# Hydraulic Spray Application of Gypchek as a Homeowner Control Tactic Against Gypsy Moth (Lepidoptera: Lymantriidae)[1]

R. E. Webb, J. D. Podgwaite[3], M. Shapiro[4]
K. M. Tatman[5], and L. W. Douglass[6]

Insect Chemical Ecology Laboratory, PSI, ARS-USDA, BARC-East
Beltsville, MD 20705



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**ABSTRACT** Gypsy moth, *Lymantria dispar* L., nuclear polyhedrosis virus (NPV) was applied by ground equipment at the rate of $2.5 \times 10^{12}$ polyhedral inclusion bodies (PIB's) per ha to the lower half of ten trees (per plot) in homeowner-sized plots in Hardford and Baltimore Counties, MD, in 1986. A laboratory bioassay of field collected larvae indicated that a highly significant ($P < 0.001$) increase in early season mortality of gypsy moth larvae due to NPV occurred in the zone of spray, compared to mortality in a similar foliage zone in untreated plots. Late-season treatment effects varied greatly, in apparent response to significant ($P < 0.05$) area effects.

**KEY WORDS** Biological control, gypsy moth, *Lymantria dispar,* nuclear polyhedrosis virus, microbial pesticide, *Ooencyrtis kuvanae, Cotesia melanoscela.*

The gypsy moth, *Lymantria dispar* L., is a serious forest pest, with recent timber losses in Pennsylvania totaling $266,000,000 (Division of Forest Pest Management, Commonwealth of Pennsylvania, 1985). It is at the forest/urban interface, however, that the gypsy moth problem has been and will continue to be most serious, due to the greater value of a tree around a home (Payne et al. 1973; Moeller et al. 1977), and the greater propensity for older, specimen oaks to die after a defoliation (Kegg 1971; Stalter and Serrao 1983). One problem facing homeowners is how to deal with gypsy moths in large shade trees. Spray equipment available to homeowners, or even communities, can reach about 10 m into trees and are typically 20 - 25 m in height. Commercial arborists are available with large hydraulic sprayers reaching 30 m in height, or the community can be aerially sprayed. However, options available for a homeowner to combat gypsy moth without resorting to outside help are limited. Effective homeowner treatments for individual trees include barrier banding of trees (Blumenthal 1983; Webb and Boyd 1983; Blumenthal and Hoover 1986) and tree implantations or injections with systemic insecticides (Webb et al. 1988). However, the use of an infectious agent would be advantageous if spread from the treated zone could be demonstrated.

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[3] Center for Biological Control, Forest Service-USDA, 51 Mill Pond Rd., Hamden, CT 06514.
[4] Insect Pathology Laboratory, ARS-USDA, Bldg. 011A, BARC-West, Beltsville, MD 20705.
[5] Maryland Department of Agriculture, 50 Harry S. Truman Parkway, Annapolis, MD 21401.
[6] Department of Animal Science, University of Maryland, College Park, MD 20742.

The present study evaluated a novel approach that might be utilized by a homeowner to control gypsy moth. This would involve applying Gypchek®, by ground equipment, to the lower portion of a limited number of shade trees. Gypchek is a nuclear polyhedrosis virus (NPV) product registered by the U. S. Forest Service with the U. S. Environmental Protection Agency (EPA Registration No. 27586-2) for gypsy moth control. In nature, gypsy moth larval mortality caused by NPV often occurs in a bimodal temporal pattern (Woods and Elkinton 1987), with a small 'first wave' of NPV-induced mortality occurring early in the season to young larvae (instars 1-3) typically followed later in the season by a larger 'second wave' of NPV-induced mortality for older larvae (instars 4-6). Our study was designed to determine if NPV (applied as Gypchek) spreads from zones of application to suppress gypsy moth populations throughout a homeowner-sized plot. Our hypotheses were the following: (1) Gypchek would provide direct early-season suppression of gypsy moth larvae feeding on foliage on which the sprays were directly applied (the lower half of treated trees); (2) the virus would spread from the treated lower half of the tree to suppress the larval population throughout the tree; and (3) the NPV would spread from the treated trees throughout the plot; The agents of spread would be (1) drift from the spray application, and (2) intra- and inter-tree movement of infected caterpillars. These caterpillars would die about 14 days after ingestion of the virus, would lyse (Glaser 1915) and release new virus, and would thus provide inoculum that would augment any naturally occurring NPV, resulting in an enhanced second wave of disease.

