Efficacy of Aerially-Applied Gypchek against Gypsy Moth (Lepidoptera: Lymantriidae) in the Appalachian Highlands[1, 2]

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ABSTRACT Gypsy moth, *Lymantria dispar* L., populations in six northern Virginia plots were aerially treated with the nucleopolyhedrosis virus product, Gypchek. Two applications of an aqueous Orzan LS-Pro Mo-Rhoplex B60A tank mix, each at 18.7 liters and 1.25×10^{12} polyhedral inclusion bodies per ha, reduced larvae by more than 92% and egg masses by more than 94% in all but one of the treated plots. Defoliation averaged 22% in Gypchek-treated plots compared to 67% in control plots.

KEY WORDS Gypchek, nucleopolyhedrosis virus, gypsy moth, *Lymantria dispar*.

The gypsy moth, *Lymantria dispar* L., has been endemic in the northeastern United States for many years and is steadily expanding its range south and west. The pest is now firmly established in the Appalachian mountains of Virginia and West Virginia and threatens the southern hardwood forests beyond. In response to the threat, the U. S. Forest Service initiated the Appalachian Integrated Pest Management (AIPM) Gypsy Moth Project (USDA 1989). A major objective of AIPM is to evaluate environmentally sound intervention tactics to minimize the spread and impact of gypsy moth within the project area.

After several years of intensive research and development (Lewis and Yendol 1981), the gypsy moth nucleopolyhedrosis virus (NPV) product, Gypchek, was shown to be effective in reducing gypsy moth populations in both ground- and aerial-application field tests in Maryland (Webb et al. 1989, 1990; Podgwaite et al. 1987, 1991). The aerially-applied tank mix contained an effective sunscreen, Orzan LS (ITT Rayonier, Inc., Seattle, WA) (Podgwaite and Shapiro 1986), a protein-molasses feeding stimulant/humectant, Pro Mo liquid supplement (Southern States Cooperative, Inc., Richmond, VA), a sticker, Rhoplex B60A (Rohm and Haas Co., Philadelphia, PA), and technical Gypchek (U. S. Forest Service, Hamden, CT) prepared from an aqueous extract of NPV-killed gypsy moth larvae. The aerial applications in Maryland were done over relatively flat terrain at elevations between 15 and 180 m above sea level. The establishment

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of gypsy moth populations in the rugged mountainous terrain (peak elevations of 450-800 m) of northern Virginia allowed us to further evaluate the Gypchek tank mix under geographical and meteorological conditions prevalent within much of the AIPM project area. This is a report of that evaluation.

Materials and Methods

Study location and design. The study area was the valley between Little Sluice and Little North Mountains in the George Washington National Forest north of Columbia Furnace, Shenandoah County, VA. The gypsy moth had first infested the general area in the two years previous to this study, and populations were causing moderate to severe defoliation. In March and April 1988, twelve 12-ha plots, separated by at least 325 m, were established along National Forest Road 88. All plots were similar in stand composition (mixed oaks, *Quercus* sp.) and understory vegetation, but varied in elevation and aspect. Plots were paired based on similar gypsy moth egg mass densities, and each plot within a pair was randomly assigned either Gypchek treatment or no spray treatment (control). Since egg mass densities ranged between ca. 1250 and 12500 per ha., the pairing assured a representative distribution of both treatments across the density. The treatments were evaluated on the basis of population trend (the ratio of egg mass counts after treatment to those before treatment), changes in larval density within the treatment plots, and defoliation of selected trees within the treatment plots.

Formulation and application. Gypchek was from production lot LDP 226 [3.47×10^{10} polyhedral inclusion bodies (PIB) per g]. Activity of this lot was estimated to be 5.4×10^{6} Gypsy Moth Potency Units per g after bioassay in second instar laboratory strain (New Jersey) gypsy moth larvae. The tank mix was essentially the same as that used in the Maryland field test in the previous year (Podgwaite et al. 1987) and contained per liter: Orzan LS, 60g (6.0% wt/vol); Pro Mo liquid supplement, 125 ml (12.5% vol/vol); Rhoplex B60A, 20 ml (2.0% vol/vol); Gypchek, 1.9 g; stream water (pH 7.8), 855 ml (85.5% vol/vol).

Aerial applications began when the average expansion of white oak (*Quercus alba* L.) foliage was ca. 20%, and most gypsy moth larvae were late-first instars. Because larval development was advanced in the plots at the lower elevations, applications to several plots were done sooner than the standard recommendations of spraying when white oak foliage is $\geq$ 50% expanded and the majority of the larvae are in the second instar. A 600 hp Grumman AgCat equipped with 8 Micronair AU5000 Mini Atomizers [Micronair (Aerial) Ltd., Isle of Wight, England) made two applications per plot. Each atomizer was adjusted (blade angle 55°) to generate droplets 250-350 nm volume median diameter at an airspeed of 160 km/h. The spray system was calibrated to deliver 1.25×10^{12} PIB in 18.7 liters per ha over a 23 m wide swath. Spraying was done on 8 and 11 May 1988 between 0600 and 0800 EDT. Conditions on both mornings were dry, (45-75% RH), bright, and cold (7 to 14C) with intermittent southerly winds $\leq$ 19km/h. Kromekote cards (Mead Corp., Dayton, OH) were used to monitor drift and confirm deposit into Gypchek-treatment plots. No attempt was made to quantify the deposit per plot.

