

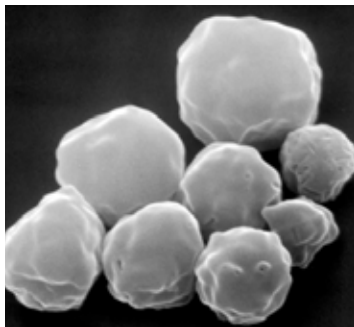


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Bioinsecticide

A ROLE FOR GROUND-BASED GYPCHEK TREATMENTS FOR GYPSY MOTH CONTROL



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Forest Service

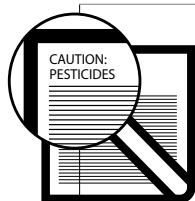
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Introduction

The gypsy moth-specific baculovirus (LdMNPV) product Gypchek was developed by the USDA Forest Service as an alternative to the use of broad-spectrum insecticides for gypsy moth control. Registration of Gypchek as a general use insecticide has provided resource managers with a safe choice for the treatment of infestations where environmental issues, such as the presence of an endangered or threatened species, are of concern (Podgwaite 1999, Reardon et al. 2016). The product is most often aerially-applied to forested areas, either by fixed- or rotary-wing aircraft, and is effective in protecting foliage and reducing larval populations in the year of treatment and the following year (Podgwaite et al. 1992a, 1992b). Aerial applications are conducted under the auspices of gypsy moth management programs that are funded and facilitated through the Federal and State Gypsy Moth Cooperative Suppression and Eradication Program. At present Gypchek is produced for the Forest Service by Sylvar Technologies, Inc., a Canadian company. Production is fixed at 8,400 acre treatments per year. Here we briefly review and discuss the research that has been conducted on ground-based Gypchek applications and offer justification for their use.

History of LdMNPV

Late in the 19th Century it became clear to European entomologists studying the gypsy moth (*Lymantria dispar*) that a disease was responsible for dramatic collapses of populations of the pest (Porchinsky, 1904). The disease, often referred to as “Wilt” due to the flaccid nature of dead larvae (Figure 1), was not observed in the United States until the early 1900’s. At that time researchers speculated that the causative agent was a “polyhedral body” associated with the dead larvae, but there was not a clear understanding of the pathological processes involved nor knowledge of the epizootiology of the disease. There were no reports of virus-killed larvae oozing their contents directly onto egg masses or of female moths depositing eggs onto virus contaminated bark (Figure 2). Although remains of dead larvae were observed on foliage, the early literature shows no evidence of that specific knowledge being useful, except in speculating that the virus itself might in some way be used to control gypsy moth (Reiff, 1911). Ultimately, with the advent of high-resolution microscopy and advanced bioassay technology, the disease was shown to be caused by a baculovirus (nucleopolyhedrovirus) and also that it was associated with gypsy moth eggs (Bergold, 1947). Subsequently, the virus was shown to passively contaminate eggs through contact with contaminated bark or leaves in the pest’s habitat and by the female moth during oviposition (Doane, 1969, 1975). More recently the virus has been shown to be covertly present within the egg, passed there by the female moth and moved to the next generation by transmission mechanisms that are yet to be completely understood (Il’inykh et al., 2004).

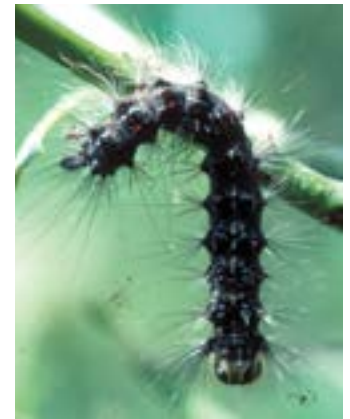


Figure 1. Gypsy moth larva killed by Gypchek.

