Nuclear Polyhedrosis Virus of the Gypsy Moth (Lepidoptera: Lymantriidae): Potency and Effects of Selected Adjuvants on Insect Feeding Behavior[1, 2]

Robert R. Farrar, Jr., Richard L. Ridgway, Stephen P. Cook, Kevin W. Thorpe, and Ralph E. Webb

Beltsville Agricultural Research Center
ARS, USDA, Beltsville, MD 20705

J. Entomol. Sci. 30(4): 417-428 (October 1995)

ABSTRACT The potency of two formulations of the nuclear polyhedrosis virus of the gypsy moth (LdMNPV) was evaluated in the laboratory. Both formulations were prepared with the same batch of LdMNPV produced in live insects by the USDA Forest Service. A Forest Service-recommended tank-mix preparation (LdMNPV, molasses, ultraviolet light screen, and sticker in water) was found to be about 20 times more potent than an experimental wettable powder preparation. The wettable powder also deterred feeding; the 20-fold difference in potency, though, is based on actual doses consumed. The addition of a stilbenedisulfonic acid derivative, Blankophor BBH, to the tank-mix and wettable powder preparations at a concentration of 1% (wt./vol.) reduced the LD_{50}s by 42- and 214-fold, respectively. Blankophor BBH was also a moderate feeding deterrent to gypsy moth, *Lymantria dispar* (L.), larvae which could limit its efficacy as an enhancer of the virus. However, the addition of molasses to Blankophor BBH at least partially overcame the feeding deterrence. Other adjuvants were also tested for effects on larval feeding behavior, including Bond (sticker), Lignosite AN (ultraviolet light screen), and Carrier 244 (spray carrier). Of the materials tested, molasses was the strongest feeding stimulant, followed by Carrier 244. Bond and Lignosite AN had no detectable effect on feeding behavior in these tests. These data demonstrate the importance of monitoring potency during any formulation process, the possibility of enhancing the virus with adjuvants, and of understanding the effects of adjuvants on feeding behavior.

KEY WORDS Insecta, gypsy moth, *Lymantria dispar,* nuclear polyhedrosis virus, enchancer, adjuvant, formulation, potency, feeding behavior.

The gypsy moth, *Lymantria dispar* (L.) (Lepidoptera: Lymantriidae), is one of the most important pests of forest and ornamental trees in the northeastern United States. Because it occurs in populated areas and diverse natural ecosystems, there is concern about the environmental effects of any measures used for control. For this reason, *Bacillus thuringiensis* Berliner is often the control agent of choice in the United States. The strains of *B. thuringiensis* used against the gypsy moth are specific to lepidopterous insects, but these include

[1] Accepted for publication 12 May 1995.

[2] Use of trade names does not imply endorsements of products named nor criticism of similar ones not mentioned.

nontarget caterpillars that may be important components of forest ecosystems. Therefore, there is considerable interest in another microbial agent, the nuclear polyhedrosis virus of the gypsy moth (LdMNPV), which has an extremely narrow host range (Barber et al. 1993). This virus is registered as *Gypchek* by the USDA Forest Service for gypsy moth control. Gypchek is usually applied aerially (Podgwaite 1985, Podgwaite et al. 1992), but it also can be applied effectively from the ground (Webb et al. 1990).

Several factors have limited the use of LdMNPV. Because it is currently produced in live insects, it is expensive and of limited availability (Podgwaite 1985). It requires as long as 14 days to kill larvae, during which time significant defoliation can occur (Podgwaite 1985), and it is most effective when applied against first or second instars (Lewis et al. 1979). Like other nuclear polyhedrosis viruses, it is susceptible to degradation by environmental factors, particularly ultraviolet light (UV) (Jaques 1977), and it must be consumed by the larvae, so it is best applied in a formulation that the larvae will readily eat and that provides some protection against UV.

A variety of adjuvants have been, or are currently being, developed for use in spray applications to overcome factors limiting the utility of LdMNPV, including stilbenedisulfonic acid derivatives as UV screens (Shapiro 1992). A number of these derivatives are used in laundry detergents to make fabrics appear brighter, chiefly by absorbing energy from ultraviolet light and re-emitting it as visible light. For this reason, these materials are commonly known as optical brighteners or fluorescent brighteners. In addition to being effective UV protectants, though, these materials were found to be strong enhancers of the activity of LdMNPV (Shapiro and Robertson 1992, Shapiro et al. 1992). The mode of action of stilbene disulfonic acid derivatives as enhancers of LdMNPV is unknown, but does not appear to be related to their fluorescence; therefore, they will be referred to herein simply as enhancers.

