Gypsy Moth (Lepidoptera: Lymantriidae) Control with Ground-Based Hydraulic Applications of Gypchek, In Vitro-Produced Virus, and *Bacillus thuringiensis*

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ABSTRACT Gypchek, a registered microbial insecticide for aerial and ground-based application against the gypsy moth, *Lymantria dispar* L., was field-tested in 1996 and 1997 at 2 doses (10^{11} and 10^{12} polyhedral inclusion bodies (PIB) per 379 liters (100 gallons)) and with and without a sunscreen. An in vitro-produced strain of gypsy moth virus was tested in 1997 at the 10^{11}-PIB dose; however, the effective dose was lower because in vitro-produced PIBs contained 5-fold fewer virions than Gypchek PIBs. Treatments were applied to overstory oak, *Quercus* spp., trees with a truck-mounted hydraulic sprayer. Larval mortality was significantly greater, and larval density was significantly lower, on trees treated with both Gypchek and in vitro-produced virus than on untreated trees. Treatment with in vitro-produced virus resulted in significantly lower mortality and higher larval density than Gypchek treatment. Defoliation was significantly reduced by all treatments in 1996 but not in 1997, when overall gypsy moth population density dropped to very low levels. Mortality was significantly higher among larvae treated at the 10^{12}-PIB dose in 1996, but there was no significant dose effect on larval density or defoliation. Mortality, density, and defoliation were not dose dependent in the 1997 test. The addition of sunscreen to Gypchek applied at the 10^{12}-PIB dose did not have a significant effect on any of the measured parameters in 1996 or 1997. In 1996, the addition of an enhancer, Blankophor BBH, to the 10^{11}-PIB dose increased mortality to a level that was not significantly different from that at the 10^{12}-PIB dose. The level of control with the 10^{12}-PIB treatment was not significantly different from that achieved with an application of *Bacillus thuringiensis* subsp. *kurstaki* Berliner (Foray 48B) at 36 billion international units (BIU) per 379 liters. These results suggest that Gypchek can be used effectively as a ground-based or arborist-applied control agent, and that it may be possible to achieve acceptable levels of foliage protection at a 10^{11}-PIB dose and without the addition of a sunscreen. It also demonstrates the effectiveness in the field of in vitro-produced gypsy moth virus.

KEY WORDS *Lymantria dispar*, Gypchek, gypsy moth nucleopolyhedrosis virus, in vitro-produced virus, formulations, arborist

THE GYPSY MOTH, *Lymantria dispar* L., is a significant defoliator of forest and shade trees in the northeastern United States. Widespread defoliation, sometimes exceeding 2×10^6 ha in a single year, often occurs during the periodic outbreaks of this pest. As the gypsy moth continues to expand its range to the south and west, its greatest economic impact is in residential areas (Leuschner 1994). Although homeowner-applied sticky trunk barriers can provide limited protection to trees in the residential landscape (Webb and Boyd 1983, Thorpe et al. 1995), the only reliable defense against this pest is the application of insecticides from the air or ground. The ground-based application of chemical insecticides and the microbial insecticide *Bacillus thuringiensis* subsp. *kurstaki* Berliner can provide effective control of gypsy moth larvae and protect trees from defoliation (Thorpe 1996). Ground-based applications of gypsy moth control materials are generally available from commercial arborists by using truck-mounted hydraulic sprayers capable of reaching into the canopies of overstory hardwood trees (Vaughn et al. 1997).

The gypsy moth nucleopolyhedrosis virus (LdMNPV) product Gypchek is registered by the United States Environmental Protection Agency as a general use insecticide for aerial and ground application to control gypsy moth (Reardon and Podgwaite 1992). Currently, Gypchek is produced in limited quantities by the United States Department of Agriculture (USDA) for research purposes and for use in certain publicly funded spray programs. Although a commercial source of the virus is not presently available, a survey of gypsy moth managers indicated that there was a high level of interest in the commercial availability of a gypsy moth virus product (Podgwaite

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation by USDA for its use.

