Efficacy of Gypchek Against the Gypsy Moth (Lepidoptera: Lymantriidae) and Residual Effects in the Year Following Treatment[1]

R. E. Webb, K. W. Thorpe, J. D. Podgwaite[2], R. C. Reardon[3], G. B. White and S. E. Talley[4]

Insect Biocontrol Laboratory, USDA-ARS, Beltsville, MD 20705 USA

J. Entomol. Sci. 34(4): 404-414 (October 1999)

Abstract Gypsy moth, *Lymantria dispar* (L.), populations in three Maryland plots and three West Virginia plots were treated aerially with the gypsy moth multienveloped nuclear polyhedrosis virus product, Gypchek® (U.S. Forest Service, USDA, Washington, DC). The study was a pilot test to demonstrate the efficacy of a single application of Gypchek suspended in the commercially-produced Carrier 038® (Abbott Laboratories, N. Chicago, IL) at 9.5 liters and 1×10^{12} polyhedral inclusion bodies per ha. This treatment resulted in virus levels that were significantly higher in the treated woodlots (58.7%) than in paired control woodlots (10.5%), with treatment effects highly significant. Results from treated plots in West Virginia (67.7% post-treatment virus infection) were clearly superior to results from Maryland (49.7% post-treatment virus infection) probably due to more favorable conditions during application in West Virginia. Defoliation averaged 15% in the treated woodlots and 32% in the control woodlots; however, a high degree of variability in the control woodlots, perhaps due to compensatory mortality, probably caused by a late-season epizootic of the fungus *Entomophaga maimaiga* Humber, Shimazu & Soper, accounted for the treatment effects being statistically non-significant at $P = 0.05$. Significantly higher levels of virus were found in treated woodlots than in control woodlots in an early-season larval collection made the year following treatment (1997) with virus levels averaging 11.7% in treated plots vs 5.0% in control plots. The second-year effects were particularly striking in the West Virginia plots (12.7% in treated plots vs 3.0% in control plots) suggesting that Gypchek applications may be particularly desirable in situations where natural virus is low or absent. The results of the pilot test now give forest managers the option of using one application (full dose) or two applications (split dose) of Gypchek against the gypsy moth.

Key Words Gypchek, nuclear polyhedrosis virus, gypsy moth, *Lymantria dispar*.

Gypchek® (U.S. Forest Service, USDA, Washington, DC), the (multienveloped) nuclear polyhedrosis virus product of the gypsy moth, *Lymantria dispar* (L.), is registered with the U.S. Environmental Protection Agency as a general use pesticide (Reardon and Podgwaite 1996). A recent survey (Podgwaite et al. 1997) indicated that a number of potential users of Gypchek were attracted by its positive environmental attributes such as high host specificity and would use Gypchek were it avail-

[1]Received 07 October 1998; accepted for publication 22 December 1998.
[2]USDA Forest Service, Northeastern Center for Forest Health Research, Harnden, CT 06514.
[3]USDA Forest Service, Northeastern Area State and Private Forestry, Morgantown, WV 26505.
[4]Rockbridge County Gypsy Moth Program, Lexington, VA 24401.

able commercially and competitively priced with other bioinsecticides. (Gypchek is currently produced in limited quantities for the U.S. Forest Service by the Animal Plant Health Inspection Service at its Otis, MA, laboratory). Based on work by Podgwaite et al. (1992), Gypchek label recommendations (prior to 1996) called for two applications of a standard tank mix (Gypchek, a lignosulfonate sunscreen, molasses, a sticker, and water), 3 days apart, with a split virus dose. Further work (Reardon and Podgwaite 1994) demonstrated that Gypchek added to a premixed carrier was as good as the "standard" formulation and was much easier to handle, but recommendations still suggested two applications, 3 days apart. More recent tests with Gypchek and a new carrier (Webb et al. 1999a) found that one application (with a full, unsplit virus dose) gave results equivalent to the double application, split dose. The single dose option has favorable economic and programmatic implications (Webb et al. 1999a), but a pilot test was required before the U.S. Department of Agriculture Forest Service would support the one application option for operational use. This is a report of the efficacy and biological evaluation of that pilot test, the results of which now gives forest managers the option of using one application (full dose) or two applications (split dose) of Gypchek against the gypsy moth (Reardon and Podgwaite 1996).

