Field Evaluation of an Improved Formulation of Gypchek (a Nuclear Polyhedrosis Virus Product) Against the Gypsy Moth (Lepidoptera: Lymantriidae)[1]

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Abstract Prior to 1994, Gypchek® (USDA Forest Service, Washington, DC), the gypsy moth nuclear multicapsid polyhedrosis virus (LdMNPV), was used in operational programs against gypsy moth *Lymantria dispar* (L.) dispersed in a standard molasses-lignosulfonate tank mix and applied twice at the rate of 5×10^{11} polyhedral inclusion bodies (PIB) per ha per application in a volume of 19 liters per ha. In 1995, we evaluated a commercially-produced carrier and operational options that would make Gypchek application more efficient and less costly without reducing efficacy. Specifically, the standard tank mix formulation and application procedure was compared against a premixed commercial carrier, Carrier 038 (Novo Nordisk, Franklinton, NC) applied by three different application options. Option 1 consisted of double applications of Gypchek at the rate of 5×10^{11} PIB per ha per application in volumes of 9.5 liters per ha. Option 2 was identical to Option 1 except that application volume was reduced to 4.8 liters per ha. Option 3 consisted of a single application of Gypchek dispersed in 9.5 liters per ha at the rate of 1×10^{12} PIB per ha. There was also a fifth treatment consisting of unsprayed control plots. All treatments were evaluated in replicated 4-ha forest plots in southwestern Virginia. Levels of LdNPV-induced mortality, of larvae collected live 6 to 11 days after treatment, among treatments receiving a double application were not significantly different at $\alpha = 0.05$ from such mortality resulting from the single application of Gypchek in Carrier 038. However, both of the double-application treatment options (but not the single application option) of Gypchek dispersed in Carrier 038 had significantly higher levels ($\alpha = 0.05$) of LdMNPV than the double-application treatment of Gypchek dispersed in the standard tank mix. Defoliation was significantly different among the four Gypchek treatments, but all were significantly lower than the controls. These results indicated that all three options using Carrier 038 provided a level of efficacy equal to or better than the standard tank mix formulation applied twice at 19 liters/ha. Economic assessment indicated that the single application option was more efficient and/or less expensive than the other options.

Key Words *Lymantria dispar* (L.), adjuvant, formulation, aerial spray, insect sampling.

Gypchek®, the U.S. Department of Agriculture-Forest Service's gypsy moth (*Lymantria dispar* (L.)) multicapsid nuclear polyhedrosis virus (LdMNPV) product, was registered in 1978 by the Environmental Protection Agency as a general use pesticide

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to control gypsy moth. In 1987, an "improved" Gypchek tank mix was developed consisting of a lignosulfonate powder, molasses, a sticker, Gypchek, and water (Reardon and Podgwaite 1996). This formulation has proven efficacious in field trials (Podgwaite et al. 1995, Webb et al. 1989) when applied under appropriate conditions as discussed in Reardon and Podgwaite (1994). Because it is active against only the gypsy moth, Gypchek has wide appeal to all who wish to minimize environmental effects of spray applications on populations of non-target organisms; however, limited availability, higher cost, and operational considerations have largely restricted its use to situations where environmental concerns are paramount (Podgwaite 1996).

Protocols for using the "improved" Gypchek tank mix call for two applications, 3 d apart, of 1.25×10^{12} polyhedral inclusion bodies (PIB) in 19 liters final mix per ha. Collaborative efforts with Entotech, a subsidiary of Novo Nordisk (Franklinton, NC) led to the development of a ready-to-use spray adjuvant (Carrier 244®) with superior handling qualities (Podgwaite et al. 1995) to the "improved" tank mix, including enhanced protection of the virus from harmful UV radiation (sunlight), improved anti-evaporative properties, and ease of mixing and spraying. Consequently, Forest Service recommendations for using Gypchek for gypsy moth suppression were revised to two applications, 3 d apart, at a reduced rate of 5×10^{11} PIB per ha in a reduced volume of 9.4 liters of Carrier 244 (Podgwaite and Reardon 1994). In 1995, Novo Nordisk replaced Carrier 244 with a closely-related spray adjuvant, Carrier 038®.