Egg mass treatments in Russia, Ukraine, Germany and Canada

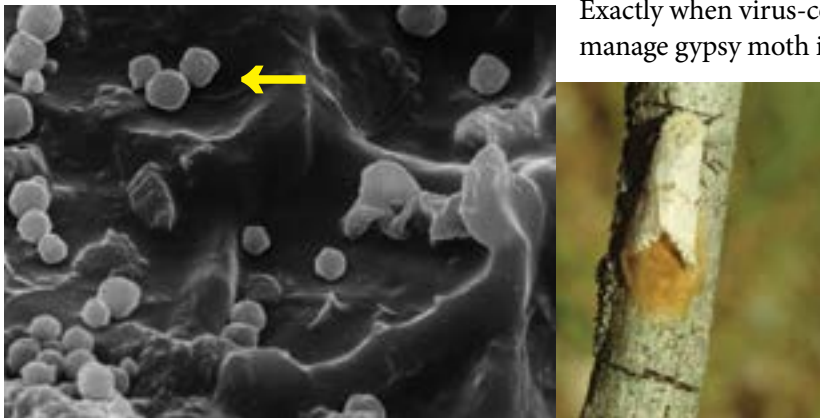


Figure 2. Scanning electron micrograph of viral occlusion bodies (arrow) on the bark (Kathleen Shields). Gypsy moth female depositing an egg mass on virus-contaminated bark (Roger Zerillo).

Exactly when virus-contaminated (sprayed) egg masses were first used to manage gypsy moth is not clear, but several field experiments were reported in the Russian and Ukrainian literature beginning in the early 1960’s. Much of this work is reviewed by Orlovskaya (1970) and abstracts of many studies can be found in Baranchikov et al. (1998). Most of the reported field experiments involved the topical treatment of individual egg masses within “foci” of gypsy moth infestations, either in forests or in fruit orchards. The expected results were that the virus would not only reduce larval populations within the foci but also spread from these foci and lead to viral epizootics, or at least some reduction in gypsy moth population density in nearby areas in the following year. Typically, a variable number of egg masses were misted with a hand-held sprayer containing a

known volume and concentration of the Russian commercial preparation of baculovirus (Virin-ENSh). Larval mortality was assessed relative to that occurring in untreated control areas. Results of some studies indicated that treatments were efficacious in significantly reducing larval populations, not only in the area of treatment but also throughout the infestation foci in the years following treatment (Yatsenko and Rudnev, 1989). Results of other studies, although showing positive results in the vicinity of egg mass treatments, were less convincing regarding effectiveness in spreading the virus disease to areas distant from the treatments (Agafonova et al., 1978). Notwithstanding the ambiguity in some of the field experiments, egg mass treatments with Virin-ENSh became a useful approach to managing gypsy moth infestations on small plots in Russia while aerial treatments were preferred for infestations on large tracts. It should be noted that the Russian virus product has not been available for use outside of government-sponsored programs, principally because over 99% of gypsy moth infested forested-lands in Russia are under government control (V.V. Martemyanov, personal communication). Likewise, Gypchek is not available for use outside of government-sponsored programs in the US, but for different reasons.



Figure 3. Topical treatment of egg masses with hand-held, pump-up hydraulic sprayer. (Roger Zerillo)

Gypsy moth is not a major pest in Germany. However, a serious infestation occurred in 1984-85 prompting German scientists to introduce LdMNPV into an infested forest (Bogenschutz, et al. 1989). Following successful laboratory tests with Gypchek supplied by the U.S. Forest Service sprayed on field-collected egg masses, egg masses in an oak stand were sprayed. The researchers calculated that virus mortality, shown by DNA analysis to be due to Gypchek, would have reached 95 % if not for concurrent mortality caused by parasitoids. The results of laboratory and field tests in Canada by Cardinal and Smirnoff (1973) led those authors to conclude that egg mass treatments would have a good chance of establishing viral epizootics and of reducing pest populations to enzootic levels. Also, the results from Lavigne and Carter (1996), who treated egg masses in three sites in New Brunswick, Canada, showed post-treatment egg mass reductions of 17, 24 and 65% in the three sites. The authors suggested that the technique had the potential for controlling gypsy moth in small isolated infestations. Other Canadian scientists treated egg masses in three urban Toronto parks and observed two waves of LdMNPV mortality in treated sites but not in untreated control sites. The result strongly suggested that virus-infected larvae emerging from egg masses were responsible for initiating the second wave of larval mortality. Unfortunately, significant larval mortality from the fungal pathogen *Entomophaga maimaiga* in the following year prevented an accurate assessment of any long-term impact the egg mass treatments may have had on gypsy moth population dynamics in the area (Thurston and Lapointe, 2008).

