

Droplet Size and Density Effects of *Bacillus thuringiensis kurstaki* on Gypsy Moth (Lepidoptera: Lymantriidae) Larvae

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J. Econ. Entomol. 88(5): 1376-1379 (1995)

ABSTRACT Differences in droplet density and spectrum of pesticide deposited on foliage after spray were examined to determine differences in gypsy moth, *Lymantria dispar* L., larval mortality. The susceptibility of 2nd, 3rd, and 4th instars of the gypsy moth to undiluted *Bacillus thuringiensis kurstaki* was examined under laboratory conditions. Droplets of known size (100, 200, and 300 μm) and density (1, 5, and 10 drops/ cm^2) of *B. thuringiensis kurstaki* were sprayed onto leaf disks of oak foliage and fed to gypsy moth larvae. Parameters tested were time required for mortality, leaf area consumed, and changes in larval weight. The time to mortality increased as droplet density and size decreased and as larval size increased. Droplet densities of 5 and 10 drops/ cm^2 were effective (> 90% mortality) against 2nd and 3rd instars. Fourth instars had high mortality rates at 5 and 10 drops/ cm^2 at the 200 μm and 300 μm droplet sizes. Low droplet densities (1 drop/ cm^2) at 100 μm were ineffective in controlling 3rd and 4th instars. Surviving treated larvae had lower post treatment weights than control larvae and exhibited weight loss during the experiment. As droplet density increased, larvae consumed less foliage. Foliage consumed by surviving treated larvae was 2-6 times less than foliage consumed by untreated larvae.

KEY WORDS *Lymantria dispar*, *Bacillus thuringiensis kurstaki*, feeding study, droplet size, droplet density

THE USE OF commercial formulations of *Bacillus thuringiensis* to control gypsy moth, *Lymantria dispar* L., populations is widespread in the northeastern United States. Approximately half of the treated forested areas are aerially sprayed with commercial formulations of *B. thuringiensis*. Currently, *B. thuringiensis* manufacturers and United States Department of Agriculture Forest Service (Reardon 1991) recommend the use of undiluted formulations for gypsy moth control. A typical application is made undiluted at a rate of 60 billion international units (BIU)/ha, 4.7 liters/ha (24 BIU/acre, 0.5 gal/acre) using a 12.5-BIU/liter formulation. Canopy deposition studies of such applications have shown forest canopy droplet densities ranging from 1.3 to 6 drops/ cm^2 of foliage (Dubois et al. 1993). Droplet sizes during these applications commonly range in size from 28 to 640 μm with an approximate volume mean diameter ($D_{V0.5}$) of 180 μm (Bryant 1988). Although several field studies attempted to determine the optimal droplet size for *B. thuringiensis*, no clear conclusions were obtained. Laboratory feeding studies conducted by Bryant and Yendol (1989) showed that the LD_{95} of a diluted *B. thuringiensis* formulation (5.8 BIU/liter) decreased as droplet size decreased. Ratcliffe and Yendol (1993) showed LD_{95} values of 21.1 IU per larva using small diluted droplets of *B. thuringiensis* at high droplet densities against 3rd instars.

The current study was conducted to determine the toxicity of undiluted *B. thuringiensis* when applied to 2nd, 3rd, and 4th gypsy moth instars with droplet densities and size distributions approximating those of aerial applications using undiluted *B. thuringiensis* formulations.

Although 2nd and 3rd instars are predominant during most spraying operations, some 4th instars are usually present in most suppression projects. Fourth instars are also commonly found in eradication projects during which *B. thuringiensis* is applied several times per year. The droplet sizes (100, 200, and 300 μm) and average densities (1, 5, and 10/ cm^2) tested in this experiment reflect those found on the forest canopy after aerial application of undiluted *B. thuringiensis* in forests (Dubois et al. 1993). Typical bioassays of insect response incorporate *B. thuringiensis* or other pesticides into a media substrate or immerse leaf material into a dilute mixture of pesticide (Keating et al. 1989, Appel and Schultz 1994). This study used both plant tissue and actual sprayed undiluted *B. thuringiensis* droplets.

Materials and Methods

Gypsy moth larvae used in the experiment were reared from eggs received from the Animal, Plant Health Inspection Service (APHIS) Gypsy Moth

Rearing Facility at Otis, MA. Eggs were kept in a growth chamber at 25°C with a photoperiod of 16:8 (L:D) h until hatch, when larvae were placed on artificial media (Bioserv, Frenchtown, NJ) until they reached the desired growth stage.

