Augmenting Nucleopolyhedrovirus Load in Gypsy Moth (Lepidoptera: Lymantriidae) Populations with Egg Mass Treatments[1]

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Abstract Previous observations show that gypsy moth, *Lymantria dispar* L., mortality induced by the fungus *Entomophaga maimaiga* Humber, Shimazu & Soper is quickly manifested as host population density increases. However, the gypsy moth nucleopolyhedrovirus (LdMNPV) lags behind the rebounding gypsy moth population. In this study, egg masses were contaminated with virus to successfully augment LdMNPV in gypsy moth populations in Virginia. Laboratory bioassays determined the approximate LdMNPV dose to apply to egg masses with and without the addition of the virus enhancer Blankophor BBH to the spray mixture. The highest dose of virus (5.3×10^5 PIBs/mL) tested without Blankophor BBH gave 82.3% mortality. Mortality for this virus dose increased to 91.8% when 1% Blankophor BBH was added. Field studies established that application of virus at an earlier date (04 April) was as efficacious as an application made at a later date (12 April); this study also included a further assessment of the addition of Blankophor BBH to the spray mixture. While application of LdMNPV + Blankophor BBH resulted in faster kill, levels of kill were similar (88.0% for early treatment and 78.8% for later treatment for virus applied alone versus 87.8% for early treatment and 89.1% for later treatment for virus + Blankophor BBH). However, a higher than expected number of cadavers in the LdMNPV + Blankophor BBH treatments had few or no polyhedral inclusion bodies (PIBs). Finally, virus infection resulting from the application of LdMNPV to pupae in June 1998 was compared with infection levels seen after the application of virus to egg masses in April 1999. The April 1999 treatment to egg masses clearly resulted in a higher kill of emerging larvae (=79.3% mortality) compared to the June 1998 treatment to female pupae (with virus incorporated into the egg masses laid by females after adult emergence) (=13.7% mortality). The virus was recovered season-long from larvae collected from populations in the treated plots (but not from control plots), indicating within season spread.

Key Words *Lymantria dispar,* biological control, Gypchek, nucleopolyhedrovirus, disease augmentation

Low levels of the gypsy moth, *Lymantria dispar* L., nucleopolyhedrosis virus (LdMNPV) often occur in populations occupying newly-infested leading-edge areas and in generally-infested areas where populations have collapsed and have been maintained at very low levels for several years (R. E. Webb, unpub. data). In 1996, Webb et al. (1999a) successfully augmented LdMNPV into rising, virus-free, leading-

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edge gypsy moth populations in West Virginia by aerial application of the virus. The year following the application, significantly higher LdMNPV levels occurred in the treated populations than in nearby untreated gypsy moth populations. The study reported herein investigated the treatment of egg masses for introducing LdMNPV into leading-edge populations or into virus-depleted populations in generally-infested areas.

Research has demonstrated that annual LdMNPV epizootics arise primarily from contaminated egg masses (Doane 1969, 1975, Dwyer and Elkinton 1993, 1995, Elkinton et al. 1990, Murray and Elkinton 1989, 1990, Woods et al. 1990). We evaluated the efficacy of virus applied to egg masses just before hatch. We also sought to augment LdMNPV levels by applying the virus to pupae massed under burlap bands just prior to female emergence from pupae, expecting that PIBs would contaminate the abdominal hairs of the adult females at emergence. We further postulated that PIBs would then be incorporated into the subsequent egg masses as the females covered the egg mass with her abdominal hairs. These PIBs would overwinter in the egg mass and be consumed by emerging larvae as they chewed their way out of the egg masses in the spring.

