Dose Responses of *in vivo*- and *in vitro*-produced Strains of Gypsy Moth (Lepidoptera: Lymantriidae) Nucleopolyhedrovirus (LdMNPV) Applied With and Without the Virus Enhancer Blankophor BBH[1]

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Abstract The gypsy moth, *Lymantria dispar* L., nucleopolyhedrovirus (LdMNPV) product Gypchek is a microbial pesticide produced by the USDA Forest Service. Gypchek is a mixture of LdMNPV genotypes produced in vivo. Commercial interests prefer to develop a stable, high-potency genotype that can be produced at low cost, preferably in vitro. We sprayed 2 LdMNPV strains and Gypchek at various doses, with and without the viral enhancer Blankophor BBH (Burlington Chemical Co., Burlington, NC), on oak foliage under field conditions to determine the relationship between application rate and larval mortality. We used strains previously isolated from Gypchek; strain 203 was produced in vivo and strain 122 was produced in vitro. When applied at a rate of 10^{12} viral occlusion bodies (OB) per 379 L of water without enhancer, mortality was 26% with strain 122 and greater than 90% with both strain 203 and Gypchek. In addition, strain 203 killed larvae faster. At an application rate of 10^{11} OB with enhancer, larval mortality was greater than 90% with all 3 viral preparations. Strain 122 produced in vitro at a lower cost than is currently possible for Gypchek production would allow compensation for its reduced virulence by the application of a higher dose. Alternatively, if applied with enhancer at a rate of 10^{11} OB, there would be no significant differences in efficacy of 122 compared with Gypchek applied at that same rate.

Key Words *Lymantria dispar*, Gypchek, gypsy moth nucleopolyhedrovirus, viral enhancer

The gypsy moth, *Lymantria dispar* L., nuc1eopolyhedrovirus (LdMNPV) product, Gypchek, is registered with the United States Environmental Protection Agency (EPA) as a general use insecticide for aerial and ground application to control gypsy moth (Podgwaite 1999, Reardon et al. 2012). Because it kills only gypsy moths, Gypchek is in demand for use in areas where there is concern over nontarget organisms. The potency of LdMNPV is enhanced by the addition of certain stilbenedisulfonic acid derivatives used commercially as optical brighteners in laundry detergents. In the laboratory, LC_{50} values

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were decreased by a factor of 1000 with the addition of certain of these enhancers (Shapiro and Robertson 1992). The viral enhancing effect of these compounds has been confirmed in field studies when they were applied with ground-based hydraulic spray equipment (Webb et al. 1990, Webb et al. 1994, Thorpe et al. 1998). It is thought that these compounds enhance virus infection in insects either by preventing the sloughing of virus-infected midgut epithelial cells (Washburn et al. 1998) or by altering the integrity of the peritrophic membrane to increase its permeability to virus (Wang and Granados 2000, Zhu et al. 2007). Both of these modes of action apply to LdMNPV infections (Dougherty et al. 2006). Cunningham et al. (1997) reported that the addition of an enhancer resulted in a significant increase in efficacy from an aerial application of gypsy moth virus, but in another aerial application test increased efficacy resulting from the addition of an enhancer did not occur (Thorpe et al. 1999). In the latter study, measurements of spray deposit indicated that the amount of enhancer deposited on leaf surfaces was below the threshold amount required for enhancement to occur. Because a much higher volume of spray per tree is used in a ground-based hydraulic application, more enhancer is deposited per unit of leaf surface (Webb et al. 1996), and the threshold is exceeded (Thorpe et al. 1999).

As the gypsy moth continues to expand its range, most of the anticipated economic impacts are in residential areas (Leuschner et al. 1996). Ground-based application of chemical and microbial control agents (primarily *Bacillus thuringiensis* subsp. *kurstaki* Berliner) play an important role in these areas (Vaughn et al. 1997). If Gypchek was available on a commercial basis, it could be an important additional tactic that could be used more widely by pest control operators and government agencies in sensitive areas. A survey of gypsy moth managers (Podgwaite et al. 1998) revealed that demand for the product is expected to rise, but that its cost could be prohibitive. Thus, the successful transfer of gypsy moth virus production from the public to the private sector may require a reduction in production costs; in vitro production is one way costs might be reduced.

