Effects of Blankophor BBH, a Virus-Enhancing Adjuvant, on Mortality of Gypsy Moth (Lepidoptera: Lymantriidae)[1]

R. E. Webb, R. A. Peiffer,[2] R. W. Fuester,[3] M. A. Valenti,[4] K. W. Thorpe, G. B. White and M. Shapiro

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

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Abstract We examined aspects of the gypsy moth, *Lymantria dispar* (L.)/nuclear polyhedrosis virus relationship, and the effects of Blankophor BBH on that relationship, that might impact the timing of virus kill in a cohort of treated larvae. We studied this relationship both for virus and enhancer applied together and separately. We found that a portion of larvae ingesting virus polyhedral inclusion bodies die later (more than 4 wk after infection) in the season, and that this can be affected by the presence of Blankophor BBH if the virus dose is above a certain level (in this study, 10^7 polyhedral inclusion bodies per 378 liters). Furthermore, the pattern of mortality resulting from virus ingestion was elucidated. This pattern was affected by Blankophor BBH, but only when the virus dose was above a certain higher level (in this study, 10^{11} inclusion bodies per 378 liters). We also found that Blankophor BBH alone had no obvious effect on the course of the disease in gypsy moth larvae that had previously ingested virus; it caused neither an increase in mortality, a decrease in time to kill, nor any obvious effect on the pattern of kill. Most larvae died between 18 and 29 d. Few larvae ingesting virus died earlier (13 to 17 d); however, about 5% of the larvae died later than 30 d after infection, which may be late enough to contribute to the second wave of mortality. A combination of Blankophor BBH at 0.5% and virus at 10^{11} inclusion bodies resulted in an increase in mortality and a decrease in time of kill compared with that seen for that level of virus without the enhancer, while eliminating the "tail" of mortality occurring 30 d after infection. However, a combination of Blankophor BBH at 0.5% and virus at 10^9 inclusion bodies still give higher mortality than expected with the virus alone, but did not decrease the time of kill or eliminate the "tail."

Key Words *Lymantria dispar,* baculovirus, adjuvant, epidemiology, forest pest.

Gypchek® (U. S. Forest Service, USDA, Washington, DC) is a product with the *Lymantria dispar* multienveloped nuclear polyhedrosis virus as the active ingredient that is registered by the USDA Forest Service with the U. S. Environmental Protection Agency as a general use insecticide for aerial and ground application against the gypsy moth, *Lymantria dispar* (L.) (Reardon et al. 1996). Successful field trials with the commercially-produced Carrier 038 (Novo Nordisk, Franklinton, NC) (Reardon et al. 1996, Webb et al. 1999) and environmental concerns over the effects of non-specific insecticides applied to forest ecosystems stimulated interest in the use of

[1]Received 30 September 1998; accepted for publication 22 December 1998.

[2]Department of Agriculture and Natural Resources, Delaware State University, Dover, DE 19901.

[3]USDA, ARS, Beneficial Insects Introduction Research, Newark, DE 19713.

[4]Delaware Department of Agriculture Forest Service, Dover, DE 19901.

Gypchek (Reardon et al. 1996). The addition of certain stilbene-derived optical brighteners enhanced the performance of this virus in the laboratory (Shapiro and Robertson 1992) and in the field (Webb et al. 1994a,b). Additional field work in 1996 (Webb et al. 1998) compared properties of this virus with standard insecticides using the "bugs-in-bags" approach developed by D'Amico and Elkinton (1995).

This is the eighth in a series of reports on efforts to adapt virus strains and formulations, with or without enhancing agents, for use by arborists and nurserymen using ground equipment. One goal of our program is to develop usage patterns for virus that take advantage of the virus' ability to persist and to spread. A knowledge of the timing and pattern of mortality resulting from the application of the virus is essential to this goal. In this study, we again used a bugs-in-bags approach to elucidate additional aspects of the relationship between the virus and Blankophor BBH under the field conditions similar to that encountered by arborists.