Materials and Methods

Determination of egg mass treatment dose. We assayed LdMNPV applied to gypsy moth eggs just before hatch. Egg masses used were from the USDA-APHIS Otis Methods Development Laboratory, NJ Standard Strain, F46, received 24 February 1998 (mating date = 1997-JD204, refrigerated 1997-JD246; + 170 days = 1998-051). On 2 March 1998, 15 egg masses were removed from storage, and individual eggs were separated and re-mixed together. Approximately 100 eggs (0.11g of loose eggs + hairs) were placed in individual 30-mL plastic cups (Solo Cup Co., Urbana, IL) with paper lids (WLMA Inc., Newark, NJ), and stored at 7 to 10°C overnight. On 3 March 1998, cups were inoculated with LdMNPV at 0900 to 1030 h.

The LdMNPV used was Gypchek® (USDA Forest Service, Hamden, CT) Batch DR25-32 which contained 4.35×10^{10} polyhedral inclusion bodies (PIBs) per gram. Six concentrations were evaluated: T-5 = 8.3×10^{2} PIBs/mL, T-4 = 8.3×10^{3} PIBs/mL, T-3 = 8.3×10^{4} PIBs/mL, T-2 = 8.3×10^{5} PIBs/mL, T-1 = 5.3×10^{6} PIBs/mL, and T-6 = distilled water controls. There were 10 replicates (cups) for each treatment. A stock suspension (T-1) was made by adding 45.98 mg Gypchek to 200 mls of distilled water. Subsequent treatment suspensions were made by serial dilution. Number of PIBs was confirmed for T1 and T2 using light microscopy and an enumeration chamber. An 0.1-mL aliquot of each treatment suspension was applied to the eggs in the cups. This slurry was stirred into the loose eggs + hairs with a toothpick. The cups were allowed to air dry for 5 h and were then capped with paper lids and placed in a plastic bag with moist paper toweling and held in a controlled-temperature cabinet at 27 to 28°C, 16:8 L: D photoperiod. Initial hatch occurred on 4 March 1998. Twenty larvae (the first 20 encountered) from each of 10 cups from each treatment (= 200 larvae/treatment) were placed, one per cup, on artificial diet (Bell et al. 1981) in 30-mL plastic cups with paper lids and returned to the controlled-temperature. Larvae were examined daily for mortality.

Data were analyzed by analysis of variance (ANOVA) using the General Linear Models (GLM) procedure (SAS Institute 1998). 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 1998). LC_{50} and LC_{90}s were computed using Probit Procedure (Log 10) (SAS Institute 1998).

Blankophor BBH additive study. The objective of this test was to assay LdMNPV dose, applied to egg masses with the addition of Blankophor BBH. Egg masses were from the same source and were handled as previously described. Gypchek (Batch DR25-32, 4.35×10^{10} PIB per g) was used as the LdMNPV source. On 17 March 1998, approximately 100 eggs were placed in individual cups between 1000 and 1200 h. Cups were inoculated between 1315 and 1400 h with 0.1 mL of virus suspension per cup. All treatments and methods were the same as in the previous test, except that 0.05 g (=1% w: v) Blankophor BBH (disodium salt of 2,2[1]-(1,2-ethanediyl) *bis* (5(4-(4-morpholinyl)-6-(phenyl-amino)-1,3,5-triazin-2-yl) amino-benzenesulfonic acid) [CAS No. 16090-02-1]) (Burlington Chemical, Burlington, NC) was mixed with 5 mls of the test suspensions from the previous study. There were 10 replicates (cups) per treatment. Twenty newly-eclosed larvae from each cup were transferred to individual cups of diet as previously described. Larvae were examined daily for mortality. Data were analyzed by analysis of variance (ANOVA) using the General Linear Models (GLM) procedure (SAS Institute 1998). 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 1998). LC_{50}s and LC_{90}s were computed using Probit Procedure (Log 10) (SAS Institute 1998).