In 1997, an in vitro-produced gypsy moth virus strain developed by the USDA, Forest Service at Delaware, OH (Slavicek et al. 2001) was field tested using ground-based hydraulic application equipment (Thorpe et al. 1998). Whereas the in vitro strain significantly reduced the density of larvae in the canopies of treated trees, virus-caused larval mortality was significantly lower than on trees treated with Gypchek. One possible reason for this was that OB of the in vitro strain were smaller than those of virus produced in living insects, and they consequently contained only 20% as many virions. To use this strain successfully, it may be necessary to increase the application rate (number of OB per liter) to compensate for their smaller size.

For production quality control issues, commercial producers would prefer to .produce virus of a single genotype. Previous work has shown that Gypchek is composed of many LdMNPV genotypic variants (Slavicek et al. 1995). It would be difficult to correlate changes in production levels, viral potency, etc. to genotypic changes if a mixture of genotypes was used for viral production. To address this issue, viral lines were purified from Gypchek, and their potency in laboratory bioassays was compared with that of Gypchek. However, further testing of these isolates is needed before they can be considered for commercial production.

The objectives of this study were: (1) to compare the efficacy of a single genotype of LdMNPV produced in vivo and the in vitro-produced strain evaluated in Thorpe et al. (1998) to Gypchek under field conditions; (2) to establish dose responses for these strains; and (3) to determine the effect of the viral enhancer, Blankophor BBH, on dose response to assist in determining the economic feasibility of combining an enhancer with lower doses or weaker strains of virus.

Materials and Methods

LdMNPV preparations. Three gypsy moth LdMNPV preparations were tested: (1) Gypchek, produced by the USDA, Forest Service, Ansonia, CT, from a New Jersey laboratory strain of gypsy moth larvae inoculated with the LDP226 virus isolate (the so-called Hamden Standard derived from field collected Connecticut LdMNPV isolation); (2) viral strain 122, produced in cell culture at USDA, Forest Service, DE, OH, as described previously (Slavicek et al. 1992, Thorpe et al. 1998); and 3) a novel viral strain, 203, produced in vivo at USDA, Forest Service, DE, OH. Strain 122 is the same in vitro-produced virus strain that was tested in Thorpe et al. (1998). Strain 203 was tested because it routinely exhibits 2-fold greater activity compared with Gypchek when tested in laboratory diet-incorporated bioassays. Strain 203 was selected for evaluation from a series of LdMNPV viral lines generated essentially as described by Smith and Crook (1988). Approximately 10^4 OB obtained from a production lot of Gypchek were applied to the surface of a 10 cm disc of gypsy moth diet in a rearing container. One hundred fourth- instar gypsy moth larvae were placed on the diet for 24 h, and then were placed on fresh diet. The larvae were checked daily, and dead larvae were placed in individual containers and frozen. Viral DNA was isolated from OB and digested with restriction endonuc1eases as previously described to identify genotypic variants (Slavicek et al.1995). One or two additional passages in fourth- instar larvae with some of the isolates were performed as described for the initial infection to increase genotypic purity. Larval mortality ranged from 1 - 26% in the first infection and from 1 - 6% in subsequent infections, indicating that a large proportion of infections were caused by a single virion. Once the isolates appeared to be pure, based on genomic restriction endonuclease analysis, they were plaque purified by standard techniques using Ld652Y cells. Occlusion bodies of the genotypic variants were produced in gypsy moth larvae, and their potencies were determined by bioassay (Slavicek et al. 1992). This analysis identified over 22 LdMNPV genotypic variants (JMS, unpubl. data). One of these isolates, 203, exhibited potency about twice that of Gypchek (LC_{50} of 2.6×10^3 and 4.7×10^3 OB per ml of diet, respectively; LC_{90} of 3.5×10^4 and 5.3×10^4 OB per ml of diet respectively), and was selected for field trials.

