. Smith et al. (1969–70) stated that a trap spacing of 15 m may be a good compromise for the most common small mammal species; however, we shortened this suggested spacing by 1.3 m to increase trapping accuracy. Each trapping grid determined the populations on 1.4 ha assuming a 2.9-m "draw." "Draw" accounts for mammals outside the plot which are "drawn" to the plot by the bait. The draw distance is based on trap recognition distance (Gentry et al. (1971)). All live traps for censusing were prepared and established the same way for all plots so that comparisons of species and populations between plots were valid. Also, all traps were set "lightly" and on several instances they caught 3 g *S. cinereus* as well as young *P. leucopus* and *C. gapperi* as light as 6.0 g.

Each plot was censused for 5 consecutive days during late May (before NPV application) in mid-June (after the 2nd NPV application), and in mid-July (after maximum defoliation, pupation, and during early emergence of adult gypsy moths). Two of the census plots had been treated (one plot twice with CIB and one plot twice with SVA); the 3rd plot was a control. Information recorded during a census included: plot number, date, temperature, weather conditions, trap number, species, animal number—identified by toe clipping (Blair 1941), age (adult, sub-adult or juvenile—identified by weight and developmental molts (Collins 1923)), weight (±0.25 g), testes position, mammary gland condition, and general condition.

Censuses were conducted on the same days for all 3 plots; they began at 6 a.m. and were completed by noon. A different plot was censused 1st each morning to avoid leaving one group of mammals in traps longer than another. These mammals were removed from the plots after the final census.

Caged Mammals

Before the 1st NPV treatment (May 28, 1975) 2 male and 2 female white-footed mice, one male opossum, and one female opossum with her litter were trapped and caged in wire mesh (0.6 cm) enclosures. These animals were then placed on the 3 census plots near the center of each census plot. Mice were caged in pairs (one male and one female)

Table 2.—Mean live weight of control and treated white-footed mice and red-backed voles censused in June and July, 1975.

Mammal	Control			Treated		
	No.	Mean (g)	SE	No.	Mean (g)	SE
White-footed mouse						
Adult male	19	19.3	0.67	44	20.3	0.49
Adult female	15	20.8	0.64	26	21.2	0.63
Young male	3	12.1	2.07	17	12.6	0.73
Young female	7	12.0	0.73	13	11.9	0.79
Red-backed vole						
Adult male	6	26.1	2.05	28	23.6	0.90
Adult female	7	25.8	0.87	26	23.2	0.93
Young male	7	15.0	1.44	10	14.5	1.44
Young female	3	15.3	2.27	9	16.7	1.15

Table 3.—Numbers of control and treated caged and field-collected mammals submitted for necropsy and histopathological examination.

Mammal	Female				Male			
	Adult		Young		Adult		Young	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Caged								
White-footed mouse	2	5	—	—	3	4	—	—
Opossum	1	2	2	6	1	2	7	12
Field-collected								
White-footed mouse	16	36	9	8	23	41	2	3
Red-backed vole	18	35	—	—	10	19	—	—
Chipmunk	1	5	—	—	3	7	—	—
Opossum	—	2	—	—	1	2	—	—
Raccoon	2	4	—	—	1	2	—	—

and were provided with cotton for nesting material. Each female opossum was caged with her litter; male opossums were caged individually. Mice were trapped near the study area, but the opossums were trapped in valleys, close to human habitation.

All caged animals were given food and water daily. Mice were fed wild bird seed and sunflower seeds; opossums were fed canned dogfood. The caged mammals were sent to the veterinary pathology laboratory at Pennsylvania State University on June 30, 1975 (4 wk after the 2nd treatment), for necropsy and histopathological examination. Caged mammals that died before June 30 were sent to the laboratory within 24 h of death. In all, 47 caged mammals were submitted for analysis.

Trapped Mammals

During the course of the study 250 free-living mammals were trapped from NPV treated (CIB and SVA) and control plots and subjected to necropsy and histopathological examination. Trapping these mammals began on June 16, 1975, when the 1st gypsy moth mortality from NPV was observed. Trapping continued on a weekly basis through Aug. 13, 1975, and ca. 66 mammals (⅔ from NPV treated

Table 4.—Results of necropsies and histopathological studies on caged *Didelphis marsupialis*—young, male, and female.

