

Evaluation of the Influence of Droplet Size and Density of *Bacillus thuringiensis* Against Gypsy Moth Larvae (Lepidoptera: Lymantriidae)

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ABSTRACT A study was conducted to determine the effect of spray droplet size and density on the efficacy of a commercial preparation of *Bacillus thuringiensis* against the gypsy moth (*Lymantria dispar* L.). A concentration of 5,795 International Units (IU)/ μ l was used. In the laboratory, various size droplets and densities were tested against larvae using 12-cm² red oak leaflets. Results show that producing relatively high densities of small (50–150 μ m) droplets of *B. thuringiensis* will increase the efficacy compared with larger droplets (>150 μ m) present at low densities at the same dose. Median lethal dose estimates are made for droplets in three size classes (50–150, 150–250, and 250–350 μ m). LD₅₀'s for the generalized size classes of 100, 200, and 300 μ m were 10.8, 2.2, and 0.9 drops/cm², respectively. Implications of these results are discussed.

KEY WORDS Insecta, *Lymantria dispar*, *Bacillus thuringiensis*, lethal dose

THE BACTERIAL-pesticide *Bacillus thuringiensis* has been used for insect control because of its environmental safety and specificity to most of the target pests. Because *B. thuringiensis* must be ingested to be effective, coverage of the food source plays an important role in its effectiveness. With some *B. thuringiensis* applications used in the suppression of the gypsy moth, *Lymantria dispar* L., inconsistent results have occurred with little reduction in posttreatment egg masses (Yendol et al. 1973, Wollam & Yendol 1976). Other applications have provided good foliage protection and population reduction (Andreadis et al. 1982, Andreadis et al. 1983). Consequently, some forest managers have questioned the effectiveness of *B. thuringiensis* as a tool to reduce populations of this serious forest pest.

Inadequate spray coverage of the host tree canopy could be an important cause for such inconsistent results. The transport to and impingement on the target oak foliage by the toxic spray droplet is one of the least understood and studied phenomena. Our preliminary observations suggest that once impacted on the leaf surface, droplet density and sizes may interact with the foraging and specific feeding characteristics of gypsy moth larvae. This may result in changes in mortality due to the droplet density and size characteristics of the dose. Smith et al. (1977) demonstrated correlations between the application parameters droplet size, droplet density, and spray concentration with insect mortality using *B. thuringiensis* against the cabbage looper, *Trichoplusia ni* (Hübner), and the bollworm, *Heliothis zea* (Boddie). Munthali (1984) also demonstrated the effect of changing the droplet size and concentration of dicofol against *Tetranychus urticae* (Koch). A series of optimum droplet size

and concentrations were found which maximized mortality.

Some research has been done to demonstrate the level of foliage deposit received from aerial application into hardwood forests. Using a fluorescent tracer method and applying *B. thuringiensis* at the rate of 16 billion International Units (IU)/acre, Dunbar et al. (1973) found *B. thuringiensis* residues ranging from 30–43 IU/cm² of leaf surface. Droplet density and size on the leaf surface was not determined. Maksymiuk & Orchard (1975) showed droplet densities of 2.8–4.2 per cm² measured from hardwood foliage after aerial application at 2 gpa and a volume median diameter (vmd) of 360 μ m. Andreadis et al. (1983) reported droplet densities of 5–6 per cm² and a droplet size range of 100–400 μ m vmd. However, lethal doses for each instar and optimal droplet sizes and densities are unknown.

Our study was done to determine the effect of spray parameters such as droplet size and density on the efficacy of *B. thuringiensis* against gypsy moth larvae under laboratory conditions. We attempted to use application rates and droplet sizes and densities that provided doses and droplet characteristics typically found when *B. thuringiensis* sprays have been applied to hardwood forests. The results of these experiments will assist forest pest managers in estimating the amount of spray deposit and optimum form of that deposit needed on oak foliage to reduce damaging gypsy moth populations.