The Gypchek used in this study was a lyophilized powder chosen randomly from lots that had been processed 2 months prior to the field test and stored frozen at -20°C. On the day prior to application, it was mixed with water, diluted to the appropriate concentration, and placed in spray bottles. The in vivo strain 203 and the in vitro strain 122 were produced 2 months prior to the field test and stored in saline at 4°C until the day of application. At that time the virus mixtures were diluted with water to the appropriate concentrations and placed in spray bottles with the specified adjuvants.

Study design and treatments. The field study was conducted along the forested edge of a cultivated field at the Cedar Swamp Wildlife Area in New Castle Co., DE during the time of year (May) that second-stage gypsy moth larvae would normally be present. No naturally-occurring gypsy moth population was evident in the immediate vicinity of the study site. A randomized complete block design was used as follows. Two hundred and eighty pairs of oak branch tips, primarily pin oak, *Quercus palustris* Muenchhausen, occurring along the edge of the field and within reach from the ground were marked with flagging. Each pair of tips was separated from adjacent pairs by at least 2 m. The pairs of tips were grouped into 8 blocks of 33 pairs, and each pair was randomly assigned 1 of the 33 treatments or controls. The treatments consisted of 5 application rates for each of the 3 virus preparations, each with and without the

enhancer Blankophor BBH (Burlington Chemical Co., Burlington, NC) (0.5% w/v) in distilled water. The adhesive Bond® (Loveland Industries, Greeley, CO) was added to all treatments at 2% (v/v). In addition, there were 3 separate controls consisting of distilled water alone, distilled water plus Bond®, and distilled water plus Bond® and Blankophor BBH. Because the objective of the experiment was to determine dose responses, a range of doses was used that was expected, based on preliminary tests, to produce very low and very high levels of mortality at the lowest and highest doses, respectively. Because of the dramatic effect of the enhancer on virus-caused mortality, a different range of virus doses was used with and without the enhancer. Each virus preparation was mixed with distilled water, and the following concentrations were prepared by serial dilution: 10^9, 10^{10}, 10^{11}, 10^{12}, and 10^{13} OB per 379 L (100 gallons) of water (without enhancer) and 10^7, 10^8, 10^9, 10^{10}, and 10^{11} OB per 379 L (with enhancer).

Each treatment was sprayed on oak branch tips to runoff using 373- ml hand-held trigger-pump sprayers (Delta Industries, Philadelphia, PA). When the foliage was dry, 10 gypsy moth second instars were placed on each branch tip, and the tip was covered with a nylon organza sleeve cage (60 cm long x 30 cm wide) which was secured to the branch with a plastic twist tie. After 1 wk, the cages were removed and the larvae were placed individually in 30- ml plastic cups with paper lids and containing artificial diet (Bell et al. 1981) in an outdoor insectary at Beltsville, MD. The cups were checked every 2 - 3 d to record larval mortality until death, pupation, or for 52 d. All dead larvae were examined microscopically at 400x for the presence of OB. If cause of mortality could not be determined 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.

Gypchek and 122 were compared in a laboratory bioassay after the completion of the field test. For this bioassay, OB were incorporated into artificial gypsy moth diet at concentrations of 10^2, 10^3, 10^4, 10^5 and 10^6 OB per ml of diet. Second-stage larvae (New Jersey laboratory strain) were placed in groups of 10 in plastic Petri dishes (100 x15-mm) containing treated diet for 2 days and then were transferred to untreated diet for another 12 - 13 d. At the end of this time, mortality was determined. There were 5 replicates for each combination of virus preparation and dose.