Materials and Methods

This pilot test was conducted at three locations near Ocean City, MD, and at three additional locations near Cameron, WV. In 1997, the areas treated in 1996 were evaluated for residual virus levels.

Plot establishment and pretreatment characterization, 1996. In the spring of 1996, 12 experimental plots were established in mixed-oak stands, 6 (17 to 61 ha) on flat Coastal Plain terrain in Worcester Co., near Ocean City, MD, and 6 (20 to 56 ha) on mountainous terrain in Marshall Co., near Cameron, WV. The six treated plots (three in each area) were paired with six untreated control plots containing similar gypsy moth populations based on preseason egg mass densities. Gypchek-treated woodlots were similar in size to those left untreated (averaging 36 and 28 ha, respectively). Elevations above sea level in the Maryland plots ranged from 3 to 15 m, while those in West Virginia ranged from 305 to 460 m. A square 4-ha sampling core area was established in the center of each plot. Sixteen fixed-radius, 0.01-ha subplots were established within each 4-ha sampling core (2 subplots per subquadrant for a total of 8 subquadrants per core sampling area). Subplots were scattered throughout the 4-ha core evaluation area and contained predominantly preferred gypsy moth host plants (*Quercus* spp.). Egg-mass surveys were conducted before season (before the appearance of leaves) and after season (after leaf-drop) in each of the 16 fixed-radius subplots as per Liebhold et al. (1994). A 1-h walk was conducted through each experimental plot and as many egg masses as possible were physically touched to establish a ratio of old to new egg masses, and the total egg mass count for each block was adjusted for this ratio.

Treatment. Treatment consisted of one application of 1×10^{12} polyhedral inclusion bodies in 9.5 liters of Carrier 038® formulation (Abbott Laboratories, N. Chicago, IL) per ha applied using a Cessna 188 Ag Truck (Cessna Aircraft, Wichita, KS) equipped with a standard boom (with inline screens removed) and a total of 45 flat fan nozzles with 8004 tips with slotted screens (Spraying Systems, Wheaton, IL) that were positioned 90° to the flight line. Before-treatment calibration of the aircraft delivery system and characterization of droplet deposit indicated that the required vol-

ume 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. The Gypchek used in this study was a lyophilized powder from production lot MRI-8 (2.45×10^{10} polyhedral inclusion bodies per g). The median lethal concentration (LC_{50}) of this lot for second-stage larvae of a gypsy moth laboratory strain (New Jersey Standard Strain, F_{42}) was estimated to be 9.0×10^3 polyhedral inclusion bodies per ml from diet incorporation bioassays (J.D.P., unpublished data). The formulation handled and mixed well. The slurry for each treatment was prepared by adding the required amount of Gypchek to a measured volume of 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.

The Maryland plots were sprayed during the afternoons of 2 May (one plot at 17:40 to 18:30 h, leaf expansion 30 to 60%, 95% first-instar, 5% second-instar larvae present, 25°C, 40% RH, wind speed 0 to 8 km/h) and 6 May (two plots at 15:00 to 16:20 h, leaf expansion 40-80%, 80% first-instar, 20% second-instar larvae present, 16°C, 79% RH, wind speed 0 to 8 km/h). The general area had repeated scattered showers of highly variable strength (0.1 to 1 cm per shower) during the period 5 May to 8 May, but rainfall occurring in the plots was not recorded. West Virginia plots were sprayed during the morning of 19 May (5:30 to 10:00 h) under clear conditions, 17 to 28°C, 57 to 88% RH, wind speed 3 to 19 km/h, with no rain reported from the general area until 23 May. Due to the seasonal differences between the two areas, foliage (30 to 80% expansion) and insect (80 to 95% first, 5 to 20% second instars) development were similar to that seen for the earlier Maryland treatment.