	Control			NPV treated		
	No. mammals	$\bar{x}$	C.I.[a]	No. mammals	$\bar{x}$	C.I.
	Weights (g)			Weights (g)		
Total body	9	132.111	124.547–139.675	18	109.472**	95.032–123.912
Heart	9	0.863	0.763–0.963	18	0.968	0.838–1.098
Kidney	9	1.348	1.277–1.419	18	1.269	1.131–1.407
Liver	9	5.258	4.735–5.781	18	4.822	4.194–5.450
Lung	9	1.875	1.640–2.110	18	1.670*	1.509–1.831
Spleen	8	0.258	0.198–0.318	18	0.295	0.231–0.359
Testes	7	0.243	0.000–0.599	6	0.157	0.123–0.191
Andrenal	9	0.036	0.033–0.039	16	0.037	0.025–0.049
	Hematology and differential cell counts (per 100 WBC)					
Packed cell volume (%)	9	40.2	37.9–42.6	15	36.1**	32.9–39.281
Hemoglobin (g/100ml)	9	12.5	12.0–13.0	15	11.7*	10.7–12.7
Erythrocytes ($\times 10^6$)	9	4.0	3.6–4.4	15	3.2**	2.7–3.7
Leukocytes ($\times 10^3$)	9	19.1	14.7–23.5	15	15.9	10.9–20.4
Nucleated red cells	8	0.8	0.0–1.6	15	5.1	0.0–13.0
Basophils	8	0.4	0.0–0.9	15	0.2	0.0–0.6
Monocytes	8	0.4	0.0–0.9	15	0.5	0.0–1.3
Eosinophils	8	3.8	0.0–8.4	15	5.7	2.6–8.9
Metamyelocytes	8	0.0	0.0–0.0	15	0.0	0.0–0.0
Bands	8	0.3	0.0–0.9	15	0.2	0.0–0.9
Segs	8	12.3	5.8–18.7	15	20.3	11.4–29.1
Lymphocytes	8	81.5	74.8–88.2	15	71.7	63.5–79.9
	Organ and tissue condition[b]					
Liver	9	1.2	1.0–1.6	18	1.1	1.0–1.4
Lung	9	1.3	1.0–1.8	17	1.5	1.0–2.1
Adrenal	9	1.6	1.1–2.1	18	1.4	1.0–1.9
G.I.	9	1.3	1.0–1.8	18	1.6	1.0–2.2
Lymph	9	1.0	1.0–1.0	17	1.4	1.0–2.0

[a] Based on one standard deviation of the mean.
[b] Based on: 1 = excellent, 2 = good, 3 = fair, 4 = poor (including only the $\bar{x}$ and C.I. of those which differed).
* Significant at 5% level.
** Significant at 1% level.

Table 5.—Results of necropsies and histopathological studies on field collected *Peromyscus leucopus*—adult males.

	Control			NPV treated		
	No. mammals	$\bar{x}$	C.I.[a]	No. mammals	$\bar{x}$	C.I.
	Weights (g)			Weights (g)		
Total body	22	17.195	14.537–19.853	41	16.449	13.981–18.917
Heart	22	0.137	0.110–0.164	41	0.131	0.105–0.157
Kidney	22	0.243	0.207–0.279	41	0.245	0.208–0.282
Liver	22	0.765	0.595–0.935	41	0.773	0.617–0.929
Lung	22	0.202	0.140–0.264	41	0.187	0.138–0.236
Spleen	22	0.031	0.016–0.046	40	0.032	0.000–0.069
Testes	21	0.339	0.203–0.475	41	0.297	0.148–0.446
Adrenal	20	0.015	0.010–0.020	41	0.021	0.000–0.073
	Hematology and differential cell counts (per 100 WBC)					
Packed cell volume (%)	19	52.8	48.8–56.9	35	51.9	48.7–55.0
Hemoglobin (g/100ml)	19	17.8	16.3–19.4	37	17.5	16.4–18.5
Erythrocytes ($\times 10^6$)	19	10.3	8.7–11.9	31	10.2	9.1–11.4
Leukocytes ($\times 10^3$)	19	17.5	5.5–29.5	31	13.5	1.7–25.2
Nucleated red cells	18	0.6	0.0–1.9	37	0.7	0.0–2.7
Basophils	18	0.7	0.0–1.7	37	0.1**	0.0–0.4
Monocytes	18	1.6	0.0–3.4	37	2.4	0.0–5.0
Eosinophils	17	3.3	0.0–7.2	37	4.0	0.0–9.1
Metamyelocytes	18	0.0	0.0–0.0	37	0.7	0.0–4.3
Bands	18	0.4	0.0–1.5	37	1.1	0.0–2.8
Segs	18	13.2	0.8–25.6	37	13.1	0.8–25.4
Lymphocytes	18	68.1	57.7–83.5	37	67.5	52.2–82.9
	Organ and tissue condition[b]					
Kidney	23	1.4	1.0–2.0	41	1.2	1.0–1.7
Lung	23	1.0	1.0–1.2	41	1.2	1.0–1.7
Adrenal	23	2.0	1.2–2.8	41	2.1	1.3–3.0

[a] Based on one standard deviation of the mean.
[b] Based on: 1 = excellent, 2 = good, 3 = fair, 4 = poor (including only the $\bar{x}$ and C.I. of those which differed).
**Significant at 1% level.

plots and ⅓ from control plots) were captured and examined weekly.