Statistical analyses. The dose-response analyses were conducted using Proc Probit (SAS Institute 1989). LT_{50} and LT_{90} values were calculated by generating a linear regression model for time to death versus dose (Proc Reg) (SAS Institute 1989) for each replicate of each combination of virus preparation and presence or absence of enhancer. These models were then used to estimate time to death at the LC_{50} and LC_{90} for each replicate of the corresponding virus preparation and enhancer combination. The resulting values were used in an analysis of variance to calculate mean LT values and test for treatment effects. Analyses of variance were conducted using Proc Mixed (SAS Institute 1996). When interaction effects were not significant, means for each virus preparation were separated using a least significant difference (LSD) procedure at a comparison-wise error rate of 0.05. When interactions were significant, means from all combinations of virus preparation x enhancer were separated by LSD at a comparison-wise error rate of 0.05.

Results

The LC_{50} and LC_{90} values of Gypchek and 122 in diet incorporation bioassays were similar (Table 1). Gypsy moth larval mortality caused by each of the virus preparations

Table 1. LC_{50} and LC_{90} values (OB/ml of diet) for Gypchek and in vitro-produce LdMNPV Strain 122.

	Log number of OB/ml of diet	
LdMNPV preparation	LC50 (95% fiducial limits)	LC90 (95% fiducial limits)
Gypchek	4.2 (4.0 - 4.3)	5.0 (4.7 - 5.3)
122	4.4 (4.2 - 4.6)	5.2 (5.0 - 5.6)

with and without enhancer over the range of virus concentrations evaluated in the field test is shown in Fig. 1A. Mortality among controls was 0.4, 0.6, and 12.0% for larvae on leaves sprayed with distilled water, distilled water plus sticker, and distilled water plus sticker plus enhancer, respectively. Without enhancer, 10^{12} OB per 379 L of both Gypchek and 203 were required to cause greater than 90% mortality. Mortality was 26% at 10^{12} OB per 379 L of 122 without enhancer, and at 10^{13} OB per 379 L, the highest dose tested, mortality was 66%. With enhancer, mortality was 98% for Gypchek and 87% for 203 at 10^{9} OB per 379 L. Mortality exceeded 95% with both Gypchek and 203 at 10^{10} OB per 379 L. With enhancer, mortality from 122 was 35% at 109, 82% at 10^{10}, and 94% at 10^{11} OB per 379 L. The shapes of the dose-mortality curves were similar for both Gypchek and 203 with and without enhancer. The response of the 122 was less steep than those of Gypchek and 203 both with and without enhancer.

The results of the analysis of variance of the LC_{50} and LC_{90} values for the 3 virus preparations with and without enhancer are shown in Table 2. The virus preparation x enhancer interaction effect was significant for LC_{50}, but not for LC_{90}. For LC_{90}, both the virus preparation and enhancer effects were significant. The LC_{50} and LC_{90} values for the 3 virus preparations with and without enhancer are given in Table 3. The LC_{50} values for Gypchek and 203 did not differ significantly regardless of whether enhancer was present or absent. However, the LC_{50} values for 122 were significantly higher than for either Gypchek or 203 both with and without enhancer. The LC_{90} values for Gypchek and 203 did not differ significantly, but both were lower than the value for 122. The presence of enhancer decreased the LC_{50}values of both Gypchek and 203 by about 3 logs (l000 x) and that of 122 by 3.75 logs (5,600 x).

The time to death of larvae treated with the 3 virus preparations with and without enhancer over the range of concentrations tested is shown in Fig. 1B. Both with and without enhancer, 203 killed larvae faster than either Gypchek or 122 at all doses tested. With enhancer, time to death decreased sharply with increasing concentration for Gypchek and 203, but not 122. The analyses of variance of the LT_{50} and LT_{90} values for the 3 virus preparations with and without enhancer are shown in Table 4. The virus preparation x enhancer interaction effect is significant for LT_{50}, but not for LT_{90}. For LT_{90}, both the virus preparation and enhancer effects are significant. The LT_{50} and LT_{90} values for the 3 virus preparations with and without enhancer are given in Table 5. Without enhancer, the LT_{50} values for 203 were significantly lower than for either Gypchek or 122. With enhancer, the LT_{50} value of Gypchek was significantly higher than those of either 203 or 122. However, it should be noted that the estimated LT_{50} value of 27.0 days for Gypchek with enhancer at the LC_{50} of 10g (7.4) is higher than the actual time to death value at that concentration (Fig. 1B) because the regression models used in the calculation of the LT_{50} value assume a linear relationship, which does not fit the data shown in Fig. 1B. The LT_{90} values differed significantly among virus preparations, with the

