# Potency of Nucleopolyhedrovirus Genotypes for European and Asian Gypsy Moth (Lepidoptera: Lymantriidae)[1]

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**Abstract** Gypchek is a gypsy nucleopolyhedrovirus (LdMNPV) product used for management of European gypsy moth (*Lymantria dispar dispar* L.) in the United States, primarily in areas where the use of broad-spectrum pesticides is not appropriate. Similar LdMNPV products are used in Russia for control of a flighted-female strain of Asian gypsy moth (*Lymantria dispar asiatica* Vnukovskij), an insect not yet established in the United States. Gypchek is a mixture of LdMNPV genotypic variants and is being developed further toward a single, high- potency genotype product that is effective against both European and Asian strains. We isolated 5 LdMNPV genotypic variants from Gypchek and, through diet incorporation bioassays, assessed their potencies for both a laboratory strain of European gypsy moth and a wild Asian gypsy moth strain. Bioassays conducted in the United States showed that 2 viral isolates, 122b and 122-HP, were about 3 times as potent as Gypchek against European gypsy moth. Bioassays conducted in Russia showed that 122-HP was as effective as a wild Siberian LdMNPV against a wild Asian (Siberian) strain of gypsy moth. Both 122-HP and 122b were shown to be at least as effective as Gypchek in killing European gypsy moth larvae when formulated at a high dose and sprayed on oak foliage in a ground-based field test. Overall results indicated that both 122b and 122-HP are potential candidates for further development as a single-genotype Gypchek product.

**Key Words** gypsy moth, nucleopolyhedrovirus, genotypic variants, bioassay

Gypchek is a gypsy moth (*Lymantria dispar* L.) -specific biopesticide, registered with the U.S. Environmental Protection Agency and produced and distributed by the USDA Forest Service. It is the only baculovirus product registered for gypsy moth management in the United States. Its primary use is for treating areas where environmental concerns outweigh the use of broad-spectrum pesticides for gypsy moth control (Podgwaite 1999, Reardon et al. 2012). Gypchek is a lyophilized powder produced from larvae that have been infected with the gypsy moth nucleopolyhedrovirus (LdMNPV). The viral inoculum for in vivo production is the so called "Hamden Standard" (Lewis and Rollinson 1978), originally isolated and partially purified from LdMNPV-killed larvae collected from a Connecticut gypsy moth population undergoing a viral epizootic. Thus, as would be expected and shown by Slavicek et al.

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(1995), the Gypchek product contains a mixture of closely-related LdMNPV genotypes which, in combination, act as active ingredients. The genotypes vary in quantity and quality (virulence) and account for the variability in viral occlusion body (OB) yield from one in vivo Gypchek production lot to the next. The number and proportion of genotypic variants within Gypchek have not been determined. Forest Service scientists and collaborators continue to evaluate virulent strains of LdMNPV that may be suitable for either large-scale in vitro- (cell culture) or in vivo- production and whose OB yield and activity can be maximized and standardized from one production lot to the next.

Currently, Gypchek is used almost exclusively for suppression of European strains of gypsy moth infesting forested areas in the United States (U.S. Department of Agriculture 2007). In the recent past there have been several accidental introductions of Asian gypsy moth (*Lymantria dispar asiatica* Vnukovskij) strains into the United States, some from Russian areas (U.S.Department of Agriculture 2003). Phenotypic characteristics and proposed revised taxonomic status of *Lymantria* spp. are described by Pogue and Schaefer (2007). Though no Asian strains have been reported as established in the United States, there is concern over future introductions given that Asian strain females fly and could rapidly disperse to a variety of susceptible forested habitats and potentially become a greater economic threat than the European gypsy moth whose females are not flighted.

In 1994, Gypchek was applied to sensitive habitat in North Carolina following an accidental introduction of Asian gypsy moth from Germany. Aerial treatments (2 applications, each at $2 \times 10^{11}$ OB/ acre) were based on the response of a quarantined Russian strain to Gypchek (JDP, unpubl. data). Though male moth trapping results indicated that Gypchek contributed to the putative eradication of the Asian strain in North Carolina (Wall 1997), studies with Russian colleagues were undertaken sometime later to more accurately assess the response of Asian gypsy moth larvae from Siberian populations to Gypchek. Results of a bioassay on birch foliage in Novosibirsk, Russia, showed that Gypchek and the multi -genotype LdMNPV-product Virin NSh (Bakhvalov et al. 2005a) were remarkably similar in their potencies for the Asian gypsy moth as judged by probit analysis (Bakhvalov et al. 2005b). Studies by other investigators also have shown the mixture of genotypes in the "Hamden Standard" LdMNPV to be active against larvae from a Russian population of gyspy moth (Ebling et al. 2004) as well as from a Mongolian population of the insect (Duan et al. 2011).