The free-living small mammals were trapped in Sherman live traps placed on transects established on the inner 10 ha of the 4 treated plots that had not been used for censuses. Larger mammals were trapped in large wire live traps baited with sardines. Larger mammal traps were placed in both censused and non-censused treated and control plots.

Necropsy and Histopathology

Individual mammals submitted for analysis were coded by species, number, age, sex, and plot and examined within 24 h of arrival at the laboratory. Before necropsy blood was drawn from each living mammal and the following hematological measurements taken: packed cell volume (PCV) (%), hemoglobin (g/100ml), total erythrocytes ($\times 10^6$), total leukocytes ($\times 10^3$) and counts of nucleated red cells, basophiles, monocytes, eosinophiles, metamyelocytes, band neutrophiles, segmented neutrophiles, and lymphocytes. Erythrocyte and leukocyte levels were determined using a Coulter® counter while differential counts were recorded by microscopic examination under oil emersion (1000×). After drawing the blood each mammal was weighed as were each of the following organs as the necropsy progressed: heart, kidney, liver, lung, spleen, testes, ovaries, and adrenal glands. Each female was noted as gravid or not; if gravid, the number and size of embryos were noted. For histopathological examination the heart, kidneys, liver, lungs, spleen, brain, uterus, adrenals, thyroid, pituitary, muscle, ovaries, testes, urinary bladder, salivary gland, gastrointestinal tract, lymph nodes, and pancreas were processed routinely (Luna 1968), embedded in paraffin, sectioned at 6 μm, stained with hematoxylin and eosin, and examined with light microscopy.

After laboratory analysis, data on individual mammals and their code (including species, number, age, sex, and plot) plus data from necropsy weights, hematological values, differential cell counts and a numerical condition ranking (1=excellent, 2=good, 3=fair, 4=poor) of the histopathological findings for each organ or tissue, plus data on organ and tissue weights and hematological values for each individual, were entered on computer cards. These measurements produced a maximum of 45 variables that could be coded for each animal.

Data on caged and free-living mammals were analyzed separately. When samples were large they were further divided into subgroups by species, treatment, sex, and age. When samples were small, mammals were often combined regardless of sex and age. We used *t*-tests and confidence intervals to compare means between treatments. Means between groups were tested only if the samples of treated or control mammals contained 9 animals or more in each group. However, means and standard deviations for all groups regardless of sample size were always examined to detect trends in values that might indicate abnormal organs, tissue weights, hematological values, differential cell counts or differences in the histopathological ranking of each organ or tissue.

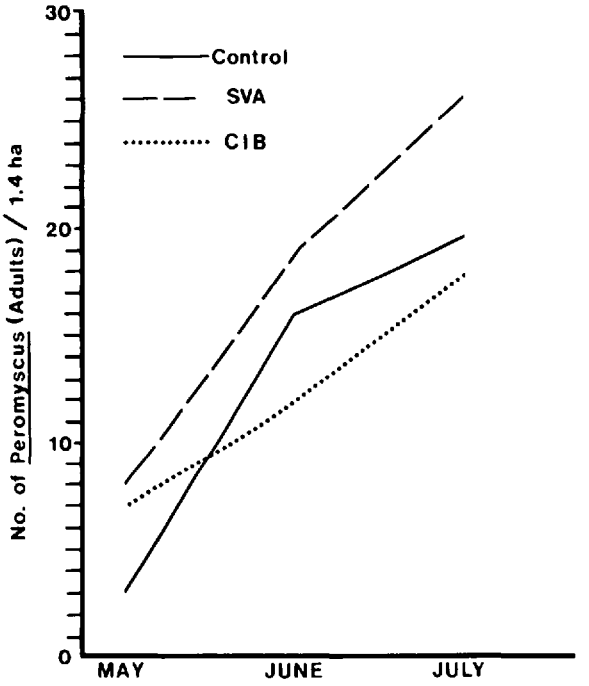

FIG. 1.—Number of individual adult white-footed mice captured monthly on control and treated census plots. (SVA=treated with NPV mixed with Sandoz Virus Adjuvant 16–B®; CIB=treated with NPV mixed with Cargill Insecticide Base®).