Materials and Methods

Insect colony and virus. Gypsy moth larvae from Newark, DE (USDA-ARS Newark stock culture) and Beltsville, MD (eggs obtained from the New Jersey Standard Strain from the APHIS rearing facility at Otis ANGB, MA) were reared in 230-ml paper cups on standard gypsy moth artificial diet (Bell et al. 1981). Larvae were reared 100 per cup for second instars, 50 per cup for third instars, and 25 per cup for fourth instars. The virus inoculum used was the Hamden isolate LPD-226.

Field plots. Ninety-six groups of 4 oak branch tips, primarily pin oak, *Quercus palustris* Muenchh., that were accessible from the ground were marked along the edge of a forest cultivated field in the Cedar Swamp Wildlife Area northeast of Smyrna, DE, in the spring of 1997. Each group of tips was separated by at least 2 m. There was no evident natural gypsy moth population in the woodlot. A randomized complete block design was used. There were eight blocks, each with all 12 treatments randomly assigned.

Host plant phenology. Leaf expansion was determined at the end of each evaluation period by removing all leaves from the branch tips taken from five of the plots (the same plots were used for all time periods), as the gypsy moth larvae were removed from the bags, and measuring the areas of these leaves using a Li-cor LI-3100 area meter (Li-cor, Inc., Lincoln, NE). The leaves from the 5-wk evaluation averaged (± SEM) 40.6 ± 7.6 cm^2 and were considered fully expanded. We found that leaf area averaged (± SEM) 1.7 ± 0.3 cm^2 at the time of treatment, indicating 4% leaf expansion based on the 5-wk measure. Leaf expansion 3 wks after treatment averaged 63%.

Treatments. Treatments 1 to 7 were as follows: (1) virus at 1×10^{12} polyhedral inclusion bodies per 378 liters final solution, with 2% Bond sticker; (2) virus at 1×10^{11} polyhedral inclusion bodies per 378 liters final solution with 2% Bond sticker; (3) virus at 1×10^{11} polyhedral inclusion bodies per 378 liters final solution +0.5% Blankophor BBH, with 2% Bond sticker; (4) virus at 1×10^{9} polyhedral inclusion bodies per 378 liters final solution +0.5% Blankophor BBH, with 2% Bond sticker; (5) virus at 1×10^{7} polyhedral inclusion bodies per 378 liters final solution +0.5% Blankophor BBH, with 2% Bond sticker; (6) no virus +0.5% Blankophor BBH, with 2% Bond sticker; and (7) untreated (control "A") tips. These first seven treatments received gypsy moth larvae produced at the Beneficial Insects Introduction Research Laboratory, Newark, DE, reared as below, and were analyzed separately from treatments 8 to 12.

In treatments 8 to 12, the virus part of the treatment was administered separate from the Blankophor BBH part of the treatment. Larvae were fed first with virus in the lab, and then, after feeding for 24 h, were put onto Blankophor BBH-treated or non-treated leaves in the field. Treatments 8 to 12 were as follows: (8) 0.5% Blankophor BBH, with 2% Bond sticker, with larvae from Beltsville infected at 10^5 inclusions/ml as per Shapiro et al. (1994); (9) no BBH, with larvae from Beltsville infected at 10^5 inclusions/ml as per Shapiro et al. (1994); (10) 0.5% Blankophor BBH, with 2% Bond sticker, with larvae from Beltsville infected at 10^3 inclusions/ml as per Shapiro et al. (1994); (11) no BBH, with larvae from Beltsville infected at 10^3 inclusions/ml as per Shapiro et al. (1994); and (12) no virus, no BBH, (control "B") with uninfected larvae from Beltsville. These last five treatments received gypsy moth larvae produced at the Insect Biocontrol Laboratory, Beltsville, MD. Treatments 8 to 11 consisted of larvae infected with virus by the method of Shapiro et al. (1994). Virus was diluted in distilled water, and the resulting virus suspensions were applied to the surface of the diet (1 ml per cup, 180-ml cup) at two concentrations during the 24-h prior to being encaged on treated or untreated foliage in the field. Larvae were dosed at concentrations meant to yield median lethal doses (LD_{50}s) (1×10^5 inclusion bodies per diet cup for treatments 8 and 9), and LD_{20}s (1×10^3 inclusions per diet cup) for treatments 10 and 11.