The purpose of this field study was to compare the standard tank mix formulation and application procedure against the premixed commercial Carrier 038 applied by three different application options with improved economic and operational features such as one versus two applications, and reduced volume.

Materials and Methods

Field experimentation, 1995. In the spring of 1995, 30 4-ha plots were established in a mixed-oak stand on the Little North Mountain Wildlife Management Area, Augusta Co., VA, at elevations ranging from 488 to 792 m. The area had not previously experienced gypsy moth defoliation. Sampling for treatment evaluation was conducted in a 1-ha core area within each plot. The central core areas were separated from those of adjacent plots by a minimum of 300 m. All plots consisted of at least 50% oak (*Quercus* spp.), mostly white (*Q. alba* L.), chestnut (*Q. prinus* L.), red (*Q. rubra* L.), southern red (*Q. falcata* Michaux) and black (*Q. velutina* Lamarck), and contained similar understory vegetation. Plots were grouped into six blocks based on gypsy moth population density as estimated from egg mass counts and geographic proximity. Egg-mass surveys were conducted January-March 1995 (before season) and February 1996 (after season) at five 0.01-ha fixed-radius egg-mass survey points (Liebhold et al. 1994) that were spaced uniformly within the central 1-ha core plot. All new egg masses on any surface within the cylinder above the survey circle were counted. Egg masses within reach were felt to determine if they were old or new. Egg masses not within reach were viewed with binoculars and judged new or old based on appearance.

To determine relative levels of natural LdMNPV occurring on or within egg masses at the beginning of the study, 10 egg masses were collected from each plot on 21 to 29 March and returned to the Beltsville Agricultural Research Center where they where held in an outdoor insectary. After eclosion, 20 larvae from each egg mass

were placed on artificial diet (Bell et al. 1981) in 30-ml plastic cups with paper lids and held in the outdoor insectary. The number of larvae dead after 28 d was determined and used to calculate the percent mortality for each plot.

There were six replicates of each of five treatments assigned in a randomized block design. The Gypchek used in this study was a lyophilized powder from production lot MR1-8 (2.45×10^{10} PIB per g). The LC50 of this lot for second-stage larvae of a standard laboratory gypsy moth strain (New Jersey, F_{42}) was estimated to be 9.0×10^3 PIB per ml from diet incorporation bioassays (J.D.P., unpublished data). There were four Gypchek treatments and a control treatment: (1) two applications of 5×10^{11} PIB in 19 liters of the Forest Service standard "improved" formulation per ha (STD-19L-2×), (2) two applications of 5×10^{11} PIB in 9.5 liters of an aqueous flowable carrier (Carrier 038, Novo Nordisk, Franklinton, NC) formulation per ha (038-9L-2×), (3) two applications of 5×10^{11} PIB in 4.8 liters of a Carrier 038 formulation per ha (038-5L-2×), (4) one application of 1×10^{12} PIB in 9.5 liters of a Carrier 038 formulation per ha (038-9L-1×), and (5) no spray (controls). Each treatment was replicated six times. The STD-19L-2× treatment consisted of 3.5 g Gypchek, 227 g (6% by weight) of the lignosulfonate sunscreen Lignosite® AN (Georgia Pacific, Bellingham, WA), 0.47 liters (12.5% by vol) of Triple Crown® Pure Cane Molasses (Equine Specialty Feed, Ada, MN), 77.6 ml (2% by vol) Bond® spreader sticker (Loveland Industries, Greely, CO) and 3.24 liters (85.5% by vol) of natural stream water (pH 7.4) per 3.79 liters of finished spray. The Gypchek was added slowly to the mix-tank as a dry powder after all other ingredients were in solution and circulating. The formulation for the Carrier 038 treatments was 3.6 liters (95% by vol) of Carrier 038 (lot no. 9508257-79) and 0.9 liters (5% by vol) of a Gypchek-water slurry. The slurry for a particular treatment was prepared by adding the required amount of Gypchek to a measured volume of stream water and blending to a homogenous consistency with a variable-speed mixer. The slurry was slowly added to a mix-tank in which the appropriate amount of Carrier 038 was circulating.