Materials and Methods

Larval Rearing. Larvae were reared from egg masses of the New Jersey strain (USDA, Animal

Table 1. Summary of mean target droplet sizes and quantity of each applied to a single leaflet with the respective equivalent calculated dose (IU/droplet) for each mean droplet treatment

| Mean larval wt (g) | Determined \bar{x} droplet size (\pm SE) (μ m) | No. droplets applied per leaflet | Dose IU applied for respective droplet no. per leaflet |
|--------------------|--|----------------------------------|--|
| 31.1 | 87.2 (15.1) | 5, 10, 20, 40 | 33, 21, 17, 34 |
| 28.8 | 98.5 (—) | 20, 40, 60, 80 | 58, 116, 174, 232 |
| 25.2 | 101.3 (11.6) | 5, 10, 20, 40 | 9, 15, 103, 226 |
| 27.1 | 108.7 (2.7) | 5, 10, 20, 40 | 22, 43, 62, 159 |
| 25.2 | 108.8 (2.2) | 5, 10, 20, 40 | 21, 43, 74, 136 |
| 31.7 | 126.3 (2.3) | 10, 20, 40, 60 | 61, 133, 260, 420 |
| 22.8 | 189.2 (3.9) | 3, 6, 12, 24 | 50, 101, 268, 673 |
| 31.5 | 204.0 (5.4) | 6, 12, 18, 24 | 159, 302, 564, 510 |
| 25.2 | 209.3 (3.1) | 1, 2, 4, 8 | 26, 51, 123, 233 |
| 26.1 | 221.4 (5.0) | 2, 4, 8, 16 | 55, 154, 265, 525 |
| 22.8 | 244.7 (11.2) | 3, 6, 12, 24 | 195, 259, 472, 848 |
| 31.8 | 268.3 (8.0) | 2, 4, 6, 8 | 146, 190, 351, 457 |
| 30.4 | 281.3 (1.6) | 1, 2, 4, 8 | 65, 132, 278, 556 |
| 29.7 | 287.3 (4.0) | 1, 2, 4, 8 | 81, 144, 280, 529 |
| 26.1 | 311.8 (5.3) | 1, 2, 4, 8 | 80, 181, 394, 804 |

and Plant Health Inspection Service Laboratory, Otis, Mass). Eggs were stored at 8°C until needed, then incubated at 27°C for hatching. At eclosion, larvae were transferred individually to a container with artificial diet (Bioserv Inc., Frenchtown, N.J.) and reared at 27°C and a photoperiod of 16:8 (L:D) until the second instar (25–30 mg). Each treatment was replicated with 20 larvae.

Treatment Leaf Substrate. The *B. thuringiensis* treatments were applied to leaflets cut from greenhouse-propagated red oak (*Quercus rubra* L.) about 30–35 d old. Each leaflet was cut in a rectangle (3 by 4 cm), leaving the petiole attached. Droplets were dispensed randomly over the leaf surface. The petioles of treated leaves were then placed in a water-filled glass vial (15 by 45 mm) and fitted with a rubber cap seal to maintain leaf turgidity. The leaf and a single larva were placed in a 475-ml waxed paper container with lid and incubated at 27°C. A moist cotton plug was positioned in the container to maintain a high relative humidity. When the leaf was totally consumed, fresh untreated material was introduced.

Droplet Application. Droplets were administered to substrates by a low speed flicking needle apparatus (Wolf 1961). This experimental technique allowed doses of a commercial *B. thuringiensis* formulation to be applied to leaves in controlled droplet sizes and numbers. Droplets could be produced at a rate of approximately one per second. Droplet diameters measured from magnesium oxide craters (May 1950) with an optical micrometer produced a coefficient of variation of about 5%. Droplets were visualized on the leaf with a fluorescent tracer (1 g/liter Brilliant sulphaflavine [BSF], Aldrich Chemical Co.). Long-wavelength (420 nm) ultra-violet (UV) illumination permitted droplets to be counted. Preliminary bioassay trials using *B. thuringiensis* solutions with and without BSF on diet medium (50 insects per treatment)

showed significant overlap of the LD₅₀'s of 95% fiducial limits after 5–9 d.

We attempted to evaluate the International Standard of *B. thuringiensis* HD-1-1980 (16,000 IU/mg) at various concentrations with the droplet dispenser, but droplets could not be obtained because of the physical properties of the suspensions. Therefore, the commercial water-based formulation of *B. thuringiensis* used in these trials was Thuricide 64LV (Zoecon Corporation, Palo Alto, Calif.). Bioassay of Thuricide 64LV against the HD-1-1980 standard *B. thuringiensis* strain (Dulmage 1973) showed a relative toxicity of 12,870 IU/mg.

