BULLETIN 807 SEPTEMBER 1982

Aerial spray tests with *Bacillus thuringiensis* for control of the gypsy moth in Connecticut

By T.G. Andreadis, N.R. Dubois, R.M. Weseloh,
R.E.B. Moore, J.F. Anderson, and F.B. Lewis

THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION NEW HAVEN

ABSTRACT

Two experimental strains of *Bacillus thuringiensis* Berliner, HD-243 and HD-263, and the commercial strain, HD-1 all formulated as Dipel 4L® were evaluated against natural infestations of *Lymantria dispar* (L.), in aerial spray trials. Two weekly applications of HD-1 at 8 BIU/0.4 ha, or a dry weight equivalent for the experimental strains, gave significant reductions in larval density and good foliage protection. HD-243 and HD-263, which had previously been identified as more potent against gypsy moth larvae in laboratory bioassays, were as effective but no better than HD-1 in the field. One application of HD-1 also was effective in reducing larval populations and protecting foliage but did not protect foliage as well as two applications of the same strain. Dry weather throughout the operation and optimal timing of applications against highly susceptible 1st-3rd instars contributed significantly to the performance of these materials. Substantial reductions in posttreatment egg mass density, ranging from 84.7-95.1% were observed in all treated as well as untreated plots. Egg mass reductions in untreated populations were attributed to an epizootic of nuclear polyhedrosis virus which reduced larval densities to the levels observed in treated plots just prior to pupation but after significant defoliation had already occurred.

Aerial spray tests with *Bacillus thuringiensis* for control of the gypsy moth in Connecticut

By T.G. Andreadis,[1] N.R. Dubois,[2] R.M. Weseloh,[1]
R.E.B. Moore,[1] J.F. Anderson,[1] and F.B. Lewis[2]

The gypsy moth, *Lymantria dispar* (L.), remains one of the most important forest defoliating insects in the northeastern United States. In 1980, it defoliated a record 2.1 million ha. of hardwood forest at an estimated cost of several million dollars (Anon. 1981). Aerial application of insecticides is the only practical means of protecting large acreages of forested land from extensive defoliation. However, public concern over the use of broad-spectrum chemicals and stringent state regulations, which prohibit aerial application of chemical pesticides to forests in Connecticut, have necessitated a closer investigation and evaluation of alternative control agents.

Commercial formulations of the bacterial insecticide, *Bacillus thuringiensis* Berliner, have been used for gypsy moth control since 1961 (see Dubois 1981a, Dubois and Lewis 1981 for reviews). Although their performance has been erratic, ground tests (Yendol et al. 1973) and aerial tests (Dunbar et al. 1973, Kaya et al. 1974, Lewis et al. 1974, Wollam and Yendol 1976) with new strains and improved formulations have shown that significant foliage protection and some population reduction could be achieved with multiple applications. However, due to the staggered egg eclosion and development of the gypsy moth and the moderate susceptibility of larvae to the bacterium (Dubois and Lewis 1981), formulations with improved residual properties and more potent strains were still needed to achieve consistently effective results.

A nonaqueous emulsifiable suspension of *B. thuringiensis* claimed to have improved residual and weatherability properties, called Dipel 4L®, has been developed by Abbott Laboratories. It has been shown to be highly effective in agricultural application but has not been extensively tested in the forest. Additionally, two new strains of *B. thuringiensis*, HD-243 and HD-263, have been identified from laboratory bioassays (Dubois 1981b) as potentially more potent against the gypsy moth than the present commercial strain, HD-1.

Our objective was to compare the effectiveness of these two experimental and one commercially available strains of *B. thuringiensis* when applied aerially against natural gypsy moth infestations in Connecticut.

Materials and Methods

Study area and plot design

The study area was located in a gypsy moth-infested, mixed hardwood forest in Harwinton, Conn. Twelve treatment and three control plots were established. Each measured 16.2 ha. and was at least 400 m from another. Ten observation points, from which data were collected, were established within each plot; these were 110 m from the plot edge and 61 m from each other.

