Response of Birds to Aerial Application of the Nucleopolyhedrosis Virus of the Gypsy Moth, *Lymantria dispar*[1,2,3]

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ABSTRACT

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Resident populations of wild birds and caged quail, *Colinus virginianus* L., were evaluated to detect 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.

Comparisons of prespray and postspray censuses of the common birds on the control and NPV-treated plots revealed no changes in populations of the wild birds that could be attributed to the NPV treatments.

Data from 23 caged quail and 53 free-living birds showed no significant differences, or differences in trends, for any species between NPV-treated and control birds in organ weights or necropsy and histopathological rankings of the condition of organs and tissues. It was concluded that the aerial application of NPV had no short-term adverse effect on birds that contacted NPV during its application or that subsequently fed on NPV-infected gypsy moths or other NPV-contaminated food sources.

The nucleopolyhedrosis virus (NPV) of the gypsy moth, *Lymantria dispar* L., is a natural component of the host's habitat, persisting naturally at high levels in soil, litter, and on bark following natural epizootics (Podgwaite et al. 1979). Lautenschlager et al. (1978) reported that the aerial application of the gypsy moth NPV on woodland plots in central Pennsylvania had no adverse effect on resident small mammals, including some important predators of the insect. However, many wild bird species, some of which also prey upon the gypsy moth (Forbush and Fernald 1896, Palmer and Fowler 1975, Smith and Lautenschlager 1978) are likely to contact NPV directly by ingesting NPV on vegetation, or by consuming infected gypsy moth larvae, pupae or adults. The beneficial effects of these species could be compromised if they responded adversely to NPV. This paper presents the results of a study to determine the demographic and pathologic changes in free-living birds and the pathologic changes in caged quail after aerial applications of NPV.

Materials and Methods

The study area, NPV formulations, and treatment methods were described by Lautenschlager et al. (1978).

Bird Census

To determine bird abundance and species composition, a "spot-mapping" census (Williams 1936, Enemar 1959, Robbins 1978) was conducted in 1975 on 2 NPV-treated plots, one with CIB (treated with NPV mixed with Cargill Insecticide Base®), and one with SVA (treated with NPV mixed with Sandoz virus Adjuvant 16-B®), and one control plot. We established a permanent 6×6 grid consisting of 36 stations spaced at 61-m intervals on the innermost 10 ha of each 14-ha plot. This allowed for the census of the total 14 ha, since the observer could hear birds singing at a distance of 30 m in any direction.

Censuses were conducted on "clear" days between 5:30 a.m. and noon. Each plot was censused at least 4 times during each of the following periods: May 20–25 (before the 1st NPV application), June 10–15 (10 days after the 2nd NPV application), and July 9–12 (during maximum defoliation, pupation, and early emergence of adult gypsy moths). No census was attempted in Aug. since most birds had stopped singing by that time. At the end of each census period, we calculated the mean number of individual male birds, by species, heard daily on the plots.

Caged Quail

Before the 1st NPV treatment (May 28), young quail, *Colinus virginianus* L., were placed in open bottom 1.2-m^3, 0.6-cm wire mesh enclosures near the center of one NPV-treated plot (CIB) and one control plot. Six female and 7 male quail were caged on the treated plot and 5 female and 5 male quail were caged on the control plot. The quail were fed a commercial pelleted poultry feed and given water daily. Quail were maintained on both plots for 3 wk following the 2nd aerial application of NPV (June 2) and were delivered to the veterinary pathology laboratory at Pennsylvania State Univ. on June 27, 1975. Quail which died before this date were delivered to the laboratory within 24 h of death.

Collecting Birds for Examination

Submitted for necropsy and histopathological examination along with the caged quail were birds that had been shot primarily within 4 treated and 2 control plots. Bird collections began one wk after the 2nd NPV application (June 3) and continued on a weekly basis through Aug. 18. Collections were restricted to those species with: (1) a history of eating significant amounts of gypsy moth larvae (Smith and Lautenschlager 1978); (2) large enough population densities to ensure that they were residents of the collection plot; and (3) similar populations on NPV-treated and control plots. After Aug. when spot-mapping had been discontinued, collections were

[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. 8, 1979.

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made from the inner 10 ha of the censused plots. Birds were shot with a 410-gauge shotgun and No. 9 shot, placed on ice, and transported within 5 h of death to Penn. State Univ.