All treatments. A total of 560 larvae from Newark and 320 larvae from Beltsville was needed for each of four evaluation periods. All treatments were sprayed to runoff in distilled water on 2 May 1997 using a backpack sprayer, and were allowed to dry prior to the encagement of the first cohort of larvae (placed approximately 1 h after treatment). Subsequent cohorts of larvae were encaged on residues aged 1 wk, 3 wk, and 5 wk. Larvae were placed in bags as per Webb et al. (1998a) that were fastened over treated branch tips. One tip from each of the 96 groups of branch tips was bagged for each evaluation date. Bags were constructed of 60 × 60 cm squares of organza cloth seamed to make a bag. Ten gypsy moth larvae from the appropriate rearing location (second instar for both the 1-h residue period and the 7-day residue period, third instar for the 3-wk residue period, and fourth instar for the 5-wk residue period) were placed in the bags, which were then tied off. Larvae were left in the bag for 1 wk, after which the branch tips bearing the bags were snipped off and the bagged tips were taken to the lab, where all larvae were removed from the bags and placed on artificial diet (Bell et al. 1981) in 30-ml plastic cups with paper lids, one larva per cup. The rearing cups were held on shelves in a wooden outdoor insectary (368 cm long, 215 cm wide, 92 cm deep, with hardware cloth covering the front to allow natural conditions of light, temperature, and humidity but not rain) at the Beltsville Agricultural Research Center, Beltsville, MD. All larvae in the insectary were observed every 2 to 3 d for mortality until death, pupation, or for 66 d. Dead larvae were labeled by date-of-death and placed in a freezer to await necropsy. Tissue samples from all of the larvae that died were examined under 400X for the presence of viral inclusion bodies. If determinations could not be made with certainty using the above procedure, smears of tissue were fixed over a flame, stained with dilute Giemsa solution (Glaser 1915), and then examined under oil emersion at 1000X.

Statistical methods. Data were analyzed by analysis of variance (ANOVA) using the General Linear Models (GLM) procedure (SAS Institute 1985). 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). An arcsine-square root transformation was used on all percentage data. Time to death

data were not normally distributed, so a $\log_{10}$ transformation was used to normalize the data. All values that were analyzed using transformation are presented in the tables back-transformed. Chi-square tests were applied to categorical data compiled for patterns of death due to virus.

Results

Virus-induced mortality, treatments 1 to 7 (virus and enhancer fed concurrently). Mortality caused by virus varied widely by treatment and by residual date within the treatment group (treatments 1 to 7) where gypsy moth larvae were fed foliage bearing residues of virus and Blankophor BBH (Table 1). Treatment effects were highly significant for the 1-h ($F = 23.41$; df = 6,42; $P < 0.0001$), the 1-wk ($F = 35.54$; df = 6,42; $P < 0.0001$), and the 3-wk ($F = 3.76$; df = 6,42; $P < 0.0001$) evaluation periods, but not significant for the 5-wk evaluation period. There was a rapid loss of effectiveness with time, and by the week 5 evaluation, only one larva (fed on Treatment 3 foliage) succumbed to virus. Treatments 1 and 2 (respectively, 10^{12} and 10^{11} inclusions of virus per 378 liters, applied without Blankophor BBH), were not significantly different for the 1-h residue evaluation despite a 10-fold increase in dose. Both treatments lost most of their effectiveness when feeding was initiated on 1-wk resi-

Table 1. Percent virus infection (SE) for gypsy moth larvae (NJ Standard strain) placed in bags on treated oak foliage in the Cedar Swamp Wildlife Management Area, DE, in 1997. Gypchek (virus, or NPV) and Blankophor BBH (BBH) applied together, along with NPV treatments lacking BBH