In laboratory bioassays (Shapiro and Robertson 1992, Shapiro et al. 1992), LC_{50}s were reduced as much as 1837-fold by the addition of enhancers, and survival time of infected larvae also was reduced. In field tests, Webb et al. (1994b) reported that the addition of 1% enhancer to a high volume, ground-based spray allowed a 10-fold reduction in the amount of virus applied per hectare without reduction in control, and that addition of the enhancer reduced survival time of infected larvae. The enhancer also can be used to extend the time period within which the virus can be applied; its use makes possible control of third and fourth instars on individual shade trees (Webb et al. 1994a). Stilbenedisulfonic acid derivatives thus have the potential to help alleviate several of the problems that currently restrict the use of LdMNPV, through reduced rates of application, increased speed of kill, widened windows of application, and lessened degradation by UV light. However, the responses of insects to varying concentrations of LdMNPV in the presence versus absence of these enhancers have not previously been studied in detail. Furthermore, all published laboratory data were based on virus applied to artificial diet, which may not accurately reflect effects in a more natural setting.

Technical grade gypsy moth virus, freeze dried, (hereafter referred to as *technical virus*) is being produced in limited quantities by the USDA Forest Service and currently is used in an aqueous tank-mix preparation with stickers, sodium lignin sulfonate (UV screen), and molasses (humectant and feeding stimulant) (Reardon

and Podgwaite 1992). Recently, an experimental wettable powder formulation of LdMNPV, designed to be mixed with water and applied without additional adjuvants, was developed by American Cyanamid Co. Herein, we report the results of dosage-mortality studies of LdMNPV in both preparations on foliage with and without a stilbenedisulfonic enhancer. The tank-mix and wettable powder were prepared to resemble as closely as possible sprays that would actually be applied in the field. Because little data on the effects of the components of the tank mix, or of the enhancer, on the feeding rates of gypsy moth larvae have been published previously, studies in which these effects were measured in detail also were conducted. Also included in these studies was an experimental carrier (Carrier 244, 1993 preparation; Novo Nordisk Bioindustrials, Danbury, CT) developed for use with insect viruses.

Materials and Methods

Insects and virus. All insects were obtained as eggs from a colony maintained at the Otis Methods Development Center, Otis ANGB, MA. The eggs were held under refrigeration at 5°C until needed. Larvae were reared through the first stadium on artificial diet (Bell et al. 1981). All larvae that were used were newly-molted second instars and had been starved for ≈ 18 h. The technical virus was provided by the USDA Forest Service, Hamden, CT; the wettable powder formulation, by American Cyanamid Co., Princeton, NJ. Both preparations were made from technical virus from the same batch produced in live insects by the USDA Forest Service.

Effects of formulation and enhancer on potency. Potency of LdMNPV was investigated through a dosage-mortality experiment with the tank-mix and wettable powder. The tank-mix preparation consisted of an aqueous solution/suspension of molasses, 12.5% (vol./vol.; feed grade, Southern States Cooperative, Inc., Richmond, VA); sodium lignin sulfonate, 6% (wt./vol; Lignosite AN, Georgia Pacific, Bellingham, WA); sticker, 2% (vol./vol.; Bond, Loveland Industries, Greeley, CO), and technical virus. The wettable powder virus formulation was suspended in water with no additional adjuvants. Similar suspensions of each preparation also were prepared with 1% (wt./vol.) enhancer (Blankophor BBH, Burlington Chemical Co., Burlington, NC). Six concentrations of virus without enhancer (5, 20, 200, 2000, 20,000, and 60,000 polyhedral inclusion bodies (PIBs/μl) and six concentrations with enhancer (0.1, 0.2, 0.5, 1, 2, and 5 PIBs/μl) were prepared by serial dilution. (Concentrations were adjusted for the third of three replicates to include 5, 20, 100, 200, 2000, and 20,000 PIBs/μl without enhancer and 0.2, 0.4, 0.6, 0.8, 1, and 2 PIBs/μl with enhancer.) Calculations of rates (mg dry material/ml) of the wettable powder were based on the reported content of PIBs in material received; those of the tank mix, on counts of PIBs made our laboratory. Concentrations of enhancer and other adjuvants were held constant over the range of virus concentrations. Selection of concentrations of virus was based on preliminary tests showing large differences in the amount needed to kill insects between treatments with and without enhancer (R. R. Farrar, unpublished). The control treatment for the tank-mix with and without enhancer included all components of the tank-mix with and without enhancer, respectively, but no