Necropsy and Histopathology

Individual birds were coded by number, species, age, sex, and plot and examined within 24 h of arrival at the laboratory. Each bird was weighed as were each of the following organs as the necropsy progressed: heart; kidneys (with and without adrenal glands); liver; lungs; spleen; testes; ovaries; adrenals; and thyroid. The reproductive condition of female quail was noted as active or inactive. Histopathological examination noted the condition of the brain, heart, tongue, duodenum, lungs, breast muscle, skeletal muscle, liver, intestine, gizzard, kidneys, air sacs, pancreas, ureter, proventriculus, thyroid, spleen, and ovaries. The presence of parasites also was noted. Organs that were mutilated by birdshot were not weighed or examined.

Necropsy and histopathological data were analyzed by a ranking technique described by Lautenschlager et al. (1978). A total of 34 variables was analyzed for each bird. Means and confidence intervals for total body weight, the weights of individual organs, and the necropsy and histopathological ranking for the organs and tissues were analyzed for all species collected. Birds from NPV-treated plots were combined and tested against the controls. Subgroups were tested only if the sample size for both the treated and control subgroups combined contained 5 or more birds. Although *t*-tests were not always practical for all subgroups because of small sample sizes, means and confidence intervals were always examined to detect trends.

Verification of Exposure to NPV

To verify that test birds had ingested either infected gypsy moth larvae or NPV on vegetation or some other food, the alimentary tracts (with contents) from 34% of the field-collected birds were bioassayed by using methods described by Lewis and Rollinson (1978). The number of polyhedral inclusion bodies (PIB) per g of alimentary tract was estimated by extrapolation from standard NPV dose-mortality curves. Results of these bioassays (Table 1) indicated a higher percentage of the treated birds had ingested NPV, and that treated birds had ingested a larger amount of NPV than control birds. NPV in control birds resulted from a natural outbreak of NPV on the control plots.

Table 1.—Incidence and amounts[a] of gypsy moth NPV in the alimentary tracts of birds from treated and control plots.

Treatment	Birds		Amount of NPV[b]
	No.	% containing NPV	
NPV	13	92	$2.85 \pm 1.54 \times 10^{7}$ [c]
Control	5	60	$5.45 \pm 0.56 \times 10^{6}$

[a] Determined by bioassay.
[b] $\bar{x} \pm$ SE of the number of polyhedral inclusion bodies of NPV/g of alimentary tract.
[c] Different from control group ($P<0.20$).

Results and Discussion

Censused Birds

A census conducted monthly on each plot revealed the number of singing males of each species that were regularly encountered on each plot (Table 2). The trend for each species was an overall decrease in the number of birds heard singing from spring to early summer. However, this decrease did not actually represent a decrease in numbers because there was a numerical increase in fledgling young entering the population. Rather, the decrease resulted from a change in the birds' territorial behavior (singing) as well as in their response to increasing defoliation in the study plots.

Changes in numbers of individual species on different plots from May to June should have indicated any immediate effect of the NPV treatment, while population changes from June to July should have indicated the effect of the NPV treatment over a longer period (6 wk after the 2nd NPV application). Although the means for individual species recorded on the 3 census plots (Table 2) were not always analogous, overall trends indicated little difference between treated and control populations. Differences which did occur were not related to the NPV treatment, but rather to habitat and behavioral changes and normal random variation.

The most dramatic effect of a habitat change occurred in yellowthroats, *Geothlypis trichas* L., which during July reached population levels 7 times greater on the SVA plot than on either the CIB or the control plot. Yellowthroats prefer dense brushy areas; since trees and shrubs in the SVA plot were not defoliated as severely as those in the CIB and control plots (Wollam et al. 1978), it seemed that defoliation reduced the potential habitat for yellowthroats.

The effect of defoliation on the brown-headed cowbird, *Molothrus ater* Boddaert, was also noted. These nest parasites (bird species which lay eggs in the nests of other bird species) appear to have been attracted to the most defoliated plot (the control), where they could more easily locate host nests.

Although changes in species population densities did occur between plots, no significant differences were observed between treated and control plots either one or 2 mo after the NPV treatment. Differences which occurred were related either to behavior, random variation, or to the reaction of individual species to defoliation, but not to the NPV application. In fact, the NPV application, by reducing defoliation, seemed to help maintain densities of yellowthroat warblers, which are usually found in thick cover.