Maryland field test. In 1999, a test was conducted on Sugar Loaf Mountain in southern Frederick Co., MD. A randomized complete block design was used, with 10 replicates blocked on location. Ten groups of egg masses, with at least 12 egg masses per group, were marked off along a trail on the north slope of Sugar Loaf Mountain. The expected egg hatch was 15 April 1999. We applied LdMNPV on two dates, 02 April and 12 April, in advance of expected hatch. Although we calculated from the previously discussed laboratory tests that, under laboratory conditions, 10^7 PIBs per liter would kill 99% of the larvae hatching from egg masses given no exposure to sunlight, and no addition of Blankophor BBH, we decided to use a higher concentration of 2.64×10^7 PIBs/mL. We concluded that this higher concentration would more likely yield a high level of mortality when applied to egg masses under field conditions, where the virus would have to survive exposure to sunlight for an extended period before hatch. The 6 treatments were LdMNPV + sticker (2%, v/v) applied on 2 April, LdMNPV + Blankophor BBH (1%, w/v) + sticker applied on 2 April, LdMNPV + sticker applied on 12 Apr., LdMNPV + Blankophor BBH + sticker applied on 12 Apr., Blankophor BBH + sticker applied on 12 April, and sticker alone applied on 12 April. The sticker was Bond® (Loveland Industries, Greeley, CO). Egg masses were sprayed using 373-mL hand-held trigger-pump sprayers (Delta Industries, Philadelphia, PA).

On 14 Apr 10 egg masses were collected with underlying bark, one per each replicate block from each of the 6 treatment groups. These egg masses were held in an outdoor insectary at BARC (Beltsville Agricultural Research Center, Beltsville, MD) (Webb et al. 2001) until larval eclosure. Twenty-five neonates from each egg mass were placed individually in 30-mL cups half-filled with gypsy moth diet (Bell et al. 1981) and held until death or 35 days. This evaluation method assessed death due to ingesting NPV while hatching from the egg mass, but not subsequent exposure to contaminated surfaces.

In addition, 25 larvae hatching from each egg mass left in the field were collected from each of 10 replicate blocks for each of the 6 treatment groups as the larvae

hatched from the masses; hatch occurred from 19 April until 28 April. These larvae were removed from the bark surface with a toothpick (a new toothpick was used for each egg mass) and placed into 30-mL plastic cups without diet. The cups were capped and returned to the BARC insectary, where the larvae were placed individually in 30-mL cups half-filled with diet and held until death or 35 days. Eclosed larvae were collected as they crawled away (typically upwards) from the egg mass. This evaluation method assessed death due to ingesting LdMNPV while hatching from the egg mass as well as from subsequent exposure to contaminated surfaces. Larval eclosion from the egg masses held in the field insectary at BARC occurred from 16 April to 27 April. Larval eclosion from egg masses remaining in the field occurred from 19 April to 1 May. The effects of collection method (collected as egg mass or larvae), presence or absence of Blankophor BBH, and collection date (4 April versus 12 April) and all possible interactions were tested by analysis of variance (SAS 1996, PROC MIXED), with the above three factors modeled as fixed.

Virginia field test. A field test was conducted in 1998-1999 in two northern Virginia counties - Fauquier and Prince William. No natural LdMNPV was detected at the sites. Burlap bands containing an estimated one-half of the female pupae at each of 3 treatment plots were treated with LdMNPV (as Gypchek) at peak pupation on 25 June 1998 (Jun-98 treatment). Burlap skirts on individual trees were lifted and the area under the burlaps was sprayed to the point of runoff with a virus solution of 2.64×10^7 PIBs/mL final suspension + 1% Bond spreader-sticker. Burlap bands with egg masses produced by the other one-half of the female pupal population was designated for treatment just prior to expected egg hatch in the spring of 1999. These egg masses were treated on 06 April 1999, and are hereafter called the Apr-99 treatment. Also on 06 April, just prior to the Apr-99 treatment, 10 egg masses were removed from under the bands to serve as controls.