A standard aqueous suspension of 5,795 IU/ μ l was prepared for the droplet trials. This concentration was comparable with typical field operational concentrations of 4,227 IU/ μ l or 6,340 IU/ μ l used for 7 liters/ha (96 fl oz/acre) or 4.7 liters/ha (64 fl oz/acre), respectively, at 29.6 BIU/ha (12 BIU/acre).

Droplet Size and Dosage Applied. For each experiment, a droplet size within the range of sizes normally found in aerially applied sprays (that is, 50–350 μ m [Yates et al. 1982]) was used. In total, each of 15 sizes was tested in a separate experiment. At each size, four doses were obtained by varying the number of droplets applied to the leaflet. Each of these four doses was applied to 20 replicates. Total time to complete treatments was 3–4 h.

Dose was calculated as $D = C \times \pi \times N \times d^3 \times 10^{-9}/6$, where C is concentration of *B. thuringiensis* in the spray solution (5,795 IU/ μ l), N is number of droplets applied per leaflet, d is mean droplet diameter (μ m), and $10^{-9}/6$ is a conversion factor.

The mean droplet diameter (d) was estimated for each dose level. A total of 60 droplets was collected on magnesium oxide slides just before and immediately after application to the 20 leaflets that constituted a dose level. This provided a total of four estimates of droplet size per experiment from the four dose levels within each experiment.

Table 1 lists treatments used for the 15 experiments. The droplet size is presented as an average (\pm SE) of the four droplet-size means within the experiment. The standard error is an expression of the change in droplet size between dose levels. While this change in diameter is small, its effect on volume and hence dose applied can be large, as seen in the 87.2 μ m experiment (Table 1, line 1). In this example, the droplet number per leaflet is increasing but the dose remains relatively constant because of a change in the mean droplet diameter between doses. However, these differences were accounted for by using individual droplet size estimates for each treatment in the calculation of dose (IU per leaflet).

The number of droplets applied was chosen to provide a range of mortalities. These values were also within the range of droplet densities typically found following aerial application at 7 liters/ha

(96 fl oz/acre) used in aerial treatment of hardwood forest.

In all cases, a series of 20 control insects was handled in an identical manner, except that *B. thuringiensis* was not applied to the leaves. Mortality in the controls was very low. Treatment mortalities were corrected with Abbott's (1925) formula when necessary. In this group, the untreated leaf was consumed within the first 2–4 d and had to be replaced with clean foliage as needed. However, consumption rate was reduced in the treatment groups, and less frequent replacement of the foliage was required. This is the typical response caused by the antifeedant properties of some component of a *B. thuringiensis* spray. Similar effects have been noted by Yendol et al. (1975), working with gypsy moth, and by Ignoffo et al. (1968), working with the cabbage looper, *T. ni*.

Mortality was transformed to logits and dose was transformed to logarithms for analysis. Droplet size was represented by allocating each of the magnesium oxide determined droplet sizes (Table 1) to one of three arbitrary size classes: 50–150, 150–250, and 250–350 μm . These three size classes were first compared using a first-order linear model with an interaction term and a weighted regression (Neter et al. 1985). The full model was

$$Y_i = \beta_0 + \beta_1 X_{1i} + \beta_2 X_{2i} + \beta_3 X_{3i} + \beta_4 X_{1i} X_{2i} + \beta_5 X_{1i} X_{3i} + \epsilon_i$$

where Y_i is logit mortality, X_{1i} is the logarithm of dose, and X_{2i} and X_{3i} are indicator variables such that $X_{2i} = 1$ for the 150–250 size class and $X_{3i} = 1$ for the 250–350 size class; otherwise X_{2i} and X_{3i} are 0. PROC GLM (SAS Institute 1979) was used to test the hypothesis that changes in the droplet size and density result in a change in efficacy. Significance of the regression coefficients was tested with the t test.