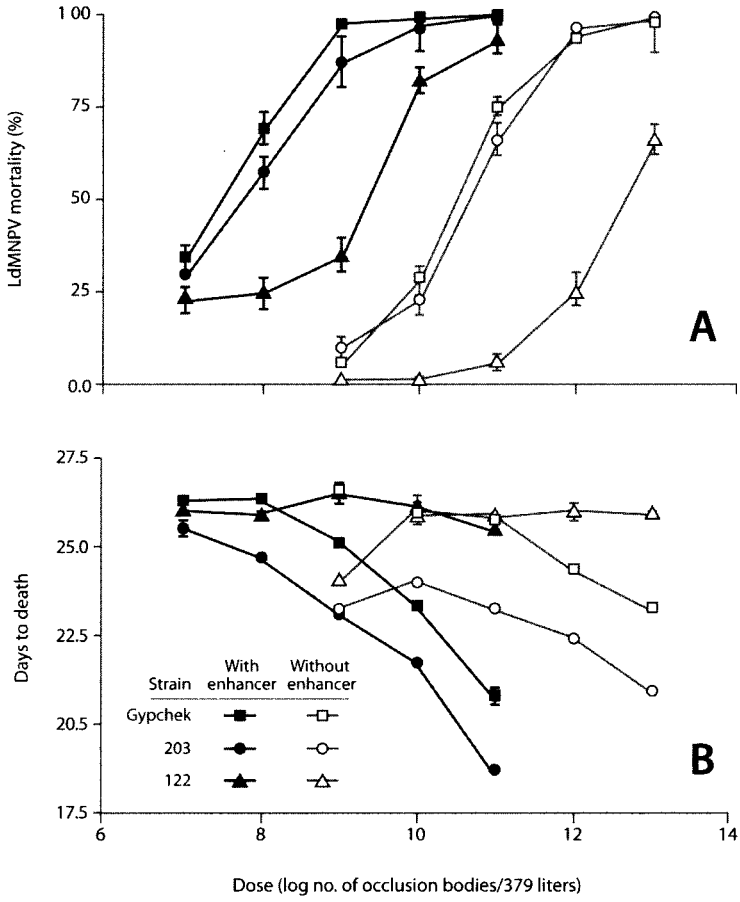

Fig. 1. Mortality (A) and time to death (B) of gypsy moth larvae caged on oak foliage treated with different doses of three preparations of LdMNPV with and without the enhancer Blankophor BBH.

values highest for the 122 and lowest for 203. The effect of the enhancer on LT_{90} was not significant. Table 6 shows gypsy moth larval mortality and time to death for the 3 virus preparations at 10^{12} OB per 379 L without enhancer (the currently-recommended rate) and at 10^{11} OB per 379 L with enhancer. Mortality was greater than 90% for all virus preparations under these treatments except for 122 at 10^{12} OB without enhancer, for which mortality was 26%. Time to death varied from 18.7 d for 203 at $10^{11}$with enhancer to 26.0 d for 122 at 10^{12} without enhancer.

Discussion

This is the first ground-based study to demonstrate that a single genotype of LdMNPV produced in vivo (203) can provide a level of gypsy moth control comparable to that of Gypchek, and that larvae are killed significantly faster. These data suggest

Table 2. Analysis of variance of LC_{50} and LC_{90} values for gypsy moth larvae on foliage treated with three LdMNPV preparations with and without the enhancer Blankophor BBH.