The probability of establishment of an Asian strain of gypsy moth in the United States will be taken into account as U.S. Forest Service scientists move toward developing a single-genotype Gypchek product whose efficacy against both European and Asian strains of the insect is maximized. Cloned LdMNPV genotypes from Gypchek have not been previously studied for their biological activity in Asian gypsy moth. However, one such cloned genotype, LdMNPV-203, has been shown to be at least as efficacious as the parent product in laboratory bioassays against a New Jersey (European) strain of gypsy moth (JDP, unpubl. data) and in limited aerial field trials against a wild Maryland gypsy moth population (Webb et al. 2005). We have continued to study LdMNPV-203 variants as well as 4 additional genotypically distinct cloned isolates from Gypchek. Here we report on the potencies of these isolates for a western Siberian strain of the gypsy moth and for the New Jersey laboratory strain of European gypsy moth. Also presented are results of a limited ground-based field test comparing 2 of the isolates with Gypchek.

## Materials and Methods

**Isolation and purification of LdMNPV genotypes.** Baculovirus genotypes (122b, 122-HP, 203, B1B, and A21-MPV) were isolated from lyophilzed Gypchek powder containing viral occlusion bodies (OBs) of the "Hamden standard" strain of LdMNPV. All exhibited a wild phenotype with respect to polyhedra formation. Additional properties of isolates A21-MPV (Slavicek et al. 1996) and 122b (Slavicek et al. 2001) have been previously described. All of these isolates were initially semipurified by the in vivo method of Smith and Crook (1987) in fourth- instar larvae, and then plaque purified 3 times in the Ld652Y cell line (Goodwin et al. 1978) using standard techniques. The viral strains were propagated in *L. dispar* 652Y cells and DNA was isolated as previously described (Bischoff and Slavicek 1994). Viral isolate genomic DNA was digested with the restriction enzyme *Bgl*II, and the fragments were separated on an agarose gel and visualized after staining with ethidium bromide (Slavicek and Hayes-Plazolles 2003). The differences in restriction endonuclease fragment lengths resulting from DNA digestion was used to distinguish viral genotypes.

**New Jersey larval bioassay.** For bioassays, the plaque purified isolates were propagated in fourth- stage gypsy moth larvae and semipurified by methods described above. Groups of 10 newly molted second- stage gypsy moth larvae (30 larvae per dose) (New Jersey colonized strain) were challenged with aqueous suspensions of Gypchek, 122b, 122-HP, 203, B1B, and A21-MPV OBs incorporated into artificial diet (Bio-Serv, Frenchtown, NJ) in serial 10-fold doses ranging from $10^2$ to $10^6$ OBs per ml diet. Larvae fed ad libitum on the virus-treated diet for 2 d and then on virus-free diet until day 14 at which time mortality was assessed and confirmed microscopically. Bioassay conditions were, with minor modifications, those described by Slavicek et al. (1992).

**Siberian larval bioassay.** The 5 LdMNPV genotypes isolated from Gypchek, as well as 1 wild viral strain (Chistoozernyy) isolated from a gypsy moth population inhabiting Western Siberia, were evaluated in Novosibirsk, Russia using a diet incorporation bioassay. Test insects were from egg masses collected in the previous autumn from an Asian gypsy moth population defoliating birch stands in the Ural area. Egg masses were held at 4°C to break diapause and then allowed to hatch at 24°C in the laboratory the following spring. Larvae were reared on artificial diet in Petri dishes (Ilyinykh 1996) until they reached second instar. Groups of 2-d-old second-instar larvae were challenged with serial dilutions of virus incorporated into diet at $10^4$, $10^5$, $10^6$ and $10^7$ OBs/ml. There were 5 replicates of 10 larvae per dose for each viral genotype. Control larvae fed on untreated diet throughout the experiment. Larvae tested for their response to viral genotypes were exposed to virus-treated diet for 2 d and then placed on virus-free diet for the remainder of the test (13 d). Larval mortality was recorded every other day during the test and NPV mortality confirmed by light microscopy at 1000x.