virus. The control treatments for the wettable powder with and without enhancer were water with 1% enhancer and water only, respectively. Fresh suspensions were prepared for each replicate.

A Levy-Hausser hemocytometer (Clay Adams, Parsippany, NY) was used to count PIBs in samples of both the wettable powder and technical virus. Counts of PIBs in the wettable powder were 45.1% of reported levels (see Results), so the data were adjusted before statistical analysis by multiplying the rates of the wettable powder by 0.451.

A bioassay was designed so that the actual dose of virus acquired by each larva could be estimated. Disks, 9 mm in diameter, were cut with a cork borer from leaves of lettuce, *Lactuca sativa* L., cv. 'Black Seeded Simpson', that had been grown in a greenhouse. Lettuce was chosen because it is readily consumed by gypsy moth larvae and is available all year. Disks were held on moist paper towels to reduce wilting. Five microliters of treatment suspension was pipetted onto each disk and the disks were allowed to dry. Larvae were placed individually in 5.5-cm diam Petri dishes lined with moist filter paper with one treated leaf disk. The dishes were sealed with parafilm (American National Can, Greenwich, CT) and held at 27°C with a photoperiod of 16:8 (L:D) for 48 h. All larvae that had consumed at least 75% of the leaf disk were then transferred to individual 30-ml plastic cups containing about 10 ml of artificial diet (Bell et al. 1981); other larvae were discarded. (Any larvae that died while on the leaf disk were assumed to have been injured in handling and were excluded.) Because the treatment suspensions only covered a small spot near the center of each disk, those larvae that consumed 75% of the disk were assumed to have eaten all of the virus applied to the disk. The doses received by each larva were thus approximately equal to the concentrations of PIBs listed above multiplied by five. Larvae in cups with diet were held under the same conditions as above for 14 d; mortality was recorded at 48 h intervals through the 14 d period.

Twenty-five larvae were offered disks with each concentration of each suspension, though only data from larvae that ate at least 75% of their disks were analyzed. The experiment was replicated three times over time. Percentage mortality was calculated based on the cumulative number of larvae that died within 14 d after being placed on diet. Doses were transformed to logarithms, and the data were analyzed by probit analysis (SAS Institute 1988). The two formulations of virus (tank-mix and wettable powder) were compared within each enhancer treatment (with and without) by factorial analysis of variance (ANOVA) with virus dose and formulation as main effects (SAS Institute 1988). Enhancer treatments within each virus treatment were not compared by ANOVA because only one concentration of virus (5 PIBs/µl) was common to both enhancer treatments; instead, enhancer treatments were compared by overlap of 95% fiducial limits of their LD_{50} values. The mean survival time of larvae that died was calculated for each treatment of each replicate; these means were then analyzed by factorial ANOVA as above.

Effects of formulations and adjuvants on feeding behavior. The effects of the tank-mix and wettable powder preparations of LdMNPV on feeding behavior were studied by measuring consumption of treated leaf disks. Two experiments were conducted. The first experiment included ten treatments: (1) technical virus, 2.7×10^7 PIBs/ml; (2) wettable powder, 2.7×10^7 PIBs/ml; (3)

molasses, 12.5%; (4) Bond, 2%; (5) Lignosite AN, 6%; (6) Blankophor BBH, 1%; (7) tank-mix, including molasses, Bond, and Lignosite AN as above, and technical virus, 2.7×10^7 PIBs/ml; (8) tank mix, 2,255 PIBs/ml, plus Blankophor BBH, 1%; (9) wettable powder, 2,255 PIBs/ml, plus Blankophor BBH, 1%; and (10) control, water only. The second experiment included six treatments; Carrier 244; molasses, 12.5%; and control, water only; each with and without Blankophor BBH, 1%.