## Materials and Methods

Eight study sites were established in the spring of 1986 in northern Maryland: four in Harford County and four in Baltimore County. These sites, 0.4 ha or less, were either isolated tree stands or discrete portions of somewhat larger isolated stands. Treatments were assigned to a site by a randomized block design whereby a site to be treated was paired with a control site on the basis of proximity. No site was within 1 km of another site, so that interaction among sites was unlikely. The number of trees per site varied from 13 to 69, the mean diameter at breast height (dbh) for trees in a site ranged from 1.3 to 4.6 m, with total dbh of these trees ranging from 30 to 137 $m^2$. The percent dbh represented by oak (*Quercus* spp.) varied from 68% to 96%.

For the preseason egg mass evaluation, all egg masses on all trees or in the ground litter were counted in four randomly selected 0.01 ha subplots at each site, representing 10% or more of the total area of the plot. In early April, five egg masses were randomly sampled from each site and returned to the laboratory. The eggs were allowed to hatch, and data were taken on number of eggs per mass, % hatch, and % parasitism by *Ooencyrtis kuvanae* (Howard). To evaluate natural NPV loads present on the sample egg masses, 40 newly-hatched larvae from each mass (= 200 per plot) were reared, one per cup, in 30cc plastic cups with snap-on plastic lids. The cups contained ca. 15 ml ICN High Wheat Germ Diet for Gypsy Moth (Bell et al. 1981). Each lid was punctured five times with a standard probe to provide air exchange and to retard excessive buildup of moisture. All larvae were rated for NPV-induced mortality after 21 days on the diet. Larval death was assumed to be due to NPV if the larvae were lysed, and due to other causes if not. A representative sample of lysed larvae were examined by light microscope to confirm the presence of polyhedral inclusion bodies (PIB's) in such larvae. Postseason egg mass counts were made in the same subplots as the preseason counts. Ten accessible egg masses (from the lower tree trunks) were randomly sampled from each of the sites in early August and brought back to the laboratory where the number of eggs per mass was determined.

NPV was applied at the rate of $2.5 \times 10^{12}$ polyhedral inclusion bodies (PIB's) per ha in water with Rhoplex B-60A sticker added at 2% (vol/vol). Sprays were applied on 7 May using a Smithco® hydraulic sprayer with a 1135-1 tank with an FMC® gun at 28 $kg/cm^2$ pressure. Only the bottom half of each of 10 trees was sprayed (to a height of ca 11 m) in each of four plots, with each tree receiving ca 38 l of spray mixture. In the four matched control sites, 10 trees were designated as sample trees and evaluated throughout the study in the same manner as the treated trees in the spray plots.

The direct mortality caused by the applied NPV (augmented by any natural NPV present in the plots) was determined by a bioassay of larvae occurring naturally in the plots. Thirty first instars were sampled from the lower foliage of the 10 sprayed trees (treated plots) and from the 10 sample trees (control plots), three larvae per tree, on the day of treatment, just prior to treatment (treated plots), or later that day (control plots) for a pretreatment bioassay. For a post-reatment bioassay, a similar collection of larvae was made three days after treatment (first instars) and also 10 days after treatment (second instars). All collected larvae were reared, one per cup, as in the previously mentioned bioassay of larvae from sampled egg masses, except in this case they were rated for NPV-induced mortality after 14 days. Late season NPV prevalence was monitored by weekly burlap-band larval counts. Comparative burlap counts of gypsy moth life stages have been used as indicators of treatment suppression in studies involving aerially applied formulations of *Bacillus thuringiensis* Berliner (Yendol et al. 1973) and aerially applied NPV (Wollam et al. 1978). Each treated tree (treated plots) or sample tree (control plots) had a burlap band placed at 1.5 m on the bole. The bands were approximately 0.3-m wide, were folded once, were wrapped completely around the tree, and were slit two or three times to facilitate monitoring gypsy moth life stages collecting under the bands. Data were recorded, at weekly intervals beginning 23 May and ending 31 July, of all live larvae and pupae, which were then left unmolested under the burlap. All dead larvae were recorded by apparent cause of death and removed. Larvae were assumed to be killed by NPV if lysed, by other causes if not. Similarly, all cocoons of the early-season parasitoid *Cotesia melanocela* (Ratzeburg), dead gypsy moth pupae, and shed gypsy moth pupal cases were counted, recorded, and removed. Populations peaked in late June. Relative population density was expressed as the peak number of immatures (live or dead larvae or pupae) found under the bands of a given plot on any date. An index (the 'virus index' of Webb et al. 1989) for the relative late season NPV-induced mortality was computed for each plot by the formula: virus index = (number killed by NPV/peak life stage count) $\times$ 100. Defoliation was estimated at peak defoliation of all treated and sample trees by two trained observers.