**Ground-based field test.** Results of the laboratory bioassays led to the design of a small-scale ground- based field test to compare the efficacy of 122b, 122-HP and Gypchek applied in a spray formulation. The test was conducted within a 20 × 40-m red-oak (*Quercus rubra* L.) plantation at the U.S. Forest Service field facility, Ansonia, CT. The plantation consists of a 4 × 8 grid of 32 trees 10 to12 m in height. Fifty branches that could be reached easily from the ground were selected from 16 trees within the plantation, and all but 30 leaves at the tip of each branch were removed.

Leaves on these individual branch tips were treated either with a high ($5 \times 10^{11}$ OBs), medium ($1 \times 10^{9}$ OBs), or low ($1 \times 10^{8}$ OBs) per ha dose of Gypchek, 122b, or 122-HP. There were 5 replicates of each dose. An aqueous formulation containing 6% w/v Lignosite AN® (Georgia Pacific, Bellingham WA) [UV screen], 15% v/v Triple Crown Pure Cane Molasses® (Equine Specialty Feed Co., Ada, MN) [feeding stimulant], and 2% v/v Bond® (Loveland Industries, Inc., Greeley, CO) [adhesive] was prepared. Ten ml of an aqueous virus suspension, containing the appropriate number of OBs, was added to 40 ml of Lignosite formulation and thoroughly mixed. Two ml of the finished formulation (the per ha dose) was applied to a branch tip using a small, hand-held atomizer (SKS, Watervliet, NY) calibrated to deliver 0.2 ml/ trigger squeeze. Five control tips received 2 ml of the formulation to which no virus was added. The 5 control tips were bagged (plastic) to prevent contamination when spraying the virus treatments. The order of the different treatments was randomly selected, and each branch tip was shielded with plastic during application to minimize the chance of drift to other branches. Foliage was allowed to dry, and 30 third-stage gypsy moth larvae (New Jersey laboratory strain) that had been starved for 24 h were released into individual mesh bags that were then secured around the treated branch tips. Treatments were started and completed during an early June morning under conditions favorable for spraying: 18 - 20°C, Relative humidity ±64%, no precipitation and wind 6 - 11 km/h. There was no precipitation in the week following the spray applications. On the morning of the 7$^{th}$ day following spraying the virus-treated and control branch tips were cut from the trees and brought to the field laboratory. The bags were opened, larval counts were made and mortality assessed. Individual live larvae were placed into plastic creamer cups (Bio-Serv, Frenchtown, NJ) with 30 ml of artificial diet and reared in the laboratory under ambient conditions. Virus-caused mortality was recorded periodically until 21 d after spraying when all larvae had either died or pupated.

**Statistical analysis.** Probit analysis (PoloPlus, 2.0, LeOra Software) (Robertson et al. 2003) of the mortality data from both the New Jersey and the Siberian larval bioassays was used to calculate relative potency of viral genotypes, at $LC_{50}$ and $LC_{90}$, to Gypchek for the New Jersey bioassay, and to Chistoozernyy for the Siberian bioassay. Mortality data from the ground-based field test was transformed (square root) and analyzed by a one-way ANOVA using the Holm-Sidak method for the pairwise comparisons of means.

## Results

**Identification of viral genotypes.** Viral isolates A21-MPV, 122b, 122-HP, and 203 were found to contain unique 9.2 kbp, 6.7 kbp, 27/7.0/6.2 kbp, and 3.5 kbp *Bgl*II DNA fragments, respectively (Fig. 1). Isolate B1B did not contain any unique fragments; however, it was the only isolate to contain fragments of 23.4, 16.1, 9.0, 7.0, 6.8, 6.1, and 3.4 kbp in length. All of the isolates contained *Bgl*II DNA fragments of 24.6, 11.1, 10.4, 9.4, 8.1, 7.9, 4.8, 3.8, 3.6, and 3.2 kbp. Four of the 5 isolates contained DNA fragments of 23.4, 9.0, 6.1, and 3.4 kbp, and 3 of the 5 isolates contained fragments of 16.1, 7.0, and 6.8 kbp. The differences in restriction endonuclease fragment lengths were due to differences in the nucleic acid sequence of the viral isolates and were not correlated with phenotypic differences (JMS, Unpubl. data) The existence of genotypic differences allowed the use of the differences in the length of restriction endonuclease digestion fragments as markers to distinguish viral variants. A determination of the specific genotypic differences