	% Mortality (SE) for larvae placed on indicated residues*		
Treatment	1-h (2nd instar)	1-wk (2nd instar)	3-wk (3rd instar)
1. NPV 10^{12} (No BBH)	77.6cd (5.0)	24.1b (7.8)	1.7a (1.7)
2. NPV 10^{11} (No BBH)	70.1cd (4.3)	7.1a (2.9)	0.0a
3. NPV 10^{11} + 0.5% BBH	84.7d (12.4)	81.2c (8.1)	17.8b (8.1)
4. NPV 10^{9} + 0.5% BBH	57.6c (10.5)	5.6a (3.7)	0.0a
5. NPV 10^{7} + 0.5% BBH	16.4b (6.2)	0.0a	0.0a
6. No NPV + 0.5% BBH	1.4ab (1.4)	0.0a	0.0a
7. Control A (untreated)	0.0a	0.0a	0.0a

* Means within columns followed by the same letter are not significantly different (GLM, LSD [SAS Institute 1985]). Five-wk data not shown because only 1 of 493 recovered larvae died.

dues. Treatment 3 (10^{11} inclusions +0.5% Blankophor BBH per 378 liters) was not significantly different from treatments 1 and 2 for the 1-h residue feeding-initiation evaluation, although it was clearly superior in the day 7 results; all replications showed high levels of NPV-induced mortality except for replicate 4, where 0% mortality was recorded, suggesting that the replicate 4 bag was inadvertently placed on an untreated branch tip. If this replicate is not considered, then mortality for treatment 3 rises to 97% for the 1-h residue evaluation. Treatment 3 induced 81.2% mortality of larvae initiating feeding on 7-d residues despite a 15-fold dilution due to leaf expansion. Some activity (17.8%) was still seen for treatment 3 for larvae that initiated feeding on leaves bearing 21-d residues. Treatment 4 (10^{9} inclusions +0.5% Blankophor BBH per 378 liters) was active against larvae initiating feeding on 1-h residues (57.6% mortality), but effectiveness was clearly reduced at this dose.

Virus-induced mortality, treatments 8-12 (virus and enhancer fed separately). It had been shown in the laboratory (Dougherty et al. 1995) that an optical brightener similar to Blankophor BBH increased the efficacy of the gypsy moth virus, but only when both brightener and virus are present concurrently in the midgut. In the present study, we examined the sequential feeding of virus and enhancer to determine if subtle effects might be manifested under field conditions that were not observed under laboratory conditions. Treatment effects were highly significant for the 1-h ($F = 22.71$; df = 4,28; $P < 0.0044$), the 1-wk ($F = 36.69$; df = 4,28; $P < 0.0001$), the 3-wk ($F = 21.04$; df = 4,28; $P < 0.0001$) and the 5-wk ($F = 27.04$ df = 4,28; $P < 0.0001$) evaluation periods. However, unlike the results seen above with treatments 1 to 7, the differences in treatment mortality for treatments 8 to 12 were due solely to the fact that larvae were fed two doses of virus on diet prior to encagement on foliage. Mortality for both higher virus-dose treatments (larvae fed in diet cups on 10^{5} inclusion bodies per ml per 180 ml diet cup, then fed 1 wk on foliage with or without Blankophor BBH) statistically separated from mortality for both of the lower virus-dose treatments (fed on 10^{3} inclusions). Treatment mortality comparisons for treatment 8 vs treatment 9 (respectively, 10^{5} inclusions with and without Blankophor BBH follow-up) and also for treatment 10 vs treatment 11 (respectively, 10^{3} inclusions with and without Blankophor BBH follow-up), were statistically equivalent for the 1-h, 1-wk and 3-wk evaluation periods for the 10^{5} treatment pair, and for all four evaluation periods for the 10^{3} treatment pair (Table 2). Thus, in agreement with previous laboratory findings (Dougherty et al. 1995), feeding the newly virus-infected larvae on leaves containing Blankophor BBH, but no additional virus, had little obvious impact on the resulting mortality. The observed decrease in mortality with increasing age of residue seen in the 0, 7, 21, and 35 d treatments were likely due to feeding the same dose of virus to increasingly larger larvae (as per Shapiro et al. 1986). Leaf expansion was about 4% when the sprays were applied. Nonetheless, high levels of virus mortality resulted from the high virus treatment (Treatment 1), and the low virus treatment (Treatment 2), indicating that virus can be applied soon after bud-break.