Necropsy and Histopathological Findings

A total of 76 caged and field-collected birds submitted for necropsy and histopathological examination was used in the analysis. Because there were no clinical standards for organ and tissue weights for these species of wild birds, the values for the control birds were used as standards in the analysis. The analysis was by species and statistical tests (*t*-test) were applied only when at least 5 individuals were in both the control and treated groups. Species and numbers (control/treated) statistically analyzed were: towhees, *Pipilo erythrophthalmus* L. (8/11);

Table 2.—Mean ± standard error of the numbers of birds heard singing on the control and treated plots.

Species	May			June			July		
	Control	CIB	SVA	Control	CIB	SVA	Control	CIB	SVA
Rufous-sided towhee[a] (*Pipilo erythrophthalmus* L.)	11.3±0.6	13.6±2.0	15.0±0.7	9.8±0.5	13.4±2.4	13.0±1.2	12.8±0.9	17.0±0.6	18.0±0.8
Ovenbird[a] (*Sciurus aurocapillus* L.)	9.8±1.5	9.2±1.1	10.4±0.8	8.3±1.5	6.4±2.0	5.8±1.1	0.5±0.5	2.0±1.0	0.8±0.5
Black-capped chickadee[b] (*Parus atricapillus* L.)	4.0±0.9	3.4±0.7	3.2±1.0	2.8±1.3	1.4±1.2	0.8±0.8	2.8±1.1	2.3±1.5	1.5±1.5
Yellowthroat (*Geothlypis trichas* L.)	3.0±1.1	2.0±0.6	0.8±0.2	2.5±0.7	1.6±0.7	2.0±1.1	0.5±0.5	1.3±0.9	7.5±2.3
Robin[b] (*Turdus migratorius* L.)	1.0±0.5	1.2±1.0	0.6±0.4	0.0±0.0	1.4±0.5	0.2±0.2	2.3±0.6	0.6±0.3	0.0±0.0
Blue jay[b] (*Cyanocitta cristata* L.)	2.8±0.2	1.0±0.6	2.2±0.4	3.3±0.9	2.6±0.7	1.5±0.7	1.0±0.4	0.7±0.3	0.5±0.5
Black-and-white warbler[a] (*Mniotilta varia* L.)	4.0±0.6	2.0±0.6	2.4±0.4	1.5±0.5	0.0±0.0	2.3±1.3	0.0±0.0	0.0±0.0	0.0±0.0
Great crested flycatcher[a] (*Myiarchus crinitus* L.)	2.3±0.7	3.2±0.9	3.0±1.0	2.0±0.8	1.2±0.5	1.8±0.8	1.0±0.4	0.3±0.3	0.5±0.5
Mourning Dove (*Zenaidura macroura* L.)	0.5±0.2	0.4±0.4	1.6±0.8	0.3±0.3	0.4±0.4	5.5±1.0	0.0±0.0	0.7±0.7	0.8±0.8
Red-eyed vireo[b] (*Vireo olivaceus* L.)	2.0±0.7	1.0±0.6	1.2±0.6	1.3±0.6	2.6±0.4	2.3±1.0	2.3±1.3	2.7±0.7	0.0±0.0
Hairy woodpecker[a] (*Dendrocopos villosus* L.)	0.5±0.2	1.6±0.4	1.4±0.4	1.0±0.4	1.0±0.3	1.3±1.0	0.5±0.3	0.7±0.3	0.3±0.3
Scarlet tanager[a] (*Piranga olivacea* Gmelin)	1.2±0.5	1.0±0.3	0.8±0.4	2,0±0.4	2.8±1.1	2.3±0.9	1.3±0.6	3.7±0.9	0.8±0.5
Cuckoos (Yellow-billed and black-billed)[b] (*Coccyzus* sp.)	0.0±0.0	0.0±0.0	0.0±0.0	3.0±0.7	4.0±0.6	4.8±2.1	0.0±0.0	0.7±0.7	0.3±0.3
White-breasted nuthatch[a] (*Sitta carolinensis* Latham)	1.3±0.2	1.2±0.6	0.0±0.0	0.3±0.3	1.8±0.7	0.5±0.5	0.0±0.0	0.7±0.7	0.0±0.0
Thrushes[a] (*Hylocichla* sp.)	0.3±0.2	0.8±0.5	0.2±0.2	1.3±1.0	2.0±2.0	0.5±0.3	4.0±0.7	0.7±0.3	0.0±0.0
Indigo bunting[a] (*Passerina cyanea* L.)	0.0±0.0	1.4±0.5	0.2±0.2	0.0±0.0	1.2±0.5	2.0±1.4	0.0±0.0	0.3±0.3	1.8±0.3
Eastern wood pewee[a] (*Contepus virens* L.)	1.3±0.6	2.2±0.5	4.0±0.8	2.8±1.1	2.0±0.9	2.0±0.9	2.5±1.3	0.7±0.7	2.8±1.3
Brown-headed cowbird[a] (*Molothrus ater* Boddaert)	1.0±0.6	0.4±0.4	0.6±0.4	3.8±1.1	2.6±1.1	4.3±1.2	3.5±1.3	1.0±1.0	1.3±0.5