Treatments were assigned in a complete randomized block with three replicates. Pretreatment gypsy

[1]Department of Entomology, The Connecticut Agricultural Experiment Station, P.O. Box 1106, New Haven, CT 06504-1104.

[2]Forest Insect and Disease Laboratory, Northeastern Forest Experiment Station, USDA-Forest Service, Hamden, CT 06514.

moth egg mass density was the criterion for block design.

Test materials and application

The strains of *B. thuringiensis* tested were: HD-1, HD-243, and HD-263. All were prepared in a 4L® formulation (Abbott Laboratories, North Chicago, Ill). The commercial strain HD-1, was formulated at 8.8×10^3 IU of potency/mg, as determined by the manufacturer, and applied at a rate of 8 BIU/0.4 ha. in 3.8 liters of finished spray containing 3% (vol/vol) Acrylocoat® sticker (Rohm and Haas, Philadelphia, PA). Experimental strains, HD-243 (ABG-6118) and HD-263 (ABG-6117) were formulated in dry weight equivalents and applied at the same volumetric rate as HD-1.

Applications were made with a Bell 47-G-2A, 260 Hp, helicopter equipped with a standard 10.4 m boom with hollow cone nozzles calibrated to deliver droplets ranging from 75-400 mmd at a rate of 3.8 liters/0.4 ha. $^{+}$ 2%. Two applications of each of the three strains of *B. thuringiensis* and a single application of HD-1 were evaluated. The first spray was applied on May 21, 1981, when most gypsy moth larvae were in the 1st and 2nd stages and when white oak, *Quercus alba* L., leaf expansion was 25-35%. The second spray was applied on May 28 when 2nd and 3rd instars were present in equal numbers.

To verify the potency of each strain, formulation samples were laboratory bioassayed against 2nd instar gypsy moths, and tank mix samples were submitted to Dr. C.C. Beegle, Cotton Insects Research Laboratory, USDA, Brownsville, Tex., for independent bioassays against *Trichoplusia ni* (Hubner).

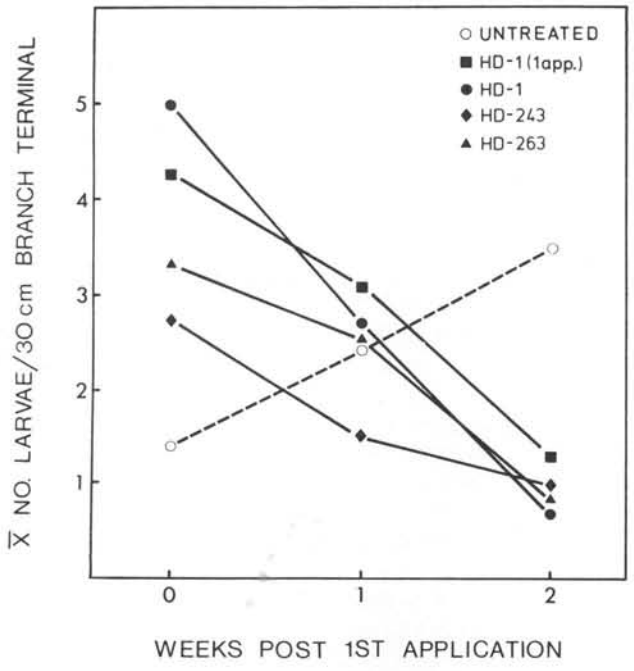

Fig. 1. Gypsy moth larval population densities as determined by 30 cm branch terminal counts following aerial application of various strains of *Bacillus thuringiensis*.