Post-treatment assessment of virus, 1996. Larval mortality was estimated from the Maryland plots by a pretreatment collection of 120 larvae per plot (15 from each of the 8 subplots) on 2 May (4 d before treatment) and a post-treatment collection of 120 larvae (15 from each of the 8 subplots) on 10 to 13 May (4 to 7 d after treatment) from understory vegetation within each plot. Similar collections were made from the West Virginia plots on 17 May (2 d before treatment) and on 24 May (5 d after treatment). All larvae were placed on artificial diet (Bell et al. 1981) in 30-ml plastic cups with paper lids and returned to the Beltsville Agricultural Research Center where they where held on shelves in a wooden outdoor insectary (368 cm long, 215 cm wide, 92 cm deep, with hardware cloth across the front to allow natural conditions of light, temperature, and humidity, but not rain). The number of larvae dead after 28 d was determined and used to calculate the percent mortality for each plot. All of the larvae that died were examined in wet mounts under 400X for the presence of nuclear polyhedrosis virus polyhedral inclusion bodies or spores of the gypsy moth-specific fungus *Entomophaga maimaiga* Humber, Shimazu & Soper (Hajek and Roberts 1992). If determinations could not be made with certainty using under 400X, smears of tissue samples were fixed over a flame, stained with dilute Giesma solution as per Glaser (1915), and then examined under oil emersion at 1000X.

Post-treatment biological assessments, 1996. An additional evaluation of *E. maimaiga* apparency was made between 18 and 26 June. We determined that the observed mortality was due to *E. maimaiga* by using the field method of Hajek and Snyder (1992). We used a 1 to 3 rating of the apparency of late-season *E. maimaiga*-killed cadavers; 1 = cadavers, if present, few and scattered; 2 = 10 to 75% of trees with 1 or more cadavers, some trees (typically <10%) noted with 20 or more cadavers; 3 = Virtually all trees with multiple cadavers, many trees (typically over 50%), with 20

or more cadavers. Defoliation was estimated on 18 June in the Maryland plots and on 26 June in the West Virginia plots as per Webb et al. (1999a).

Field experimentation, 1997. The 12 experimental blocks examined in 1996 were re-examined in the spring of 1997 for residual virus activity. Early-instar larval collections were made from the Maryland plots on 23 April and from the West Virginia plots on 15 May. An attempt was made to collect 100 larvae from each plot, but this was not possible for all plots (collections averaged 80 from Maryland treated plots, 77 from Maryland control plots, 85 from West Virginia treated plots, and 77 from West Virginia control plots). All larvae were reared on artificial diet in 30-ml cups and held in the outdoor insectary as in 1996. The number of larvae dead after 28 d was determined and used to calculate the percent mortality for each plot. All of the larvae that died were examined under 400X for the presence of polyhedral inclusion bodies or *E. maimaiga* spores as in 1996.

Data analysis. Mixed model analysis of variance (SAS, Version 6.12) (SAS Institute 1996) was used to model the effects of location and treatment. The fixed portion of the model included sources of variation for location (Maryland, West Virginia), treatment (Gypchek, control), and the interaction between these two factors. The random portion of the model was specified as the variation due to replicates within location. The best fitting variance-covariance structure was found to be constant variance. Therefore, this covariance structure was specified in all analyses.

Results

Preseason egg mass density, 1996. Egg mass density was similar in treated and control plots with 865 (SE = 178) per ha in treated plots and 829 (SE = 216) per ha in control plots (Table 1). The large standard errors reflect the fact that the treatments were blocked on egg mass numbers with plots in replicates 1, 2, and 3 averaging 1,834 (SE = 116), 817 (SE = 140), and 347 (SE = 79) egg masses per ha, respectively. The result of this blocking was that the preseason egg mass populations were well balanced among the treatments and areas, and that ANOVA treatment effects, area effects, and treatment x area interactions were all non-significant at $P = 0.05$ for this parameter.

Virus levels-pretreatment, 1996. Pretreatment larval collections from the Ocean City, MD, woodlots revealed a considerable level of natural nuclear polyhedrosis virus in all plots, while virus was absent from similar pretreatment collections made in the Cameron, WV, woodlots. Area effects were significant ($F = 11.51$; df = 1,8; $P < 0.0095$) (Table 2). However, treatment effects, and treatment x area effects were not significant at $P = 0.05$, indicating that the blocking was such that naturally-occurring virus was appropriately distributed between the treated and control plots.