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et al. 1997). The development of procedures for the effective use of Gypchek, and the testing of spray adjuvants to improve its effectiveness, have been the subject of much research effort over the past 2 decades (Reardon and Podgwaite 1994). Demonstrations of the effectiveness of Gypchek applications, and the acceptance of and demand for this product by users and the public, should increase the chances that it will become commercially available.

One way to increase the commercial attractiveness of a gypsy moth virus product is by reducing its production cost. Gypchek is currently produced in vivo at a large-scale, federally funded insectary (Reardon and Podgwaite 1992). In vitro production has the potential to reduce production costs by eliminating the need to rear insect hosts. In a previous study (Webb et al. 1993a), an industry-produced experimental in vitro gypsy moth virus product was field tested along with Gypchek at an equivalent dose with favorable results. However, this product did not become commercially available and is no longer produced. Efforts on development of in vitro gypsy moth virus production systems have focused on generating virus strains that can be produced in the Ld652Y gypsy moth cell line. This cell line exhibits good growth characteristics in bioreactors and can withstand sparging shear-stress. However, the Ld*M*NPV viral line used in the production of Gypchek exhibits a high frequency of few polyhedra (FP) mutant formation when produced in the Ld652Y cell line (Slavicek et al. 1995). Consequently, Ld*M*NPV viral lines were developed that exhibit a lower frequency of FP mutant formation compared with wild-type virus (Slavicek et al. 1996). The Ld*M*NPV isolates A21-MPV (Slavicek and Mercer 1995) and 122b1a (Slavicek and Hayes-Plazolles 1995, patent pending) are two such isolates. Isolate 122b1a exhibits a lower frequency of FP mutant formation compared with isolate A21-MPV and was used in our study.

Previous investigations of ground-based applications of Gypchek demonstrated that significantly higher levels of mortality occur in larvae collected from Gypchek-treated trees (Webb et al. 1990, Podgwaite et al. 1991), and that mortality increases with increasing dose (Webb et al. 1993a, b; 1994a, b). Gypchek is subject to damage from ultraviolet (UV) radiation, and the addition of a sunscreen is considered essential to achieve consistent results in aerial spray programs (Podgwaite et al. 1991). Webb et al. (1993a) found that the addition of a sunscreen had no effect on mortality in a ground-based application of Gypchek. All of the studies of ground-based Gypchek applications mentioned above demonstrated that the treatment significantly increased the mortality of larvae collected from treated trees, but none reported a significant effect on defoliation from unenhanced Gypchek. In some cases, this was probably attributable to low overall levels of defoliation due to low gypsy moth populations. However, in 2 studies, the addition of the gypsy moth virus enhancer Blankophor BBH significantly reduced defoliation below control levels (Webb et al. 1994b, 1996).

The objectives of this study were to assess the impacts of ground-based Gypchek applications at different doses and with or without a sunscreen on gypsy moth populations, and to compare the effectiveness of an in vitro-produced virus with that of Gypchek. In addition to measurements of larval mortality in samples collected from treated foliage and assessments of defoliation, this study reports the direct effects of the treatments on gypsy moth larval population density in the tree canopy as measured by frass samples (Liebhold and Elkinton 1988a). Also reported is a direct comparison of the effects of ground-based hydraulic applications of Gypchek and *B. thuringiensis*.

Materials and Methods

Ld*M*NPV was obtained from 2 sources. Gypchek was produced from gypsy moth larvae (NJ-42 strain) inoculated with virus (LDP226 strain). The in vitro virus was produced from Ld652Y cells (Goodwin et al. 1978) that were propagated as previously described (Slavicek et al. 1992). Flasks (T150, Corning, Corning, NY) were seeded with 6.75×10^6 Ld652Y cells and infected with 6.5 $TCID_{50}$ units of isolate 122b1a per cell. Seven days after infection the cells were harvested, sonicated, and the polyhedral inclusion bodies collected by centrifugation and quantified by visual counting in a hemacytometer. The PIBs were resuspended in phosphate-buffered saline prior to mixing for field application.