Larvae were weighed and placed individually in inverted 5.5-cm plastic Petri dishes lined with moist filter paper. A cork borer was used to cut disks of lettuce foliage, 19 mm in diam, in pairs from opposite sides of the midvein. Disks were dipped in treatment suspensions and held on moist paper towels long enough for liquid residue from the dip to dry but not long enough for the leaf material to wilt. One disk from each pair was placed in a Petri dish with a larva; the other disk was dried to constant weight at 70°C and weighed. This value was used as an estimate of the starting weight of the food for that larva. The test was held at 27°C for 24 h with a photoperiod of 16:8 (L:D) h, then terminated by freezing. For each repetition of the test, 10 additional larvae (not fed leaf disks) were weighed, killed by freezing, dried and weighed again to determine percentage moisture. Later, uneaten leaf material remaining in each Petri dish was dried and weighed; this weight was subtracted from the starting weight of the food to determine the approximate dry weight of food consumed. Relative consumption rate (RCR), defined as the dry weight of food consumed divided by the initial dry weight of the larva (Farrar et al. 1989), was calculated for each larva.

Ten individually-fed larvae were randomly assigned to each treatment for each experiment, and each experiment was repeated six times for each treatment for a total of 60 larvae per treatment per experiment. Larvae that died during the 24 h of the test were assumed to be have been injured in handling and were, therefore, excluded. The first experiment was analyzed by ANOVA and treatment means were separated by the least significant difference (LSD) test (SAS Institute 1988). RCR was the response variable, with each individual larva treated as the experimental unit. The six repetitions of each experiment were treated as blocks, with 10 larvae randomly assigned to each treatment in each block. The second experiment was analyzed as a 2×3 factorial ANOVA, with the presence or absence of enhancer as one main effect and the other adjuvants as the other main effect. Because no significant interaction between the main effects was found (see results), means of adjuvant treatments across enhancer treatments were separated by LSD, and means of enhancer treatments across adjuvant treatments were separated by the F test (SAS Institute 1988).

Results

Effects of formulation and enhancer on potency. The tank-mix preparation was found to be about 20 times more potent (without enhancer) in terms of LD_{50}s than the experimental wettable powder preparation (Table 1). Our counts of PIBs were lower for the wettable powder formulation than the values reported on the container of virus that we received; our counts averaged 45.1% of reported values. However, data reported herein are adjusted for this difference. The addition of 1% Blankophor BBH greatly increased the potency of both virus preparations. The LD_{50}s were reduced by about 42- and 214-fold for the tank-mix and

Table 1. LD_{50} and LD_{95} values (PIBs per larva) for a tank-mix preparation and an experimental wettable-powder preparation of LdMNPV with and without the addition of a stilbenedisulfonic acid derivative, Blankophor BBH, as an enhancer, fed to second instar gypsy moth larvae.

Preparation	Enhancer	N*	LD_{50} ± 95% fiducial limits (PIBs/larvae)		LD_{95} ± 95% fiducial limits (PIBs/larvae)	
Tank-mix	0%	462	113.1	73.8 – 169.0	5827.7	$3038.8 - 1.41 \times 10^4$
	1%	425	2.7	1.8 – 3.9	16.0	9.1 – 50.8
Wettable powder	0%	444	2273.5	$316.8 - 3.06 \times 10^4$	2.11×10^6	$9.75 \times 10^4 - 2.54 \times 10^{10}$
	1%	455	10.6	6.4 – 25.0	258.3	75.8 – 3554.4

* Total number of larvae tested with indicated combination of virus and enhancer.

wettable powder preparations, respectively (Table 1). Fiducial limits of LD_{50}s at the 95% level with and without enhancer did not overlap for either formulation. In the ANOVA, significant differences in potency were found between the tank-mix and the wettable powder preparations, both without and with enhancer (F = 30.37; df = 1, 31; $P < 0.0001$ without enhancer and F = 22.11; df = 1, 31; $P < 0.0001$ with enhancer).