Virus levels-post-treatment, 1996. Post-treatment larval collections indicated that virus was significantly higher in the treated woodlots (58.7%) than in the control woodlots (10.5%) with treatment effects highly significant ($F = 85.49$; df = 1,8; $P < 0.0001$) (Table 2). Although area effects were not significant at $P = 0.05$, virus infection in West Virginia (67.7%) was higher than in Maryland (49.7%), and treatment x area effects were significant ($F = 12.61$; df = 3,8; $P < 0.0075$).

Parasitoids early-season, 1996. A few gypsy moth parasitoids emerged from the pre- and post-season larval collections. The 1,440 larvae collected in Maryland yielded 14 *Cotesia melanoscela* (Ratzeburg) (Hymenoptera: Braconidae) cocoons and 2 *Phobocampe* sp. (Hymenoptera: Ichneumonidae) pupae, while the 1,440 lar-

Table 1. Average (n = 3 plots per location) plot size and preseason gypsy moth egg mass numbers recorded from Gypchek-treated and untreated (control) plots, Worcester Co., MD and Marshall Co., WV, in 1996

Parameter	Controls			Gypchek			Significance by effect*		
	MD	WV	Avg	MD	WV	Avg	Area	Treat	A X T
Plot size (ha)	19	37	28	32	39	36			
(SE)	(1)	(10)	(7)	(12)	(8)	(7)			
Preseason egg masses per ha	812	845	829	994	735	865	NS	NS	NS
(SE)	(270)	(401)	(216)	(255)	(268)	(178)			

* ANOVA (Proc MIXED) (SAS Institute 1996)

Table 2. Levels of nuclear polyhedrosis virus found the year of treatment. Average and SE (computed from 8 subplots for each plot) virus levels in Gypchek-treated or untreated (control) woodlots 1-d pretreatment and 7-d post-treatment at two locations, Worcester Co., MD and Marshall Co., WV in 1996

	% virus, MD plots				% virus, WV plots				Combined
Virus evaluation	I	II	III	Avg	I	II	III	Avg	Avg
Pretreat. larval collection (n = 120 per plot)*									
Gypchek plots	27	6	20	17.7	0	0	0	0.0	8.8
(SE)	(5.5)	(2.4)	(4.9)	(1.4)					(1.8)
Control plots	20	5	4	9.7	0	0	0	0.0	4.8
(SE)	(2.8)	(1.7)	(2.1)	(2.0)					(1.2)
Post-treat. larval collection (n = 120 per plot)**									
Gypchek plots	57	39	53	49.7	73	70	60	67.7	58.7
(SE)	(4.0)	(5.9)	(7.4)	(3.7)	(2.8)	(4.1)	(7.0)	(2.9)	(2.6)
Control plots	35	8	17	20.0	2	1	0	1.0	10.5
(SE)	(4.8)	(1.6)	(3.4)	(3.0)	(1.2)	(0.9)	(0.0)	(0.5)	(2.0)

* Area effects significant ($F = 11.51$; df = 1,8; $P = 0.0095$); treatment effects, and treatment x area effects not significant at $P = 0.05$ (ANOVA, Proc GLM [SAS Institute 1996]).

** Treatment effects significant ($F = 85.49$; df = 1,8; $P = 0.0001$); treatment x area effects significant ($F = 12.61$; df = 3,8; $P = 0.0075$); area effects not significant at $P = 0.05$ (ANOVA, Proc MIXED [SAS Institute 1996]).

vae collected in West Virginia yielded 4 *C. melanoscela* cocoons, 5 *Phobocampe* pupae, and one unidentified tachinid (Diptera: Tachinidae) prepupa. No attempt was made to evaluate late-season parasitism.