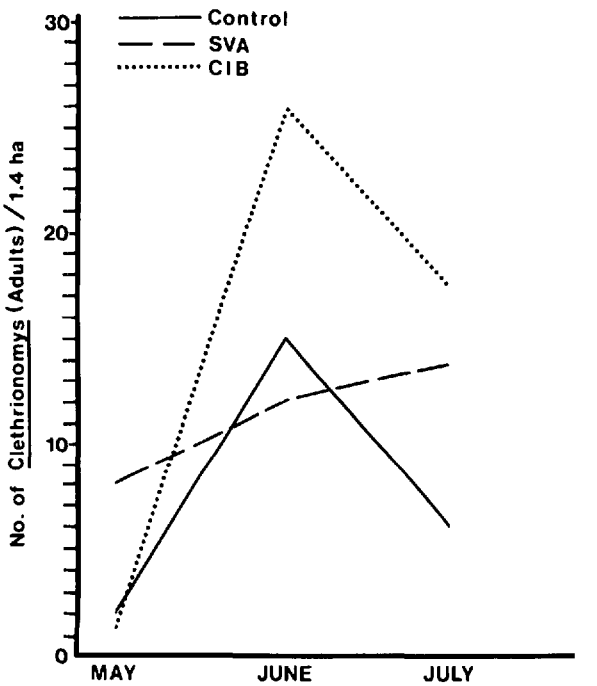

FIG. 3.—Number of individual adult red-backed voles captured monthly on control and treated census plots. (SVA=treated with NPV mixed with Sandoz Virus Adjuvant 16–B®; CIB=treated with NPV mixed with Cargill Insecticide Base®).

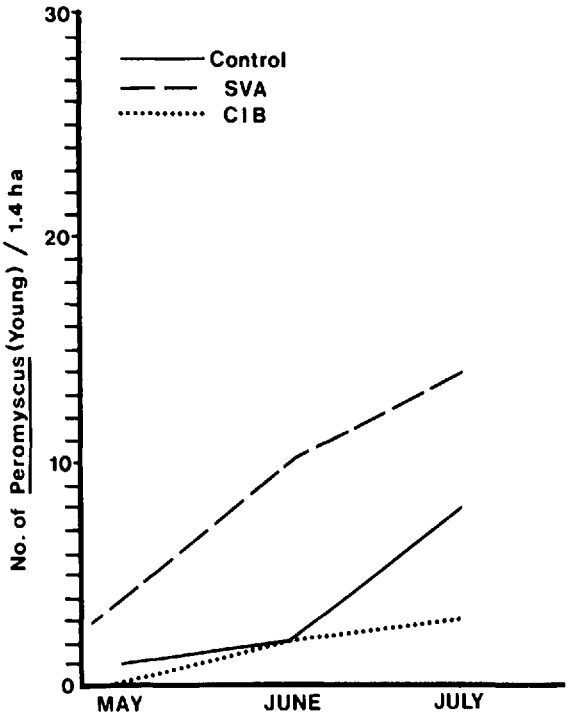

FIG. 2.—Number of individual young (juveniles and subadults) white-footed mice captured monthly on control and treated census plots. (SVA=treated with NPV mixed with Sandoz Virus Adjuvant 16–B®; CIB=treated with NPV mixed with Cargill Insecticide Base®).

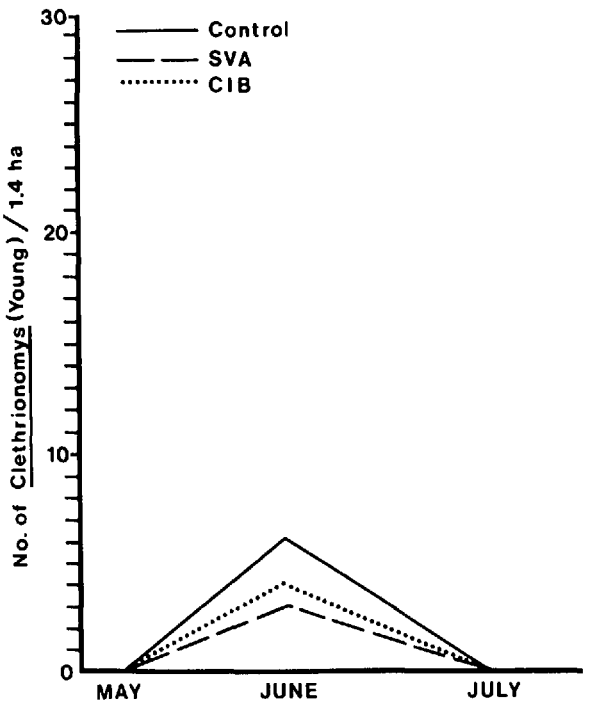

FIG. 4.—Number of individual young (juveniles and subadults) red-backed voles captured monthly on control and treated census plots. (SVA=treated with NPV mixed with Sandoz Virus Adjuvant 16-B®; CIB=treated with NPV mixed with Cargill Insecticide Base®).