Survival time of larvae that were killed by the virus (Fig. 1) was significantly affected by both formulation and dose in the absence of enhancer (F = 11.53; df = 1,30; $P = 0.0019$ for formulation and F = 22.41; df = 1, 30; $P < 0.0001$ for dose), but not in its presence (F = 0.52; df = 1,31; $P = 0.4778$ for formulation and F = 2.42; df = 1, 31; $P = 0.1298$ for dose). Without enhancer, those larvae that died from the virus did so in less time at higher doses of virus. Larvae that died after receiving comparable doses of the two formulations died sooner if they received the tank-mix. However, none of these differences were apparent in treatments with enhancer.

Effects of formulations and adjuvants on feeding behavior. Rates of food consumption (RCR) of lettuce leaf disks treated with virus and/or adjuvants in the first experiment varied significantly (F = 16.02; df = 9, 577; $P < 0.0001$) among treatments (Table 2). Relative to the control, RCR was increased by molasses, decreased by enhancer, and not affected by Bond and Lignosite AN. Consumption of foliage treated with the wettable powder formulations of virus was greatly reduced; that of a lower rate of wettable powder plus enhancer was also reduced, but to a lesser degree. The technical virus, without adjuvants, did not affect RCR relative to the control, but the addition of the tank-mix adjuvants did significantly increase consumption of the virus-treated foliage. Although enhancer applied alone reduced RCR, the reduction of RCR by addition of enhancer to the tank-mix preparation with the other adjuvants (about 15%) was not significant ($P > 0.05$).

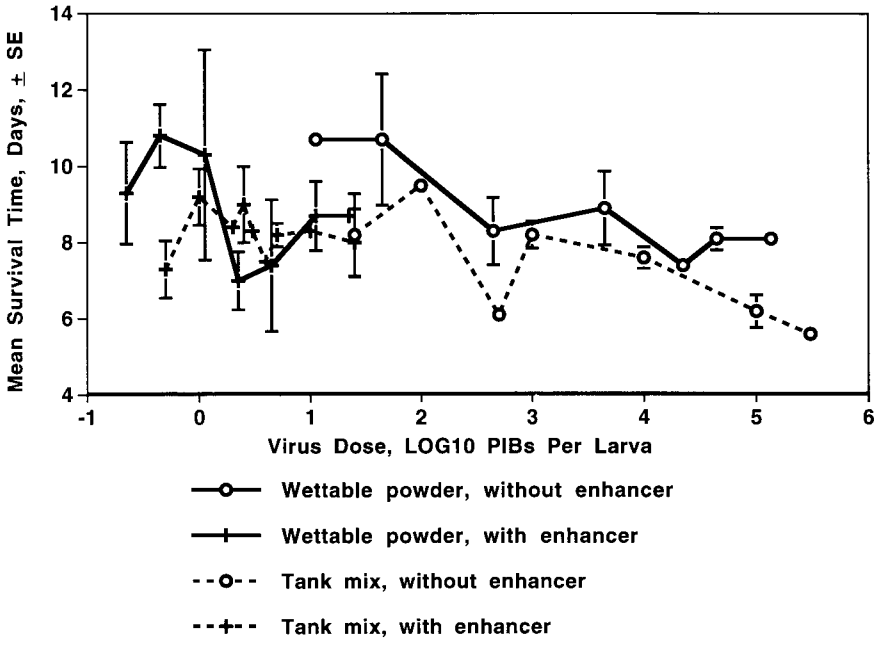

Fig. 1. Survival times of second-instar gypsy moth larvae that died as a result of being fed measured doses of LdMNPV in tank-mix and wettable powder preparations with and without the addition of a stilbenedisulfonic acid derivative, Blankophor BBH, 1%, as an enhancer. Points with no standard error bars represent rates included in only one replicate.

More variability was evident in the response of larvae to the tank-mix preparations, with and without enhancer, than in their response to other treatments that were readily consumed. This variability can be seen in the standard errors, which are more than twice as large for these preparations than for the control. The residue of the tank-mix preparations tended to absorb moisture in the Petri dishes, creating sticky surfaces that may have deterred some larvae from feeding.