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Egg mass treatments in the United States

Though there are only a few published studies on field treatments of gypsy moth egg masses in the United States, there are unpublished laboratory and field data supporting the potential of this treatment method. Campbell (1983) tested egg mass treatments separately and in combination with several other control measures in small forest plots in Eastford, CT and Lisbon, NJ. In general, there were no significant differences between the egg mass treatments and untreated control plots in reducing populations or protecting foliage. However, the lack of treatment effect was most likely due to the low potency of the virus suspension used in the field. The survival rate of larvae emerging from eggs treated with the same suspension in the laboratory was quite high (74%) compared with 95% survival larvae emerging



Figure 4. Gypsy moth larvae emerging from an egg mass. Nearly 90% of the first instar will die when the egg mass is treated with Gypchek. (Roger Zerillo)

from un-treated egg masses. Podgwaite et al. (1981) treated egg masses in two forest plots in Connecticut with a concentrated suspension of viral occlusion bodies (OBs) using hand-held pump-up sprayers (Figure 3) to test the feasibility of integrating virus introduction with small mammal management for control of the gypsy moth. Unfortunately, an area-wide gypsy moth population collapse in the second year of the study made it impossible to evaluate efficacy of the combined tactics; however, treated egg masses brought back to the laboratory in both years showed 87% mortality in emerging larvae (Figure 4) and indicated that egg mass treatments most likely would have value where populations were not likely to be overwhelmed by naturally occurring disease. An effective tank-mix for the treatment of individual egg masses is shown in Table 1.

Table 1. Gypchek tank mix for application directly to egg masses¹

Ingredient	Amount per 3.79 liters (1 gal)
Tween 80 (polysorbate 80) ²	1.5 ml (0.5 fl oz) (0.04% v/v)
Nonchlorinated water	3.78 liters (~1 gal) (99%)
Gypchek	378 billion OBs

¹ Soak egg masses with about 1 fl. oz. of tank mix just prior to hatch

² Wetter/spreader (many commercial sources, e.g., Lowes, Walmart, Amazon.com)

Broadcast treatment of egg masses with manual backpack sprayers (Figure 5) or ground-based hydraulic sprayers (Figure 6) does not appear to offer the same degree of efficacy as observed from the topical treatment of individual egg masses. In 1983 backpack mist-blower applications of concentrated suspensions of Gypchek to low and moderate egg mass-density populations in West Virginia woodlots resulted in no statistically significant changes in egg-mass densities in the following year. However, the trend in egg density (post treatment/pretreatment) was lower in the moderate density treated plots (1.90) than in moderate density untreated control plots (5.34) (Podgwaite, unpublished data). In Maryland in 1985, prior to gypsy moth egg hatch, four small woodlots were treated with Gypchek using ground-based hydraulic equipment. The following year, egg mass density decreased in three of four treated plots but increased in three of four untreated control plots (Podgwaite, unpublished data). In Massachusetts in the spring of 1991, prior to gypsy moth oviposition, the boles of trees in seven 400 m² forest plots were treated with Gypchek using a backpack mistblower. In 1992, viral mortality of larvae emerging from egg masses collected from these sites was compared that of larvae from egg masses collected from untreated sites. Mortality due to virus was significantly higher in larvae emerging from eggs collected from the treated sites, but subsequent weekly collections of larvae from both treated and untreated sites showed no significant differences in mortality caused by LdMNPV. The authors speculated that the lack of treatment effect may have been caused by infected first-stage larvae ballooning from treated plots into control plots (D'Amico, et al., unpub-



Figure 5. (left) Backpack mist blower treatment of Gypchek to egg masses. (Roger Zerillo) Figure 6. (right) Ground-based hydraulic treatment of Gypchek to egg masses. (Roger Zerillo)

lished report).