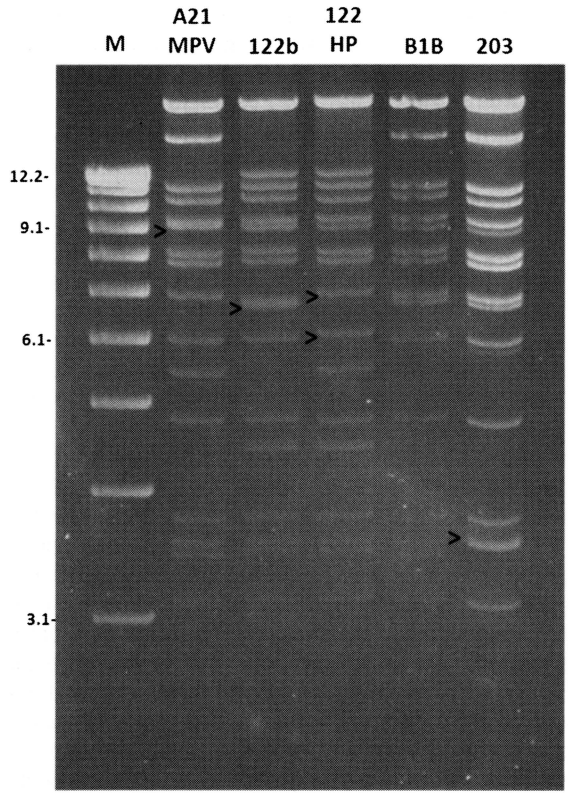


**Fig. 1. Genotypic analysis of LdMNPV isolates. Viral isolate genomic DNA was digested with the restriction enzyme *Bgl*II, and the fragments were separated on an agarose gel and visualized after staining with ethidium bromide. The lane labeled M contains DNA size markers, and the lanes containing viral DNA are labeled with the viral designation. Unique DNA fragments are indicated by the > mark.**

that may be related to differences in potency among the viral variants was outside the scope of this study.

**New Jersey larval bioassay.** Statistics for the probit analysis of the New Jersey larval bioassay are presented in Table 1. Larval mortality data from the bioassay are presented in Table 2 ($LC_{50}$) and Table 3 ($LC_{90}$). Based upon calculated 95% confidence limits, both 122-HP ($LC_{50}$=1568 OBs) and 122b ($LC_{50}$=2128 OBs) were equally as potent and more active against the New Jersey colonized strain of gypsy moth than either the parent product Gypchek ($LC_{50}$=6896 OBs) or any of the other genotypes tested; 203 ($LC_{50}$=7414 OBs), A21- MPV ($LC_{50}$=13113 OBs), B1B ($LC_{50}$=18079 OBs). Gypchek and 203 were equally potent as were A21-MPV and B1B; the latter pair being the least potent of all genotypes tested, and significantly less potent than either Gypchek, 203, 122b or 122-HP. Differences in potencies were supported by the tests for parallelism and equality of slopes of the regressions. These differences can be

**Table 1. Statistics from a probit analysis of viral mortality data from a bioassay of LdMNPV genotypes in second instar gypsy moth larvae from a colonized New Jersey strain.**

| Genotype | n | Slope (± SE)* | Chi-square | Heterogeneity |
|---|---|---|---|---|
| 122-HP | 30 | 1.60 (0.25) | 0.10 | 0.04 |
| 122b | 30 | 1.40 (0.21) | 0.46 | 0.15 |
| 203 | 30 | 1.47 (0.22) | 5.59 | 1.86 |
| B1B | 30 | 0.97 (0.13) | 4.38 | 1.46 |
| A21-MPV | 30 | 1.54 (0.23) | 1.13 | 0.37 |
| Gypchek | 30 | 1.36 (0.20) | 0.34 | 0.11 |

* Hypothesis of equal slopes and intercepts rejected ($P < 0.05$); hypothesis of parallel slopes not rejected ($P > 0.05$).

seen in the $LC_{50}$, and $LC_{90}$ potency ratios of the genotypes relative to Gypchek . The $LC_{50}$ data are the most reliable on which to base any conclusions. From that data it appears that 122b and 122-HP are about 3X as potent as Gypchek.