Isolate 122b1a PIBs produced in the Ld652Y cell line and Gypchek PIBs generated in gypsy moth larva were prepared and sectioned for electron microscopic analysis as previously described (Slavicek et al. 1992). Photographs of PIB cross sections were generated and the diameter was determined by measurement of PIBs occurring on 25 photographs for each isolate. Because all PIB samples were handled similarly, and the sectioning was done at random, the measurements provide an accurate relative measurement of PIB diameter (J.M.S., unpublished data).

The study was conducted during 1996 and 1997 at the Glassboro Wildlife Management Area, Glassboro, NJ. Oak, *Quercus* spp., trees within 6 m of unpaved roads within the Wildlife Management Area and separated by a minimum of 50 m were selected for inclusion in the study. In 1996, only white oak, *Quercus alba* L., trees were included. In 1997 there were not enough suitable white oak trees that had not previously been used, so white oak, chestnut oak, *Quercus prinus* L., and black oak, *Quercus velutina* Lamark, trees were included. Although the study areas overlapped, no trees treated in 1996 were used in 1997. In 1997, treatments were balanced with respect to tree species to prevent confounding of treatment and tree species effects. The species composition, average height, diameter at breast height (dbh), and the preseason mean number of new egg masses per tree are given in Table 1. Preseason egg mass density, estimated using the method described in Liebhold et al. (1994), was 5,766 egg masses per hectare and 2,953 per hectare in 1996 and 1997, respectively.

Table 1. Species, diameter, and height of trees included in experiments, and foliage expansion at the time of spray application, Glassboro, NJ, 1996 and 1997

Year	Species composition, %	dbh, cm	Height, m	Preseason egg masses per tree	Foliage expansion, %
1996	White oak (100)	43.0 ± 2.1	18.8 ± 0.4	62.3 ± 11.8	38.3 ± 1.9
1997	White oak (16.7) Chestnut oak (22.2) Red oak group (61.1)	42.8 ± 2.4	17.3 ± 1.9	48.8 ± 12.1	41.1 ± 2.6

Values are mean ± SEM. dbh, diameter at breast height (1.5 m). $n = 36$.

Six treatments were applied in 1996: (1) Gypchek (USDA, Forest Service, Hamden, CT) at 10^{12} PIB per 379 liters (100 gallons) of tank mix + sunscreen (Lignosite AN, Georgia Pacific, Bellingham, WA) at 6% wt:vol; (2) Gypchek at 10^{12} PIB per 379 liters; (3) Gypchek at 10^{11} PIB per 379 liters + sunscreen; (4) Gypchek at 10^{11} PIB per 379 liters + sunscreen + enhancer (Blankophor BBH, Burlington, Burlington, NC) at 0.5% wt:vol; (5) *B. thuringiensis* (Foray 48B, Abbott, Chicago, IL) at 36 billion international units (BIU) per 379 liters; and (6) untreated control. All Gypchek treatments also included a sticker (Bond, Loveland, Greeley, CO) at 2% vol:vol. In 1997, the first 3 treatments were repeated, along with a 4th treatment consisting of Gypchek at 5×10^{11} PIB per 379 liters + sunscreen + sticker, and a 5th treatment consisting of in vitro-produced virus at 10^{11} PIB per 379 liters + sunscreen + sticker. Each year, 6 blocks, each consisting of 6 trees, were established in a randomized block design with proximity and preseason egg mass density as the blocking factor. Each of the 6 trees in each block received a different treatment, thus providing 6 replicates of each treatment. The treatments were applied by the same commercial arborist on 14 May in 1996 and on 13–14 May in 1997 by using an FMC gun operating at a pressure of 5.5 MPa. Complete coverage required a volume of 100–150 liters per tree. Larvae were predominantly 2nd instars at the time of the treatments. Average foliage expansion at the time of treatment is given in Table 1.