Dependent Variable	Source	*F*	df	*P>F*
LD50	LdMNPV preparation	192.8	2, 14	< 0.0001
	Enhancer	785.9	1, 7	< 0.0001
	LdMNPV preparation x enhancer 8.32 2, 14 0.004			
LD90	LdMNPV preparation	78.1	2, 14	< 0.0001
	Enhancer	264.4	1, 7	< 0.0001
	LdMNPV preparation x enhancer	0.9	2, 14	0.44

that an efficacious viral gypsy moth control product that consists of a single viral genotype can be generated commercially.

In the laboratory bioassay, there was no difference in virulence between Gypchek and the in vitro strain (122). However, in the field experiment, which approximated conditions that would occur with an actual ground-based hydraulic application of virus against gypsy moth larvae, there were distinct differences between the in vivo and in vitro preparations. In a previous study (Thorpe et al. 1998), a ground-based hydraulic application of the in vitro-produced strain 122 resulted in lower levels of larval mortality and higher larval densities in the canopies of treated trees than did Gypchek. In that study, it was reported that the average volume of 122 OB was one fifth that of Gypchek OB and, therefore, putatively contained 80% fewer virions. This is likely to have contributed to the reduced efficacy of 122, both in that study and in the present field study. There may be other factors contributing to the reduced effectiveness of 122 in the field, possibly including greater susceptibility to UV (UV) radiation. Whereas a sunscreen does not appear to improve the performance of hydraulically-applied Gypchek (Thorpe et al. 1998), it might increase the effectiveness of other virus strains if they are more susceptible to UV damage. To compensate for the reduced effectiveness of 122 compared with Gypchek, it might be necessary to use a higher dose (number of OB). If the cost of in vitro production could be reduced below that of current in vivo Gypchek production, in vitro production could still be economically favorable.

Both Gypchek and the in vivo-produced strain 203 caused significantly higher levels of mortality than did 122, both with and without the enhancer. There were no obvious differences between Gypchek and 203, except that 203 appeared to kill larvae more quickly than did Gypchek. As has been reported in the past (Webb et al. 1996, Thorpe et al. 1998), these strains were as effective at 10^{11} PIB per 379 L with enhancer as they were at 10^{12} PIB without enhancer. The addition of enhancer increased the mortality caused by the in vitro strain from 26% at 10^{12} PIB without enhancer to 94% at 10^{11} PIB with enhancer. Thus, the inclusion of enhancer in a gypsy moth virus tank mix may permit the use of a virus strain that otherwise would not be effective. Whereas the addition of enhancer did not decrease the LT_{90} of any of the strains,

Table 3. LC_{50} and LC_{90} values (OB/379 L of tank mix) for gypsy moth larvae on foliage treated with three preparations of gypsy moth LdMNPV with and without the enhancer Blankophor BBH.

		Log number of OB/379 L			
LdMNPV Preparation	Blankophor BBH (0.5%)	LC_{50} (95% fiducial limits)	LC_{50} mean separation*	LC_{90} (95% fiducial limits)	LC_{90} mean separation* (preparations)
Gypchek	-	10.47 (10.35 - 10.59)	c	11.70 (11.53 - 11.90)	10.15 a
	+	7.42 (7.27 - 7.55)	a	8.60 (8.43 - 8.81)	
203	-	10.43 (10.41 - 10.66)	c	11.33 (11.66 - 12.04)	10.55 a
	+	7.67 (7.50 - 7.83)	a	9.26 (9.06 - 9.51)	
122	-	12.63 (12.46 - 12.83)	d	14.14 (13.86 - 14.63)	12.61 b
	+	8.88 (8.70 - 9.05)	b	11.03 (10.73 - 11.40)	

* Means within a column followed by the same letter are not significantly different at a comparison-wise error rate of 0.05.
A mean separation test was not performed on LC50 for preparations because the preparation x enhancer effect was significant.

Table 4. Analysis of variance of LT_{50} and LT_{90} values (days to death at LC_{50} and LC_{90}) for gypsy moth larvae on foliage treated with three LdMNPV preparations with and without the enhancer Blankophor BBH.