**Siberian larval bioassay.** Statistics for the probit analysis of the wild Ural-Siberian larval bioassay are presented in Table 4. Larval mortality data from the bioassay are presented in Table 5 ($LC_{50}$) and Table 6 ($LC_{90}$). Based upon calculated 95% confidence limits, strain 122-HP ($LC_{50} = 1.00 \times 10^4$ OBs) and strain B1B ($LC_{50}=1.64 \times 10^4$ OBs) were at least as potent as the Siberian viral strain Chistoozernyy ($LC_{50}=1.34 \times 10^4$ OBs) against wild Siberian larvae. Comparisons of $LC_{90}$ values were somewhat more difficult to evaluate because of a significant overlap of confidence limits. However, it seems clear that none of the viral isolates from Gypchek were as effective as Chistoozernyy ($LC_{90}=7.45 \times 10^5$ OBs) in killing 90% of the larvae challenged. But, of the strains tested, 122-HP ($LC_{90}=1.55 \times 10^6$ OBs) and B1B ($LC_{90}=9.50 \times 10^6$) appeared to be the most effective at that level.

**Table 2. Comparison of $LC_{50}$ values of LdMNPV genotypes from Gypchek and the genotype potency ratios relative to Gypchek.**

| Genotype | $LC_{50}$* | Upper CL** | Lower CL | Potency ratio† | Upper CL | Lower CL |
|---|---|---|---|---|---|---|
| 122-HP | 1.57 | 2.61 | 0.94 | 4.40 | 9.10 | 2.12 |
| 122b | 2.13 | 3.66 | 1.24 | 3.24 | 6.86 | 1.53 |
| 203 | 7.41 | 36.31 | 1.82 | 0.93 | 1.91 | 0.44 |
| B1B | 18.08 | 81.12 | 4.48 | 0.38 | 0.88 | 0.17 |
| A21-MPV | 13.11 | 22.13 | 7.86 | 0.53 | 1.09 | 0.25 |
| Gypchek | 6.90 | 11.86 | 3.99 | 1.00 | — | — |

* Number of viral occlusion bodies (x $10^3$) per ml of gypsy moth diet resulting in an $LC_{50}$ in second instar larvae from a colonized New Jersey strain of gypsy moth.

** 95% confidence limits.

† Potency ratio is the $LC_{50}$ value of Gypchek divided by the $LC_{50}$ value of a particular genotype.

**Table 3. Comparison of $LC_{90}$ values of LdMNPV genotypes and the genotype potency ratios relative to Gypchek.**

| Genotype | $LC_{90}$* | Upper CL** | Lower CL | Potency ratio† | Upper CL | Lower CL |
|---|---|---|---|---|---|---|
| 122-HP | 9.97 | 27.25 | 5.38 | 5.90 | 18.05 | 1.93 |
| 122b | 17.47 | 50.76 | 8.96 | 3.37 | 10.73 | 1.06 |
| 203 | 55.10 | 541.31 | 15.70 | 1.07 | 3.41 | 0.33 |
| B1B | 378.25 | 18,635.21 | 83.56 | 0.16 | 0.60 | 0.04 |
| A21-MPV | 88.53 | 245.16 | 46.96 | 0.53 | 2.06 | 0.22 |
| Gypchek | 58.84 | 167.05 | 30.21 | 1.00 | — | — |

* Number of viral occlusion bodies (x $10^3$) per ml of gypsy moth diet resulting in an $LC_{90}$ in second instar larvae of a colonized New Jersey strain of gypsy moth.
** 95% confidence limits.
† Potency ratio is the $LC_{90}$ value of Gypchek divided by the $LC_{90}$ value of a particular genotype.

**Table 4. Statistics from a probit analysis of viral mortality data from a bioassay of LdMNPV genotypes in second instar gypsy moth larvae from wild Ural-Siberian Strain.**

| Genotype | n | Slope (± SE)* | Chi-square | Heterogeneity |
|---|---|---|---|---|
| 122-HP | 50 | 0.56 (0.55) | 0.001 | 0.00 |
| 122b | 50 | 0.28 (0.08) | 0.58 | 0.03 |
| 203 | 50 | 0.46 (0.09) | 1.15 | 0.58 |
| B1B | 50 | 0.46 (0.09) | 0.007 | 0.004 |
| A21-MPV | 50 | 0.89 (0.11) | 0.012 | 0.006 |
| Chistoozernyy | 50 | 0.74 (0.12) | 0.03 | 0.02 |

* Hypothesis of equal slopes and intercepts rejected ($P < 0.05$); hypothesis of parallel slopes rejected ($P < 0.05$).