Fungus early- and late-season, 1996. Levels of the gypsy moth fungal pathogen *E. maimaiga* were assessed three times in both Maryland and Virginia during 1996 (Table 3). Early-season fungus levels in the Maryland woodlots rose from trace levels (2%) in the pretreatment collections made on 2 May to about 9% for post-treatment larvae collected 10 to 13 May. No fungus was detected in the two analogous collections from the West Virginia woodlots made on 17 May and 24 May. However, when rated for fungus mortality between 18 June and 26 June, a major epizootic of *E. maimaiga* was underway in the untreated West Virginia woodlots. In two of the three West Virginia control plots, numerous (often hundreds) fungus-killed cadavers were present on virtually all trees (rated 3 on a 1 to 3 scale), while the third control block had several cadavers on most trees, with some trees having numerous cadavers (rated 2). In contrast, two of the three treated West Virginia woodlots had but a few fungal-killed cadavers. They received a rating of 1, while the third woodlot received a rating of 2. The late-season fungal epizootic was less apparent in the Maryland woodlots. Two of the treated woodlots and 2 of the control woodlots received a rating of 1, the third treated woodlot received a 2, and the third control woodlot received a rating of 3.

Defoliation, 1996. Defoliation averaged 15% in the treated woodlots and 32% in the control woodlots (Table 4). However, a high degree of variability, especially in the control woodlots, may account for the treatment effects being non-significant. The degree of foliage protection was considerably more impressive in the West Virginia plots (12% in treated plots vs 34% in control plots) than in the Maryland plots (18% in treated plots vs 29% in control plots), perhaps reflecting the higher treatment efficacy seen in the post-treatment larval collections.

Table 3. Average (n = 3 plots per location) fungus (*Entomophaga maimaiga*) levels measured at three times during the season recorded from Gypchek-treated and untreated (control) plots at two locations, Worcester Co., MD, and Marshall Co., WV, in 1996

Parameter	Control plots			Gypchek plots		
	MD	WV	Avg	MD	WV	Avg
% fungus in pretreatment collection	2	0		0	0	
(SE)	(0.3)					
% fungus in posttreatment collection	9	0		8	0	
(SE)	(3.7)			(2.8)		
Late-season fungus rating*	1.7	2.7	2.2	1.3	1.3	1.3

* Based on a 1 to 3 rating of late-season *E. maimaiga*-killed cadavers: 1 = cadavers, if present, few and scattered; 2 = 10 to 75% of trees with 1 or more cadavers, some trees (typically <10%) noted with 20 or more cadavers; 3 = virtually all trees with multiple cadavers, many trees (typically over 50%), with 20 or more cadavers.

Table 4. Average (n = 3 plots per location) postseason gypsy moth egg mass numbers and late-season defoliation recorded from Gypchek-treated and untreated (control) plots at two locations, Worcester Co., MD, and Marshall Co., WV, in 1996

Parameter	Controls			Gypchek			Significance by effect*		
	MD	WV	Avg	MD	WV	Avg	Area	Treat	A X T
% Late-season defoliation	29	34	32	18	12	15	NS	NS	NS
(SE)	(7.3)	(22.8)	(10.8)	(4.4)	(0.9)	(2.5)			
Post-season egg masses per ha	552	415	469	669	180	424	NS	NS	NS
(SE)	(310)	(119)	(319)	(656)	(169)	(151)			

* ANOVA (Proc MIXED) (SAS Institute 1996)

Post-season egg mass levels, 1996. Post-season egg mass levels averaged 424 egg masses per ha in the treated woodlots and 469 egg masses per ha in the control woodlots (Table 4). Once again, a high degree of variability, especially in the control woodlots, may account for the treatment effects being non-significant. Results from West Virginia were again superior (180 egg masses per ha in treated plots vs 415 in control plots) to those of the Maryland blocks (669 egg masses per ha in treated plots vs 552 in control plots), again reflecting the higher treatment efficacy seen in the post-treatment larval collections.

Virus levels early-season, 1997. Significantly higher levels of nuclear polyhedrosis virus were found in our treated woodlots (vs control woodlots) in our early-season larval collection made the year following treatment (1997), with treatment effects significant (F = 13.21; df = 1,8; $P < 0.0066$). Virus incidence averaged 11.7% in treated plots vs 5.0% in control plots (Table 5). Area effects and treatment x area effects were not significant at $P = 0.05$. The second-year effects were particularly striking in the West Virginia plots (12.7% in treated plots vs 3.0% in control plots) compared to the Maryland plots (10.7% in treated plots vs 7.0% in control plots).