On 13 April, three plots in Prince William Co. were sampled (ten egg masses per plot) as untreated control blocks to assess the extent of natural LdMNPV in evidence in the region during 1999. Also on 13 April, ten Jun-98 treated, and ten Apr-99 treated egg masses (where possible) were collected from each of the 3 treatment blocks. Egg masses were held in an outdoor insectary at BARC until egg hatch. Twenty larvae from each egg mass were placed individually in 30-mL diet cups half-filled with gypsy moth diet (Bell et al. 1981) and held until death or 35 days. Not all Apr-99 treated egg masses were sampled. Therefore, 50 larvae from the gypsy moth populations in each plot were sampled from leaves on 04 May, 26 May, 09 June, and 24 June 1999. Larvae were placed individually in 30-mL cups half-filled with gypsy moth diet and held until death or 35 days. This allowed us to follow the course of the developing epizootic in treated vs control plots that might have resulted from the treated egg masses left in the field interacting with the general gypsy moth populations in the plots.

Data were analyzed by analysis of variance (ANOVA) using the general Linear Models (GLM) procedure (SAS Institute 1998). 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 1998).

Results and Discussion

Laboratory bioassays. The highest dose tested ($=5.3 \times 10^5$ PIB/mL) caused an 82.3% level of mortality (Table 1). Efficacy decreased rapidly to 33% at 8.3×10^4

Table 1. Mortality of neonate gypsy moth larvae after emergence from eggs treated in laboratory dishes with the indicated dose of LdMNPV per 30-ml cup. Beltsville, Md, 1998

	Test 1 (no BBH)					Test 2 (+BBH)				
	%	Instar at death				%	Instar at death			
Treatment	Mortality*	1st	2nd	3rd	4th	Mortality*	1st	2nd	3rd	4th
5.3×10^5	82.3a	143	15	1	0	91.8a	175	2	0	2
8.3×10^4	33.0b	56	6	3	1	63.6b	124	2	0	0
8.3×10^3	5.5c	8	3	0	0	19.5c	38	1	0	0
8.3×10^2	0.5c	1	0	0	0	3.0d	6	0	0	0
8.3×10^1	0.5c	1	0	0	0	0.0d	0	0	0	0
distilled water	0.0c	0	0	0	0	0.0d	0	0	0	0

* Means within columns followed by the same letter are not significantly different (GLM, LSD [SAS Institute 1998]).

PIBs/mL, and to 5.5% at 8.3×10^3 PIBs/mL. Treatment effects were significant ($F = 181.79$; df = 5, 54; $P < 0.0001$). As expected from previous studies (Shapiro and Robertson 1992, Webb et al. 1994), the addition of Blankophor BBH resulted in higher levels of mortality at most concentrations tested (91.8% at the highest concentration, 5.3×10^5 PIBs/mL). Mortality then fell to 63.6% at 8.3×10^4 PIBs/mL, and to 19.5% at 8.3×10^3 PIBs/mL. Treatment effects were significant ($F = 236.85$; df = 5, 54; $P < 0.0001$). The LC_{50} and LC_{90} for LdMNPV without Blankophor BBH were calculated to be 1.4×10^5 PIBs/mL, and 1.5×10^6 PIBs/mL, respectively. The LC_{50} and LC_{90} for LdMNPV with Blankophor BBH were calculated to be 4.2×10^4 PIBs/mL, and 5.1×10^5 PIBs/mL, respectively.

Maryland field study. The 2.64×10^7 PIBs/mL concentration caused high levels of mortality for both application timings (88.0% for the 04 April treatment, 78.8% for the 12 April treatment) for both treatment timings (Table 2). Mortality was slightly higher with the addition of Blankophor BBH (Table 2) for the 12 April timing (89.1%), but not for the 4 April treatment (87.8%). Treatment effects were not significant. There was no indication that field exposure of the treatments for an additional 8 days led to decreased efficacy of the treatments with or without Blankophor BBH. During necropsy we characterized the cadavers as having many PIBs, a few PIBs, or no PIBs (Table 2). Mortality was low for both the surfactant control (2.0% and 3.2% for larvae from sampled egg masses and for larvae collected from the field, respectively) and for the Blankophor BBH control (4.4% and 3.6% for larvae from sampled egg masses and for larvae collected from the field, respectively). In both cases, only one dead larva was positive for PIBs.