**Ground-based field test.** Mortality data resulting from testing 3 doses each of formulated 122b, 122-HP and Gypchek are shown with standard error bars in Fig. 2. Low-, medium-, and high-dose mean percent mortality values (± SEM) 22 d after treatment were 8.2 (5.2), 19.1(7.9) and 46.9 (6.5), respectively, for viral strain 122b, 38.7 (13.5), 27.2 (8.1), and 68.8 (6.0), respectively, for viral strain 122-HP, and 32.99 (16.0), 31.04 (19.4), and 76.0 (7.3), respectively, for Gypchek. There was no mortality in control (untreated) larvae. The ANOVA showed a significant dose effect ($F = 3.41$, df = 8, 44, $P = 0.005$), but the only significant differences (Holm-Sidak) in mean larval mortalities were between the Gypchek high dose and the 122b low dose ($P < 0.0010$) and between the 122-HP high dose and the 122b low dose ($P < 0.001$).

## Discussion

Gypchek has been produced by the U.S.Forest Service and distributed for use in European gypsy moth suppression programs since the early 1980s. Several biopesticide producers have been encouraged to commercialize Gypchek, but high production costs and an unstable market generally have dampened their interest. Commercial production costs could be eased using an LdMNPV strain that is more potent than the genotypic mixture in the current product. Results presented here indicate that both LdMNPV strain 122-HP and 122b may provide the added potency and, based on its activity against an Asian strain of gypsy moth, 122-HP likely would be useful in suppressing flighted Asian strains should any become established in the United States. We had hoped that the results of the ground-based field test would mirror those seen in the laboratory bioassays, i.e., that 122b and 122-HP would kill significantly more larvae than Gypchek. However, because of the variability often seen in "bugs-in bags" experiments, and this one was no exception, the higher mortality was not seen. However, 122b and 122-HP performed at least as well as Gypchek at all doses tested and we are encouraged to move forward with more definitive testing on both of these strains.

Given the uncertainty in the availability of a commercial product, either 122-HP or 122b could be produced in vitro as inoculum for a single-genotype, in vivo- Gypchek produced either by the Forest Service or a toll producer. To date, commercial

**Table 5. Comparison of $LC_{50}$ values of LdMNPV genotypes isolated from Gypchek and genotype potency ratios relative to the Siberian LdMNPV strain Chistoozernyy.**

| Genotype | $LC_{50}$* | Upper C** | Lower CL | Potency ratio† | Upper CL | Lower CL |
|---|---|---|---|---|---|---|
| 122-HP | 10.01 | 26.57 | 1.85 | 1.34 | 6.24 | 0.29 |
| 122b | 69.94 | 298.41 | 4.24 | 0.19 | 1.32 | 0.03 |
| 203 | 38.29 | 101.23 | 8.21 | 0.35 | 1.54 | 0.08 |
| B1B | 16.44 | 48.65 | 2.35 | 0.82 | 4.26 | 0.16 |
| A21-MPV | 260.32 | 446.33 | 150.35 | 0.05 | 0.15 | 0.02 |
| Chistoozernyy | 13.43 | 29.02 | 4.05 | 1.00 | — | — |

* Number of viral occlusion bodies (x $10^3$) per ml of gypsy moth diet resulting in an $LC_{50}$ in second instar larvae of a wild Ural-Siberian gypsy moth strain.

** 95% confidence limits.

† Potency ratio is the $LC_{50}$ value of Gypchek divided by the $LC_{50}$ value of a particular genotype.