Applications began when the majority of gypsy moth larvae were in the first stadium and oak leaf expansion approximated 25% in all plots, except that in plots above 600 m, white oaks had barely broken bud. Applications were made using a Cessna 188 Ag Truck (Cessna Aircraft, Wichita, KS) equipped with a standard boom and flat fan nozzles (Spraying Systems, Wheaton, IL) that were positioned 90° to the flight line. Forty-five 8008-nozzles, 45 8004-nozzles and 23 8004-nozzles were used for the 19 liter/ha, 9.5 liter/ha and the 4.8 liter/ha applications respectively. Before-treatment calibration of the aircraft delivery system and characterization of droplet deposit indicated that the required volume of spray and the desired droplet spectrum were achieved by spraying 15 m above the canopy at an air speed of 193 km/h and a boom pressure of 2.8 kg/cm^2 and using a lane separation of 23 m.

Treatment applications were made on 6, 7, 8 and 9 May 1995 between 0625 and 0915 hours EDT. The first of the double applications was on either 6 or 7 May; the second was 2 d later. The single treatment was applied on 7 May. Weather conditions during all applications were dry with a broken overcast sky. Winds were westerly at 0 to 8 km/h with occasional gusts of 12 to 16 km/h, temperatures were between 6 and 14°C and the relative humidity was between 57 and 83%. Separate applications (see below) were made between 0925 and 1100 hours EDT on 7 and 8 May to assess deposit. Conditions were as above except temperatures were higher (12 to 21°C) and the relative humidities were lower (34 to 54%).

Because the dye had the potential for altering the evaluation of treatment efficacy by acting as an ultraviolet sun screen as per Shapiro and Robertson (1990), separate plots were used for the spray-deposition evaluations. To determine the amount of spray deposit on leaves in plots sprayed with STD-19L-2X; 038-9L-2X, and 038-5L-2X, a study was conducted using an additional two plots of each spray treatment as replicated sampling areas. Formulations for the spray-deposition assessment were prepared as above except that an aqueous concentration of the fluorescent dye, Rhodamine WT (Keystone Aniline, Chicago, IL) was added (0.4% by vol) to each tank mix. Leaf samples were collected by shooting down small branches of 30 dominant or codominant overstory trees from each plot. Leaf samples were taken from two different sides of each tree in the mid-canopy; the leaves were placed in paper bags, one bag per side, for storage prior to analysis. Thirty trees in a non-sprayed area also were sampled for use as controls. Leaves were then taken to the laboratory and frozen (–30°C) until they were analyzed.

A Sequoia-Turner (Sequoia-Turner Corporation, Mountain View, CA) fluorometer was used to determine the amount of Rhodomine occurring in washoff from each sample. Samples were taken from each tank mix in order to develop a standard curve for calculating Rhodamine concentration as a percentage of the tank mix. Because some of the leaves were small, samples consisted of from 1 to 4 leaves. Leaf area for each sample was measured with a Li-Cor leaf area meter (Model 3100, Licor Corporation, Lincoln, NE). Also, the tree species was recorded at this time. Each sample was then placed in a 125-ml Nalgene (Fisher Scientific, Pittsburgh, PA) plastic bottle. Fifty ml of deionized water was added to each bottle which was then closed. The bottles were then placed on a Burrell Wrist Action Shaker (Burrell Inc., Pittsburgh, PA) for 30 min. Preliminary work established that 30 min of shaking time was sufficient to remove all of the Rhodomine. The unsprayed control leaves were similarly washed and measured with the fluorometer. No species had measurable background contamination at the fluorometer or leaf-size ranges used in this experiment. After shaking, 10 ml of each sample was removed using a 10-ml syringe. A hose was then connected to the syringe and a filter holder containing Fisher C5 filter paper (Fisher Scientific, Pittsburgh, PA) was added to the hose. The sample was forced through the filter until 6 ml of filtrate was delivered to a cuvette placed under the filter holder. The cuvettes were then inserted into a special holder located in the door of the fluorometer. The samples were read on the 3X scale and compared daily to standards.

