Laboratory Assessment of the Effects of *Bacillus thuringiensis* on Native Lepidoptera

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ABSTRACT The effect of 2 formulations of *Bacillus thuringiensis* subsp. *kurstaki* (Foray 48B and Dipel 8AF) was evaluated on 42 species of native Lepidoptera in laboratory bioassays using instars that are present in the field at the time of gypsy moth suppression applications. Mortality was significant for 27 of the 42 species evaluated against Foray 48B, and 8 of 14 species evaluated against Dipel 8AF. Susceptible species were noted in 5 of 6 families assayed—Papilionidae, Nymphalidae, Geometridae, Lasiocampidae, Saturniidae, and Noctuidae. The 1 species treated in the Lymantriidae family was not susceptible to *B. thuringensis*. Treated individuals that survived for a week were likely to reach adulthood. Intrageneric differences in susceptibility to *B. thuringiensis* were recorded among 8 species of *Catocala* and 3 species of *Lithophane* assayed. Of the 18 species assayed as 1st or 2nd instars, mortality was significant, usually exceeding 95%. By contrast, 9 of 11 species not susceptible to *B. thuringiensis* were assayed as penultimate or ultimate instars. However, species susceptible to *B. thuringiensis* were found in both early and late instars.

KEY WORDS *Bacillus thuringiensis*, nontarget Lepidoptera, microbial control

Bacillus thuringiensis subsp. *kurstaki* is one of the insecticides most commonly used to control forest insect pests. *B. thuringiensis* has been used extensively in suppression and eradication programs against gypsy moth, *Lymantria dispar* (L.). From 1980 to 1993, >1.9 million ha (4.9 million acres) were sprayed with *B. thuringiensis*. In 1994, >151,000 ha were treated with *B. thuringiensis*, including >57,000 ha in Michigan and 20,000 ha in both Pennsylvania and Maryland. In 1994, *B. thuringiensis* was used on >55% of the area sprayed for gypsy moth suppression (Anonymous 1994). Besides suppression activities aimed at the European gypsy moth, eradication efforts for both the European and Asian strain of the gypsy moth also occur yearly. In 1992, efforts to eradicate the Asian gypsy moth in certain locations in Oregon, Washington, and the Vancouver area of British Columbia resulted in >67,000 ha being sprayed with *B. thuringiensis* (Anonymous 1992). In 1994, >56,000 ha were sprayed near Wilmington, NC, in attempts to eradicate the Asian gypsy moth (McGovern 1994).

Although there is a growing concern over the nontarget effects of *B. thuringiensis*, there has been limited research aimed at determining its effects on native North American species of Lepidoptera. In Oregon, Miller (1990) demonstrated that both richness and diversity of native Lepidoptera associated with Garry oak, *Quercus garryana* Dougl., were reduced following treatment of oak habitat as part of a gypsy moth eradication program. Sample et al. (1996) also reported a significant reduction in species abundance and richness in both larval and adult nontarget Lepidoptera from field studies in eastern West Virginia. In west-central Virginia, Wagner et al. (1996) found that the abundance of micro- and macrolepidopteran caterpillars was consistently lower in treated plots. However, the differences were modest, and nearly every lepidopteran species recovered within a year of treatment. James et al. (1993) demonstrated that *B. thuringiensis* is toxic to late, but not early, instars of the cinnabar moth, *Tyria jacobaeae* (L.), an introduced biocontrol agent.

Significant reductions in numbers of larvae of nontarget lepidopterans following the use of *B. thuringiensis* can indirectly affect other animals that rely on lepidopterous larvae as a primary source of food. Rodenhouse and Holmes (1992) found that a significant reduction in biomass of larvae following *B. thuringiensis* application led to significantly fewer nesting attempts by certain birds. Bellocq et al. (1992) showed that the use of *B. thuringiensis* increased emigration rates and caused dietary shifts in shrews in treated areas.

Krieg and Langenbruch (1981) presented an extensive summary of the toxicity of *B. thuringiensis* to Lepidoptera, which suggests extremely broad impacts within the order. Navon (1993) reported on bioassay techniques and results for various agricultural and forest lepidopteran pests. Faust and Bulla (1982) reported on the susceptibility of 57 lepidopteran species, most of which also were agricultural and forest pests. Again, broad taxonomic effects were evident within the order. Most bioassays have been conducted to determine the susceptibility of *B. thuringiensis* to lepi-

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dopterous pests, but there have been few attempts to evaluate the toxicity of *B. thuringiensis* against native, nontarget species in the laboratory. We report the results of laboratory bioassays aimed at determining the effect of *B. thuringiensis* on larvae of native, nontarget Lepidoptera.

Materials and Methods

Selection of Species. Forty-two species of native Lepidoptera were evaluated in the laboratory assays. These included 1 papillionid species, 3 nymphalids, 7 geometrids, 1 lasiocampid, 3 saturniids, 1 lymantriid, and 26 noctuids. Most of these are forest species sympatric with gypsy moths and, more importantly, are present as larvae at the time *B. thuringiensis* is applied to control gypsy moth. *Actias luna* (L.), *Antheraea polyphemus* (Cramer), *Eutrapela clemataria* (J. E. Smith), and *Papilio glaucus* (L.) also were assayed to add taxonomic diversity, although some spring brood larvae of these species usually hatch late enough to escape impact from *B. thuringiensis* applications.