Data were converted to $\log_{10}$ (count + 1), while all percentages were arcsine square root transformed before being analyzed using analysis of variance (ANOVA). Means were separated using a protected least significant difference test at the P = 0.05 level (SAS Institute 1985).

## Results and Discussion

**Preseason and Pretreatment Site Measurements.** The study was well balanced for plot size, preseason egg mass numbers, and egg mass size, with ANOVA area effects, treatment effects, and interactions all nonsignificant at P = 0.05 (Table 1). The bioassay of larvae hatching in the laboratory from the sampled egg masses indicated that NPV titers on the egg masses were low, with virus-induced mortality averaging 2% for larvae from egg masses sampled from plots to be treated versus 4% for those from control plots, with all ANOVA effects nonsignificant. Egg parasite (*O. kuvanae*) numbers were high (10 - 20%) for egg masses sampled from all plots, averaging 157 per mass.

**Pretreatment and Posttreatment Larval Bioassays.** The pretreatment bioassay of larvae sampled from the plots demonstrated that the natural NPV levels were low (5% mortality for larvae sampled from treated plots versus 8% for those from control plots) (Table 2). Treatment, area, and area-treatment interaction effects were all nonsignificant, demonstrating that there was no pattern to the low levels of NPV detected by the pretreatment larval bioassay.

The posttreatment bioassay (3-day and 10-day samples combined) of larvae sampled from the lower foliage of NPV-treated trees or control trees provided our estimated early season mortality due to natural NPV-levels (control plots) or due to natural NPV + applied NPV + interactions (treated plots). Mortality due to NPV averaged 80% in treated plots and 9% in control plots, with treatment effects significant ($F = 164.71$, df = 1, $P < 0.001$), and area effects significant ($F = 10.86$, df = 1, $P < 0.030$), but with treatment-area interaction effects nonsignificant (Table 2). Mortality due to NPV was significantly higher in the posttreatment bioassay for larvae sampled from the Harford County treated plots (93.8%) than for those from Baltimore County treated plots (66.3%); the treated plots (both areas) had significantly higher mortality levels than for larvae sampled from the corresponding untreated plots (11.8% and 8.4% respectively, for the Harford County and Baltimore County control plots) (Table 2). Harford County plots were sprayed in the morning under calm conditions while the Baltimore County plots were treated in the afternoon under windy conditions (wind speeds unmeasured). We felt at the time of spray that the windy conditions would help spread the virus throughout the plots; however, the results of our bioassay indicated that any such advantage was offset by decreased effectiveness in the zone of the spray. Thus, while our first hypothesis, that direct suppression would be otained in the zone of spray, was supported by the results of our posttreatment bioassay; these results suggest the desirability of applying the sprays under calm conditions.

**Late-season burlap counts.** Our second hypothesis was that the virus would spread from the treated lower half of the tree to infect the gypsy moth larvae throughout the tree. If speard occurred, it should be manifested by a higher percentage of large (instars 4-6) larvae dying of NPV under the burlap bands than occurred in comparable control plots. This was not seen in Harford County treated plots, possibly because the high initial kill left too few survivors to transmit the disease. However, a significant increase ($F = 25.10$; df = 1; $P < 0.038$) in late season virus levels was seen in the Baltimore County plots, possibly because the less effective initial treatment left enough survivors to transmit the disease. Late season NPV-induced mortality, as represented by the computed virus indices (number of larvae killed by NPV under burlap bands indexed against the peak