Within the week prior to the treatment application, 30 larvae were collected from within a 10-m radius of each tree. Larvae were put in 30-ml cups that were ¼ full of artificial diet (Bell et al. 1981) with a cardboard lid. One week after treatment, up to 30 larvae were collected from the canopies of the treatment and control trees by using a bucket truck to access the canopy. These larvae also were put into 30-ml cups with diet. A 30-min time limit was set for the larval collections. Because it was not possible to find 30 larvae within the allotted time on all trees, mortality estimates are missing for some trees or are based on <30 observations. Larval collections were made 1 wk after treatment to allow enough time for larvae to acquire a lethal dose, but prior to the onset of virus-caused mortality. By the time of the collection, the *B. thuringiensis* treatment had apparently reduced larval density such that sufficient numbers of larvae could not be found, so mortality data based on collected larvae are not available for this treatment. Cups with larvae were placed in an outdoor insectary at the Beltsville Agricultural Research Center, Beltsville, MD, for 3 wk, at which time they were examined to determine the fate of the larvae.

Larval population density was estimated for each tree by using the frass drop/frass yield method (Liebhold and Elkinton 1988a, b). Frass falling from the canopy was sampled with 10 plastic buckets (21 cm diameter by 15 cm high) per tree. The number of frass pellets falling into the buckets during a single 24-h period was determined and used to estimate the number of frass pellets falling per square meter of ground surface beneath the canopy. Frass yield (the number of frass pellets produced per larva during the sampling period) was determined by collecting 50 larvae from the study area and placing them individually in 177-ml plastic cups with cardboard lids. The cups were each provisioned with 1 or 2 oak leaves, and were then left in a shaded area near the experimental trees. These larvae were removed from the cups at the same time that the frass samples were recovered, so that the sampling duration and temperature conditions experienced by larvae in the cups and in the canopy were similar. The mean density of larvae in each tree (number of larvae per square meter) was estimated using the equation density $= C \cdot (x_d/x_y)$ (Liebhold and Elkinton 1988b), where $C = 1/$ (area sampled by each bucket), x_d = mean drop (frass/bucket), and x_y = mean yield (frass/larva). Samples were conducted at 14 and 21 d after treatment in 1996 and 1997, respectively, when larvae were predominantly 4th or 5th instars.

Pretreatment egg mass density was determined by counting egg masses on each tree with the aid of binoculars prior to egg hatch. Posttreatment egg mass density was determined by counting egg masses on each tree after the treated population had oviposited. Defoliation was subjectively estimated with the aid of binoculars in 10% increments on each of the trees after larval feeding had ended but before refoliation occurred. Larval mortality, larval density, defoliation, egg mass trend (posttreatment divided by pretreatment number of egg masses per tree), and PIB diameter data were analyzed by analysis of variance (ANOVA) by using the generalized linear models (GLM) procedure (SAS Institute 1985). When the treatment effect was significant, means were separated at a comparisonwise error rate of 0.05 by using the least significant difference (LSD) procedure (SAS Institute 1985). When required to stabilize the variance, the data were transformed to logarithms prior to

Table 2. Effects of ground-applied Gypchek and *B. thuringiensis* on gypsy moth mortality, density, and damage, Glassboro, NJ, 1996