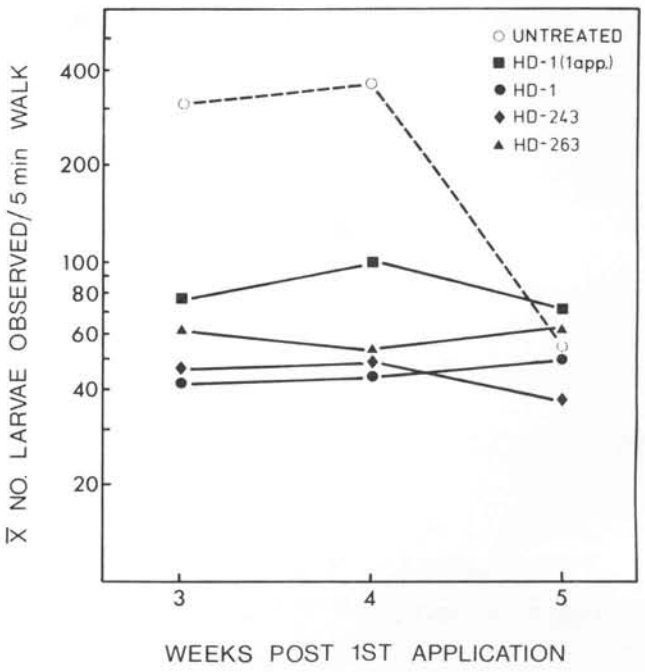

Fig. 2. Gypsy larval population densities as determined by 5 min larval counts following aerial application of various strains of *Bacillus thuringiensis*.

Evaluation

Criteria for evaluating treatment effectiveness were: egg mass density, larval density, and defoliation.

Pretreatment egg mass counts were made prior to egg eclosion at 10 observation points within each plot. Estimates of egg mass density were determined by using the fixed and variable radius sampling technique of Wilson and Fontaine (1978). Sample overstory trees at each observation point were selected with a 20 factor wedge prism. Posttreatment egg mass counts were made on the same trees in November 1981, following leaf fall. The net egg mass change was used to assess treatment effects on the gypsy moth population.

Weekly larval counts measured the initial and residual treatment effects on the populations. Beginning on the day of the first application, the number of living larvae present on two, 30-cm branch terminals of a preferred host plant at each of the 10 observation points in each plot was recorded. This procedure was followed for 3 weeks until the majority of larvae were in the 3rd instar, when three, 5-min larval counts (Connola et al. 1965) were begun in the central 180 x 120 m area of each plot and were continued for an additional 3 weeks until pupation occurred.

Defoliation estimates were obtained in each plot from each overstory tree previously selected in the egg mass count ($\bar{X}$ trees/plot = 33 ± 5 SD). Pretreatment estimates were made on the day of the first application and posttreatment estimates at the cessation of larval feeding (July 1).

Results

Weather conditions were favorable on both application dates: foliage was dry, skies were overcast and windspeed did not exceed 6.5 km/h. Precipitation readings at a nearby weather station in Burlington, Conn. showed no rainfall for the period between May 21 and 28; rainfall of 10.2 and 4.6 mm was recorded on May 29 and 30, respectively.

Counts of gypsy moth larvae on branch terminals prior to treatment with *B. thuringiensis* showed populations among plots were variable but not significantly different (Fig. 1). One week after the first application, larval densities in all treated plots declined while those in untreated plots increased. By the second week, significantly fewer larvae were counted in all treated plots when compared to untreated plots where larval densities continued to increase markedly. Differences in larval densities between treated and untreated plots were detected in the 5-min counts (Fig. 2) for two additional weeks, and although numbers were slightly higher in those plots receiving only one application of HD-1, no significant differences in larval density were observed among the treatments. Just prior to pupation, however, substantial larval mortality was observed in all untreated plots where an average of 64% of all larvae observed on week 5 exhibited symptoms of infection with a nucleopolyhedrosis virus (NPV). As a result of this natural epizootic, larval densities in untreated plots were greatly reduced and equivalent to those in treated plots on that date. Some virus-induced larval mortality also was observed in the *B. thuringiensis*-treated plots, but much less (HD-1, 38%; HD-243, 16%, HD-263, 8%; HD-1 (1 application), 18%).

Table 1. Estimated tree defoliation caused by the gypsy moth in untreated and *Bacillus thuringiensis*-treated 16.2 ha. plots in Harwinton, Conn.