**Table 1. Plot size, pre- and postseason egg mass numbers (and number of eggs per mass), and virus load of preseason egg masses, for plots treated with Gypchek or left untreated as controls, Baltimore County and Harford County, MD, 1986.**

| Treatment | Rep (County) | Plot size (ha) | Egg Masses per 0.04 ha* Pre-season | Post-season | Preseason egg masses $\bar{x}$ eggs | (n = 5) %NPV† | $\bar{x}$ eggs, postseason egg masses (n = 10) |
|---|---|---|---|---|---|---|---|
| Gypchek | I (Harford) | 0.4 | 40 | 70 | 423 | 0 | 572 |
| (2.5 × $10^{12}$ | II (Harford) | 0.3 | 96 | 6 | 363 | 2 | 530 |
| PIB's/ha | III (Baltimore) | 0.3 | 52 | 714 | 507 | 1 | 361 |
| | IV (Baltimore) | 0.2 | 231 | 939 | 447 | 5 | 318 |
| | Avg. | 0.3 | 105 | 432 | 435 | 2 | 445 |
| Controls | I (Harford) | 0.4 | 400 | 1,966 | 426 | 1 | 516 |
| | II (Harford) | 0.2 | 150 | 1,040 | 302 | 11 | 424 |
| | III (Baltimore) | 0.2 | 32 | 1,467 | 475 | 1 | 400 |
| | IV (Baltimore) | 0.4 | 33 | 146 | 444 | 2 | 372 |
| | Avg. | 0.3 | 154 | 1,155 | 412 | 4 | 428 |
| Significant by effect‡: | | NS | NS | NS | NS | NS | NS |
| | Area: | NS | NS | NS | NS | NS | NS |
| | Treatment: | NS | NS | S | NS | NS | S |
| | A × T: | | | | | | |

* Total egg masses counted in four 0.01 ha subplots

† % Mortality (due to NPV) of 40 larvae bioassayed from each of 5 egg masses (n = 200) per plot.

‡ Analysis of variance, $P \leq 0.05$, S = effect is significant at P = 0.05, NS = nonsignificance.

**Table 2. Bioassay results (% mortality due to NPV) of larvae sampled from the plots pretreatment and posttreatment, late season burlap bands counts (peak number of immatures, number of larvae dying of NPV, and the calculated virus index), and % defoliation, from plots treated with Gypchek or left untreated as controls, Baltimore County and Harford County, MD, 1986.**

| | | Bioassay (% mort., NPV) | | Life stages under burlaps | | | |
|---|---|---|---|---|---|---|---|
| Treatment | Rep (County) | Pre-treatment (n = 30) | Post-treatment (n = 60) | Peak no. immatures | No. killed, NPV | Virus index* | % defoliation |
| Gypchek | I (Harford) | 3 | 94 | 1,412 | 20 | 1.4 | 7 |
| (2.5 × $10^{12}$ | II (Harford) | 3 | 94 | ,312 | 3 | 1.0 | 2 |
| PIB's/ha | III (Baltimore) | 10 | 55 | 8,773 | 1,475 | 16.8 | 23 |
| | IV (Baltimore) | 3 | 78 | 7,163 | 1,997 | 27.9 | 31 |
| | Avg. | 5 | 80 | 4,415 | 874 | 11.8 | 16 |
| Controls | I (Harford) | 7 | 10 | 9,267 | 302 | 3.3 | 10 |
| | II (Harford) | 7 | 14 | 4,976 | 138 | 2.8 | 5 |
| | III (Baltimore) | 10 | 7 | 7,798 | 1,090 | 14.0 | 35 |
| | IV (Baltimore) | 7 | 6 | 3,285 | 376 | 11.4 | 20 |
| | Avg. | 8 | 9 | 6,332 | 477 | 7.9 | 18 |
| Significant by effect‡: | | | | | | | |
| Area: | | NS | S | NS | S | S | S |
| Treatment: | | NS | S | NS | NS | NS | NS |
| A × T: | | NS | NS | S | S | NS | NS |

* Virus index = (number killed by NPV ÷ peak number of immatures) × 100.

† Analysis of variance, $P \leq 0.05$, S = effect is significant at $P = 0.05$, NS = nonsignificance.

