Biological Efficacy of Gypchek Against a Low-Density Leading-Edge Gypsy Moth Population

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ABSTRACT: *The USDA's Slow-the-Spread (STS) program seeks to retard the continued spread of the gypsy moth using ecologically desirable treatments such as Gypchek. At "trace" population levels, evaluation of treatment success by defoliation reduction, egg mass reduction, burlap counts, or larval collection is not feasible. We adapted the "bugs-in-bags" technique to evaluate an operational application of Gypchek against trace populations of gypsy moths in Wisconsin, an STS area. Late first- or early second-instar gypsy moth larvae were placed, 1 per bag or 10 per bag, in sleeve cages placed over treated foliage one hour posttreatment. Mortality observed for larvae placed 10 per bag was equivalent to that recorded for larvae placed 1 per bag, and both should approximate the mortality occurring to the larvae scattered in nature. A single application of Gypchek applied in 9.5 liters of Carrier 038 at 10^{12} polyhedral inclusion bodies per hectacre was found to induce a higher rate of infection in blocks treated in the early morning than in blocks treated later in the morning, correlating significantly with a lowering of relative humidity and an increase in temperature and wind speed. Recorded levels of efficacy (24–67%) did not meet quarantine objectives; however, Gypchek, which kills only the gypsy moth, remains a product of choice by many land managers for use in certain environmentally sensitive areas. These results provide such land managers with a realistic assessment of the level of efficacy that can be expected from this formulation of Gypchek used at the currently recommended dose. North. J. Appl. For. 21(3):144–149.*

Key Words: *Lymantria dispar*, Gypchek, nuclear polyhedrosis virus, forest pest, Slow the Spread.

Gypchek (USDA Forest Service, Washington, DC), the (multienveloped) nuclear polyhedrosis virus product of the gypsy moth, *Lymantria dispar* (L.), is registered with the US Environmental Protection Agency as a general use pesticide (Reardon and Podgwaite 1996). Virus-infected cadavers are provided in limited quantities for the USDA Forest Service's production of Gypchek by the Animal Plant Health Inspection Service from its Otis, MA, laboratory. Until recently, Gypchek label recommendations called for two applications, 3 days apart, of a molasses-based tank-mixed formulation (Reardon and Podgwaite 1994). Recent tests with Gypchek and a "new" commercially produced carrier (Webb et al. 1999a) found that one application at 10^{12} polyhedral inclusion bodies/ha gave results statistically equivalent to a double split-dose application of the "old" molasses-based tank-mix formulation. Although the single application of the "new" formulation was statistically less efficacious than a double application of the "new" formulation, the single-dose option has favorable economic and programmatic aspects (Webb et al. 1999a) that makes the single application attractive.

Two recently published studies evaluated the aerial application of Gypchek against high gypsy moth populations (Podgwaite et al. 1992a, 1992b). These studies used foliage protection and egg-mass reduction as the primary indicators of application success. A study of the aerial application of Gypchek against "low-density," but measurable (59–272 egg masses/ha) populations (Podgwaite et al. 1993) used

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egg masses reduction and counts of live larvae under burlap bands as measures of efficacy; as might be expected in such low populations, defoliation differences between sprayed and control plots were not significant. Webb et al. (1999a, 1999b), working in populations of various densities, used the virus-induced mortality calculated for resident larvae collected 6–11 days after treatment from treated or control plots as the primary indicator of treatment success. Measuring treatment effects soon after treatment avoided the confounding influence of late-season population collapse due to the fungal entomopathogen *Entomophaga maimaiga* Humber, Shimazu, and Soper.

The present study was designed to address the specific problem of evaluating the operational efficacy of Gypchek application against populations of gypsy moth in "transition" areas, defined as the area between the uninfested and generally infested area; populations are discontinuous, population outbreaks do not occur, and defoliation is uncommon (USDA Forest Service 1998). The primary measurable gypsy moth life stages found in the transition area are adult male moths caught in pheromone traps. Use of Gypchek in the transition area would support the USDA's Slow-the-Spread (STS) Program (Leonard and Sharov 1995, Sharov et al. 1998), which seeks to retard the continued spread of the gypsy moth using ecologically desirable treatments such as Gypchek. STS, which was started in 1993 and became fully operational in 1999 (see Sharov et al. 2002 for a map of the STS project area), initially used *Bacillus thuringiensis* (BT) as its primary treatment. However, the "mating disruption technique," based on applying a synthetic version of the gypsy moth sex pheromone disparlure, has proven more effective than BT (Sharov et al. 2002) and is now the most heavily used control material in STS. Gypchek is potentially a third environmentally friendly control material for use by STS.