Larval population density was estimated in the central core of each plot on 31 May using the frass drop/frass yield method (Liebhold and Elkinton 1988a, b). Frass drop over a 24-h sampling period was estimated from 50 plastic buckets (21 cm diam × 15 cm high) distributed randomly beneath the oak canopy in each plot. Frass yield (the number of frass pellets produced per larva) was determined during the sampling period by holding 150 larvae (50 from each of three control plots) individually with 1 to 2 oak leaves in 177-ml plastic cups with cardboard lids. The mean density of larvae per plot was estimated using the equation:

$$\text{Density} = (1/A)\cdot(\bar{x}_d/\bar{x}_y)$$

where A = the area sampled by each bucket; $\bar{x}_d$ = mean drop (Number of frass pellets per bucket); and $\bar{x}_y$ = mean yield (number of frass pellets per larva). Frass samples were conducted when larvae were predominantly in the fourth instar.

Larval mortality was estimated by collecting 50 larvae on 2 to 4 May (2 to 8 d

before treatment) and 100 larvae on 16 to 17 May (6 to 11 d after treatment) from understory vegetation within each plot and rearing them on artificial diet in 30-ml cups with paper lids. The rearing cups were held on shelves in a wooden outdoor insectary (368 cm long, 215 cm wide, 92 cm deep, with hardwear cloth covering the front to allow natural conditions of light, temperature, and humidity but not rain) at the Beltsville Agricultural Research Center. Collections were made only within the central core area. The number of larvae dead after 28 d was determined and used to calculate the percent mortality for each plot.

Tissue from all of the larvae that died were examined under 400X for the presence of LdMNPV PIBs or spores of the fungus *Entomophaga maimaiga* Humber, Shimazu, and Soper (Hajek and Roberts 1992). If determinations could not be made with certainty using the above procedure, smears of tissue samples were fixed over a flame, stained with dilute Giesma solution, and then examined under oil emersion at 1000X.

Larval samples (50 per plot where possible) were taken 17, 24, and 31 d after treatment from all control plots and from all plots receiving the 038-9L-1X treatment. Larvae were held in individual diet cups in an outdoor insectary as above. Mortality was assessed 7 d after collection, with the cause of death determined by light microscopy as above.

Defoliation was estimated on 25 June within the central core area of each plot according to the following procedure. Two experienced workers walked a transect that started at a randomly chosen point and extended across the central core area of the plot. Both workers examined the same 20 trees along the transect with binoculars and estimated the percent defoliation in 10% increments. The average of the two estimates was used as the estimate of defoliation for each tree.

Data analysis. The significance of the treatment effects was tested by analysis of variance (ANOVA) using the General Linear Models (GLM) procedure of the SAS statistics package (SAS Institute 1985). In all analyses, the block x treatment interaction provided the error term to test the treatment effect. An arcsine-square root transformation was used on all percentage data. All other dependent variables were checked for homogeneity of variance. When necessary, a log transformation of the form ($Y_{transformed}$) = log (Y + constant) was used to stabilize the variance (Berry 1987). For each analysis, a constant resulting in the most homogeneous variance was used for the transformation (Carrol and Ruppert 1988). When treatment effects were significant, means were separated at a comparison-wise error rate of 0.05 using the least significant differences (LSD) procedure (SAS Institute 1985).