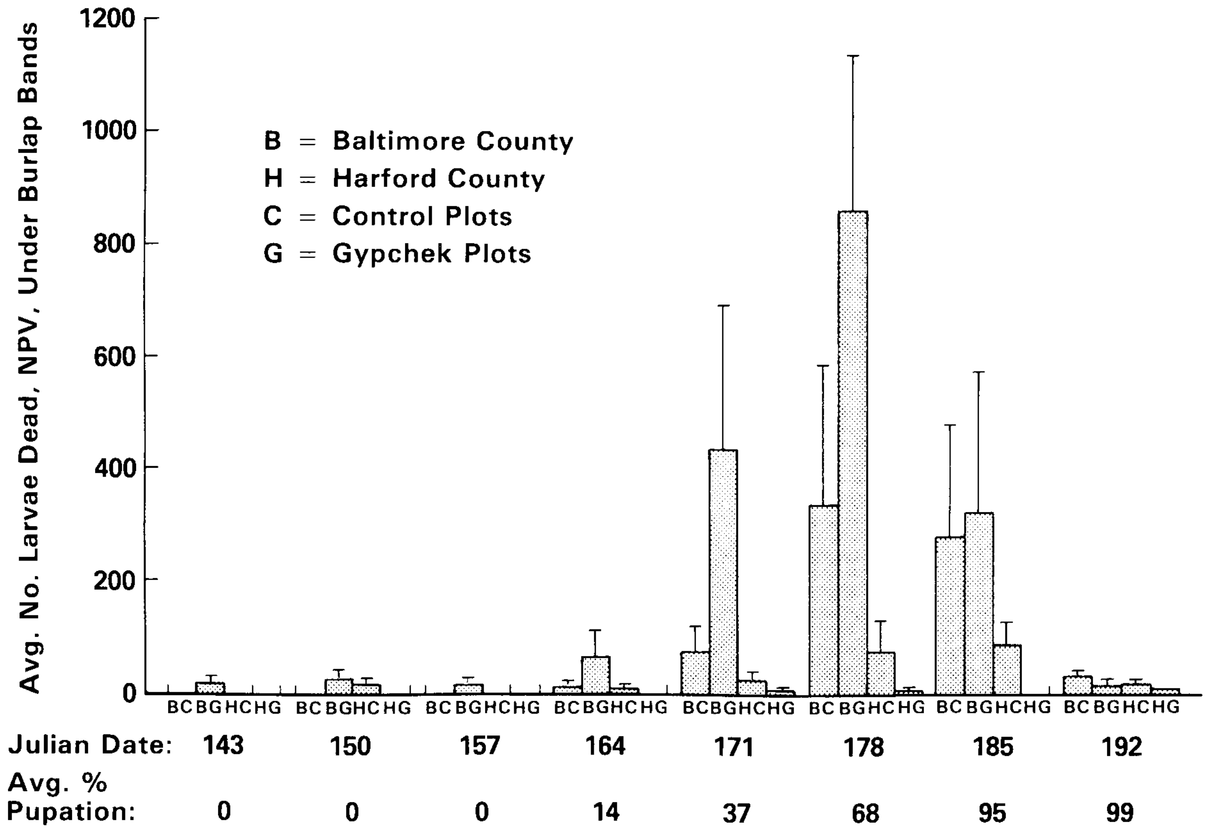


Fig. 1. Progression over time of gypsy moth pupation, and of the second wave of NPV-induced gypsy moth larval mortality, in Gypchek-treated or control plots in Harford or Baltimore counties in 1986.

number of immatures counted under burlaps) for the plots, differed in intensity according to area (area $F = 64.40$; df = 1; $P < 0.0013$) but not treatment (treatment effects nonsignificant at $P = 0.05$). The higher virus kill in the Baltimore County treated plots was associated with an earlier onset of late season NPV mortality (Fig. 1) that occurred a week before the second wave occurred in the corresponding untreated plots, at the time of first pupation. If gypsy moth larvae infected with NPV manage to pupate before succumbing to the virus, some will die as pupae, but many will survive to adulthood (Bakhvalov et al. 1982; Shapiro and Robertson 1987). The later onset of the second wave in the Baltimore County treated plots allowed a greater percentage of the population to escape (into the pupal stage) the effect of virus. The results suggest that late season 'second wave' responses to applied NPV are quite variable, which agrees with the findings of Webb et al. (1989).

**Postseason egg mass numbers.** The egg mass survey points were independent of the treated trees, and thus the relative increase or decrease of egg mass numbers (preseson versus postseason), in treated plots versus control plots, provided a test of our third hypothesis, that the virus would spread from the treated trees and suppress gypsy moth population throughout the plot. The postseason egg mass results are given in Table 1. Perhaps reflecting the variable early and late season NPV occurrences, area-treatment interactions were significant ($F = 7.84$; df = 1; $P < 0.049$). The Harford County treated plots had significantly fewer egg masses than did their corresponding untreated plots (Table 3), but the Baltimore County treated plots did not have significantly fewer egg masses than their corresponding untreated plots. Thus, the inconsistent results do not permit conclusions to be drawn about hypothesis 3.