[a] Birds reported to have eaten gypsy moth larvae, pupae, or adults.
[b] Birds reported to have eaten significant amounts of gypsy moth larvae, pupae, or adults.

Table 3.—Results of necropsies and histopathological studies on caged quail.

	Male		Female	
	Control	Treated	Control	Treated
	Weight (g)		Weight (g)	
	$\bar{x}$ ± SE (no.)	$\bar{x}$ ± SE (no.)	$\bar{x}$ ± SE (no.)	$\bar{x}$ ± SE (no.)
Total body	181.10±6.23 (4)	180.90±4.67 (7)	193.70±4.90 (4)	227.30±8.50 (6)
Heart	0.91±0.07 (5)	1.05±0.09 (7)	0.91±0.07 (5)	1.12±0.08 (6)
Kidney only	1.19±0.06 (4)	1.31±0.06 (5)	1.37±0.11 (5)	1.89±0.13 (5)
Kidney and adrenal	1.30 (1)	1.47±0.09 (2)	—	—
Liver	2.82±0.27 (5)	3.17±0.16 (7)	3.34±0.28 (5)	5.53±0.57 (5)
Lung	1.08±0.08 (5)	1.28±0.07 (7)	0.80±0.04 (5)	1.09±0.12 (6)
Spleen	0.04±0.01 (5)	0.04±0.01 (7)	0.05±0.01 (5)	0.06±0.01 (5)
Testes	0.93±0.16 (5)	1.19±0.17 (7)	—	—
Ovary active			4/5 80% active	5/6 83% active
Adrenal	0.020±0.004 (4)	0.025±0.002 (5)	—	—
Thyroid	0.024±0.004 (5)	0.026±0.003 (6)	0.025±0.008 (4)	0.024±0.002 (5)
	Organ and Tissue Condition[a]			
Nutritional condition	2.0±0.0 (5)	1.9±0.1 (7)	1.8±0.2 (5)	1.8±0.2 (6)
Brain	2.0±0.0 (5)	2.3±0.3 (7)	2.0±0.1 (5)	2.1±0.1 (6)
Lung	2.6±0.5 (5)	2.1±0.2 (7)	3.0±0.5 (5)	2.2±0.1 (6)
Liver	2.6±0.2 (5)	2.6±0.3 (7)	3.2±0.5 (5)	2.2±0.1 (6)
Pancreas	2.0±0.0 (5)	2.1±0.2 (7)	2.0±0.2 (5)	2.0±0.1 (6)
Ovary	—	—	2.2±0.2 (5)	2.0±0.0 (6)

[a] Based on: 1 = excellent, 2 = good, 3 = fair, 4 = poor

chickadees, *Parus atricapillus* L. (5/7); and quail (10/13). No statistical analysis was attempted on data from the species which had smaller sample sizes, (catbirds, *Dumetella carolienesis* L. (3/2); blue jays, *Cyanocitta cristata* L. (3/3); and robins (3/8), but means and confidence intervals for these species were examined to detect trends. The analysis showed no significant differences in organ weights or the necropsy and histopathological rankings of the organs and tissues between NPV-treated and control quail (Table 3), towhees, or chickadees. Comparisons of trends in histopathological data not statistically analyzed revealed no differences between treated and control birds.

To reveal histopathological abnormalities that may have been masked by a limited number of observations on an individual species, data for all treated and control birds (regardless of sex and species) were combined. There were no significant histopathological differences between the treated and control group.

Conclusion

Population trends, as indicated by the monthly bird censuses, indicated no significant differences between the treated and control plots either one or 2 mo after the NPV treatments. Analysis of the necropsy and histopathological findings revealed no significant differences between the NPV-treated and control birds, indicating that the NPV treatment had no adverse effect on the caged quail or on resident birds. Therefore we conclude that aerial applications of NPV did not have a short-term adverse effect on birds that contracted NPV from the sprays, or eating NPV on or in their foods.

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