Results

Biological assessment of formulation and treatment options. A lack of significance at $\alpha = 0.05$ for treatment effects ($F = 0.2$; df = 4, 20; $P = 0.96$) indicated that plots were well-balanced among treatments with respect to before-season egg mass density (Table 1). Levels of natural LdMNPV and *E. maimaiga* mortality were low and evenly distributed among the treatment plots (Table 1) prior to treatment. LdMNPV levels were 1% or less in larvae reared from egg masses sampled from the plot; no *E. maimaiga*-induced mortality occurred among these larvae. LdMNPV was found at similarly low levels in larvae sampled from the plots 2 to 8 d prior to treatment. *Entomophaga maimaiga* was present, but at less than 1% in any of the plots.

The measured amount of spray deposit on leaf surfaces increased as spray ap-

Table 1. Parameters ($\bar{X}$ ± SEM) measured before treatment, including number of egg masses per ha, percent LdMNPV mortality of larvae eclosing from sampled egg masses, and mortality due to LdMNPV and *E. maimaiga* for larvae collected 2 to 8 d before treatment

Treatment*	Egg masses per ha	% LdMNPV mortality from egg masses	% Mortality in larvae collected prior to treatment due to:	
			LdMNPV	*E. maimaiga*
Control	2968 ± 1196	0.3 ± 0.1	0.7 ± 0.4	0.7 ± 0.4
038-9L-2X	3316 ± 1774	1.0 ± 0.2	1.3 ± 1.0	0
038-5L-2X	3482 ± 1658	0.4 ± 0.3	0	0.3 ± 0.3
038-9L-1X	3193 ± 1228	0.3 ± 0.2	0.7 ± 0.7	0.3 ± 0.3
STD-19L-2X	3158 ± 1448	0.2 ± 0.1	0	0.3 ± 0.3
F (df)	0.2 (4,20)	2.9 (4,17)	0.9 (4,20)	0.5 (4,20)
P > *F*	0.96	0.06	0.48	0.73
Transformation	log(x + 10)	arcsine$\sqrt{x}$	arcsine$\sqrt{x}$	arcsine$\sqrt{x}$

* Control = untreated plots; 038-9L-2X = two applications of 5×10^{11} PIB in 9.5 liters of Carrier 038 per ha; 038-5L-2X = two applications of 5×10^{11} PIB in 4.8 liters of Carrier 038 per ha; 038-9L-1X = one application of 1×10^{12} PIB in 9.5 liters of Carrier 038 per ha; STD-19L-2X = two applications of 5×10^{11} PIB in 19 liters of the Forest Service standard formulation per ha.

plication volume increased, with means of 13.9 ± 3.9 nl/cm^2, 32.9 ± 14.5 nl/cm^2, and 59.8 ± 3.3 nl/cm^2, respectively, for the 038-5L, the 038-9L, and the STD-19L treatments.

Statistically significant treatment effects ($F = 21.1$; df = 4, 20; $P < 0.0001$) were obtained for acute early-season total larval mortality as estimated by the 6 to 11 d larval bioassay (Table 2). Necropsy of the cadavers revealed that LdMNPV and *E. maimaiga* were the two major sources of mortality. Some (72) cadavers contained both pathogens. Treatment effects were not significant at $\alpha = 0.05$ for *E. maimaiga* ($F = 0.7$; df = 4, 20; $P < 0.63$). However, treatment effects for LdMNPV were highly significant ($F = 26.9$; df = 4, 20; $P < 0.0001$), and LdMNPV was the major component of the total mortality (Table 2). All Gypchek treatments resulted in significantly higher LdMNPV-induced early-season larval mortality than the controls. There were no significant differences at $\alpha = 0.05$ between the Carrier 038-based treatments and the standard (STD-19L-2X) treatment for early-season larval mortality due to LdMNPV. While there were no significant differences at $\alpha = 0.05$ among any of the three double application treatments (038-5L-2X, 038-9L-2X, STD-19L-2X), the single application (038-9L-1X) treatment resulted in significantly less LdMNPV-induced early-season larval mortality than did the either of the two Carrier 038-based double applications. A comparison of the two Carrier 038-based double applications revealed that treatment volume made no apparent difference, with the 038-9L-2X and the 038-5L-2X treatments resulting in statistically equal levels of early-season LdMNPV-induced larval mortality.