Analysis of variance of the data for total mortality indicated that the date of treatment and presence or absence of Blankophor BBH effects were not significant. Also, all interactions were non-significant. However, collection method was significant ($P = 0.042$). Mortality was consistently higher for larvae that emerged from egg masses collected prior to hatch than for larvae collected in the field after hatch. Analysis of

Table 2. Mortality (total, with PIBs, and with many PIBs) of neonate gypsy moth larvae after emergence from egg masses treated in the field with LdMNPV (2.6×10^7 PIBs/mL), at two different dates, with or without 1% Blankophor BBH (BBH). All treatments applied with 2% Bond. N = 250 for larvae from collected eggs (eggs), and for larvae collected as larvae (larvae), and N = 500 for the combined total (Total). Sugar Loaf Mountain, MD, 1999

Treatment time	LdMNPV	BBH	Stage collected	N	Total mortality	Mortality with PIBs	Mortality with many PIBs
4 April	+	0	eggs	250	89.6	83.2	79.2
			larvae	250	86.4	74.0	62.4
			Total	500	88.0	78.6	70.8
4 April	+	+	eggs	250	88.0	72.8	58.8
			larvae	250	87.6	64.4	54.8
			Total	500	87.8	68.6	56.8
12 April	+	0	eggs	250	80.4	78.4	74.4
			larvae	250	79.2	73.6	64.0
			Total	500	78.8	76.0	69.2
12 April	+	+	eggs	250	93.2	77.6	68.4
			larvae	250	85.0	51.2	42.4
			Total	500	89.1	64.4	55.4
12 April	0	0	eggs	250	2.0	0.4	0.4
			larvae	250	3.2	0.0	0.0
			Total	500	2.6	0.2	0.2
12 April	0	+	eggs	250	4.4	0.4	0.4
			larvae	250	3.6	0.0	0.0
			Total	500	4.0	0.2	0.2

variance of the data for larvae positive for PIBs again found that the date-of-treatment effect and all interactions were non-significant. However, the presence-or-absence-of-Blankophor-BBH effect was significant ($P = 0.0006$). This was apparently due to less mortality "with PIBs" occurring for larvae from egg masses treated with LdMNPV + BBH than for larvae treated with LdMNPV alone. Interestingly, collection method was highly significant ($P = 0.0001$). Mortality "with PIBs" was notably and consistently higher for larvae that emerged from egg masses collected prior to hatch than for larvae collected in the field after hatch. It should be noted that this effect of collection method is not reflected in the surfactant controls or in the BBH controls, and thus seems to be an effect of the treatments. When only larvae "containing many PIBs" are considered, the above effects are strengthened. We again found that there was no

significant difference for date of treatment, and all but one of the interactions were non-significant, the exception being that the Date*BBH*Collection interaction was now significant at $P = 0.035$. The presence or absence of Blankophor BBH was now even more significant ($P = 0.0001$), with even less mortality "with high PIBs" consistently noted for larvae from egg masses treated LdMNPV + BBH than for larvae treated with LdMNPV alone. Collection method was again highly significant ($P = 0.0001$). Mortality differences "with high PIBs" were even higher for larvae that emerged from egg masses collected prior to hatch than for larvae collected in the field after hatch, again with the effect especially prominent for egg masses treated with LdMNPV + BBH. In Table 3, the data from this study are examined in a different way, with larvae dying in 14 days or less separated from larvae dying in 15 to 35 days. Here we see that the excess of larvae dying "without PIBs" largely occurs to larvae that die within 14 days of hatch. A few such early deaths were to be expected because not all larvae placed on diet will settle and feed on artificial diet, and 23 dead larvae (out of 1000) were observed for the surfactant and BBH controls. However, the high number of such larvae in the LdMNPV + Blankophor BBH treatments may represent larvae dying of viremia before the onset of PIB formation, because Blankophor BBH + LdMNPV is known to cause death significantly faster compared to LdMNPV applied alone (Webb et al. 1999b). The high number of early deaths, and of deaths "without PIBs", associated with larvae collected from the field versus larvae emerging from egg masses sampled prior to hatch may represent sick larvae succumbing earlier due to the stress of extra handling.