Egg mass estimates. A 4 × 5 grid of 20 fixed-radius 0.01-ha subplots was established in the center of each 12 ha plot. The grid design ensured representative posttreatment evaluations of an inner 4.4-ha core of each Gypchek and control plot that was protected from late season re-invasion of gypsy moth from adjacent, untreated forest. All new egg masses occurring on any surface within the cylinder determined by the subplot circumference were counted. New egg masses within reach were verified by palpation; those out of reach were verified using binoculars to differentiate new, light brown masses from old, gray masses. After-spray/before-spray egg masses counts in Gypchek and control plots were compared by a nonparametric sign test (Lehmann and D'Abrera 1975) appropriate for the paired-plot design. Abbotts (1925) method was used to estimate percent population reduction due to Gypchek treatment.

Larval reduction estimates. A 0.09m^2 burlap flap was attached at breast height to each of two oak trees (*Quercus* sp.) located at least 15 m from the center of each 0.01ha subplot. NPV-killed and live larvae under flaps were counted twice weekly, beginning when the majority of larvae were late third-instars and ending when all surviving larvae had pupated. Percentage larval reduction in Gypchek-treated plots, relative to reductions in control plots, were calculated using Abbott's (1925) method. Incidence of NPV-killed larvae occurring under burlap over time were compared using a nonparametric sign test, as referenced above.

Defoliation estimates. Defoliation of the two burlap-flapped trees was measured after all surviving larvae had pupated but before trees had refoliated. Estimates were made in 10% increments with the aid of binoculars and were based on the entire foliage on each tree. The nonparametric sign test, referenced above, was used to detect differences in defoliation between Gypchek-treated and control plots.

Results and Discussion

Applications of Gypchek were done under atmospheric conditions and temperatures generally considered to be less than ideal, i.e., turbulent and cold. Nevertheless, spray deposit in Gypchek-treated plots was considered acceptable, as judged from visual observations of kromekote cards placed in plots the morning of each application. There was no spray deposit on cards similarly placed in control plots.

The evaluation of data was complicated by naturally occurring NPV-induced larval mortality in control plots. Before treatment, egg mass densities ($\bar{X} \pm$ SEM) in Gypchek-treated plots and in control plots were similar; 6192 ± 1605 and 6155 ± 1685 per ha, respectively. After treatment, egg mass densities averaged 50 ± 22 ha in Gypchek-treated plots and 1415 ± 551 in control plots (Table 1). Though several control plots suffered a precipitous drop in egg mass density, an analysis of population trend (pretreatment/posttreatment egg masses) revealed a significant treatment effect ($P < 0.016$). Further, despite the naturally occurring NPV-caused mortality, net egg mass population reduction ($\bar{X} \pm$ SEM) (Abbott 1925) due to sprayed virus ranged from 94.0 ± 3.0 to 99.3 ± 0.6% in all but one (27.1 ± 36.2%) of the Gypchek-treated plots (Table 1).

Table 1. Pre- and Posttreatment egg mass densities* per ha and percentage egg mass population reduction in Gypchek-treated and control plots.

	Gypchek			Control			
Plot Pair	Pre	Post	% Reduction†	Pre	Post	% Reduction†	Net % Reduction‡
1	10700 ± 2537	5 ± 5	99.5 ± 0.1	11490 ± 2794	109 ± 17	99.1 ± 0.2	95.1 ± 5.3
2	9480 ± 2682	59 ± 22	99.4 ± 0.3	10369 ± 1932	99 ± 16	99.1 ± 0.2	27.1 ± 36.2
3	7711 ± 714	40 ± 17	99.5 ± 0.2	6881 ± 1242	588 ± 147	91.5 ± 2.2	94.0 ± 3.0
4	6215 ± 1191	153 ± 31	97.5 ± 0.6	3898 ± 785	2361 ± 479	39.4 ± 8.9	95.9 ± 1.1
5	1882 ± 240	10 ± 7	99.5 ± 0.4	2924 ± 640	1976 ± 566	29.2 ± 24.2	99.3 ± 0.6
6	1166 ± 203	35 ± 11	97.0 ± 0.9	1368 ± 358	3359 ± 667	-146.0 ± 54.2	98.8 ± 0.5
All	6192 ± 1605	50 ± 22	98.7 ± 0.5	6155 ± 1685	1415 ± 551	35.4 ± 38.4	85.0 ± 11.6

* Mean densities (± SEM) in 20, 0.01 ha subplots per plot.
† % reduction = Pre - Post/Post × 100.
‡ % reduction adjusted for population reduction in control plots using the formula of Abbott (1925).