Table 6.—Results of necropsies and histopathological studies on field collected *Peromyscus leucopus*—adult females.

	Control			NPV treated		
	No. mammals	$\bar{x}$	C.I.[a] Weights (g)	No. mammals	$\bar{x}$	C.I. Weights (g)
Total body	16	18.112	14.260–21.964	35	17.294	13.623–20.965
Heart	16	0.134	0.108–0.160	35	0.138	0.100–0.176
Kidney	16	0.251	0.200–0.302	35	0.265	0.219–0.311
Liver	16	0.939	0.653–1.225	35	0.832	0.582–1.082
Lung	16	0.205	0.161–0.249	35	0.235	0.088–0.382
Spleen	16	0.033	0.020–0.046	35	0.030	0.011–0.049
Ovary	—	—	—	2	0.027	0.020–0.034
Adrenal	16	0.014	0.009–0.019	34	0.016	0.011–0.021
			Reproductive condition			
No. gravid	4/16	—	—	6/36	—	—
Embryos	4	3.5	2.4–4.6	6	3.8	2.5–5.2
			Hematology and differential cell counts (per 100 WBC)			
Packed cell volume (%)	10	51.9	49.1–54.7	22	51.7	47.5–55.8
Hemoglobin (g/100ml)	13	17.3	15.9–18.6	26	16.8	14.7–18.9
Erythrocytes ($\times 10^6$)	8	9.8	9.3–10.4	19	10.2	9.3–11.0
Leukocytes ($\times 10^3$)	8	13.7	4.3–23.1	19	13.0	2.3–23.8
Nucleated red cells	13	0.2	0.0–0.7	26	0.7	0.0–2.6
Basophils	13	0.1	0.0–0.3	26	0.4	0.0–1.0
Monocytes	13	2.5	0.7–4.4	26	2.5	0.0–5.2
Eosinophils	13	3.9	0.7–7.2	26	5.9	0.0–12.6
Metamyelocytes	13	0.2	0.0–0.5	26	0.2	0.0–1.2
Bands	13	1.2	0.0–2.6	26	0.8	0.0–2.5
Segs	13	16.9	3.7–30.1	26	12.2	4.8–19.6
Lymphocytes	13	54.6	36.7–72.5	26	68.8	56.5–81.1
			Organ and tissue condition[b]			
Lung	16	1.2	1.0–1.3	36	1.3	1.0–1.7
Uterus	16	1.2	1.0–1.7	36	1.1	1.0–1.3
Adrenal	16	2.1	1.3–2.8	36	1.9	1.1–2.8
Muscle	16	1.3	1.0–1.8	36	1.0	1.0–1.0

[a] Based on one standard deviation of the mean.
[b] Based on: 1 = excellent, 2 = good, 3 = fair, 4 = poor (including only the $\bar{x}$ and C.I. of those which differed).

Verification of Exposure to NPV

To verify that test mammals had ingested either infected gypsy moth larvae, NPV on vegetation, or NPV on some other food, the alimentary tracts (with contents) from 48% of the free-living mammals trapped for analysis were bioassayed against 2nd-stage gypsy moth larvae. Results of these bioassays (Table 1) indicated that a higher percentage of the treated mammals than of the controls had ingested NPV, and that the treated mammals had ingested roughly 10 times as much NPV as the control mammals. NPV in the control animals, seen clearly in the red-backed voles, resulted from a natural outbreak of NPV on the control plots.

Results and Discussion

Censused Mammals

A census conducted monthly on each plot indicated that the small mammals most common to the study area were white-footed mice, red-backed voles, and chipmunks. Raccoons, opossums, and shrews (both short- and long-tailed) were also trapped in limited numbers.

The number of white-footed mice (adults and young, Fig. 1, 2) and red-backed voles (adults and young, Fig. 3, 4) captured monthly on the 3 census plots shows the populations (late spring and early summer) of both these species on these plots. Chipmunks were caught in fewer numbers and with less consistency than mice and voles, therefore no population figures have been presented for this species. Monthly population changes of white-footed mice and red-backed voles gave no indication that the NPV sprays adversely effected the populations of either species. In fact, demographic parameters between the treated and the control mammals were similar. Populations of all species were lowest in May but increased in June after parturition. The number of white-footed mice continued to increase in each plot through the period of maximum defoliation (July census). The number of red-backed voles decreased on both the control and CIB treated plot during maximum defoliation (July census) while the number of adult red-backed voles increased slightly on the SVA treated plot.