The number of larvae per m^2 of ground surface beneath the canopy on day 20 after treatment as estimated by frass sampling is given in Table 2. Treatment effects were highly significant ($F = 9.3$; df = 4, 20; $P = 0.0002$). Results are consistent with those

Table 2. Parameters ($\bar{X}$ ± SEM) measured after treatment, including mortality due to LdMNPV and *E. maimaiga* for larvae collected from the field 6 to 11 d after treatment, number of live larvae in canopy 20 d after treatment, percent defoliation, and residual egg mass numbers*

	% Mortality in larvae collected after treatment				
Treatment**	LdMNPV	*E. maimaiga*	No. live larvae†	% Defoliation	Residual egg masses
Control	18.9 ± 4.9 a	7.5 ± 1.1	125.9 ± 39.8 a	37.4 ± 2.5 a	86.0 ± 12.7
038-9L-2X	85.8 ± 3.7 c	4.4 ± 1.6	20.2 ± 13.3 b	17.7 ± 1.4 b	41.7 ± 12.7
038-5L-2X	83.2 ± 2.2 c	10.9 ± 6.9	19.6 ± 8.1 b	19.7 ± 1.1 b	59.1 ± 14.2
038-9L-1X	67.6 ± 10.4 b	4.0 ± 1.3	56.7 ± 36.5 b	21.0 ± 1.5 b	54.7 ± 12.7
STD-19L-2X	80.2 ± 4.9 bc	7.6 ± 2.8	24.7 ± 9.4 b	22.7 ± 1.5 b	42.9 ± 14.2
F (df)	26.9 (4,20)	0.7 (4,20)	9.3 (4,20)	3.3 (4,20)	1.9 (4,18)
P > *F*	<0.0001	0.63	0.0002	0.03	0.15
Transformation	arcsine$\sqrt{x}$	arcsine$\sqrt{x}$	log(x + 50)	arcsine$\sqrt{x}$	none

* Means within a column followed by the same letter are not significantly different ($P > 0.05$) by unprotected LSD (SAS Institute 1985). Co-infected larvae were credited to both pathogens.

** Control = untreated plots; 038-9L-2X = two applications of 5×10^{11} PIB in 9.5 liters of Carrier 038 per ha; 038-5L-2X = two applications of 5×10^{11} PIB in 4.8 liters of Carrier 038 per ha; 038-9L-1X = one application of 1×10^{12} PIB in 9.5 liters of Carrier 038 per ha; STD-19L-2X = two applications of 5×10^{11} PIB in 19 liters of the Forest Service standard formulation per ha.

† Number of live larvae per m^2 of ground surface beneath the canopy 20 days after treatment.

of the 6 to 11 d bioassay indicating that the high levels of mortality predicted by that bioassay had actually occurred in the field by day 20 after treatment. Means for all treatments were statistically lower than the average for untreated control blocks. Consistent with the 6 to 11 d bioassay (though not significant at $\alpha = 0.05$), the average number of larvae in plots receiving the 038-9L-1X treatment was considerably higher (56.7 ± 36.5) than in those plots receiving two applications (19.6 ± 8.1, 20.2 ± 13.3, and 24.7 ± 22.7, respectively, for the 038-5L-2X, 038-9L-2X, and STD-19L-2X treatments).