Larval Collections and Rearing. Larvae of most of the species that we evaluated were reared from field-collected ova or from ova obtained from wild females that were taken at bait or light most often in southern New Jersey. In most assays, larvae were the progeny of a single female. If progeny of 2 females were used, offspring were similarly represented in the control and treatment lots. Most of the *Abagrotis alternata* (Grote) were collected as wild late instars; the 1993 *Malacosoma disstria* Hübner were collected as wild third instars. Details of larval collections and rearing can be obtained from D.F.S.

Larvae were assayed on a plant species (or at least genus in the case of oak feeders) that is a known host in the field. Polyphagous species that commonly use oaks were usually assayed on oaks because they are often dominant or codominant in forests sprayed for gypsy moth control. Species not assayed on potted red oaks (usually *Quercus rubra*) and the food plants used are as follows: *Papilio glaucus, Limenitis arthemis* (F.), *Euchlaena obtusaria* (Hübner), *Eupsilia vinulenta* (Grote), *Lithophane unimoda* (Lint), and *L. petulca* Grote on potted *Prunus serotina; L. lemmeri* (B & Benjamin) on *Juniperus virginiana* bouquets; *Ennomos magnaria* Guenée, *Eutralepa clemataria, Lithophane grotei* Riley, and *Sunira bicolorago* (Guenée) on potted *Acer rubrum; Actias luna, Catocala obscura* Strecker, and *C. vidua* (J. E. Smith) on *Carya ovata* bouquets; *C. praeclara* Grote & Robinson on mostly potted *Aronia arbutifolia; C. sordida* Grote, *Eupsilia vinulenta, Metaxaglaea semitaria* Franclemont, *Xylotype capax* (Grote), and *Abagrotis alternata* on a potted *Vaccinium coymbosum* cultivar; *Psaphida resumens* Walker, and *Antheraea polphemus* on *Quercus alba* bouquets; *Malacosoma disstria* (2nd only) and *Hemileuca maia* (Drury) (2nd only) on potted *Q. alba; Asterocampa clyton* (Boisduval & Leconte) on potted *Celtis occidentalis,* and *Speyeria diana* on *Viola* sp.

Several polyphagous species were reared on *Prunus serotina* Ehrh. before assay but were switched to another host (usually oak) for the assays. These included all *Sericaglaea, Chaetaglaea, Eupsilia, Orthosia, Amphipyra,* and *Alsophila.* Oligophagous and monophagous species were reared and assayed on the same species.

Larvae were bioassayed in an instar in which they occur in the field at the time when *B. thuringiensis* applications typically are scheduled. To obtain appropriate instars for assay, all adults and eggs were held outdoors in a screened, shaded enclosure at Port Norris, Cumberland County, New Jersey, to ensure normal eclosion times. Except for species assayed as 1st or 2nd instars, larvae were reared outdoors in muslin sleeves. For most species, wild larvae of similar age were observed at the rearing site.

Gypsy moth larvae from the continuous laboratory culture maintained at the Northeastern Forest Experiment Station at Hamden, CT, were reared on synthetic diet (BioServ, Frenchtown, NJ) before the assays.

Treatment of Foliage, Bioassay Procedure. Foliage treatments consisted of undiluted applications of Foray 48B or Dipel 8AF formulations of *B. thuringiensis* subsp. *kurstaki* to potted seedlings or foliage bouquets at rates equivalent to the aerial application of 89 (Foray) or 99 (Dipel) BIU/ha in field programs. All species were assayed against Foray, and 14 of the 42 species also were assayed against Dipel. Multiple assays for a species were conducted in different years with different batches of Foray. Bioassays with 2nd- and 4th-instar gypsy moth on treated and untreated foliage on each bioassay date confirmed the insecticidal activity of the Foray and Dipel preparations.

The pesticide was applied using a pro-mist system with a mini-Beecomist nozzle (Beecomist, Telford, PA) rotated at 12,000 RPM to generate drops at 75–125 units VMD in a cylindrical spray tower (Hubbard and Lewis 1973). The tower was ≈2.5 m in diameter and 4 m high, with the nozzle positioned in the center of the tower at a height of ≈3.5 m above the plant material to be treated. Foliage bouquets or potted seedlings were placed on a rotating table that revolved 4 times during the 1-min spray period. Kromecote spray cards (Mead, Dayton, OH) were placed next to each seedling or bouquet to monitor droplet size and distribution. For this spray procedure, the average number of drops/cm^2 generally ranges from 60 to 300 (N.R.D, unpublished data) An autoclaved sample of Foray 48B was applied at a rate equivalent to 89 BIU/ha to suitable host foliage and bioassayed against *Hemileuca maia, M. disstria, Alsophila pometaria* (Harris), *Amphipyra pyramidoides* Guenée, and *Egira alternans* (Walker). Because there was no larval mortality in any of the species assayed against the autoclaved sample, foliage to be used as controls was not sprayed with innocuous material.