At such "trace" population levels, evaluation of treatment success by defoliation reduction, egg mass reduction, burlap counts, or larval collection is not feasible. Webb et al. (1998, 1999c) compared this virus against standard insecticides using the "bugs-in-bags" approach developed by D'Amico and Elkinton (1995). This article now reports the successful adaptation of the "bugs-in-bags" approach as a technique to evaluate Gypchek application against trace populations of gypsy moths. Of concern is the number of larvae to be placed in each bag. In trace populations, we assume that the larval population consists of widely scattered individuals existing one per branch on a few branches on a few trees. Thus, one larva per bag would reflect reality in the field. However, multiple larvae per bag would give more information, allow the computation of standard errors, and provide the added confidence that corresponds with higher numbers of experimental animals. Therefore, we designed the study to determine whether the overall mortality observed for larvae placed 10 per bag was equivalent to that recorded for larvae placed 1 per bag.

We report the successful use of this technique to evaluate an operational program designed to use Gypchek to suppress low-density gypsy moth populations to levels too low to sustain reproduction. Although this level of suppression was not achieved, an insight into Gypchek performance under operational conditions was gained.

Materials and Methods

Plot Establishment

In the spring of 1999, five mixed-oak stands (176–280 ha) on flat terrain in Portage and Waushara Counties, Wisconsin, were chosen for treatment by the State of Wisconsin gypsy moth control program. These five plots were paired with five similarly sized untreated control plots based on proximity and gypsy moth population size, defined by male trap catch. Elevations ranged from 250 to 350 m above sea level. A transect of 10 evaluation sites, each site about 20 m apart, was established in the center of each plot. At each of the 10 sites, two accessible branch tips on preferred gypsy moth host plants (*Quercus* spp.) were selected on the day prior to treatment and marked 300 cm from the end of the tip with a ribbon bearing identifying information.

Treatment

Gypchek used in this study was from lot GR1–14, which contained 3.8×10^{10} polyhedral inclusion bodies/g. Treatment consisted of one application of 1×10^{12} polyhedral inclusion bodies in 9.5 liters of Carrier 038 formulation (Abbott Laboratories, N. Chicago, IL) per hectare. A slurry was prepared by adding the required amount of Gypchek to a measured volume of water and blending to a homogenous consistency. The slurry was slowly added to a mix-tank in which the appropriate amount of Carrier 038 was circulating. Applications were made using a turbine-powered Air Tractor AT-502B (Pratt and Whitney, Olne, TX) equipped with a standard boom (with inline screens removed) and a total of eight Micronair AU5000 nozzles (VRU setting = 13). The aircraft sprayed 15 m above the canopy at an air speed of 233 km/h and a boom pressure of 2.8 kg/cm^2, with a lane separation of 53 m. The aircraft was guided using SATLOC Foreststar (SATLOC Inc., Tempe, AZ) Differentially Corrected Global Positioning System (DGPS).

The plots were sprayed beginning 06:05 on the morning of May 15 and ending 12:10 that afternoon. Measurements made within the spray blocks recorded that leaf expansion was 40–60%, with 90% first-instar and 10% second-instar larvae present, with temperatures ranging from 12 to 22° C, relative humidity measured at 53–75%, and wind speeds of 0–13 km/h recorded (see Table 1 for specific plot parameters). Instar determination was base on the size of the head capsule, while leaf expansion was based on estimates of experienced observers in the plots at the time of spray. Leaf expansion varied by plot and by host species, while larval-instar determinations were necessarily made at the few sites where larval populations were known. The general area of the plots had 1.7 cm of rain on the afternoon of May 16.

Post-Treatment Assessment of Virus

Larval encagement began approximately 1 hour after treatment in each block. Larvae (late first, early second instars) used in this study were supplied from a colony (New Jersey Generation 43) maintained at the Center for

Table 1. Association of treatment time (of Gypchek), temperature, relative humidity, and wind speed on the mortality of gypsy moth larvae placed 10 per bag or 1 per bag on treated foliage in five Wisconsin operational spray blocks, 1999.