Treatment	No. applications	x̄ % Defoliation[1,2] Pretreatment	Final	Net
HD-1	2	6.3a	14.8a	8.5a
HD-243	2	4.5a	24.0ab	19.5ab
HD-263	2	11.2a	30.5ab	19.3ab
HD-1	1	5.6a	36.6 b	31.0 b
Untreated	-	7.6a	61.0 c	53.4 c

[1]Includes all tree species.

[2]Means within colums followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test. Percentages transformed to arcsin $\sqrt{\%}$ for analysis.

Pretreatment defoliation was generally 10% or less and was similar in all plots (Table 1). Significant foliage protection was achieved with all treatments and there was a significant positive linear correlation ($r = 0.88$, $p<0.01$) between larval population densities on week 4 and the degree of defoliation in each plot. Although no significant differences were observed with any of the three strains which were applied twice, less defoliation occurred in plots treated with HD-1. Two applications of HD-1 also gave significantly better foliage protection than one application, but the level of defoliation with one application of HD-1 was not significantly greater than that observed with two applications of HD-243 or HD-263.

Table 2. Estimated gypsy moth egg mass density before and after aerial application of various strains of *Bacillus thuringiensis*

Treatment	No. applications	x̄ No. Egg Masses/ha Pretreatment	Post-treatment	% Egg mass reduction[1]
HD-1	2	24,868	1,663	93.3a
HD-243	2	33,695	1,658	95.1a
HD-263	2	27,574	3,452	87.5a
HD-1	1	26,400	4,045	84.7a
Untreated	-	27,673	2,083	92.5a

[1]Numbers followed by the same letter are not significantly different ($p<0.05$) according to Duncan's multiple range test. Percentages transformed to arcsin $\sqrt{\%}$ for analysis.

Substantial reductions in egg mass density, ranging from 84.7-95.1%, were observed in all plots. (Table 2). There was no correlation between these reductions in egg mass density and observed reductions in larval density or the degree of defoliation.

Table 3. Laboratory bioassay against 2nd instar gypsy moths of the 4L formulations of HD-1, HD-243 (ABG-6118) and HD-263 (ABG-6117) with 3% Acrylocoat[1].

	Treatment Strain HD-1	HD-243	HD-263
LC_{50} (x10²) IU/ml[2]	6.6	9.6	6.0
± 95% range	5.0-8.8	6.6-13.9	4.4-8.3
Slope	1.92	1.77	3.16
Ratio[3]	1	1.45	0.91

[1]For comparative purposes HD-243 and HD-263 were assigned the same potency (8.8 x 10³ IU/mg) as HD-1 since all three formulations were prepared on the same weight basis.

[2]The bioassay consisted of six doses with 50 larvae/dose. Mortality was recorded after 5 days. LC_{50} estimates were made using the procedure described by Litchfield and Wilcoxon (1949).

[3]$\frac{LC_{50}\ \text{Test Strain}}{LC_{50}\ \text{HD-1}}$

Laboratory bioassays of the formulation samples against 2nd instar gypsy moths (Table 3) showed a decrease in potency for the HD-243 formulation; but due to the variability in the bioassays, all three strains were considered equally potent. Independent bioassays against *T. ni* with samples from the spray tank also showed a loss of potency for the HD-243 formulation, whose average potency was 3.7 BIU/3.8 liters as compared to 6.1 and 10.8 BIU/3.8 liters for HD-1 and HD-263, respectively. (The original powder preparations of these strains using *T. ni* indicated that HD-243, with a potency of 43,300 IU/mg, was slightly more potent than either HD-1 or HD-263 which were 39,000 and 39,600 IU/mg respectively (H.T. Dulmage, personal communication).

Discussion

Significant reductions in larval density and good foliage protection were achieved with each strain of *B. thuringiensis* tested. The experimental strains, HD-243 and HD-263, which were more potent against the gypsy moth in laboratory bioassays (Dubois 1981b) performed equally well in the field but no better than the commercial strain, HD-1. This apparent change in potency may have occurred during fermentation or formulation of these strains into the commercial prototype. Our laboratory bioassays against gypsy moth larvae with the formulated products showed no differences in potency between HD-1 and HD-243 or HD-263. These findings were also supported with standard potency determinations against *T. ni*. Improved fermentation and formulation of HD-243 and HD-263 are apparently still needed.