Beginning with the larval collection made 17 d after treatment, large numbers of gypsy moth cadavers appeared in all plots. Most of the cadavers showed symptoms characteristic of mortality caused by *E. maimaiga.* Over the next 2 wks numbers of live larvae dropped noticeably in the plots, until by the collection made 31 d after treatment, numbers of living larvae were so low that no further collections were made. Necropsies conducted on larvae that were alive when collected but that died after being placed on artificial diet indicated that *E. maimaiga* was a significant source of late-season gypsy moth mortality in all plots. For example, necropsies of larvae that were collected from a single application plot 10 d after treatment indicated that 98% of the cadavers contained LdMNPV but only 2% contained *E. maimaiga.* Necropsies of larvae from the same plot, but collected 2 wks later revealed that 23% of the cadavers contained LdMNPV and 92% contained *E. maimaiga.*

Despite the late-season population collapse, measurable levels of defoliation occurred in many of the plots. Defoliation in the control plots averaged 37.4 ± 2.5%, which was significantly higher than the defoliation that occurred in the Gypchek treatment plots (17.7 ± 1.4% to 22.7 ± 1.5%). It is likely that the defoliation levels would have been higher in the both the control and Gypchek-treatment plots if late-season mortality from *E. maimaiga* had been less severe. Reflecting the late-season collapse, residual egg mass numbers declined considerably in all plots compared with before-season counts (Table 2). Although the mean number of egg masses were higher in the untreated control plots than in the treatment plots (Table 2), the differences were not significant at $\alpha = 0.05$.

Discussion

Gypsy moth control costs are highly variable because, when conducted by public agencies, costs are usually subject to the unpredictable bid process (Straka et al. 1997). They found that the cost per ha of controlling gypsy moths for the Maryland Department of Agriculture's Gypsy Moth Program for the years 1990-1992 (material costs averaged for several control products) ranged from an average of $37.41 for rural areas treated by fixed-wing aircraft to $50.46 for rural areas treated by rotor aircraft to $61.12 for urban areas treated by rotor aircraft. Total costs were divided into material costs, application costs, support cost, and overhead. Table 3 gives cost and efficiency comparisons for the standard tank mix formulation and application procedure compared against the premixed commercial Carrier 038 applied by three different application options. Reardon and Podgwaite (1996) estimated that Gypchek can be produced at a cost of $20.00 for 1×10^{12} PIB, and this cost is the same for the standard formulation and all three options. Based on current price lists, the other ingredients for the standard formulation can be assembled at a cost of $4.47 per ha per application = $8.95 for the two required applications. The standard formulation is operationally inefficient in that high application volumes are required, and 100 liters of

Table 3. Cost and efficiency comparisons for the standard tank mix formulation and application procedure compared against a premixed commercial carriar, Carrier 038 applied by 3 different application options

Cost/Efficiency Parameter (per ha)	Standard Formulation and Proceedure	Carrier 038		
		Option 1	Option 2	Option 3
Total quantity Gypchek	1×10^{12} split into 2 applns	1×10^{12} split into 2 applns	1×10^{12} split into 2 applns	1×10^{12} applied in 1 appln
Cost of Gypchek	$20.00	$20.00	$20.00	$20.00
Volume of spray:				
a. Per application	19 liters	9.5 liters	4.8 liters	9.5 liters
b. Total	38 liters	19 liters	9.5 liters	9.5 liters
Cost of formulation	$8.95	$59.15	$29.58	$29.58
Application/support costs				
(rural fixed-wing)	$57.12	$57.12	$57.12	$28.56
(rural-rotor)	$83.42	$83.42	$83.42	$41.71
(urban-rotor)	$104.34	$104.34	$104.34	$52.17
Total costs				
(rural fixed-wing)	$86.07	$136.27	$106.70	$78.14
(rural-rotor)	$112.37	$162.57	$133.00	$91.29
(urban-rotor)	$133.29	$183.49	$153.00	$101.75
No. completed ha per 100 liters spray solution	2.6	5.3	10.5	10.5