Virginia field tests. Mortality of larvae hatching from the egg masses sampled from the 3 untreated "control" plots in April 1999 were 0% for two plots and 0.3% for the third (Table 4). Mortality caused by LdMNPV for untreated egg masses removed from the three "treatment" plots prior to the spring egg-mass treatment, but after the June 1998 treatment, were 0% for two plots and 3% for the third. Results from these 6 plots indicate that LdMNPV levels were extremely low in this region at the beginning of 1999. In the 3 treatment plots, LdMNPV had been applied under bands that harbored female gypsy moth pupae in June 1998. The expectation was that some of the applied PIBs would adhere to the abdominal hairs of females emerging from these pupae as they crawled over the treated surface. These hairs which would then be incorporated in their resulting egg masses as per the reports of Murray and Elkinton (1989, 1990). Mean mortality of larvae emerging from these masses in late Apr 1999 was 13.7% (range = 7% to 26%). In the 3 treatment plots, there were also bands under which gypsy moth egg masses had been oviposited but that had not been treated in June 1998. LdMNPV was applied to these egg masses in early April 1999. Mean mortality of larvae emerging from these masses in late April 1999 was 79.3% (range = 65% to 85%). The April 1999 treatment resulted in a significantly ($F = 62.37$; df = 3, 8; $P < 0.0001$) higher kill of larvae emerging from the egg masses than did the Jun 1998 treatment; however, the Jun 1998 treatment imitates the natural incorporation of PIBs into egg masses and warrants further research. The "right dose" may vary from situation to situation, because when and where the larvae die may be more important than how many of those emerging from the eggs die.

As stated in the procedures, some LdMNPV egg masses were left unsampled in the 3 treated blocks. Larvae that hatched from these egg masses were free to interact with the larvae from untreated egg masses in the block and to spread the virus. Only one larva (2%), collected on 09 June, was positive for LdMNPV in the three non-treated plots (Table 5). In contrast, LdMNPV-positive larvae were collected for all four

Table 3. Comparison of early (<14 days) versus later (15-35 days) mortality (total, with PIBs, and with many PIBs) of neonate gypsy moth larvae after emergence from egg masses treated in the field with LdMNPV (2.6 x 10^7 PIBs/mL), at two different dates, with or without 1% Blankophor BBH (BBH), Sugar Loaf Mountain, MD, 1999

Treatment time	LdMNPV	BBH	Stage collected	Died <14 d of hatch				Died 15-36 d of hatch			
				N	no PIBs	few PIBs	many PIBs	N	no PIBs	few PIBs	many PIBs
4 April	+	0	eggs	9	9	0	0	215	7	15	193
			larvae	45	14	15	16	163	9	14	140
			Total	54	23	15	16	378	16	29	333
4 April	+	+	eggs	45	34	11	0	175	4	24	147
			larvae	73	51	16	6	145	6	8	131
			Total	118	85	27	6	320	10	32	278
12 April	+	0	eggs	2	2	0	0	199	3	10	186
			larvae	23	7	6	10	175	7	19	149
			Total	25	9	6	10	374	10	29	335
12 April	+	+	eggs	31	31	0	0	202	8	23	171
			larvae	80	60	14	6	112	4	8	100
			Total	111	91	14	6	314	12	31	271

Table 3. Continued.

Treatment time	LdMNPV	BBH	Stage collected	Died <14 d of hatch				Died 15-36 d of hatch			
				N	no PIBs	few PIBs	many PIBs	N	no PIBs	few PIBs	many PIBs
12 April	0	0	eggs	3	3	0	0	2	1	0	1
			larvae	7	7	0	0	3	3	0	0
			Total	10	10	0	0	5	4	0	1
12 April	0	+	eggs	7	7	0	0	5	4	0	1
			larvae	6	6	0	0	4	4	0	0
			Total	13	13	0	0	9	8	0	1

All treatments applied with 2% Bond. N = 250 for larvae from collected eggs (eggs), and for larvae collected as larvae (larvae), and N = 500 for the combined total (Total).