The effectiveness of the Gypchek applications was also reflected in dramatic net larval reductions (Abbott 1925) under burlap in Gypchek-treated plots (Table 2). Initial total larval counts ($\bar{X} \pm$ SEM) were 23 ± 9 in Gypchek-treated plots and 83 ± 15 in control plots. Counts remained relatively static in the Gypchek-treated plots during the sampling period and averaged 109 ± 25 immediately preceding pupation. During the same period, counts in the control plots rose to a pre-pupation total of 2444 ± 332. Net larval reductions due to Gypchek ranged between 93 ± 1.5% and 98 ± 0.4% in 5 sprayed plots and was 86 ± 2% in the other. The average (± SEM) reduction for all sprayed plots was 95 ± 2% (Table 2).

The incidence of NPV-killed larvae under burlap was not significantly different ($P = 0.05$) between Gypchek-treated and control plots for the study, as a whole, except on the last sampling date when percent NPV mortality ($\bar{X} \pm$ SEM) was significantly higher ($P < 0.0005$) in control plots (51 ± 11%) than in Gypchek-treated plots (17 ± 5%) (Table 2). The data supports the hypothesis of a "second wave" of mortality (Woods and Elkinton 1987) in gypsy moth populations undergoing natural epizootics but are inconclusive with regard to the effect of a Gypchek treatment on natural NPV dynamics. The first wave of mortality in Gypchek-treated plots was not measured directly but implied by the negligible numbers of surviving larvae observed in the plots 16 days after spray. However, it was not possible to determine the relative contributions of either natural or sprayed-NPV to the second wave of mortality in Gypchek plots. The second wave may have been reduced in the Gypchek-treated plots because of the low density of larvae surviving treatment.

Defoliation of sample trees in Gypchek-treated plots was estimated to be 22 ± 4% ($\bar{X} \pm$ SEM) compared to 67 ± 13% in control plots (Table 3). A nonparametric sign test on plot pair data established that control plots suffered significantly more ($P < 0.016$) defoliation than treated plots.

At the time of spray, many first instar gypsy moth larvae were seen feeding on understory trees, shrubs, and oak seedlings. This observation is consistent with the hypothesis that early instar gypsy moth larval populations are skewed toward the understory (Ticehurst and Yendol 1989). Most of the Gypchek applications were made through relatively open canopies, and spray was well distributed throughout the understory stratum. We suggest that Gypchek will be most effective when applied in a manner that maximizes deposit in the understory where the majority of the most susceptible larval stages are feeding.

Results of the field test clearly demonstrated the effectiveness of the Gypchek tank mix in protecting foliage and reducing larval and egg mass populations. Egg mass population reduction was similar to that found after treating Maryland woodlots with Gypchek in 1987 (Podgwaite et al. 1987). Further, efficacy was demonstrated under the kind of marginal spray conditions likely to be encountered during the operational use of Gypchek over the rugged terrain of the Appalachian and Allegheny mountains.

The use of pesticides to combat high density gypsy moth populations is often eschewed in favor of no treatment. Dense populations often are expected to collapse through naturally occurring mortality factors, particularly NPV, and any interruption in these natural processes by ill-timed pesticide application is thought to be unwise (White et al. 1981). The data presented here suggest that

Table 2. Total larvae, percentage NPV-killed larvae and percentage larval population reduction from burlap flap sampling in Gypchek-treated and control plots.

	Total larvae†			% NPV killed larvae£	
Day*	Gypchek	Control	% larval reduction‡	Gypchek	Control
27	23 ± 9	83 ± 15	72 ± 11	11 ± 7	5 ± 2
30	56 ± 22	405 ± 90	85 ± 5	3 ± 2	5 ± 3
33	54 ± 20	285 ± 69	76 ± 11	10 ± 3	15 ± 6
37	131 ± 39	1585 ± 573	84 ± 8	4 ± 2	8 ± 3
39	123 ± 35	2791 ± 919	93 ± 3	4 ± 1	8 ± 3
42	109 ± 25	2444 ± 332	95 ± 2	17 ± 5	51 ± 11

* Days after first Gypchek application.
† Data are means (± SEM) of counts of the number of living plus dead larvae found under 40 burlap flaps in each of 6 Gypchek-treated plots and 6 control plots.
‡ % larval reduction in Gypchek-treated plots adjusted for larval reduction in control plots using the formula of Abbott (1925).
£ Data are means (± SEM) of % NPV-killed larvae found under 40 burlap flaps in each of 6 Gypchek treated plots and 6 control plots.

Table 3. Defoliation estimates in Gypchek-treated and control plot pairs.

	% Defoliation*	
Plot pair	Gypchek	Control
1	22 ± 2	100 ± 0
2	20 ± 3	99 ± 1
3	28 ± 2	44 ± 5
4	36 ± 2	52 ± 3
5	16 ± 1	82 ± 4
6	12 ± 1	25 ± 3
All	22 ± 4	67 ± 13

* Data are means (± SEM) of 40 trees per plot.

collapsing gypsy moth populations do not always fall below economic thresholds (250 egg masses per ha) and further, that Gypchek treatments can protect foliage while reducing these populations below economic thresholds. Thus, Gypchek can be considered as an alternative to either no treatment or, to other broad-spectrum pesticide treatment, in the management of dense gypsy moth populations.

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