Treatment[a]	Mortality, %	Larvae/m^2	Defoliation, %	Egg mass trend[b]
Gypchek @ 10^{12} PIB + sunscreen + sticker	94.4 ± 3.6a	18.1 ± 4.2ab	8.3 ± 3.1a	0.53 ± 0.25a
Gypchek @ 10^{12} PIB + sticker	81.9 ± 6.0ab	18.1 ± 3.8ab	6.7 ± 4.9a	0.24 ± 0.08a
Gypchek @ 10^{11} PIB + sunscreen + sticker	65.3 ± 11.0b	41.1 ± 17.1b	8.3 ± 4.8a	0.33 ± 0.14a
Gypchek @ 10^{11} PIB + sunscreen + sticker + enhancer	90.5 ± 4.9a	36.9 ± 16.2b	11.7 ± 3.1a	0.61 ± 0.24a
B. thuringiensis	[c]	12.6 ± 5.6a	5.0 ± 2.2a	0.57 ± 0.11a
Control	15.6 ± 4.3c	207.6 ± 88.9c	46.7 ± 15.6b	0.70 ± 0.22a

Values are mean ± SEM, $n = 6$. Means within a column followed by the same letters are not significantly different at a comparisonwise error rate of 0.05.

[a] PIB, polyhedral inclusion bodies. Gypchek dose is for a volume of 379 liters (100 gallons). Sunscreen = Lignosite at 6% wt/vol. Sticker = Bond at 2% vol/vol. Enhancer = Blankophor BBH at 0.5% wt/vol. *B. thuringiensis* dose = 36 billion international units (BIU) per 379 liters.

[b] Post- divided by pretreatment number of egg masses per tree.

[c] Larval numbers on trees treated with *B. thuringiensis* at the time of the sample were too low for the collection of sufficient numbers to quantify mortality.

analysis. Untransformed values and standard errors are reported in the tables.

Results

The relative sizes of PIBs generated by isolate 122b1a and Gypchek were determined through measurements of electron microscopic photographs of PIB cross sections. Each beam contained 120 PIBs that were sectioned randomly with respect to the cutting plane thereby generating representative cross sections from all areas of the PIBs. The Gypchek PIBs were found to be significantly larger than isolate 122b1a PIBs ($F = 44.9$; df = 1, 48; $P < 0.0001$). The relative diameters of isolate 122b1a and Gypchek PIBs were 1.4 ± 0.5 μm and 2.4 ± 0.5 μm (mean ± SD), respectively. The volume of the average Gypchek PIB is therefore 5-fold greater than the volume of the average 122b1a PIB. Because the virions are distributed evenly throughout the PIB, the reduced volume of isolate 122b1a PIBs results in a 5-fold reduction in the number of virions present in 122b1a PIBs compared with Gypchek PIBs.

Results from the spray applications in 1996 and 1997 are given in Tables 2 and 3, respectively. Mortality among larvae collected <1 wk before treatment averaged 8.1% in 1996 and 12.6% in 1997. In 1996, the treatment effect on posttreatment larval mortality was significant ($F = 29.7$; df = 4, 20; $P < 0.0001$). Mortality among larvae collected from untreated trees averaged 15.6%. Among treated trees, mortality was lowest among larvae treated with Gypchek at 10^{11} PIB + sunscreen (65.3%). This value was significantly lower than mortality among larvae treated with Gypchek at 10^{12} PIB + sunscreen (94.4%) or Gypchek at 10^{11} PIB + sunscreen + enhancer (90.5%). The addition of sunscreen to Gypchek at 10^{12} PIB did not have a significant effect. In 1997, mortality was significantly higher under all treatments than in controls ($F = 50.9$; df = 5, 22; $P < 0.0001$). Mortality of larvae treated with the in vitro-produced virus was significantly lower than among those treated with Gypchek. Mortality ranged from 10.0% among untreated larvae to 98.3% among larvae treated with Gypchek at 10^{12} PIB + sunscreen. Based on independent samples of larvae collected concurrently from the same area, mortality from the fungal pathogen *Entomophaga maimaiga* Humber, Shimazu & Soper was negligible (R.E.W., unpublished data).