In the second experiment, RCR was significantly affected by both the presence of enhancer ($F = 11.09$; df = 1, 348; $P = 0.0010$) and by the other adjuvant treatments ($F = 9.19$; df = 2, 348; $P < 0.0001$), but not by interaction between enhancer and the other adjuvants ($F = 2.37$; df = 2, 348; $P = 0.0950$) (Table 3). Consumption was increased by molasses and, to a lesser degree, by Carrier 244. Enhancer tended to reduce consumption regardless of the presence of other adjuvants. As with the tank-mix treatments, the response of larvae to Carrier 244 was more variable than that of larvae to the other treatments. This treatment formed a thick, heavy coating on the disks, and tended to absorb moisture and become

Table 2. Relative consumption rates for second instar gypsy moth larvae on lettuce leaf disks (19 mm diam.) treated with tank-mix or experimental wettable powder preparations of LdMNPV or individual adjuvants, including a stilbenedisulfonic acid-derived enhancer, Blankophor BBH.

Treatment*	RCR** ± SE
Molasses, 12.5%	3.66 ± 0.264 a
Tank-mix (virus, 27×10^6 PIBs/ml + molasses, 12.5% + Bond, 2% + Lignosite AN, 6%)	2.67 ± 0.421 b
Tank-mix (virus, 2255 PIBs/ml + molasses, 12.5% + Bond, 2% + Lignosite AN, 6%) + enhancer, 1%	2.26 ± 0.401 bc
Control	2.11 ± 0.195 bc
Bond, 2%	1.89 ± 0.201 c
Technical virus (27×10^6 PIBs/ml)	1.72 ± 0.194 c
Lignosite AN, 6%	1.71 ± 0.263 c
Wettable powder (virus, 2255 PIBs/ml) + enhancer, 1%	0.97 ± 0.145 d
Enhancer, 1%	0.91 ± 0.116 d
Wettable powder (virus, 27×10^6 PIBs/ml)	0.22 ± 0.103 e

* Indicated percentages for the preparation ingredients are vol./vol., except for Lignosite and enhancer, which are wt./vol.

** Relative consumption rate = dry weight of food eaten in 24 h divided by initial dry weight of insect; means with the same letter are not significantly different by LSD ($P > 0.05$.)

sticky. Many larvae consumed very little of it, but some consumed relatively large amounts.

Discussion

Results presented herein confirm that a stilbenedisulfonic acid derivative used as an enhancer can increase mortality caused by LdMNPV. These results are consistent with those of Shapiro and Robertson (1992), who used only artificial diet and did not measure actual doses, although the degree of reduction of LD_{50}s was less than that of the LC_{50}s of Shapiro and Robertson (1992). Results also show that the enhancer can effectively increase mortality due to formulations that have relatively low potency, such as the wettable powder formulation, although the enhanced tank-mix preparation was still about four times as potent as the enhanced wettable powder. Limited overlap in the range of doses of virus treatments with enhancer with those without enhancer limits statistical comparisons of survival times of larvae that died. However, survival times of larvae that died after ingesting ≈ 25 PIBs with the enhancer were comparable to those of larvae that died after ingesting ≈ 50,000 PIBs without enhancer.

Table 3. Consumption rates of lettuce leaf disks (19-mm diam) treated with adjuvants of LdMNPV with and without a stilbenedisulfonic acid derivative, Blankophor BBH, as an enhancer.

	RCR* ± SE at indicated rate of enhancer		
Adjuvant	0%	1%	mean
Molasses, 12.5%	2.25 ± 0.235	1.49 ± 0.209	1.87 ± 0.161 a
Carrier 244	1.85 ± 0.366	0.91 ± 0.191	1.38 ± 0.212 b
Control	0.98 ± 0.143	0.93 ± 0.168	0.95 ± 0.110 c
Mean	1.69 ± 0.157	1.11 ± 0.111	

* Relative consumption rate = dry weight of food eaten divided by dry weight of insect; means of adjuvant treatments across enhancer treatments with the same letter are not significantly different by LSD ($P > 0.05$).

While technical virus without adjuvants did not affect feeding rates, the wettable powder formulation was a strong feeding deterrent (Table 2). RCR of larvae feeding on the wettable powder was only about 10% of that of larvae feeding on the control, and about 13% of that of larvae feeding on the technical virus. Though the treatment with both the enhancer and wettable powder was less deterrent than the wettable powder alone, the treatment with both materials contained only 0.008% as much wettable powder as the treatment with wettable powder alone. The different rates were used because much less virus is needed to kill larvae with the enhancer. Most of the feeding deterrence of this combination was probably due to the enhancer, because RCR of larvae feeding on the enhancer alone was similar to that of larvae feeding on the combination. Because LdMNPV must be ingested to be effective, a formulation that deters feeding is clearly undesirable.