Time to death. With one exception, time to death of larvae infected with virus varied little among the treatments or with the residual dates, within the treatment group where gypsy moth larvae fed on foliage bearing residues of virus and Blankophor BBH (treatments 1 to 7) (Table 3). Treatment effects were highly significant only for the 1-h residual assessment ($F = 14.44$, df = 5, 193, $P < 0.0001$). Treatment effects for the 1-wk, 3-wk, and 5-wk evaluation periods were nonsignificant. The exception was that the addition of 0.5% Blankophor BBH to Gypchek at 10^{11} inclusions resulted in reduced time to death compared to Gypchek alone at 10^{11} inclusions or 10^{12}

Table 2. Percentage virus infection (SE), following treatment, of gypsy moth larvae fed on oak foliage at the Cedar Swamp Wildlife Management Area, DE, in 1997. Gypchek fed to gypsy moth larvae (Newark strain) 24 h before placement on foliage treated with Blankophor BBH

Treatment	% Mortality (SE) for larvae placed on indicated residues*			
	1-h (2nd instar)	1-wk (2nd instar)	3-wk (3rd instar)	5-wk (4th instar)
8. 10^5 NPV + BBH	64.5c (9.9)	52.3c (6.4)	25.1b (7.3)	29.8c (5.4)
9. 10^5 NPV (no BBH)	60.0c (6.4)	53.8c (9.0)	29.3b (5.8)	10.4b (2.7)
10. 10^3 NPV + BBH	16.8b (4.6)	8.0b (2.7)	0.0a (0.0)	3.8a (1.8)
11. 10^3 NPV (no BBH)	9.0ab (4.6)	4.3ab (2.7)	3.8a (1.8)	0.0a (0.0)
12. Control B (no BBH)	0.0a (0.0)	0.0a (0.0)	0.0a (0.0)	0.0a (0.0)

* Means within columns followed by the same letter are not significantly different (GLM, LSD [SAS Institute 1985]).

inclusions. However, time to death of the lower-dose treatment of 0.5% Blankophor BBH + Gypchek at 10^9 inclusions was comparable to Gypchek at 10^{12} inclusions with no Blankophor BBH. The general trend was for death to occur more rapidly in "stronger" (higher virus dosage, less age of residue) situations than in "weaker" (lower virus dosage, longer age of residue) situations. Treatment effects were not significant for treatments 8 to 12 for any evaluation period (Table 4).

Temporal pattern of death. For the Newark larvae used in treatments 1 to 7, most larvae (67%) molted once before death regardless of the presence or absence of BBH; some (27%) molted twice before death, while 6% died without molting (Table 5), while no larvae died after 3 molts. The larvae, held under conditions of ambient outdoor temperatures, died over a range of 13 to 45 days, but most died 17 to 29 d after encagement on treated foliage (Table 5). Deaths of a few (5.6%) larvae were recorded after >30 d. The temporal pattern of death for unenhanced virus applied at 10^{12} inclusions was 1.4% dying between 13 to 17 d, 77.5% dying between 18 to 24 d, 14.1% dying between 25 to 29 d, and 7.0% dying after 30 d or more; corresponding percentages for unenhanced virus applied at 10^{11} inclusions was 0%, 60%, 31.1%, and 8.9%, respectively. This 10-fold difference in virus dose had little effect on the pattern of death ($\chi^2 = 5.803$; df = 3; $P > 0.10$). The pattern of temporal death shifted towards earlier death under the influence of Blankophor BBH: over all dosages and residual times, 11.9% died in 13 to 17 d, 71.4% in 18 to 24 d, 12.4% in 25 to 29 d, and 4.3% in 30 d or more. Corresponding percentages for virus without Blankophor BBH were 0.9%, 70.7%, 20.7%, and 7.8%. Despite the fact that lower doses of virus were associated with Blankophor BBH (Table 5), these patterns differed significantly ($\chi^2 = 15.95$; df = 3; $P < 0.01$). The most compact pattern of temporal death was treatment