Block	Time of treatment	Temperature (°C)	Relative humidity	Wind speed (k/h)	% Mortality (SE)[a] (10 larvae/bag)	% Mortality[b] (1 larva/bag)
W-1	6:05–7:23	12	75	Calm	61.1 (7.6)	62.5
W-2	7:35–8:45	15	73	Calm	66.8 (5.9)	90.0
P-6	8:54–9:49	18	70	8–11	58.6 (4.9)	60.0
P-3	9:55–10:56	22	55	10–13	47.6 (7.1)	10.0
P-5	11:15–12:10	21	59	10–11	24.2 (5.4)	40.0
Ave. (SE)					51.3 (3.4)	53.2 (7.4)

[a] Results for 10 larvae per bag at 10 sites per block = 100 larvae per block except for block W-1, where two bags were not recovered, resulting in 80 recovered larvae for block W-1.

[b] Results for 1 larva per bag at 10 sites per block = 10 larvae per block except for block W-1, where two bags were not recovered, resulting in 8 recovered larvae for block W-1. Since there was only 1 larva per bag, an SE was not calculated for individual blocks.

Biological Control, Hamden, CT. Each of the branch tips labeled the day prior to treatment received a bag consisting of a 60 × 60 cm^2 of organza cloth seamed to make a bag as per Webb et al. (1998, 1999c). Two bags were placed at each of the 10 sites per block; 1 bag received a single larva and its companion bag received 10 larvae; bags then were tied off. Larvae were left in the bags for 3 days, after which the branch tips bearing the bags were snipped off past the identifying ribbon and taken to the laboratory, where all larvae were removed from the bags and placed on artificial diet (Bell et al. 1981) in 30-ml plastic cups with paper lids, one larva per cup. The rearing cups were taken to Beltsville, MD, where they were held on shelves in a wooden outdoor insectary (368 cm long, 215 cm wide, 92 cm deep, with hardware cloth covering the front to allow natural conditions of light, temperature, and humidity but not rain) at the Beltsville Agricultural Research Center. All larvae in the insectary were observed every 2–3 days for mortality until death, or for 35 days. Dead larvae were labeled by date-of-death and placed in a freezer to await necropsy. Tissue samples from all of the larvae that died were examined under 400× magnification for the presence of viral inclusion bodies. If determinations could not be made 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 1,000× magnification.

Data Analysis

SAS ver. 6.11 (SAS Institute1996) was used for the statistical analyses. A paired-difference *t* test was performed to compare mortality in the groups of 1 larva per bag and 10 larvae per bag (PROC UNIVARIATE). Mortality among groups containing 1 and 10 larvae per bag was correlated with time, temperature, relative humidity, and wind speed at the time of application (PROC CORR). Pearson product-moment correlation coefficients are reported in all cases below.

Results and Discussion

Treatment Efficacy

Mortality of caged larvae was negligible (average mortality ± SE = 3.6 ± 1.3%) in the five paired untreated plots. Mortality in the treated plots averaged 51.3 ± 3.4% for larvae caged 10 per bag and 53.2 ± 7.4% for larvae caged 1 per bag (Table 1). Based on a paired *t* test, there was no difference in mortality recorded between the two bag-densities ($t = -0.28$; $P = 0.78$). The mortality recorded for the 1 larva per bag group was highly correlated ($r = 0.37$; $df = 47$; $P = 0.0096$) with that recorded for 10 larvae per bag (Table 2). This supports a conclusion that the overall mortality observed for larvae placed 10 per bag followed the same pattern as that recorded for larvae placed 1 per bag, and both fairly well reflect mortality previously recorded for this treatment regimen from natural populations (Webb et al. 1999a, 1999b). Our larvae were taken from treated leaf surfaces and placed in individual diet cups. This raised the possibility that surfaced-contaminated insects were capable of infesting themselves by treading on their diet. However, the larvae had been in the field on treated foliage for 3 days before being removed from the bags and placed on the diet, so that most unconsumed virus in the field would have been inactivated by solar irradiation by this time. Moreover, 93.7% of virus-killed larvae were dead after 22 days, and 99.5% were dead after 26 days, with one more larva dead after 35 days. This pattern of mortality is consistent with that reported by Webb et al. (1994) for larvae held in a field insectary at ambient temperatures.