The differences in potency and relative consumption rates between the two formulations cannot be explained at this time. Reduction in potency of the virus in, and consumption of, the wettable powder may be related to processes or adjuvants used in its production, but information on these factors was not provided by the manufacturer. The differences between reported and measured concentrations of PIBs in the wettable powder may be related to settling, inadequate mixing, or differences in counting techniques. The difference in potency between the tank-mix and wettable powder formulations, however, is evidently due to factors other than differences in numbers of PIBs and feeding deterrence, since the probit analyses were based on doses consumed that were adjusted prior to analysis for PIB counts. Even without the 45.1% adjustment for counts of PIBs, however, the differences in potency between the formulations would still be great. These data illustrate the necessity of avoiding procedures in a formulation process that may cause loss of activity of the virus, of avoiding the addition of feeding deterrents, and of obtaining accurate counts of PIBs.

Both tests of feeding rates indicated that Blankophor BBH acts as a feeding deterrent to gypsy moth larvae. No reports of this effect on any insect has previously been published. Although in the first test (Table 2) reduction of RCR by Blankophor BBH relative to the control was over 50%, that in the second test (Table 3) was only about 5%. There was, however, a significant effect of the enhancer ($P = 0.0010$) but no effect of the enhancer by other adjuvant interaction ($P = 0.0950$) in the second test. Lack of a difference between control and enhancer-only treatments should have resulted in a significant interaction; the considerable variability of the data may have limited our ability to detect this interaction. The lack of a large reduction in RCR by the enhancer relative to the control in the second test may be related to the quality of the foliage, which could have held down consumption of the control foliage. The enhancer nevertheless clearly reduced feeding on foliage treated with molasses or Carrier 244. In general, thus, the data do indicate that Blankophor BBH is a moderate feeding deterrent to gypsy moth larvae.

Because the virus must be ingested, feeding deterrence might tend to reduce the utility of Blankophor BBH as an enhancer of LdMNPV. However, because the effect of Blankophor BBH as an enhancer of LdMNPV is so strong, the benefits of its use probably outweigh any feeding deterrent effects on gypsy moth larvae. In addition, feeding stimulants, particularly molasses, at least partially overcame the deterrent effect of Blankophor BBH. The other adjuvants, Bond and Lignosite AN, did not affect feeding behavior in this study. Thus, although these data indicate that feeding deterrence could reduce the efficacy of Blankophor BBH, the addition of feeding stimulants should help overcome this problem. Further research is needed on other enhancers, host plants, and insects before the generality of this effect can be evaluated.

It should be noted that both the potency of these virus formulations and the effects of adjuvants on feeding behavior may not be the same on natural host plants of the gypsy moth, such as oak, as they are on lettuce. Schultz and Keating (1991), for example, found strong effects of host plants on the activity of LdMNPV. Little is known of the effects of host plants on the efficacy of feeding stimulants, but significant effects would not be surprising.

Enhancers and other spray adjuvants thus have the potential to help overcome some of the problems that have limited the use of LdMNPV in the management of the gypsy moth. By increasing potency, enhancers may both improve levels of control and increase the area that can be treated with a limited amount of virus. Molasses, and possibly Carrier 244, have the potential to allow enhancers to work better by helping overcome feeding deterrence, while Bond and Lignosite AN do not seem to interfere. Careful selection of adjuvants, based both on their intended functions and their effects on insect feeding behavior, is thus a necessary step in the development of insect viruses as effective and environmentally compatible pest management tools.

Acknowledgment

We thank G. White, T. Sukontarak, R. Bennett (USDA-ARS) and S. Roper (University of Maryland) for technical assistance; G. Bernon (USDA-APHIS) for supplying insects; J. D. Podgwaite (USDA Forest Service) for providing the technical virus; G. G. Kennedy (North Carolina State University), M. R. Bell (USDA-ARS), B. Smith (American Cyanamid), and J. D. Podgwaite (USDA Forest Service) for comments.