One application of HD-1 was also effective in reducing larval populations and protecting foliage. It did not, however, protect foliage as well as two applications of the same strain.

The following two factors contributed significantly to the foliage protection achieved in this experiment: optimal timing of applications in terms of larval development and dry weather. The initial spray was applied against highly susceptible 1st and 2nd instars before much defoliation had occurred. Furthermore, at the time of the second application, larvae had not yet exceeded the 3rd instar and were still susceptible at our application rate of 8 BIU/0.4.ha. Dry weather prevailed throughout: there was no rainfall during the week between the first and second application, and light rain occurred 1 day following the 2nd application.

The importance of dry weather and early application of *B. thuringiensis* against young instar gypsy moths have been emphasized in other studies (Yendol et al. 1973, Lewis et al. 1974), and it would appear that the lack of foliage protection and inconsistency in some field trials have in large part been caused by operational problems and adverse weather which prevented application at the proper time or inclement weather following application that diminished the effects of the bacterium. Yendol et al. (1973) felt initial application at 50% leaf expansion of oaks may have been too late to allow for infection of early-hatched larvae and that better control might have been achieved if the first application was made when leaf expansion was 30-40%, followed by a second application within 7-10 days. Kaya et al. (1974) concluded that some foliage protection could be achieved with aerial application of *B. thuringiensis*, but pretreatment defoliation in that study was nearly 40% before the application was made. Lewis et al. (1974) attributed variable results in New York and New Jersey to wet weather that not only delayed application but also diminished the effects of the sprays. In contrast, good control was achieved in a simultaneous trial with the same materials in Pennsylvania, where virtually no rain fell during the operation.

Because of the mortality attributed to an epizootic of NPV in untreated populations in our experiment, it was not possible to assess the suppressive effects of *B. thuringiensis*-treatment on the subsequent gypsy moth population. This mortality, which occured just prior to pupation but after significant defoliation had occurred, was reflected in the number of residual egg masses, which was similar in both treated and untreated plots. A similar decrease in egg mass density regardless of treatment was observed by Kaya et al. (1974), who also conducted their spray tests against a gypsy moth population that was beginning to collapse. Conversely, Dunbar et al. (1973), working with a building population, reported that egg masses increased markedly in both treated and untreated plots. Even in those studies where some population reduction has been achieved (Yendol et al. 1973, Wollam and Yendol 1976), the residual egg masses in the *B. thuringiensis*-treated plots have been sufficiently abundant to cause significant defoliation the following year. These findings suggest that while substantial larval reduction and acceptable foliage protection can be achieved with *B. thuringiensis*, this microbial insecticide may be of limited use as a population suppressant, and that increases or decreases in subsequent gypsy moth generations will occur independent of treatment.

On the other hand, *B. thuringiensis* has been shown to substantially increase parasitism of gypsy moth larvae by *Apanteles melanoscelus* (Ratzeburg) (Hymenoptera: Braconidae) in the field (Dunbar et al. 1973, Kaya et al. 1974). Gypsy moth larvae that ingest a sublethal dose of *B. thuringiensis* are delayed in development, and *A. melanoscelus* attacks the small larvae more successfully than large ones (Weseloh and Andreadis 1982, Weseloh et al. 1982). The phenomenon has implications for the effectiveness of *B. thuringiensis*, and more study of it is desirable. Further studies with *B. thuringiensis* are also needed in building gypsy moth populations to evaluate its potential in preventing these populations from attaining the levels which cause severe defoliation.

Our aerial tests show that significant larval reduction and good foliage protection can be achieved with two applications of *B. thuringiensis* when applied against early instars in dry weather. Unfortunately, two applications with these time and weather limitations make treatment of large acreages both difficult and expensive. Therefore, the development of strains or formulations of higher potency which would provide adequate control with one application are still desirable to make *B. thuringiensis* more economical for practical gypsy moth control in the Northeast.