Table 3. Average days to death (SE). Gypchek and Blankophor BBH applied together, with gypsy moth larvae placed (as second-instars for the 1-h and 1-wk residues, and as third instars for the 3-wk residues) on treated foliage. Cedar Swamp Wildlife Management Area, DE, 1997

Treatment	Days to death (SE) for larvae placed on indicated residues*		
	1-h (2nd instar)	1-wk (2nd instar)	3-wk (3rd-instar)
1. NPV 10^{12} (No BBH)	23.7a (0.4)	24.2 (1.0)	25.0 (0.0)
2. NPV 10^{11} (No BBH)	25.0a (0.5)	26.0 (2.2)	no dead
3. NPV 10^{11} + 0.5% BBH	20.4b (0.3)	21.9 (0.6)	24.4 (0.9)
4. NPV 10^{9} + 0.5% BBH	24.2a (1.1)	22.5 (1.3)	no dead
5. NPV 10^{7} + 0.5% BBH	25.0a (0.7)	no dead	no dead
6. No NPV + 0.5% BBH	29.0a (0.0)	no dead	no dead
7. Control A (untreated)	no dead	no dead	no dead

* Means within columns followed by the same letter are not significantly different (GLM, LSD [SAS Institute 1985]). Five-wk data not shown because only 1 of 493 recovered larvae died.

3 for 1-h encagement (25% died between 13 to 17 d, while 75% died between 18 to 24 d). However, somewhat "weaker" treatment/date combinations (treatment 3, 7-d; treatment 4, 1-h) were characterized by the wider spread of mortality seen for unenhanced virus.

The Beltsville larvae (treatments 8 to 12) provide an interesting contrast in that encaged larvae for all four time periods were dosed prior to encagement, so that there is no problem of interpretation due to virus inactivation or leaf expansion. The Beltsville larvae died over a range of 13 to 34 d. The overall pattern of death (Table 6) was similar to that seen for Newark larvae (Table 5), including a "tail" of mortality occurring after 30 d. Patterns of death did not differ between treatments ($\chi^2 = 10.00$; df = 3; $P > .30$).

Discussion

Specific questions addressed in this study were: (1) Will a portion of larvae ingesting virus die later (more than 4 wk after infection) in the season? (2) Would this be affected by the presence of Blankophor BBH? (3) What is the pattern of mortality resulting from NPV ingestion, and is this pattern affected by Blankophor BBH? (4) If Blankophor BBH were sprayed alone, would it cause gypsy moth larvae that had ingested sublethal levels of virus to develop a killing viremia? (5) Is the course of the

Table 4. Average days to death (SE). Gypchek fed to gypsy moth larvae 24 h before placement on foliage treated with Blankophor BBH. Cedar Swamp Wildlife Management Area, DE, 1997

Treatment	Avg days to death (SE) for larvae placed on indicated residues*			
	1-h (2nd instar)	1-wk (2nd instar)	3-wk (3rd instar)	5-wk (4th instar)
8. 10^5 NPV + BBH	24.2 (0.5)	20.3 (0.4)	21.0 (0.4)	22.1 (0.5)
9. 10^5 NPV (no BBH)	24.5 (0.5)	20.8 (0.6)	21.3 (0.5)	22.4 (1.5)
10. 10^3 NPV + BBH	26.9 (1.3)	19.5 (1.0)	no dead	no dead
11. 10^3 NPV (no BBH)	25.8 (0.9)	23.3 (0.7)	21.3 (0.7)	no dead
12. Control B (no BBH)	no dead	no dead	no dead	no dead

* Treatment effects not significant at $P = 0.05$.