Foliage for the experiment was harvested from midsize (10 m high) trees of white oak, *Quercus alba* L. Leaves were rinsed with deionized water and leaf disks of 2.0 cm² were cut using a number 12 cork borer. To obtain droplet deposit on leaves for the study, leaf disks were placed on clean paper in the target zone of the droplet generation apparatus.

The spraying apparatus consisted of a spinning disk atomizer driven by compressed air. A commercial formulation of *B. thuringiensis* (Foray 48B, Novo Nordisk, Danbury, CT) was supplied to the system by a syringe pump (Model 341B, Orion, Boston, MA) with a flow rate of 0.5 ml/min. A fluorescent tracer (Brilliant Sulfaflavine, Organic Dyestuffs, East Providence, RI) was added to the *B. thuringiensis* to aid in the counting of droplets on the leaf disk surface at a rate of 0.4% wt:vol. Different droplet sizes were obtained by adjusting the speed of the spinning disk by changing the flow rate of compressed air. A hand-held tachometer (Model MT-1, Sticht, New York, NY) was used to measure the speed of the spinning disk after the air flow was adjusted. Before the experiment, spinning disk speeds that generated droplets of ≈ 100 , 200, and 300 μm were calculated from a data set of 7 disk speeds regressed against actual droplet diameters. To accurately measure sizes, droplets at each speed were captured in silicone fluid (Accu-metric, Elizabethtown, KY), which enables the spherical shape of a droplet to be maintained, and measured under a microscope. A sample of 100 droplets from each of the 3 selected spinning disk speeds were measured by microscope to insure the proper droplet size was maintained. Actual droplet sizes for the droplet categories were (mean \pm SEM) 109.8 \pm 8.4 μm , 212.2 \pm 12.9 μm , and 311.7 \pm 10.6 μm .

A hand-held UV lamp (Lite-Mite, Stocker and Yale, Beverly, MA) was used to examine the number of droplets on each leaf disk. The actual droplet densities for the 3 density categories were (mean \pm SEM) 1.0 \pm 0, 5.05 \pm 0.87, and 10.2 \pm 1.24 drops/cm². Once the desired range of droplet numbers were obtained on a leaf disk, the disk was transferred with forceps to a feeding cell (Hopple Plastics, Florence, KY). One larvae was placed in each feeding cell with a treated leaf disk. To maintain high humidity inside the feeding cells, moist cotton balls were also placed in each feeding cell. The cell was then sealed with clear mylar film to help minimize moisture loss and placed in a growth chamber at 25°C with a photoperiod of 16:8 (L:D) h. Each treatment was replicated with 30 larvae. Treatments consisted of *B. thuringiensis* spray deposits with droplets of 100, 200, and 300 μm diameters at mean densities of 1, 5, and 10

Table 1. Percentage of mortality of *L. dispar* larvae when exposed for 144 h to different droplet densities and sizes of Novo Foray 48B

Droplet size, μm	Drops/ cm ²	Instar		
		2nd	3rd	4th
100	1	56	43	23
	5	100	80	47
	10	100	97	73
200	1	87	72	50
	5	100	93	87
	10	100	100	100
300	1	97	87	60
	5	100	100	97
	10	100	100	100
Control		10	3	0

droplet/cm². The treated leaves were fed to 2nd, 3rd, and 4th instars. Control groups of larvae were fed untreated oak foliage.

Larvae were examined every 24 h for mortality. At these times, fresh untreated leaf disks were added to cells where larvae had consumed the entire treated disk. The experiment was concluded after 144 h. Weights of instars before treatment were compared with weights of instars after treatment as well as with control larvae. The leaf area consumed was calculated using image analysis equipment (Optomax V, Burlington, MA). The lethal dose of *B. thuringiensis* and lethal time needed to kill 95% of larvae (LC₉₅ and LT₉₅, respectively) were calculated by probit analysis (LeOra Software 1987) and are based on values on a per larva basis. Analysis of variance (ANOVA) for comparing means of treatment values of LT₉₅ were calculated with an unprotected least significant difference test (Steel and Torrie 1980).

To test for possible alteration of the *B. thuringiensis* by the fluorescent tracer used in visualizing deposits, or by UV light, a separate bioassay was conducted using 3rd instars. The control larvae were fed foliage of white oak sprayed with *B. thuringiensis* and tracer and then exposed to the UV light for 30 s. An experimental group of larvae were fed white oak foliage sprayed with *B. thuringiensis*, which did not contain dye and which had not been exposed to UV light. The foliage given to both groups were sprayed with equal densities and sizes of *B. thuringiensis* droplets. No significant differences in mortality were recorded between the two groups.