References Cited

Anonymous. 1981. Why fight the gypsy moth? USDA IBP 24 revised). 4 pp.

Connola, D.P., F.B. Lewis, and J.L. McDonough. 1965. Experimental field techniques used to evaluate gypsy moth, *Porthetria dispar*, control in New York. *J. Econ. Entomol.* 59:284-287.

Dubois, N.R. 1981a. *Bacillus thuringiensis*. pp. 445-453. *In* C.C. Doane (ed.) The gypsy moth: Research Toward Integrated Pest Management. USDA Tech. Bull. 1584. 757 pp.

Dubois, N.R. 1981b. Spectrum of *Bacillus thuringiensis* against *Lymantria dispar* L. *In* H.T. Dulmage, L.C. Lewis, and H.D. Burges (eds.) Spectrum of activity of *Bacillus thuringiensis*. USDA SEA Bull. (in press).

Dubois, N.R., and F.B. Lewis. 1981. What is *Bacillus thuringiensis*? J. Arboriculture 7:23-40.

Dunbar, D.M., H.K. Kaya, C.C. Doane, J.F. Anderson, and R.M. Weseloh. 1973. Aerial application of *Bacillus thuringiensis* against larvae of the elm spanworm and gypsy moth and effects of parasitoids of the gypsy moth. Conn. Agric. Exp. Stn. Bull. 735. 23pp.

Kaya, H.D., Dunbar, C. Doane, R. Weseloh, and J. Anderson. 1974. Gypsy moth aerial tests with *Bacillus thuringiensis* and pyrethroids. Conn. Agric. Exp. Stn. Bull. 744. 22pp.

Lewis, F.B., N.R. Dubois, D. Grimble, W. Metterhouse, and J. Quimby. 1974. Gypsy moth: Efficacy of aerially-applied *Bacillus thuringiensis*. J. Econ. Entomol. 67:351-354.

Litchfield, J.R., and F. Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. and Exp. Therap. 96:99-113.

Weseloh, R.M., and T.G. Andreadis. 1982. A possible mechanism for synergism between *Bacillus thuringiensis* and the gypsy moth parasitoid, *Apanteles melanoscelus*. Ann. Entomol. Soc. Am., 75:435-438.

Weseloh, R.M., T.G. Andreadis, N.R. Dubois, R.E.B. Moore, J.F. Anderson, and F.B. Lewis. 1982. Field confirmation of a mechanism causing synergism between *Bacillus thuringiensis* and the gypsy moth parasitoid, *Apanteles melanoscelus*. J. Invertebr. Pathol., (in press).

Wilson, R.W., Jr., and G.A. Fontaine. 1978. Gypsy moth egg-mass sampling with fixed and variable-radius plots. USDA Agric. Handb. 523. 46 pp.

Wollam, J.D., and W.G. Yendol. 1976. Evaluation of *Bacillus thuringiensis* and a parasitoid for suppression of the gypsy moth. J. Econ. Entomol. 69:113-118.

Yendol, W.G., R.A. Hamlen, and F.B. Lewis. 1973. Evaluation of *Bacillus thuringiensis* for gypsy moth suppression. J. Econ. Entomol. 66:183-186.

Acknowledgment

We express our appreciation to the following individuals who assisted in various aspects of this project. X. Asbridge, D. Brown, J. Miner, A. Ross, S. Sandrey and P. Trenchard of The Connecticut Agricultural Experiment Station; H. Hubbard, P. Huntley, D. Newman, and W. Rollinson of the USDA Forest Service; R. Garrepy, W. Kearns, J. Pronovost, L. Rousseau, D. Smith, and F. Trafidlo of the Connecticut Department of Environmental Protection; and B. Bellew of the Bristol Water Company.

We also thank the Town of Harwinton, Conn.; Drs. H.T. Dulmage and C.C. Beegle of the Cotton Insect Research Laboratory, USDA, Brownsville, Tex., for the bioassay data on *T. ni*; and pilot C. Voss, Ag-Rotors Inc., Gettysburg, Penn.