References Cited

Barber, K. N., W. J. Kaupp and S. B. Holmes. 1993. Specificity testing of the nuclear polyhedrosis virus of the gypsy moth, *Lymantria dispar* (Lepidoptera: Lymantriidae). Can. Entomol. 125: 1055-1066.

Bell, R. A., C. O. Owens, M. Shapiro and J. R. Tardif. 1981. Mass rearing and virus production, Pp. 599-655. *In* C. C. Doane, and M. L. McManus [eds.] The gypsy moth: research toward integrated pest management. U. S. Dept. Agric. For. Serv. Tech. Bull. 1584.

Farrar, R. R., Jr., J. D. Barbour and G. G. Kennedy. 1989. Quantifying food consumption and growth in insects. Ann. Entomol. Soc. Am. 82: 593-598.

Jaques, R. P. 1977. Stability of entomopathogenic viruses, Pp. 99-116. *In* C. M. Ignoffo and D. L. Hostetter [eds.]. Environmental stability of microbial insecticides. Misc. Publ. Entomol. Soc. Am. 10.

Lewis, F. B., M. L. McManus and N. F. Schneeberger. 1979. Guidelines for the use of Gypchek to control the gypsy moth. USDA, Forest Service, Northeastern Forest Experiment Station, Forest Service Research Paper NE-441. Broomall, PA..

Podgwaite, J. D. 1985. Strategies for field use of baculoviruses, Pp. 775-797. *In* K. Maramorosch and K. E. Sherman [eds.]. Viral insecticides for biological control. Academic Press, New York, NY.

Podgwaite, J. D., R. C. Reardon, G. S. Walton, L. Venables and D. M. Kolodny-Hirsch. 1992. Effects of aerially applied Gypchek on gypsy moth (Lepidoptera: Lymantriidae) populations in Maryland woodlots. J. Econ. Entomol. 85: 1136-1139.

Reardon, R. and Podgwaite, J. 1992. The gypsy moth nucleopolyhedrosis virus product. Appalachian Integrated Pest Management, USDA Forest Service, Northeastern Area NA-TP-02-92.

SAS Institute. 1988. SAS/STAT User's Guide, Release 6.03 Edition, Cary, NC; SAS Institute, Inc.

Schultz, J. C. and S. T. Keating. 1991. Host-plant mediated interactions between the gypsy moth and a baculovirus. Pp. 489-506. *In* P. Barbosa, V. A. Krischik and C. G. Jones [eds.]. Microbial mediation of plant-herbivore interactions. John Wiley & Sons, Inc., New York.

Shapiro, M. 1992. Use of optical enhancers as radiation protectants for gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus. J. Econ. Entomol. 85: 1682-1686.

Shapiro, M. and J. L. Robertson. 1992. Enhancement of gypsy moth (Lepidoptera: Lymantriidae) baculovirus activity by optical enhancers. J. Econ. Entomol. 85: 1120-1124.

Shapiro, M., E. Dougherty and J. J. Hamm. 1992. Compositions and methods for biocontrol using fluorescent enhancers. U. S. Patent 5,124,149.

Webb, R. E., J. D. Podgwaite, M. Shapiro, K. M. Tatman and L. W. Douglass. 1990. Hydraulic spray application of Gypchek as a homeowner control tactic against gypsy moth (Lepidoptera: Lymantriidae). J. Entomol. Sci. 25: 383-393.

Webb, R. E., N. H. Dill, J. D. Podgwaite, M. Shapiro, R. L. Ridgway, J. L. Vaughn, L. Venables and R. J. Argauer. 1994a. Control of third and fourth instar gypsy moth (Lepidoptera: Lymantriidae) with Gypchek combined with a stilbene disulphonic acid additive on individual shade trees. J. Entomol. Sci. 29: 82-91.

Webb, R. E., M. Shapiro, J. D. Podgwaite, R. L. Ridgway, L. Venables, G. B. White, R. J. Argauer, D. L. Cohen, J. Witcosky, K. M. Kester, and K. W. Thorpe. 1994b. Effect of optical brighteners on the efficacy of gypsy moth (Lepidoptera: Lymantriidae) nuclear polyhedrosis virus in forest plots with high or low levels of natural virus. J. Econ. Entomol. 87: 134-143.