The SVA treated plot suffered the least defoliation (16%) as compared to 39% in the control plot and 27% in the CIB treated plot (Wollam et al. 1978). The red-backed vole populations decreased less on the plot which received the least defoliation; however, compared to populations of the nocturnal white-footed mouse, which showed monthly increases (both adults and young) on all plots, the diurnal voles appeared less able to cope with the gypsy moth defoliation. The relative consistency in population trends of both white-footed mice and red-backed voles between plots indicated that these populations were not affected by the NPV application.

We compared the mean live weight of white-footed mice and red-backed voles captured on CIB and SVA plots after the 2nd NPV application. There were no significant differences between treated animals, so these data were combined and tested against those of control white-footed mice and red-backed voles. The mean live weight of mice and voles (Table 2) was based on the avg weight of individual animals caught during a census week. Analysis (*t*-tests of means) showed no statistical differences in live weight between the NPV treated and control white-footed mice and red-backed voles. Also, field notes taken on these individuals during the census periods indicated there were no gross external differences between treated and control mammals.

Pathological Examination

Table 3 shows, by sex, age, and treatment, the number of caged and field collected mammals submitted for necropsy and histopathological examination. Because no clinical standards for organ and tissue weights and hematological values were available for these species of wild mammals, the values of the control mammals were used as the standards in the analysis.

Caged Mammals

Data on the caged mammals from the CIB and SVA treated plots were combined and compared to those for caged control mammals. Of the 47 caged mammals submitted for necropsy and histopathological analysis, only the sample of young opossums (Table 4) was large enough for statistical analysis. However, the young control opossums captured for caging were significantly heavier, and contained a smaller proportion of females than the treated group. Upon final analysis these initial differences in weight, and sex accounted for the larger values of spleen weight, PCV, hemoglobin, and erythrocyte counts in the control group. However, neither these data, nor the comparison of the ranking of necropsy and histopathological comments on organs and tissues indicated that either group was in better condition.

Apart from the young opossums, the number of caged mammals in the control and treated groups: adult male white-footed mice (3 vs. 4); adult female white-footed mice (2 vs. 5); adult male opossums (1 vs. 2) and adult female opossums (1 vs. 2) was too small for statistical analysis; however, an overview of all parameters indicated that there were no radical differences in organ weight, hematological data, or necropsy and histopathological rankings between groups. Differences which did occur were considered to be related to either differences in body weight, random variation or small sample size.

Field-Collected Mammals

Examination of data on 250 field-collected mammals revealed no major differences between control and treated mammals. As with the caged mammals, data on histological, hematological parameters and organ weights were combined and compared to those of control mammals.

Table 7.—Results of necropsies and histopathological studies on field collected *Peromyscus leucopus*—young, male, and females.

	Control			NPV treated		
	No. mammals	$\bar{x}$	C.I.[a]	No. mammals	$\bar{x}$	C.I.
		Weights (g)			Weights (g)	
Total body	11	11.864	9.547–14.181	11	11.691	9.767–13.615
Heart	11	0.102	0.090–0.114	11	0.094	0.069–0.119
Kidney	11	0.183	0.145–0.221	11	0.185	0.146–0.224
Liver	11	0.485	0.369–0.601	11	0.580	0.441–0.719
Lung	11	0.131	0.096–0.166	11	0.155	0.105–0.205
Spleen	11	0.019	0.009–0.029	11	0.018	0.010–0.026
Testes	2	0.095	0.052–0.138	3	0.115	0.065–0.165
Adrenal	11	0.009	0.006–0.012	11	0.007	0.005–0.009
	Hematology and differential cell counts (per 100 WBC)					
Packed cell volume (%)	9	52.1	50.7–53.5	7	51.1	47.7–54.6
Hemoglobin (g/100ml)	8	17.9	17.3–18.5	10	17.4	16.2–18.6
Erythrocytes ($\times 10^6$)	8	9.7	8.4–11.0	8	9.9	8.8–11.0
Leukocytes ($\times 10^3$)	8	22.8	5.8–39.8	7	18.6	1.8–35.3
Nucleated red cells	8	0.5	0.0–1.5	10	1.2	0.0–2.7
Basophils	8	0.5	0.0–1.2	10	0.3	0.0–1.2
Monocytes	8	1.0	0.0–2.0	10	3.0	0.6–5.4
Eosinophils	8	4.8	0.7–8.8	10	3.5	0.5–6.5
Metamyelocytes	8	0.1	0.0–0.5	10	0.1	0.0–0.4
Bands	8	0.4	0.0–1.1	10	2.5	0.0–5.9
Segs	8	9.6	4.2–15.1	10	13.9	3.7–24.1
Lymphocytes	8	74.1	65.3–82.9	10	67.5	58.3–76.7
	Organ and tissue condition[b]					
Kidney	11	1.1	1.0–1.4	11	1.4	1.0–1.8
Liver	11	1.3	1.0–1.9	11	1.2	1.0–1.8
Lung	11	1.2	1.0–1.6	11	1.1	1.0–1.4
Brain	11	1.2	1.0–1.8	11	1.0	1.0–1.0
Adrenal	11	1.5	1.0–2.2	11	1.8	1.0–2.7