For practical purposes, a threshold deposit against which field applications can be judged is needed. These data were therefore used to provide estimated lethal dose thresholds required from a given droplet sized application. Lethal dose estimates were obtained by PROC PROBIT (SAS Institute 1979) for each experiment mean droplet size. These dose estimates represent the dose required on a 12-cm² area per leaflet. The lethal dose estimates were then regressed against experimental mean droplet size using a linear model ($Y_i = \beta_0 + \beta_1 X_i + \epsilon_i$) where Y_i is the lethal dose estimate and X_i is the experiment mean droplet size (Neter et al. 1985). Unbiased point estimators of lethal doses were made for sprays applied as droplets of 100, 200, and 300 μm from these regressions. The droplet density equivalent to these estimated lethal doses were calculated using the following equation: average droplet density (number per square centimeter) = $D/(C \times V \times A)$, where D is the estimated lethal dose (IU), C is the concentration of *B. thuringiensis* in the spray solution (IU/ μl), V is the volume per

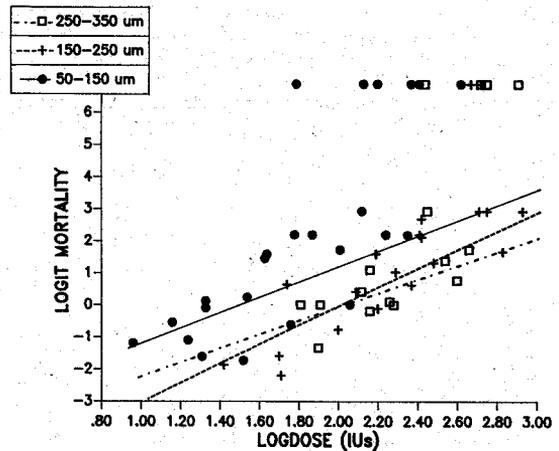


Fig. 1. Dose/mortality relationship of *Bacillus thuringiensis* with second-instar gypsy moth for doses applied at three different size classes. Droplet size classes: ●, 50–150 μm ; +, 150–250 μm ; □, 250–350 μm .

droplet (μl), and A is the area of the original target (cm^2). Confidence intervals were calculated using the Working-Hotelling confidence band for simultaneous estimates of a mean response (Neter et al. 1985).

Results and Discussion

The mortality from the 20 larvae treated at each dose level for the 15 experiments is shown in Fig. 1. The slopes of the three regression lines in Fig. 1 were not significantly different ($\beta_4 = \beta_5 = 0$). Also, the intercepts for the 150–250 and 50–150 μm classes were not significantly different ($\beta_2 = \beta_3$). Therefore, the 150–250 and the 250–350 classes were pooled and compared to the 250–350 μm class. This comparison showed strong evidence that the intercept for the 50–150 class was significantly higher than the two pooled classes ($t = 1.58$; $df = 1$; $P = 0.12$). Thus, for a given level of mortality, a lower dose will be needed if that dose is applied in droplets between 50 and 150 μm than if the same dose were presented in droplets of $>150 \mu\text{m}$.

The linear regression equations for the lethal dose and droplet size relationship were $LD_{50} = -1.241 + 0.461 \text{ size}$ ($n = 15$, SE slope = 0.162, 95 CL slope = 0.349) and $LD_{95} = 113.908 + 1.732 \text{ size}$ ($n = 15$, SE slope = 0.928, 95 CL slope = 2.005). Slopes significantly greater than 0 were found for the LD_{50} ($F = 8.139$; $df = 1, 13$; $P = 0.013$) and LD_{95} ($F = 3.481$; $df = 1, 13$; $P = 0.085$), suggesting increasing dose requirement with increase in droplet size. Similar results were found by Munthali & Scopes (1982) using dicofol against *T. urticae*. Table 2 shows point estimates for lethal dose taken from these regressions for droplet sizes of 100, 200, or 300 μm . Droplet densities (number per unit area) corresponding to the lethal dose estimates were also calculated. These droplet densities compared well with the droplet densities ac-

Table 2. Mean lethal dose estimates (IU) of *B. thuringiensis* applied in three generalized size classes, and the equivalent droplet density assuming a *B. thuringiensis* concentration of 4,227 IU/ μ l (12 BIU/acre at 96 fl oz)

| Droplet size (μ m) | LD ₅₀ (IUs) | Equivalent density (per cm ²) | LD ₅₀ (IUs) | Equivalent density (per cm ²) |
|-------------------------|--------------------------|---|----------------------------|---|
| 100 | 44.9 (44.6) ^a | 1.69 | 287.1 (256.0) ^a | 10.83 |
| 200 | 90.9 (29.1) | 0.43 | 460.3 (165.9) | 2.17 |
| 300 | 137.1 (50.7) | 0.19 | 633.5 (290.6) | 0.88 |

^a Numbers in parentheses are 90% confidence intervals.

tually found from field experimentation, namely 2.8 and 4.2 per cm² at 360 μ m (Maksymiuk & Orchard 1975) and 5–6 per cm² at 100–400 μ m vmd (Andreadis et al. 1983).