Higher levels of mortality were recorded for the earlier-treated blocks than for the later-treated blocks (Table 1), with larval mortality negatively correlated with time of treatment for both the 10 larvae per bag readings ($r = -0.55$; $df = 48$; $P = 0.0001$), and the 1 larva per bag readings ($r = -0.33$; $df = 47$; $P = 0.022$) (Table 2). Readings from the spray blocks indicated that the temperature and wind speed were increasing and that the relative humidity was decreasing as the morning progressed, with time of application positively correlated with temperature ($r = 0.95$; $df = 50$; $P = 0.0001$) and wind ($r = 0.88$; $df = 50$; $P = 0.0001$), and negatively correlated with relative humidity ($r = -0.87$; $df = 50$; $P = 0.0001$). The negative correlations recorded here for insect mortality and application sequence may simply reflect the weather conditions existing at the time of application rather than time per se. The weather parameters recorded by the spray manager at the time of treatment in the last spray block were 21° C, 59% relative humidity, with wind speeds of 10–11 km/h.

Table 2. Matrix of correlation statistics for variables in five Wisconsin operational spray blocks, 1999. Pearson product-moment correlation coefficients (parametric), probability of no difference, and number of observations for time of Gypchek treatment, temperature, relative humidity, wind speed, and the mortality of gypsy moth larvae placed 10 per bag or 1 per bag on treated foliage.

Parameter	Time of treatment	Temperature (°C)	Relative humidity	Wind speed (k/h)	% Mortality (10 larvae/bag)	% Mortality (1 larva/bag)
Time of treatment	1.00					
	0.00					
	50					
Temperature	0.95	1.00				
	0.0001	0.00				
	50	50				
Relative humidity	–0.87	–0.94	1.00			
	0.0001	0.0001	0.00			
	50	50	50			
Wind speed	0.88	0.93	–0.83	1.00		
	0.0001	0.0001	0.0001	0.00		
	50	50	50	50		
% Mortality, 10/bag	–0.55	–0.46	0.49	–0.44	1.00	
	0.0001	0.0009	0.0004	0.0018	0.00	
	48	48	48	48	48	
% Mortality, 1/bag	–0.33	–0.41	0.47	–0.41	0.37	1.00
	0.022	0.0042	0.0009	0.0038	0.0096	0.00
	47	47	47	47	47	47

Sanderson (1991) recommended that aerial spray operations stop when wind speeds exceed 16 km/h, temperatures exceed 27° C, and relative humidity drops below 50%, although Mierzejewski and Bryant (1991) recommend that spray calibration tests be halted if relative humidity drops below 50% and the temperature is above 16° C. Thus, the decision to continue spraying reflected accepted practice.

Weather Effects

These results are from an operational program. To establish with confidence the role of weather on the performance of a treatment regimen, an elaborate array of sensors would be needed to record temperatures, humidities, and wind speeds in various parts of the canopy as well as above the canopy in the spray release zone. However, the present data found that weather parameters as measured operationally were associated with subsequent larval mortality. Temperature was negatively correlated with larval mortality for both the 10 larvae per bag readings and the 1 larva per bag readings (Table 2). Wind speed was also negatively correlated with larval mortality for both the 10 larvae per bag readings ($r = -0.44$; $df = 48$; $P = 0.0018$) and the 1 larva per bag readings ($r = -0.41$; $df = 47$; $P = 0.0038$). In contrast, relative humidity was positively correlated with larval mortality for both the 10 larvae per bag readings ($r = 0.49$; $df = 48$; $P = 0.0004$) and the 1 larva per bag readings ($r = 0.47$; $df = 47$; $P = 0.0009$). As pointed out by Miller et al. (1995), any droplet released by an aircraft must fall through many meters of air, and air speed, direction, turbulence, temperature, and humidity all affect the droplet's path and evaporation rate. These characteristics of air above or in the canopy are never constant. Ground measurements of relative humidity can be deceptive. Measurements of relative humidity taken on the ground beneath the canopy may be as much as double the relative humidity above the canopy where the spray is being released (Miller et al. 1995).