**Other mortality factors.** Parasitism due to *C. melanoscela* averaged 14.6 and 38.9 cocoons per burlap band in treated and untreated plots, respectively. Gypsy moth pupal mortality (all sources) averaged 26.8 and 47.3 pupae per burlap band in treated and control plots, respectively. Webb et al. (1989) found that treatment with NPV for gypsy moth control severely reduced levels of *C. melanoscela* in the treated plots. Other mortality factors were negligible. The compensatory mortality due to *C. melanoscela* and pupal mortality factors, with higher levels seen in control plots than treated plots, reduced the apparent spray efficacy.

**Percent defoliation.** As seen in Table 2., % defoliation was related to area rather than to treatment, and this is reflected by a significant area effect ($F = 24.44$; df = 1; $P < 0.008$), with other effects being nonsignificant. Defoliation ranged from 2 - 10% in Harford County plots and from 20 - 35% in Baltimore County plots. Defoliation was low in Harford County even in untreated Plot I, where peak number of immatures averaged 927 per burlap, and 1,966 egg masses were counted postseason in the four 0.01 ha egg mass survey subplots. This does not seem to reflect tree size or species composition, because treatment, area, and interaction effects were all nonsignificant for tree number, tree size, and % oak. We have no explanation for this counterintuitive result.

**Eggs per mass, postseason.** The effects for number of eggs per mass, postseason, were significant for area-treatment interaction ($F = 23.86$; df = 1; $P < 0.039$). Egg masses were significantly larger in Harford County treated plots than in other plots, at least partially negating the control achieved by the sprays (Table 3). The significantly lower number of eggs per mass in all Baltimore County plots may represent stress due to the higher NPV titers seen in Baltimore County plots versus Harford County plots, or a response to defoliation-induced changes in leaf quality as postulated by Lance et al. (1986).

**Table 3.** Averages (n = 2) for plot size, pre- and postseason egg mass numbers (and numbers of eggs per mass), bioassay results (% mortality due to NPV) of larvae sampled from the plots pretreatment and posttreatment, late season burlap counts (peak number of immatures, number of larvae dying of NPV, calculated virus index), and % defoliation, from plots treated with Gypchek or left untreated as controls, grouped by area and by treatment, Baltimore County and Harford County, MD, 1986.

| | Controls | | Gypchek | |
|---|---|---|---|---|
| Parameter* | Baltimore Co. | Harford Co. | Baltimore Co. | Harford Co. |
| Plot size (ha) | 0.28 | 0.28 | 0.26 | 0.36 |
| Preseason egg masses per 0.04 ha | 32.4 | 275.2 | 141.6 | 68.0 |
| Eggs per mass, preseason | 460 | 364 | 477 | 393 |
| Postseason egg masses per 0.04 ha | 808ab | 1,504a | 828a | 38b |
| Eggs per mass, postseason | 386a | 470b | 340a | 551c |
| Pretreatment larval bioassay, % mortality due to NPV | 8.4 | 6.7 | 6.6 | 3.3 |
| Posttreatment larval bioassay, % mortality due to NPV | 8.4a | 11.8a | 66.3b | 93.8c |
| Burlap counts: | | | | |
| Peak number of immatures | 5,541a | 7,121a | 7,968a | 862b |
| Number dead, NPV | 997a | 220a | 1,736a | 12b |
| Virus index† | 12.7a | 3.1b | 22.4a | 1.2b |
| % defoliation | 27.5a | 7.5b | 27.0a | 4.5b |

* Means within rows, followed by the same letter, are not significantly different ($P < 0.05$), according to the Least Squares Difference test. Parameters without letter had nonsignificant F's.

† Virus index = (number killed by NPV ÷ peak number of immatures) × 100.

**Overall Assessment.** The average level of mortality estimated in the zone of treatment, 80%, is sufficient to prevent significant defoliation in most situations. However, numerous residual egg masses may remain unless late season natural enemies, such as a late season epizootic of NPV, provide further population reduction. The present study agrees with results from our previously published study (Webb et al. 1989) that a late season NPV epizootic often, but not always, follows application of gypsy moth NPV. Therefore, Gypchek should be applied to maximize its immediate, first wave, effectiveness, since a late season epizootic cannot be reliably assumed.

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