Results

Droplet densities of 5 and 10/cm² were effective against 2nd instars (100% mortality) (Table 1). Droplet density of 1 drop/cm² was effective against 2nd instars (>87% mortality) at 200 and 300 μm sizes and not effective with 100- μm droplets (56% mortality). A similar trend was observed with 3rd and 4th instars. Droplets of 200 and 300 μm at 5 and 10 drop/cm² killed >90% of 3rd and 4th instars, but droplet densities of 1 drop/cm² at 100

Table 2. Lethal time (in hours) for 95% larval mortality (LT₉₅) to occur for 3 instars of the gypsy moth under differing droplet densities and sizes.

Instar	Droplet density	Size, μm	n	Slope \pm SEM	LT	χ^2
2nd	1	100	27	1.09 \pm 0.44	766.5	1.02
	5	100	30	4.27 \pm 0.59	87.3	1.53
	10	100	30	5.27 \pm 0.91	58.7	1.38
	1	200	30	2.52 \pm 0.41	242.8	0.72
	5	200	30	4.22 \pm 0.54	106.7	11.48
	10	200	30	4.46 \pm 0.9	55.5	1.53
	1	300	30	2.4 \pm 0.42	166.4	1.11
	5	300	29	4.16 \pm 0.96	51.8	1.2
	10	300	30	3.51 \pm 0.51	102.3	15.89
	3rd	1	100	30	2.84 \pm 0.65	613.4
5		100	30	3.26 \pm 0.46	188.3	2.88
10		100	30	4.09 \pm 0.53	126.4	2.58
1		200	29	3.73 \pm 0.54	195.8	14.55
5		200	30	3.49 \pm 0.48	124.8	6.59
10		200	30	5.64 \pm 0.75	91.1	2.01
1		300	30	3.74 \pm 0.53	210.5	1.07
5		300	29	4.38 \pm 0.56	114.2	1.18
10		300	30	4.36 \pm 0.56	123.3	4.37
4th		1	100	30	4.36 \pm 1.35	470.5
	5	100	30	3.84 \pm 0.91	448.8	2.36
	10	100	30	4.95 \pm 0.81	235.9	1.33
	1	200	28	6.16 \pm 1.4	265.1	0.06
	5	200	30	4.25 \pm 0.59	183.0	1.65
	10	200	30	6.71 \pm 0.95	102.2	1.63
	1	300	30	3.77 \pm 0.66	299.2	2.07
	5	300	30	6.53 \pm 0.86	149.1	7.03
	10	300	30	5.7 \pm 0.76	122.0	3.4

μm caused only 43% mortality. Fourth instars had lower mortalities when exposed to 100- μm droplets at all densities than to droplet sizes of 200 and 300 μm . Mortality was reduced for droplet densities of 1 drop/cm² for 4th instars at 200- and 300- μm droplet sizes when compared with mortalities of 2nd and 3rd instars at the same droplet densities, indicating that low densities of droplets become less effective as larval size increases.

Droplets of 100- μm diameter had lower LT₉₅ values than 200- and 300- μm droplets at droplet densities of 5 and 10 drops/cm² but not at 1 drop/cm² (Table 2). In most cases, LT₉₅ values de-

creased as droplet density increased. As with LT₉₅, LC₉₅ values increased as larval size increased. LC₉₅ values also increase as droplet size increased (Table 3). The LC₉₅ values (in IU) for 100-, 200-, and 300- μm droplets were 61.7, 206.3, and 760.2 IU/cm², respectively, when averaged by instar.

The before and after treatment weights for larvae are shown in Table 4. Surviving treated larvae showed a universal weight loss throughout the treatment period. During the same time however, untreated larvae either maintained their weight (2nd instar) or increased their weight (3rd and 4th instars). The leaf area consumed by larvae is shown in Table 4. Surviving treated larvae consumed between 16 and 40% of that eaten by surviving untreated larvae.