Table 4. Mortality (and n) of neonate gypsy moth larvae after emergence from egg masses treated in the field with LdMNPV (2.6 x 10^7 PIBs/mL), treated at two different dates, 25 June 98 and 06 April 99. All treatments applied with 2% Bond. Northern Virginia, 1998, 1999

Location	%NPV-April, 99	%NPV-June, 98	(within-plot) %NPV-Control	(untreated plot) %NPV-Control
Fauquier-2	85 (300)	26 (300)	0 (300)	
PW-2				0 (270)
PW-3	88 (300)	8 (210)	3 (120)	
PW-4				0.3 (300)
PW-5				0 (300)
PW-6	65 (300)	7 (300)	0 (300)	
Avg.*	79.3a	13.7b	1.0b	0.1b

* Means within rows followed by the same letter are not significantly different (GLM, LSD [SAS Institute 1998]).

Table 5. Percentage mortality (n = 50 per sample date per plot) from LdMNPV (NPV) or from *E. maimaiga* (E. m.) of gypsy moth larvae collected from plots in which egg masses had been treated in the field with LdMNPV (2.6 x 10^7 PIBs/mL), or from untreated control plots, on the four indicated dates. Northern Virginia, 1998, 1999 treatments, 1999 collections

	4 May		26 May		9 June		24 June	
Location	NPV	E. m.	NPV	E. m.	NPV	E. m.	NPV	E. m.
Treated plots								
Fauquier-2	6	0	4	2	6	0	8	0
PW-3	18	0	2	2	4	0	6	0
PW-6	0	0	0	2	0	0	2	0
Untreated plots								
PW-2	0	0	0	2	0	0	0	0
PW-4	0	0	0	4	0	0	0	0
PW-5	0	0	0	0	2	0	0	0

sampling periods in 2 treated blocks and one LdMNPV-infected larva was collected on 24 June from the third block. (The latter block was located on the summit of Bull Run Mountain, and the low return of LdMNPV-positive larvae from this block may have been due to the larvae ballooning off the mountain upon hatch.) LdMNPV-infected larvae emerging from treated egg masses would have all died by 01 June, so

the LdMNPV-infected larvae sampled from all three treated blocks 24 June most likely resulted from virus spread within the blocks. This test was designed to develop application technique, and was not designed as a release study with precisely-measured parameters. However, the season-long recovery of virus from populations in the treated plots vs the control plots provide proof-of-concept that LdMNPV can be inoculated into gypsy moth populations by egg mass treatment. Another field experiment across a wider landscape is a logical follow-up study. Larvae infected with the fungus *Entomophaga maimaiga* Humber, Shimazu and Soper were collected from 5 of the 6 blocks, but only on 26 May. One larva infected with *E. maimaiga* was collected from 4 of the plots, and 2 larvae so infected were collected from the fifth plot. Clearly, *E. maimaiga* was not a factor in this study.