Typically *B. thuringiensis* has been applied to a hardwood forest at 29.6 BIU/ha (12 BIU/acre). If this dose were to be applied to a surface of 1 ha, it would result in a deposit level of 296.5 IU/cm². We have estimated that 1 ha of typical eastern oak forest contains about 5 ha of leaf area (silhouette) at 50% expansion. Consequently, if 29.6 BIU/ha were applied evenly to the upper leaf surface of all leaves in the canopy, the deposit would be one-fifth of 296.5 IU/cm², or 59.3 IU/cm². This is in reasonable accord with the measurements of 30–43 IU/cm² found by Dunbar et al. (1973). From Table 2, an LD₉₅ requirement will be 287.1, 460.3, and 633.5 IU per leaflet for the 100, 200, and 300 μ m classes, respectively. This dose per leaflet is equivalent to an average of 23.9, 38.4, and 52.8 IU/cm². Thus, the desired average dose per leaf falls in the range of the dose predicted under an ideal application of 59.3 IU/cm². However, under field application conditions, the distribution of deposit between leaves is likely to be very heterogeneous because of the overlapping and shadowing influence of neighboring leaves and the filtering effects of successive layers of foliage. Because of these canopy effects, much of the foliage may receive doses below that which is required to give high mortality levels.

The combination of application parameters (that is, droplet size and droplet density) that was the major factor causing these changes in lethal dose is not known. A decrease in droplet size would increase the droplet density per leaflet if the dose levels for each experiment were to be kept similar. In our experiments, droplet density ranged from 80 per leaflet for the small droplet size class and highest dose to only one droplet per leaflet for the large droplet and low dose. Changing droplet density on the leaflet would undoubtedly change the probability of the gypsy moth larvae encountering a droplet during feeding. A greater density on the leaf through the use of small droplets would mean a higher probable rate of droplet encounter and ingestion.

The effect of changes in droplet size and density has been demonstrated using conventional insecticides.

Fisher & Menzies (1976) showed a more rapid effect of carbaryl on larvae of the Oriental fruit moth, *Grapholitha molesta* (Busck), when applied at high droplet densities. Increased mortality of *Heliothis virescens* (F.) larvae was shown by Wofford et al. (1987), who used increased deposit density with permethrin in water, although droplet size was not thought to contribute to this effect. Alm et al. (1987) used bifenthrin with 120- μ m droplets and found a greater reduction of egg production from *T. urticae* than 200 μ m droplets. Field application of small droplets has also proved increased efficacy can be achieved. Mortality of *Anthonomus grandis* (Boheman) was increased by applying 100- and 140- μ m droplets compared with 200- and 300- μ m droplets of azinphosmethyl (Smith et al. 1977).

Theories explaining the increased efficacy of small droplets have been proposed based on the increased probability of physical contact between insect and deposit (Graham-Bryce 1977, Spillman 1980). Because *B. thuringiensis* requires oral ingestion and acts as a stomach poison, it is possible that the changes in contact rate caused by droplet size and density produce additional biological effects because of the different rates of introduction of *B. thuringiensis* into the midgut.

With spruce budworm, *Choristoneura fumiferana* (Clemens), Fast & Régnière (1984) showed that *B. thuringiensis*-induced mortality sharply increased with both length of exposure to the toxin and dose. The increased mortality was thought to be caused by a cumulative response of the insect through depletion of regenerative capacity of the midgut epithelium. A similar effect may have occurred in our trials, whereby the more frequent but lower dose of the small droplets produces a cumulative toxic impact.

Our results suggest a potential for optimizing efficacy by choice of application parameters. However, spray delivery under field conditions constitutes a more complex system. *B. thuringiensis* delivery from the nozzle to the insect is a two-phase system—first, delivering the spray to the leaf, and second, larval foraging and contact with and ingestion of the toxin. Our work has suggested that a droplet in the 50–150 μ m range would be beneficial in the second phase of application for increasing the efficacy from a given volume of spray. However, controlling deposits from droplets in the 50–150 μ m range can be difficult from an aircraft releasing at 50 ft above the canopy because of the risk of off-target drift and increased rates of evaporation. Therefore, before this droplet range can be generally recommended for gypsy moth control, extensive field testing should be required for its verification.

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