The effect of the treatments on larval density as measured by frass collections was significant in 1996 ($F = 19.8$; df = 5, 25; $P < 0.0001$) and 1997 ($F = 13.2$; df = 5, 25; $P < 0.0001$). In 1996, there was no significant difference among the Gypchek treatments (18.1–41.1 larvae per square meter), but larval density on trees treated with Gypchek was significantly higher

Table 3. Effects of ground-applied Gypchek and in vitro-produced virus on gypsy moth mortality, density, and damage, Glassboro, NJ, 1997

Treatment[a]	Mortality, %	Larvae/m^2	Defoliation, %	Egg mass trend[b]
Gypchek @ 10^{12} PIB + sunscreen + sticker	98.3 ± 1.7a	3.0 ± 1.2a	2.5 ± 1.7a	0.74 ± 0.09a
Gypchek @ 10^{12} PIB + sticker	86.4 ± 9.9a ($n = 3$)	6.9 ± 2.4a	2.5 ± 2.5a	0.66 ± 0.19a
Gypchek @ 10^{11} PIB + sunscreen + sticker	90.2 ± 3.9a	6.2 ± 1.9a	3.3 ± 3.3a	3.31 ± 2.16a
Gypchek @ 5×10^{11} PIB + sunscreen + sticker	88.7 ± 3.4a	8.6 ± 2.4a	4.2 ± 2.7a	1.08 ± 0.18a
In vitro virus @ 10^{11} PIB + sunscreen + sticker	51.1 ± 7.0b	20.5 ± 4.9b	4.2 ± 3.3a	0.24 ± 0.21a
Control	10.0 ± 4.6c ($n = 5$)	41.4 ± 9.4c	8.3 ± 3.1a	1.95 ± 1.61a

Values are means ± SEM, $n = 6$ except as indicated in parentheses. Means within a column followed by the same letters are not significantly different at a comparisonwise error rate of 0.05.

[a] PIB, polyhedral inclusion bodies. Dose is for a volume of 379 liters (100 gallons). Sunscreen = Lignosite at 6% wt/vol. Sticker = Bond at 2% vol/vol. PIB volume and total number of virions is 5-fold less in the in vitro-produced virus compared with Gypchek.

[b] Post- divided by pretreatment number of egg masses per tree.

than on trees treated with *B. thuringiensis* (12.6 larvae per square meter) and significantly lower than on untreated trees (207.6 larvae per square meter). In 1997, larval density did not differ among the Gypchek-treated trees (3.0–8.6 larvae per square meter), but was significantly lower than on trees treated with in vitro-produced virus (20.5 larvae per square meter). Larval density on untreated trees (41.4 larvae per square meter) was significantly higher than on treated trees.

Defoliation was significantly lower in 1996 on treated than on untreated trees ($F = 6.2$; df = 5, 25; $P = 0.0007$), but there were no significant differences among the insecticide treatments. Defoliation ranged from 46.7% on untreated trees to 5.0% on trees treated with *B. thuringiensis*. In 1997, defoliation averaged 8.3% on untreated trees, and the treatment effect was not significant ($F = 1.0$; df = 5, 25; $P = 0.44$). Egg mass trend did not differ significantly among treatments during either year ($F = 1.0$; df = 5, 25; $P = 0.46$ and $F = 1.74$; df = 5, 24; $P = 0.16$ for 1996 and 1997, respectively).

Discussion

Although larval mortality was lower with the in vitro-produced virus than with Gypchek, it did result in significant reductions in larval densities. The lower effective dose of the in vitro-produced virus because of its smaller size is a likely explanation for its reduced effectiveness. As production of in vitro-produced virus is scaled up, the dose response of the product will need to be established to determine how many PIBs are needed to provide effective control.