Discussion

The West Virginia virus treatments were considerably more successful than the Maryland treatments. The significant treatment x area effects probably reflected both differences in weather conditions during and after spray, and background virus mortality (20% in Maryland, 1% in West Virginia). This was likely due to weather conditions during and immediately following application. The West Virginia treatments were made in the morning with relatively high RH. The Maryland treatments were made in

Table 5. Levels of nuclear polyhedrosis virus found the year following treatment. Average (3 plots per location) 1997 early-instar virus levels recorded from larval collections made from Gypchek-treated (in 1996) and untreated (control) plots at two locations, Worcester Co., MD, and Marshall Co., WV

	% virus, MD plots				% virus, WV plots				Combined
Virus evaluation*	I	II	III	Avg	I	II	III	Avg	Avg
Early larval collection (n variable)**									
Gypchek plots	12	10	10	10.7	9	15	14	12.7	11.7
(SE)				(0.7)				(1.9)	(1.0)
Control plots	13	2	6	7.0	4	0	2	3.0	5.0
(SE)				(3.2)				(1.2)	(1.9)

* Treatment effects significant (F = 13.21; df = 1,8; $P < 0.0066$); area effects, and treatment x area effects not significant at $P = 0.05$ (ANOVA, Proc MIXED [SAS Institute 1996]).

** An attempt was made to collect 100 larvae from all plots, but this was not always possible (collections averaged 80 from Maryland treated plots, 77 from Maryland control plots, 85 from West Virginia treated plots, and 77 from West Virginia control plots). Because larvae were sampled from the whole plot rather than from subplots as in 1996, individual plot variation could not be computed.

the late afternoon when high evaporation due to low RH can retard deposition (Miller et al. 1995), and scattered light showers in the area may have resulted in some washoff. This demonstrates the desirability of applying this product, or any product, under favorable conditions. Unfortunately, program managers are often forced to spray under less-than-perfect conditions. However, delaying application until good conditions occur also incurs a penalty because Gypchek's effectiveness decreases as larval age increases (Webb et al. 1998). The Maryland treatments were applied under unfavorable conditions because the weather forecasts for the immediate future were not favorable, and the larvae were growing larger. This narrow window for treatment is an obvious problem with using Gypchek operationally. The negative impact of suboptimal application conditions may be lessened in the future by further improvements in formulation involving stickers, sunscreens, and anti-evaporation materials.

The difference in fungal ratings between the treated and control woodlots in West Virginia probably reflected the fact that most larvae in the treated woodlots were killed by the Gypchek application, leaving far fewer to succumb to the fungus. Moreover, lack of significance in treatment effects noted for defoliation and post-season egg mass numbers may have been due to compensatory mortality from the late-season epizootic of *E. maimaiga* that was much more apparent in the control plots. This fungal epizootic was expected in the Maryland plots because *E. maimaiga* had been prevalent in the general area the previous year, (R.E.W., unpubl. data), and the beginning of the epizootic was recorded in our early season data. However, the virulence of the West Virginia fungal epizootic was unanticipated because gypsy moth was new to the area and there was no indication of its presence in our early-season data. The Cameron, WV, fungal epizootic may have been due to a cloud of windborne *E. maimaiga* conidia from higher gypsy moth populations to the east similar to the one reported by Webb et al. (1999b) in 1995 in Lexington, VA. The collection data indicated that early-season parasitoids were present, but had little impact. Variable host type (unassessed) may have played a role in results, but random assignment of treatments should have balanced this parameter for treatment vs control comparisons, although host variability could have influenced area effects.

The significantly higher levels of virus found in treated plots the year following treatment were especially noticeable in the West Virginia plots because the virus was at that time just beginning to appear in the control plots. These results suggest that Gypchek application may be particularly desirable in areas where gypsy moth is newly established and natural virus is absent, or in situations where gypsy moth populations are rebounding and residual virus levels are still low. The virus can then be expected to spread into surrounding untreated gypsy moth populations by natural processes as described by Reardon et al. (1996).

Acknowledgments

We thank R. Zerillo, D. Hamilton, H. Hubbard, W. Rollinson and P. Dusha for technical assistance, B. Radsick for the aerial treatment of Gypchek, A. Hickman, P. Leasure, and T. Sukontarak for help with the field experimentation, and L. W. Douglass for statistical support.

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