Larval treatments in the United States

Four major field experiments conducted between 1986 and 1997 demonstrated the efficacy of Gypchek against gypsy moth larval populations when they were treated using ground-based hydraulic equipment. The first treatment was application to trees (Figure 7) in homeowner-sized lots in Maryland that resulted in an average of 80% viral mortality in larvae collected after treatment and reared in the laboratory (Table 2). Mortality in larval collections from control plots averaged 7%. (Webb et al. 1990). Larval mortality from different strains, doses, and formulations of LdMNPV were evaluated in 1990 following hydraulic applications to small forested plots in western Maryland. Larval mortality from Gypchek ($75 \pm 8\%$) applied at 2.5×10^{12} OBs per ha was not significantly different from larval mortality caused by a similar dose of an Abington, MA strain of the virus ($80 \pm 2\%$), or treatments with a Gypchek formulation containing the sunscreen Orzan LS ($73 \pm 6\%$) (Webb et al. 1993).

In 1992, a high dose and a low dose formulation of Gypchek, each with and without the addition of the activity enhancer Blankophor BBH (Shapiro and Robertson, 1992), were evaluated in eastern Maryland, again using ground-based hydraulic equipment. Applications of both low and high dose formulations containing Blankophor resulted in significantly more larval mortality than applications of those formulations without enhancer (Webb et al. 1994). However, more environmental testing of the enhancer will be required before EPA will sanction its use as an inert ingredient in biopesticide formulations. The addition of the enhancer to a Gypchek formulation would allow a 10-fold drop in OB dose/acre, significantly reduce productions costs, and would make more product available for general use. Finally, in 1996 and 1997, various doses and formulations of Gypchek were applied by ground hydraulic equipment to individual trees in the Glassboro Wildlife Management Area, New Jersey (Thorpe et al. 1998). In 1996, the application of a tank mix containing 1×10^{12} OBs/gal and a sunscreen (Orzan LS) resulted in larval mortality ($94.3 \pm 3.6\%$) not significantly different from either the same dose without Orzan ($81.9 \pm 6.0\%$) or a 10-fold lower dose to which Blankophor BBH had been added ($90.5 \pm 4.9\%$). In 1997 no significant differences were seen in larval mortality resulting from applications of tank mixes containing per gal: 1×10^{12} OBs ($98.3 \pm 1.7\%$), 5×10^{11} OBs ($88.7 \pm 3.4\%$) and 1×10^{11} ($90.2 \pm 3.9\%$). Also, in both years, live larval density was significantly lower on treated trees than on control trees following Gypchek applications. In 1996, defoliation



Figure 7. Hydraulic treatment of Gypchek to a larval infested tree on a home-owner's property in Maryland. (Roger Zerillo)

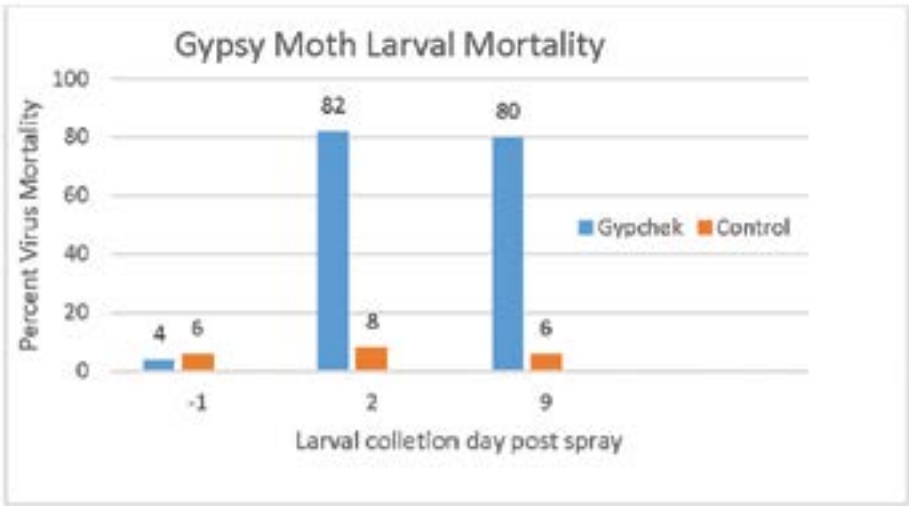


Table 2. Average virus mortality in gypsy moth larvae collected from Maryland home-owner plots sprayed with Gypchek. Larvae were collected after Gypchek treatments and reared in the laboratory until they either died or pupated.

of treated trees averaged 8%, significantly lower than 47% defoliation of control trees. Defoliation was much lower in 1997, 3% on treated trees and 8% on control trees, and not significantly different.