Summary. It has recently been stated (Richards et al. 1993) that "most baculovirus control programs have been strongly influenced by technologies developed for the chemical control of pest species and have tended to be based on large-scale inundative release of virus at high host densities. However, because entomopathogens frequently have the capacity to persist within the host population and in the environment which the host occupies, approaches which exploit and enhance virus transmission pathways, such as autodissemination and environmental manipulation, are more likely to be more successful in the longer term." Trans-ovum transmission of NPV (Bird 1961, Nordin 1976, Tatchell 1981) is common in the Lepidoptera, and virus-contaminated egg masses are responsible for most season-to-season transmission of LdMNPV in gypsy moth populations (Doane 1969, 1975, Elkinton et al. 1990, Murray and Elkinton 1989, 1990, Woods et al. 1990). Gypsy moth egg masses acquire LdMNPV contamination primarily from the surface on which they are deposited, that is, contamination occurs during the process of oviposition. The female becomes externally contaminated as she drags her abdomen over the bark surface. Alternatively, the egg mass may be deposited onto a contaminated surface. Murray and Elkinton (1990) sprayed LdMNPV onto bark surfaces and allowed uninfected lab females to oviposit on the contaminated surfaces or on similar uncontaminated surfaces. Neonates from contaminated surfaces had much higher levels of LdMNPV. Neonates from the innermost part of the egg masses near the bark surfaces had a higher level of mortality than larvae from the outer layer of the egg masses. Elkinton et al. (1990) suggested that the egg mass may provide a protective cover for the polyhedral inclusion bodies that otherwise might not survive exposure to UV radiation or other factors over the 9 month interval between oviposition and hatch. Models of Dwyer and Elkinton (1993, 1995) demonstrated the importance of trans-ovum transmission for the induction of LdMNPV epizootics. Limited but promising studies have used LdMNPV as egg mass treatments against the gypsy moth (Cardinal and Smirnoff 1973, Orlovskaya et al. 1979, Podgwaite et al. 1981, Trzebitzby et al. 1988). Orlovskaya (1979) found that treatment of egg masses was more effective in the year of treatment, and was more economical, than was the aerial application of the virus. Podgwaite et al. (1981) introduced LdNPV into sparse gypsy moth populations by treating egg masses. In addition to an estimated 85 to 90% mortality induced by LdMNPV in larvae hatching from treated egg masses, there was a 20% incidence of LdMNPV in fourth to sixth-instar larvae in the year of treatment. However, it should be noted that neonates could also acquire lethal infections by crawling over LdMNPV-contaminated surfaces (Weseloh and Andreadis 1986, Woods et al. 1990). Thus, in nature, vertical (year-to-year) transmission of LdNPV is largely due to contaminated abdominal hairs from gypsy moth females being incorporated into overwintering egg

masses. Larvae ingest these PIBs while hatching from egg masses in the spring. We sought to compare this natural avenue of introducing LdMNPV into gypsy moth populations with low viral loads with the more direct method of egg mass treatment just prior to egg hatch.

Our ultimate objective is the introduction of LdMNPV into NPV-deficient gypsy moth populations to cause an NPV epizootic a year or more before its expected natural occurrence. Objectives of this study were to investigate the role of application timing and the use of a sunscreen on the percentage of emergent neonate larvae succumbing to virus. The virus in nature is protected by melanins from the disintegrated host insect. However, the applied virus is susceptible to inactivation by sunlight. The chosen sunscreen was Blankophor BBH, a stilbene optical brightener. Stilbene optical brighteners have been demonstrated to have radiation protectant properties (Shapiro 1992), as well as causing considerable enhancement of LdMNPV activity (Shapiro and Robertson 1992).

Podgwaite et al. (1979) found that LdMNPV liberated from larval cadavers onto bark overwintered at high levels; however, LdMNPV in spray deposits survived at measurable quantities for only 3 to 15 days. Loss of viral activity was attributed to deactivation by sunlight and/or physical removal by rain. Therefore, the persistence of virus applied to egg masses in nature was a concern. Thus, our finding in the Sugar Loaf Mountain study that LdMNPV can be applied several weeks before expected egg-hatch without apparent loss of activity provides considerable operational freedom. Our expectation was that the addition of Blankophor BBH would be desirable by providing protection from sunlight (Shapiro 1992) and by potentiating virus kill. However, at the virus dosage tested, LdMNPV alone gave as high a kill as LdMNPV + Blankophor BBH, and most of the cadavers resulting from applying LdMNPV alone contained a high number of PIBs. While application of LdMNPV + Blankophor BBH resulted in faster kill, and perhaps a slightly higher level of kill, the high number of cadavers with few or no PIBs is a problem. The purpose of treating the egg masses with the virus is to get PIBs into the field. We, therefore, cannot at this time recommend that Blankophor BBH be added to LdMNPV as an egg mass treatment, although further research is needed to resolve this question.

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