**Table 6. Comparison of $LC_{90}$ values of LdMNPV genotypes isolated from Gypchek and genotype potency ratios relative to the Siberian LdMNPV strain Chistoozernyy.**

| Genotype | $LC_{90}$* | Upper CL** | Lower CL | Potency ratio† | Upper CL | Lower CL |
|---|---|---|---|---|---|---|
| 122-HP | 1,550.87 | 8,816.36 | 574.16 | 0.48 | 2.38 | 0.10 |
| 122b | 2,416,259.96 | n/a | 73,989.17 | 0.00 | 0.08 | 0.00 |
| 203 | 22,357.80 | 490,780.34 | 49,041.58 | 0.03 | 0.32 | 0.003 |
| B1B | 9,507.17 | 157,118.62 | 2,379.41 | 0.08 | 0.64 | 0.01 |
| A21-MPV | 7,066.88 | 22,456.36 | 3,240.70 | 0.11 | 0.42 | 0.03 |
| Chistoozernyy | 745.09 | 2,763.19 | 330.72 | 1.00 | — | — |

* Number of viral occlusion bodies (x $10^3$) per ml of gypsy moth diet resulting in an $LC_{90}$ in second instar larvae of a wild Ural-Siberian gypsy moth strain.
** 95% confidence limits.
† Potency ratio is the $LC_{90}$ value of Gypchek divided by the $LC_{90}$ value of a particular genotype.

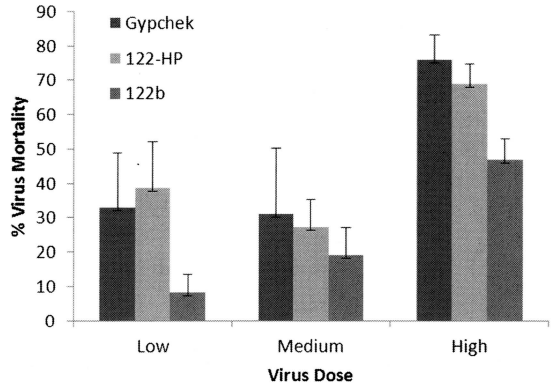


**Fig. 2. Mean mortality of gypsy moth larvae following treatment with low, medium and high doses of LdMNPV strains and Gypchek. Bars represent cumulative percent LdMNPV mortality (±SE) 22 d after treatment when all larvae had either died or pupated.**

baculovirus products have been produced only in vivo; this is due principally to the vagaries of cell culture production that often are responsible for the loss of viral virulence, genetic stability, and culture-cell viability (Szewczyk et al. 2011). Of the genotypic variants studied here, only A21-MPV (a wild-A21 mutant) and 122b have been passaged in vitro to determine their ability to maintain a high yield and stable OB production. Both genotypes do maintain high OB yield and activity after several passages (Slavicek et al. 1996, 2001). However, in this study A21-MPV was shown to be significantly less potent than either 122b or 122-HP against wild Ural-Siberian larvae and probably would not be a candidate for further development. Clearly, 122-HP will need to be studied in culture to determine if it retains potency and genetic integrity after in vitro passage. Further, 122b and 122-HP have not been bioassayed against gypsy moth strains from different geographical regions of Asia, e.g., the Russian far east, and areas of China, Korea, and Japan. Gypsy moth larvae and egg masses from these regions have been intercepted on ships arriving at ports in the western United States and Canada. Female moths from many of these Asian strains possess flight capability (Keena et al. 2008) and vary genetically (Bogdanowicz et al. 2000).They also are likely to vary in their response to different LdMNPV strains. All confirmed introductions of Asian gypsy moth have been declared eradicated before the pest could become established (U.S. Department of Agriculture 2003). *Bacillus thuringiensis* (*Bt*) Berliner products were used for all eradications except for some treatment areas in North Carolina that were supporting species determined to be sensitive to *Bt.* Those areas were treated successfully with Gypchek as noted above. It is reasonable to expect that, despite the best efforts of regulatory agencies, an Asian gypsy moth introduction into the United States will eventually go undetected and the pest will establish and spread. Thus, it is important to have an efficacious LdMNPV product available for use in suppression programs should Asian gypsy moth become established in sensitive habitats of the United States.

Currently, the Forest Service is cooperating with a Canadian company, Sylvar Technologies, Inc., Fredericton, NB, and their partner, Andermatt Biocontrol AG, Grossdietwil, Switzerland, toward the development of an improved in vivo- produced Gypchek. Notwithstanding the problems associated with large-scale cell culture production of baculoviruses, Andermatt Biocontrol AG has expressed interest in moving toward an in vitro-produced LdMNPV product. Hopefully the Sylvar-Andermatt efforts will bring to market an LdMNPV product effective against both European and Asian gypsy moth.

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