Table 3. Lethal concentration (LC₉₅) in international units needed for 95% larval mortality to occur for 3 instars of the gypsy moth for 3 droplet sizes

Instar	Size, μm	n	Slope \pm SEM	LC	χ^2
2nd	100	87	11.6 \pm 2,315,475.8	9.1	0.0
	200	90	10.2 \pm 3,063,086.6	60.7	0.0
	300	87	9.1 \pm 2,721,623.8	172.9	0.0
3rd	Combined	294	1.56 \pm 0.320,66	61.7a	10.9
	100	90	2.03 \pm 0.42	51.2	0.013
	200	89	1.63 \pm 0.52	242.3	0.82
	300	89	10.28 \pm 4,843,165.1	201.8	0.0
4th	Combined	298	1.13 \pm 0.19	206.3b	13.2
	100	90	1.36 \pm 0.39	77.0	0.95
	200	88	2.03 \pm 0.46	382.4	1.55
	300	90	2.45 \pm 0.65	692.1	0.17
4th	Combined	296	1.31 \pm 0.18	760.2c	13.5

Lethal concentration values followed by different letters are significantly different ($P = 0.05$) as calculated by an unprotected LSD test. (Steel and Torrie 1989).

Discussion

The differences in mortality in response to instar, droplet density, and droplet size may have an effect on management decisions for gypsy moth control. This study shows that droplets of *B. thuringiensis* of 100 μm diameter effectively control 2nd- and 3rd-instar gypsy moths if applied at droplet densities of at least 5 drops/cm². Dubois et al. (1993) showed that an application rate of 7 liters/ha of *B. thuringiensis* resulted in 53% of foliage having droplet densities >5 drops/cm² and an overall D_{V0.5} (volume mean diameter) of 103 μm . Control of 4th instars, however, is obtained with only with >200 μm drops (D_{V0.5} = 200 μm) at a rate of 5 or more drops/cm². Although drops of

Table 4. Before and after treatment weights (grams) and amount of leaf material eaten (cm²/d) of 3 instars of the gypsy moth when exposed to Foray 48B

Instar	Before treatment wt	After treatment wt, control	After treatment wt, treated	Foliage consumed	
				Treated	Control
2nd	0.0211	0.0199	0.0167	0.04	0.11
3rd	0.0786	0.0852	0.0499	0.12	0.77
4th	0.1773	0.1959	0.1372	0.34	1.36

300 μm had better control than 200- μm drops at all densities for 3rd and 4th instars, ≈ 3 times as many 200 μm drops can be generated per liter of *B. thuringiensis* than 300 μm drops. Greater numbers of drops may allow a greater number of larvae to ingest a lethal amount of *B. thuringiensis*.

The LD₉₅ values, which increase as droplet density and instar size increase, are higher than those obtained (check) by Rattcliffe and Yendol (1993), who used dilute *B. thuringiensis* droplets containing fewer international units per droplet than an equal size droplet in this study.

The LT₉₅ values obtained from this experiment show that 2nd instars are more quickly killed than 3rd or 4th instars at equal droplet densities and is in agreement with Bryant (1989). The increased time needed to kill large numbers of 3rd and 4th instars may be a result of the reduction of efficacy of *B. thuringiensis* over time through UV degradation (Morris and Moore 1975).

The reduction in leaf area consumption may be caused either by the feed-inhibiting effect of *B. thuringiensis* consumed late in the experiment, which can occur when 2nd instars feed on low droplet densities (Rattcliffe and Yendol 1993) or by the reduced feeding of recovering larvae that were exposed to a sublethal dose early in the experiment. The probability of a late instar receiving a sublethal dose of *B. thuringiensis* may be greater if many small droplets were present on foliage.

The surviving treatment larvae received sublethal doses of *B. thuringiensis*, but suffered weight loss similar to that seen by other investigators (Dulmage et al. 1978, Fast and Règnière 1984). The weight loss and feeding reduction of surviving larvae demonstrate the ability of *B. thuringiensis* to protect foliage even at low rates.

These data indicate that pest control managers should be aware of the larval stage at the time of spray when determining the application rate and droplet size to be used to maximize the efficiency of *B. thuringiensis*. Second instars may be more effectively controlled at standard rates per hectare using an average droplet size of 100 μm . Higher application rates and a larger average droplet diameter may be necessary for effective control of 3rd and 4th instars.

Acknowledgments

We thank William Brilla and Beckie Lease for their assistance during the course of the experiment and Richard Reardon and A. Temple Bowen for their thoughtful advice. This research was supported in part by Cooperative Agreement 42-686, between Pennsylvania State University and the U.S. Forest Service, Forest Pest Management in Morgantown, WV.

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Received for publication 4 May 1994; accepted 10 April 1995.