(NPV) disease affected by addition of BBH? Additionally, we elucidate the residual activity of Blankophor BBH/virus combinations as it might affect larvae moving onto treated foliage at various times after treatment. Finally, the findings of this study are discussed in the context of the previous 7 reports (Thorpe et al. 1998, Webb et al. 1990, 1993, 1994a, 1994b, 1996, 1999).

The virus-induced mortality seen for larvae that initiated feeding on leaves bearing 1-h residues treated with 10^7 virus inclusion bodies +0.5% Blankophor BBH per 378 liters (treatment 5) was low, but significantly different from the untreated controls. This dose was equal to approximately 25 inclusion bodies per ml of solution, and approaches the minimum level of virus that would cause mortality if fed to second-instar gypsy moth larvae in combination with Blankophor BBH; the virus would probably not cause mortality in the absence of the enhancing agent. This information might be useful in assessing natural virus levels in gypsy moth populations with very low virus loads by spraying a swath of gypsy moth-infested foliage with Blankophor BBH alone, and sampling larvae from such foliage a week after treatment. There were no deaths due to virus in the control treatment (Treatment 7), indicating that any treatment drift was below that needed to cause lethal infection when unenhanced; however, one larva died from virus in the Blankophor BBH control treatment (Treatment 6) in the 1-h residue evaluation. Death occurred after 29 d, and was probably due to contamination with a few inclusion bodies (by drift, hands, birds?) working in concert with the enhancer.

We were interested in examining the temporal pattern of death (and how this might be affected by Blankophor BBH) in part to learn if a portion of "first wave" mortality occurs later in the season as part of the "second wave" [see Woods and Elkinton

Table 5. Pattern of virus death and average number of molts until death. Gypchek and Blankophor BBH applied together, with gypsy moth larvae placed (as second instars for the 1-h and 1-wk residues, and as 3rd instars for the 3-wk residues) on treated foliage. Cedar Swamp Wildlife Management Area, DE, 1997.

Treatment	Age of Residue*	Larvae Dead within Indicated Period				Avg New Molts at Death
		13–17	18–24	25–29	30–45	
1. NPV 10^{12}	1-h	0	43	8	3	1.5
	1-wk	1	12	1	2	1.4
	3-wk	0	0	1	0	1.0
2. NPV 10^{11}	1-h	0	25	12	3	1.1
	1-wk	0	2	2	1	1.6
	3-wk		(no dead)			
3. NPV 10^{11}	1-h	16	48	0	0	1.0
+ 0.5% BBH	1-wk	4	49	4	4	1.2
	3-wk	0	7	5	1	1.2
4. NPV 10^{9}	1-h	1	19	5	3	1.1
+ 0.5% BBH	1-wk	0	3	1	0	1.0
	3-wk		(no dead)			
5. NPV 10^{7}	1-h	0	6	7	0	1.5
+ 0.5% BBH	1-wk, 3 wk		(no dead)			
6. No NPV	1-h	0	0	1	0	1.0
+ 0.5% BBH	1-wk, 3 wk		(no dead)			
7. Control A	1-hr, 1-wk, 3 wk		(no dead)			

* Five-wk data not shown because only 1 of 493 recovered larvae died.

(1987) for a discussion of the bimodal pattern of virus in gypsy moth populations]. It would generally be advantageous to the virus to be expressed as early as possible to facilitate horizontal transmission. However, expression in older larvae later in the season would result in far more inclusion body production (Shapiro et al. 1986), facilitating vertical transmission. This might be especially useful to the virus in lower gypsy moth populations where horizontal transmission may be less likely to occur. We did find examples of delayed virus-induced death, but in numbers too few to significantly affect waves of virus-induced mortality. Working soon after the appearance of gypsy moth virus in North America, Chapman and Glaser (1916) found a similar the pattern of death (to our findings) of experimental gypsy moth larvae fed virus from three different natural sources, except that they did not report any mortality occurring after 30 d (that was seen in the present study).