All of the aforementioned field studies clearly showed that extensive foliar coverage of the Gypchek formulations was provided by ground hydraulic applications (Figure 8). Also, droplet deposition on the undersides of leaves provided more shielding of the virus from deactivation by sunlight and a higher probability of larvae ingesting a lethal dose than had been observed for aerial applications of the product. The current recommended application protocol for Gypchek includes the hydraulic application to early instar larvae as soon as hatch is complete (Reardon et al. 2016). One application of a water-based tank mix (Table 3) at 1 x 10¹² OBs and 100 gal/acre is recommended for woodlots, roadsides and small acreages, and one application of 15-25 gallons of the same tank mix is recommended for individual trees in yards.



Figure 8. Hydraulic application of Gypchek to gypsy moth larval infestation on a New Jersey roadside resulting in extensive coverage throughout the overstory and understory and a saturation of the undersides of leaves. (Roger Zerillo)

Table 3. Gypchek water-tank-mix for ground-hydraulic treatments of gypsy moth larvae.

Ingredient	Amount per 3.79 liters (1 gal.)
Tactic ¹	77.6 ml (2.5 fl.oz.) (2% v/v)
Nonchlorinated water	3.71 liters (125 fl. oz) (98% v/v)
Gypchek	10 billion OBs

¹Adhesive, Synthetic latex, Loveland Industries, Greeley, CO

Discussion

Based on the results of the field tests reviewed here, it is reasonable to assume that topical treatments of egg masses with LdMNPV using hand-held pump-up sprayers will result in the death of a significant, if variable, portion of larvae emerging from the treated egg masses. If infected first-instar larvae disperse within and beyond the area treated and die on foliage it is likely that they will become the source of infection for healthy larvae within and beyond the immediate treatment area. However, it is difficult to predict the level of virus-induced mortality that will occur within the area of treatment and in the surrounding area. Mortality will be dependent upon the density, rate of development and dispersal of live larvae in the vicinity of the treatment area, and favorable weather conditions following viral applications. The application of LdMNPV to egg masses, either by manual backpack mist-blower or ground-based hydraulic equipment may be less effective in killing larvae and protecting foliage because penetration of the egg masses with virus is lower than when using small, hand-held delivery devices. In any case, both the direct, topical treatments of individual egg masses and indirect broadcast treatments will have a measureable negative impact on the larval population, mitigate defoliation and, through environmental persistence (Podgwaite et al. 1979), provide a reservoir of virus available for potential impact in the following year. The aforementioned studies have also shown that ground-based hydraulic applications of Gypchek that target gypsy moth larvae on individual trees or in small forested plots can be effective in saving foliage and reducing larval numbers and perhaps providing positive impact in succeeding years. However, there have not been a sufficient number of small home-owner properties treated with Gypchek to predict the impact on future year infestations. Clearly, based upon what is known about LdMNPV persistence in the environment and viral epizootiology, there is good reason to believe that Gypchek treatments of individual properties, particularly those in close association with one another, would provide a source of virus from

which larvae could become infected. Of course, more studies are necessary to show the extent to which this may occur, but absent those studies there is no downside and little expense incurred when treating properties in anticipation of a certain degree of success.

Why is there a reluctance to institute this technology as part of an overall gypsy moth management strategy? It appears to be a logical tool for the gypsy moth management toolbox, particularly in rural and urban environments. The Gypchek label states: "Only for use as a biological insecticide to manage gypsy moth infestations in wide-area public pest control programs sponsored by government entities". The generic statement ".....in wide-area public pest control programs sponsored by government entities" implies that Gypchek needs to be applied under government supervision due to safety concerns. Gypchek has been documented as having no effects on non-target life forms; it is specific to gypsy moth larvae (Durkin, 2004). Additionally, ground-based applications do not qualify for cost share as part of the Gypsy Moth Cooperative Suppression and Eradication Program. Both these constraints make it difficult to include ground-based applications as part of state and federal requests through the Gypsy Moth Cooperative Suppression and Eradication Program. Gypchek, the only gypsy moth-specific insecticide, was developed for use in those areas where the use of broad spectrum products would not be appropriate, or perhaps severely restricted. It is best suited for use both in large and in small environmentally-sensitive areas that are managed to protect beneficial species and their habitat.

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