[a] Based on one standard deviation of the mean.
[b] Based on: 1 = excellent, 2 = good, 3 = fair, 4 = poor (including only the $\bar{x}$ and C.I. of those which differed).

Table 8.—Results of necropsies and histopathological studies on field collected *Clethrionomys gapperi*—adult males.

	Control			NPV treated		
	No. mammals	$\bar{x}$	C.I.[a]	No. mammals	$\bar{x}$	C.I.
		Weights (g)			Weights (g)	
Total body	10	20.830	16.041–25.619	19	20.521	14.921–26.121
Heart	10	0.153	0.123–0.183	19	0.155	0.109–0.201
Kidney	10	0.252	0.203–0.301	18	0.243	0.183–0.303
Liver	10	1.000	0.652–1.348	19	1.009	0.763–1.255
Lung	9	0.298	0.220–0.376	19	0.285	0.166–0.404
Spleen	9	0.051	0.000–0.105	19	0.039	0.009–0.069
Testes	9	0.209	0.000–0.444	17	0.085	0.000–0.225
Adrenal	10	0.006	0.005–0.007	19	0.009	0.000–0.019
	Hematology and differential cell counts (per 100 WBC)					
Packed cell volume (%)	8	50.1	46.8–53.5	7	45.7	40.9–50.5
Hemoglobin (g/100ml)	7	16.7	15.5–17.9	12	16.4	15.2–17.5
Erythrocytes ($\times 10^6$)	3	10.2	9.7–10.7	5	9.4	8.7–10.2
Leukocytes ($\times 10^3$)	3	20.1	14.2–26.0	5	9.7	5.2–14.1
Nucleated red cells	8	0.3	0.0–0.7	11	0.1	0.0–0.4
Basophils	8	0.0	0.0–0.0	11	0.0	0.0–0.0
Monocytes	8	1.6	0.3–2.9	11	2.7	0.7–4.7
Eosinophils	8	1.3	0.0–2.7	11	1.7	0.0–3.8
Metamyelocytes	8	0.0	0.0–0.0	11	0.2	0.0–0.6
Bands	8	0.0	0.0–0.0	11	0.1	0.0–0.4
Segs	8	37.1	21.2–53.1	11	26.9	8.5–45.3
Lymphocytes	8	49.6	30.1–69.2	11	64.0	42.1–85.9
	Organ and tissue condition[b]					
Kidney	10	1.6	1.0–2.4	19	1.3	1.0–2.0
Lung	10	1.6	1.0–2.6	18	1.2	1.0–1.8
Brain	10	1.2	1.0–1.6	18	1.0	1.0–1.0
Adrenal	10	1.1	1.0–1.4	18	1.1	1.0–1.6
Salivary gland	10	1.2	1.0–1.6	18	1.1	1.0–1.3

[a] Based on one standard deviation of the mean.
[b] Based on: 1 = excellent, 2 = good, 3 = fair, 4 = poor (including only the $\bar{x}$ and C.I. of those which differed).

Because the white-footed mouse is an important predator of the gypsy moth (Campbell 1967, Campbell and Sloan 1977 and Smith and Lautenschlager, in press), a large sample was collected. We felt that any effect of spraying NPV in the wild might best be observed in this abundant rodent. Subgroups of white-footed mice included adult males (Table 5), adult females (Table 6), and young mice (male and female (Table 7)). Analysis of all parameters for these 3 groups indicated significant differences ($P < 0.05$) in only one parameter, basophile levels between control and treated adult male mice. Basophile levels, which indicate the amount of histamine and anticoagulants being carried to inflamed tissues, were lower in the treated males than in the control male mice. Neither this decrease in basophile levels in the controls nor any other tested parameter indicated that treated males were less healthy than the control males.

A total of 82 free-living adult red-backed voles were collected and analyzed. No young (sub-adults or juveniles) were used for analysis. Subgroups tested included adult males (Table 8) and adult females (Table 9). No significant difference ($P > 0.05$) in any of the measured parameters was found between the control and treated voles.