From these data, one can surmise that gypsy moth neonates, ingesting virus

Table 6. Pattern of virus death and average number of molts until death. Gypchek fed to gypsy moth larvae 24 h before placement on foliage treated with Blankophor BBH. Cedar Swamp Wildlife Management Area, DE, 1997

Treatment	Age of BBH Residue	Larvae Dead within Indicated Period				Avg New Molts at Death
		13–17	18–24	25–29	30–45	
8. NPV 10^5	1-h	2	29	10	4	2.0
+ BBH	1-wk	5	28	0	0	1.7
	3-wk	0	18	2	0	1.3
	5-wk	0	17	4	0	1.6
9. NPV 10^5	1-h	0	29	12	2	2.1
No BBH	1-wk	6	29	1	2	1.8
	3-wk	0	20	3	0	1.5
	5-wk	1	5	1	1	1.6
10. NPV 10^3	1-h	0	5	2	3	2.2
+ BBH	1-wk	1	5	0	0	1.0
	3-wk, 5-wk			(no dead)		
11. NPV 10^3	1-h	0	4	3	0	2.0
No BBH	1-wk	0	3	0	0	1.7
	3-wk	0	3	0	0	1.5
	5-wk		(no dead)			
12. Control B	1-h, 1-wk, 3 wk, 5 wk			(no dead)		

inclusion bodies while chewing their way out of an egg mass, will generally die in a "first wave" event 3 to 5 wks thereafter, earlier under warm conditions, later under cool conditions. A few infected larvae, perhaps having ingested fewer inclusions will survive to die in the interlude between the first and second waves. The second wave is the result of first wave cadavers releasing inclusions (Doane 1975, Woods and Elkinton 1987), and will begin 6 to 7 wks after egg hatch and peak at perhaps 10 wks after egg hatch. When a virus-based spray is applied about 2 wks after egg hatch, an anomalous "wave" is created when affected larvae die 6 to 8 wks after egg hatch. This might be too late to influence second wave virus mortality. Adjuvants that increase the speed of kill (such as Blankophor BBH) might enhance second wave effects. Also, early virus application might enhance second wave activity; thus, our findings show that a virus application can be applied soon after bud break (see above) are encouraging.

Our results suggest that two things happen when a critical dose of virus is fed together with a critical dose of Blankophor BBH. First, the percentage of larvae succumbing to virus increases compared to that seen for controls fed that particular dose of virus alone. Secondly, the time to death of larvae succumbing to virus decreases compared to that seen for controls fed that particular dose of virus alone. However, if either the dose of BBH is decreased below a certain point (as per Webb et al. 1996), or if the dose of virus is decreased below a certain point (as seen here), then the percentage of larvae succumbing to virus still increases compared to that seen for treatment groups fed that particular dose of virus alone, but the decrease in time to death of larvae succumbing to virus is less pronounced. On the other hand, it may be that, the number of larvae dying at lower doses being smaller, it becomes impossible to discern a difference in the temporal distribution of deaths with and without Blankophor BBH.

The fact that treatment effects were not significant for treatments 8 to 12 for any evaluation period (Table 4) suggests that newly virus-infected larvae feeding on leaves containing Blankophor BBH, but no additional virus, had little obvious impact on the resulting viremia. The results for treatments 1 to 7, where larvae molted only once or twice before death, agrees with the observation of Doane (1967) that if larvae survive two molts after exposure to virus, subsequent mortality is light. The fact that weaker treatment/date combinations were characterized by a wider spread of mortality may indicate a lack of uniformity of inclusion body distribution due to reduced dosage and erosion with time [caused by the inactivation of virus due to exposure to the ultra violet portion of sunlight (David 1969)] and/or leaf expansion. Because neither the virus dosage nor presence-absence of Blankophor BBH appeared to influence the resulting pattern of death, the hastening effect of Blankophor BBH on death of gypsy moth larvae appears to be lost when ingestion of the virus and enhancer do not occur contemporaneously. Finally, these results suggest that the combination of Gypchek at 10^{11} inclusions and Blankophor BBH at 0.1 to 0.5% per 378 liters is still recommended as per Webb et al. (1996), although the cost effectiveness of a dose of 10^{10} inclusions + Blankophor BBH at various doses needs to be explored.

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