spray solution will treat just 2.6 finished ha (5.2 ha per application). This would tend to lead to higher bids by applicators. The negotiated cost of Carrier 038 to the USDA Forest Service for the 1996 pilot test amounted to $29.58 per ha per application. Thus, formulation costs are considerably higher for all three application options for Carrier 038 than for the standard formulation. Application, support, and overhead costs are based on those reported by Straka et al. (1997), and are $28.56, $41.71, and $52.17 per ha per application, respectively, for rural area treated by fixed-wing aircraft, rural areas treated by rotor aircraft, and urban areas treated by rotor aircraft. When application costs are considered, Option 3 becomes the most economical of the four treatment programs because of its requirement for only one application, and its economic superiority becomes more evident when more costly application procedures (rural fixed-wing vs rural rotor vs urban rotor) are considered; Option 3 is also more efficient to apply, with 100 liters of spray solution treating 10.5 finished ha. Option 1 has the disadvantage of high formulation costs, the high cost of the second application, and poor application efficiency, with 100 liters of spray solution treating 5.2 finished ha (10.5 ha per application). Option 2 saves formulation costs and has greater operational efficiency compared with Option 1, but its requirement for a second application renders it considerably more costly than Option 3.

The purpose of this study was to evaluate modifications of a standard Gypchek aerial application regimen. The study was conducted in natural gypsy moth populations under operational conditions. We assume that the number of PIB per droplet would reflect the volume differences by being greater per unit drop as spray volume decreased. Dubois et al. (1993) had found that increased spray volume of *Bacillus thuringiensis* Berliner applied aerially to oak canopies significantly increased the number of drops per cm^2, but not the total volume of spray deposited. Assuming that droplets of Gypchek behave similarly, the number of PIBs per unit foliage ingested should have been comparable for the three treatment volumes. Treatment efficacy in the present study was measured in four ways: acute after-treatment mortality, larval population reduction, foliage protection, and year-to-year egg-mass population reduction. Because a variety of biotic and abiotic factors were operating on natural gypsy moth populations throughout the season, we expected that treatment differences would be least confounded when measured soon after treatment, and would be increasingly confounded as the season progressed. While we anticipated that a major natural mortality factor to gypsy moth in the study area would likely be the fungus *E. maimaiga,* this fungus primarily attacked later (stadia 5-6) instars (Hajek and Roberts 1991). Therefore, the 6 to 11 d after-treatment larval bioassay should most accurately reflect treatment differences, and indeed, the value of a double application could be statistically verified using this measure. The 20-d frass counts were in general agreement with the after-treatment bioassay data, with fewer larvae in the double application treatment plots than in plots receiving the single application, but, perhaps reflecting the developing fungal epizootic, these differences were no longer statistically significant. No doubt, the fungal epizootic reduced defoliation levels, but since *E. maimaiga* killed larvae late in the season, enough defoliation occurred so that Gypchek-treated plots could be statistically separated from control plots, but not from each other. The results of the larval bioassay demonstrated that a double application of LdMNPV in Carrier 038 was biologically superior to a single full application as measured by resulting LdMNPV levels; however, perhaps because of the late-season compensatory mortality caused by *E. maimaiga,* this biological superiority did not translate into increased foliage protection for the double application over that measured in plots receiving a single full application. Because two applications are considerably more costly than one, managers may wish to accept the slightly less immediate biological efficacy of a single application in exchange for the reduced cost. The presence of *E. maimaiga* throughout the range of the gypsy moth in the eastern United States increases the difficulty in designing experiments that estimate effects of treatments versus controls; however, this study demonstrated that such comparisons can be made if at least part of the efficacy measurements are made early in the season while the fungal epizootic is still low.

Although the "improved" standard tank mix was effective, gypsy moth managers have had difficulty in using the product because of the additional time and effort required to pre-mix formulation ingredients. The major finding of this study was that Gypchek applied with Carrier 038 was equally as effective as Gypchek applied in the "improved" standard tank mix, thus eliminating the concerns of managers. The finding that application volume can be reduced to 4.8 liters per ha with no observed loss of biological efficacy should lead to a further increase in program efficiency and reduced operational costs. However, a pilot project should be conducted to confirm the effectiveness of the reduced volume application under operational conditions.

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