The results of the 1996 portion of this study are in agreement with those of Webb et al. (1993a, b) and Webb et al. (1994a, b) in that higher rates of mortality among gypsy moth larvae collected from the foliage of treated trees occurred at a dose of 10^{12} versus 10^{11} PIB per 379 liters. However, as in the other studies cited above, no direct dose effect on defoliation was demonstrated. Direct estimates of larval density in the canopy by using the frass collection technique also failed to show a significant dose effect. In 1996, both the 10^{11} and the 10^{12} PIB doses lowered defoliation below that which occurred in untreated trees. In 1997, mortality among collected larvae did not differ significantly between the low- and high-dose treatments. An intermediate dose of 5×10^{11} PIB per 379 liters, which was included because mortality differed between the 10^{11} and the 10^{12} PIB doses in the previous year, resulted in mortality that was not different from the high and low doses. Larval gypsy moth populations were very low in 1997, which may account for the lack of a significant difference in defoliation between the treated and untreated trees.

In aerial applications, most of the spray is deposited on the upper surfaces of the leaves (Reardon and Roland 1991). An effective sunscreen is needed with aerial applications to retard the loss of activity of the virus, probably because of the exposed position of most of the virus. With a ground application, the spray is applied from beneath, and most leaves receive large amounts of deposit on their undersides (K.W.T., unpublished data). For this reason, it has been suspected that an effective sunscreen may not be important in tank mixes for ground applications. Eliminating the use of a sunscreen would reduce costs and mess associated with currently used sunscreens. Prior to this study, only 1 field test comparing ground-based Gypchek treatments with and without sunscreen had been reported (Webb et al. 1993a). In that study, the sunscreen was found not to have a measurable effect. In both years of the current study, ground-based applications of Gypchek at 10^{12} PIB per 379 liters were tested with and without sunscreen. The sunscreen had no detectable effect in either of the years.

Webb et al. (1996) suggested that the use of Gypchek at 10^{11} PIB per 379 liters with an optical brightener-based enhancer could provide, at a lower cost, control as effective as that provided by a 10-fold higher dose without the enhancer. In 1996, Gypchek treatments at 10^{12} and 10^{11} PIB per 379 liters were compared with a Gypchek treatment at 10^{11} PIB + 0.5% enhancer. Mortality among larvae collected from treated foliage was not significantly different between the treatment with Gypchek at 10^{12} PIB and the treatment with Gypchek at 10^{11} PIB + enhancer, but mortality from both of these treatments was significantly higher than that from the Gypchek at 10^{11} PIB without enhancer. These differences in mortality support the suggestion of Webb et al. (1996), although there were no differences in larval density or defoliation among the treatments.

In 1996, a *B. thuringiensis* treatment was included in the test to provide a direct comparison of this commercially available and widely used microbial insecticide and Gypchek. As has been reported previously for ground-based applications of *B. thuringiensis* (Thorpe 1996), very effective gypsy moth control was obtained on treated trees. Because of the difficulty in finding larvae on foliage treated with *B. thuringiensis*, mortality of collected larvae could not be assessed for this treatment. However, subsequent larval density in treated trees was significantly lower than in trees treated with Gypchek at 10^{11} PIB per 379 liters (regardless of whether or not enhancer was present), but not significantly different from larval density in trees treated with Gypchek at 10^{12} PIB. Defoliation levels did not differ among any of the treatments, including the *B. thuringiensis* treatment.

The results of this study further demonstrate that Gypchek can be used effectively as a ground-based or arborist-applied gypsy moth control agent. Although somewhat higher levels of mortality occur at a dose of 10^{12} PIB per 379 liters than at 10^{11} PIB, this difference may not be great enough to result in consistently higher levels of foliage protection, and therefore such treatment may not be necessary. Similarly, the use of a sunscreen with ground-based applications of Gypchek did not increase the level of mortality or foliage protection obtained, and may not be warranted. Currently, the key constraint to the operational use of Gypchek is its lack of commercial availability. The results of this study, along with those of

previous studies on the ground-based use of Gypchek, provide information necessary to develop procedures for the effective use of Gypchek if and when it becomes available for use by arborists. The significant levels of larval mortality resulting from the use of a reduced dose of an in vitro-produced virus in this field test are encouraging, and they suggest that a lower-cost alternative to the current in vivo production process may be feasible.

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