Dependent Variable	Source	*F*	df	*P>F*
LT_{50}	LdMNPV preparation	26.1	2, 14	< 0.0001
	Enhancer	14.6	1, 7	0.007
	LdMNPV preparation x enhancer	4.0	2, 14	0.04
LT_{90}	LdMNPV preparation	61.4	2, 14	< 0.0001
	Enhancer	0.02	1, 7	0.9
	LdMNPV preparation x enhancer	0.6	2, 14	0.4

there were differences in LT_{90} among the strains. LT_{90} was lowest for in vivo strain 203 and greatest for the in vitro strain. The difference in LT_{90} between Gypchek and 203 was 2.5 days. So, even though both strains kill 90% of the treated larvae at their LC_{90}, Gypchek would allow additional defoliation to occur prior to larval death. The decreased LT_{90} of 203 could be an important positive characteristic of this strain compared with Gypchek. The in vitro-produced 122 would not only require more virus than 203 to cause >90% mortality, but would allow even more time for defoliation to occur.

The USDA Forest Service currently recommends that Gypchek be applied at a rate of 10^{12} OB per 379 L of tank mix when delivered as a ground-based hydraulic application such as is commonly used by arborists (Reardon et al. 2012). The results of this

Table 5. LT_{50} and LT_{90} values (days to death at LC_{50} and LC_{90}) for gypsy moth larvae on foliage treated with three preparations of gypsy moth LdMNPV with and without the enhancer Blankophor BBH.

LdMNPV preparation	Blankophor BBH (0.5%)	LT_{50}	LT_{50} mean separation*	LT_{90}	LT_{90} mean separation (preparations)
Gypchek	-	26.1 ± 0.3	bc	24.8 ± 0.2	25.0 b
	+	27.0 ± 0.4	c	25.2 ± 0.2	
203	-	23.6 ± 0.2	a	22.4 ± 0.2	22.5 a
	+	25.4 ± 0.4	b	22.5 ± 0.2	
122	-	25.9 ± 0.1	b	26.0 ± 0.7	25.9 c
	+	26.0 ± 0.2	b	25.7 ± 0.2	

Values are mean ± SE. Means within a column followed by the same letter are not significantly different at a comparison-wise error rate of 0.05.

* A mean separation test was not performed on LT50 for preparations because the preparation x enhancer effect was significant.

Table 6. Percent mortality of gypsy moth larvae treated with three LdMNPV preparations at a rate of 10^{12} OB/379 L without enhancer and 10^{11} OB/379 L with enhancer.

Treatment				
No. OB/379 L	Enhancer	LdMNPV preparation	Mortality (%)	Time to death (days)
10^{11}	+	Gypchek	100 a	20.8 ± 0.2 b
	+	203	99.4 ± 0.6 a	18.7 ± 0.2 a
	+	122	93.6 ± 3.9 a	25.4 ± 0.2 e
10^{12}	-	Gypchek	94.6 ± 2.0 a	24.4 ± 0.2 d
	-	203	96.0 ± 1.3 a	22.5 ± 0.2 c
	-	122	25.9 ± 4.6 b	26.0 ± 0.2 e

Values are mean ± SE. Means within a column followed by the same letter are not significantly different at a comparison-wise error rate of 0.05.

study under field conditions (larvae caged on foliage in the field) continue to support this recommendation. Based on the mortality rates observed in this experiment, an application rate of 10^{11}OB for Gypchek used in conjunction with an enhancer (Webb et al. 1996, Thorpe et al. 1998) is also supported (greater than 90% mortality). It appears that the effective use of the in vitro-produced 122 without enhancer at the recommended rate of 10^{12}OB would not be effective based on the mortality rates observed in this study (less than 30%). However, when used with the enhancer, strain 122 could be used at the rate of 10^{11}OB with greater than 90% mortality and no significant reduction in mortality compared with Gypchek applied at the same rate. Furthermore, the results of this study suggest that viral strain 203, which consists of. a single genotype of LdMNPV and would, therefore, be more amenable than the genetically heterogeneous Gypchek to quality control procedures required for commercial production would also be effective at these application rates with or without enhancer.

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