The level of control achieved by the current program was similar to that achieved in previous studies. Webb et al. (1999a) found that a single application of Gypchek applied in 9.5 liters of Carrier 038 at 10^{12} polyhedral inclusion bodies/ha under experimental conditions in Virginia resulted in about 68% mortality of larvae collected 6–11 days subsequent to application. In a second study, Webb et al. (1999b) found that the above treatment regime resulted in an average of about 68% mortality to field-collected larvae when applied under excellent conditions in West Virginia. Both these results are consistent with results obtained in the first two plots treated in the current studies. However, Webb et al. (1999b) also found that the above treatment regimen applied under marginal weather conditions (due to rain fall soon after application) in Maryland resulted in about 30% mortality above background. These results combined with results from the late-morning application blocks in the current study suggest that a single application of Gypchek applied in 9.5 liters of Carrier 038 at 10^{12} polyhedral inclusion bodies/ha may be rather sensitive to weather conditions. Webb et al. (1999a) found that a double application of Gypchek applied in 9.5 liters of Carrier 038 at a split dose of 5×10^{11} polyhedral inclusion bodies/ha/application resulted in about 86% mortality of sampled larvae. If the program manager is willing to pay considerably greater program costs (in both time and money), the double application option should result in higher larval mortality. Reversing the order of spray block sequence for the second application will increase the probability that all blocks will be treated under favorable conditions for at least one of the applications.

A "quarantine treatment" should ideally kill 100% of a target population because the aim of such a treatment is to eliminate the population. Treatments in the generally infested areas need only reduce gypsy moth populations to a level where significant defoliation is prevented, although high levels of mortality obviously are welcome. Successful treatments in transition or leading edge areas do not need to kill 100% of the pest population because eventual reinfestation is expected. However, the goal of treatments in leading edge areas is to "slow the spread" of the advancing gypsy moth population, which is presumably facilitated by high levels of treatment mortality. Gypchek has an advantage over traditional pesticides when used in the generally infested area in that it is a true disease that can spread in time and space. Indeed, Webb et al. (1990) found that a large late-season viral epizootic occurred in plots in which disappointing early-season virus mortality was recorded following treatment, while a much smaller epizootic was recorded from plots from which high levels of early-season virus-induced mortality was record. Apparently, population levels after a highly successful virus application were reduced to a point too low to sustain an epizootic later in the season. Previous work has established that Gypchek can provide effective control when well-applied against appropriate population structures of the gypsy moth. Since Gypchek is active only against gypsy moth, it should be a material of choice, if effective, when nontarget effects are of concern. It has not been established that Gypchek is of value against trace (barely detectable) populations of gypsy moth, possibly because of the difficulty in measuring changes in such populations. The use of Gypchek is unlikely to kill all larvae present in a trace population, but it may reduce the population below the level needed to reproduce. The impact of the Gypchek application against trace populations should only be insecticidal, killing the insects in a density-independent (Knipling 1979) manner with no residual effects or spread. This is because such populations are too low to support the spread of the virus after application [such spread has been seen in higher populations (Webb et al. 1990)]. However, it is possible that enough larvae will survive in "hot spots" of gypsy moths somewhere in the treated area so that the baculovirus will be introduced as a permanent feature of the gypsy moth natural enemy complex ahead of its natural spread (as was reported by Webb et al. (1999b) for Gypchek application in areas of West Virginia found free of detectible virus prior to treatment).

In summary, this is the third in a series of studies on the aerial application of Gypchek mixed in Carrier 038. The first study (Webb et al. 1999a) was an experimental examination of treatment options such as single versus double application and reduced versus standard treatment volume. The results suggested that the one-application option, while suffering a modest decrease in effectiveness compared with a split-dose double application, nonetheless had attractive cost and programmatic considerations. The second study (Webb et al. 1999b) evaluated a pilot test that was required before the USDA Forest Service would support the one-application option for operational use. The results of this study suggested that the one-application option, when successfully implemented, would cause viral infections in about two-thirds of the resident larvae, but that extreme conditions (in this case, rain soon after application) should be avoided. This third study evaluates the use of the one-application option as part of a state operational program, the State of Wisconsin Gypsy Moth Control Program. Since Wisconsin lies on or near the leading edge of the expanding gypsy moth population, much of the control effort consists of negatively impacting trace populations. The use of Gypchek against trace populations required new evaluation technology. Our analysis of program efficacy used findings from studies 1 and 2 to explain results of study 3 and integrates the findings from all three studies to suggest future improvements. These studies have implications for all federal, state, local, and private users of Gypchek.

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