The number of other free-living mammals collected for analysis, from control and treated plots (sexes combined) included: adult opossums (1 vs. 4); adult chipmunks (4 vs. 12); and adult raccoons (3 vs. 6). The sample sizes for these species were not large enough for statistical analysis; however, as in the caged mammals, an overview of all parameters (organ weights, hematological data and necropsy and histopathological rankings of the organs and tissues) indicated no radical differences between control and treated mammals. Based on our findings for the larger samples of other species of free-living mammals described above and an overview of these parameters, we conclude that any differences that did occur in these species were due to either differences in body weight, random variation, or small sample size, and not to the NPV treatment.

Conclusion

Aerial application of NPV for gypsy moth control at 2.5×10^{12} polyhedral inclusion bodies/ha produced no significant differences, in population trends, or live weights of free-living censused white-footed mice and red-backed voles between populations on control and treated plots. Also, when sample sizes were large and mean total weight similar between tested groups, no significant differences were observed in organ and tissue weights, hematological values or necropsy and histopathological rankings of caged and free-living mammals from control and treated plots. Aerial applications of NPV did not produce any short term adverse effects in mammals that contacted the virus from the sprays, eating infected gypsy moths, eating NPV on vegetation, or eating NPV on other food sources.

Acknowledgment

We thank Wayne S. Sanders, U.S. Fish and Wildlife Service; Glen L. Bowers and John S. Schuler, Pennsylvania Game Commission; and Les Johnson, Pennsylvania De-

partment of Environmental Resources, Bureau of Forestry, for permits and assistance. We also thank Harvey R. Smith, Gerald S. Walton, and Richard M. DeGraaf, Northeastern Forest Experiment Station; Swend W. Nielsen, University of Connecticut; and John L. George, Pennsylvania State University, for critical reviews of the manuscript. We especially thank Craig S. Scharf for his untiring technical assistance during all phases of this study.

Table 9.—Results of necropsies and histopathological studies on field collected *Clethrionomys gapperi*—adult females.

	Control			NVP treated		
	No. mammals	$\bar{x}$	C.I.[a]	No. mammals	$\bar{x}$	C.I.
		Weights (g)			Weights (g)	
Total body	18	21.611	17.94–25.28	32	19.094	14.18–24.01
Heart	18	0.156	0.120–0.192	35	0.155	0.117–0.193
Kidney	18	0.283	0.189–0.377	34	0.235	0.188–0.282
Liver	18	1.136	0.857–1.415	35	0.930	0.694–1.166
Lung	18	0.263	0.189–0.337	35	0.274	0.130–0.418
Spleen	18	0.058	0.000–0.128	35	0.043	0.000–0.101
Ovary	—	—	—	2	0.035	0.028–0.042
Adrenal	17	0.009	0.006–0.012	35	0.009	0.006–0.012
		Reproductive condition				
No. Gravid	2/18	—	—	0/35	—	—
Embryos	2	4.5	3.0–6.0	—	—	—
		Hematology and differential cell counts (per 100 WBC)				
Packed cell volume (%)	8	50.8	45.3–56.2	15	47.1	43.6–50.7
Hemoglobin (g/100ml)	8	16.8	14.0–19.6	14	17.1	13.3–20.9
Erythrocytes ($\times 10^6$)	3	9.9	9.3–10.5	13	8.6	6.4–10.8
Leukocytes ($\times 10^3$)	3	27.5	24.3–30.7	13	11.758	2.7–20.8
Nucleated red cells	8	0.1	0.0–0.5	14	1.5	0.0–4.8
Basophils	8	0.0	0.0–0.0	14	0.071	0.0–0.3
Monocytes	8	1.4	0.5–2.2	14	1.9	0.1–3.7
Eosinophils	8	3.4	0.0–7.4	14	1.9	0.0–4.0
Metamyelocytes	8	0.0	0.0–0.0	14	0.1	0.0–0.7
Bands	8	0.3	0.0–0.7	14	0.9	0.0–2.6
Segs	8	26.4	9.3–43.4	14	31.6	17.7–45.4
Lymphocytes	8	61.4	39.5–83.2	14	54.2	35.3–73.2
		Organ and tissue condition[b]				
Kidney	18	1.3	1.0–1.9	35	1.2	1.0–1.7
Lung	18	1.4	1.0–2.2	35	1.2	1.0–1.7
Brain	18	1.2	1.0–1.5	35	1.0	1.0–1.2
Adrenal	18	1.2	1.0–1.7	35	1.1	1.0–1.5
G.I.	18	1.2	1.0–1.7	35	1.1	1.0–1.3

[a] Based on one standard deviation of the mean.
[b] Based on: 1 = excellent, 2 = good, 3 = fair, 4 = poor (including only the $\bar{x}$ and C.I. of those which differed).

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