Freshly collected foliage bouquets from native trees were used for assays in 1991 involving *Carya ovata* (Mill) K. Koch., *Juniperus virginiana* L., and *Quercus alba* L. The bouquets were placed in tubes of water to maintain freshness and turgidity, then placed in Plexiglas chambers (9 by 16 by 24 cm) before the addition

of larvae to treated or untreated foliage. For most assays, larvae were confined on foliage of potted seedlings in bags constructed of fine-mesh nylon. Muslin sleeves were used for some 1st instars. Larvae were placed on treated and untreated foliage within 1 h after application of *B. thuringiensis* to treated foliage.

Except for the gregarious *H. maia*, 50–100 of which were placed in a sleeve, a replication consisted of 5–15 larvae confined to treated or untreated foliage in each Plexiglas chamber or sleeve. There were 1–4 replications on untreated foliage (controls) and 2–6 replications (10 for *A. luna*) on treated foliage for each species. The number of larvae per replication and the number of replications varied because of the limited availability of larvae for certain species.

Monitoring Larvae. Larval mortality on treated and on untreated foliage was recorded over a period of 5–7 d. If selected for long-term monitoring, surviving larvae were removed from the foliage and confined in sleeves on wild-growing, unsprayed plants of the host species used before assay. Mortality for these larvae was observed at least weekly.

Pupating or aestivating larvae that normally would enter the soil were supplied with ≈10 cm of heat-sterilized, damp peat moss as a pupation medium in a plastic or wax cardboard container. Species that pupate above ground were given crumpled paper towel and dead leaves in which to spin cocoons, or were allowed to spin cocoons in their rearing sleeves. Pupae were held under ambient conditions until adult emergence. Containers with overwintering pupae were kept outdoors on the ground in a sheltered location in Port Norris, NJ. Dates of adult eclosion were recorded. Days to pupation, pupal mortality, and live pupal weights or adult dry weights also were recorded for a subset of the species.

Data Analysis. Fisher's exact test (Fisher 1958) was used to compare the mortality at day 5 (day 7 or pupation for some species) on treated versus control foliage. Mortality between treatment and control larvae was considered significantly different (and thus susceptible to *B. thuringiensis*) if $P < 0.05$.

We also categorized species as highly sensitive or insensitive to *B. thuringiensis*. Species considered as highly sensitive met the following criteria: there was at least 95% mortality in treated larvae and no more than 5% mortality in controls by days 5 or 7 or by pupation. Insensitive species met the following criteria: (1) At least 90% of the treatment and control larvae were alive on day 7, (2) at least 90% of these treatment survivors completed feeding, and (3) eclosion success was at least 75% for treatment pupae.

Results

Foray 48B Assays. Significant mortality was recorded for 27 of 42 (64.3%) species. This included the Papilionidae, all 3 Nymphalidae, 5 of 7 Geometridae, the single Lasiocampidae, all 3 Saturniidae, and 14 of 26 Noctuidae but not the lone Lymantriidae (Table 1). Mortality of treated larvae was 90–100% for the saturniid and 4 butterfly species. Mortality rates in other families were highly variable. For example, both the Geometridae and Noctuidae had species which suffered no mortality along with species which had 100% mortality among treated larvae (Table 2). For 21 of the 27 affected species, mortality was significant 5–7 d after treatment. For 5 others, mortality was not significant until day 6 through pupation, and mortality in *Limenitis arthemis astyanax* occurred during the prepupal and pupal stages (Table 3). Despite substantial mortality among the treated larvae, 14 of the significantly affected species had some larvae that recovered and reached adulthood. Eight species were assayed more than once in the same instar always giving very similar results (Table 2 and 4).

Table 1. Susceptibility to Foray 48B, by family, for 42 species of nontarget Lepidoptera

Family	No. species assayed	Early instar[a]		Late instar[b]	
		n	No. susceptible[c]	*n*	No. susceptible[c]
Papilionidae	1	1	1	—	—
Nymphalidae	3	1	1	2	2
Geometridae	7	4	4	3	1
Lasiocampidae	1	1	1	1	1
Saturniidae	3	3	3	—	—
Lymantriidae	1	—	—	1	0
Noctuidae	26	8	8	18	6
Total[d]	42	18	18	25	10

[a] First to 3rd instar.
[b] Fourth to last instar.
[c] Significant mortality ($P < 0.05$) for treated larvae.
[d] Some species assayed in both early and late instars.

Intrageneric Differences in Larval Mortality. Mortality was significant in 6 of 8 species of the noctuid genus *Catocala* that were evaluated (Table 4). These included all 4 species assayed as 1st and 2nd instars—*obscura* Strecker, *vidua*, *ilia* (Cramer), and *lineella* Grote—and 3 others that were assayed as late instars—*ilia*, *coccinata* Grote, and *praeclara*. *C. ilia* was evaluated as both early (1st/2nd) and mid (3rd/4th) instars, even though it would be present as late instar only during field applications of *B. thuringiensis*. *C. ilia* larvae in both age groups showed significant mortality after consuming *B. thuringiensis*-treated foliage, with mortality greatest in early instars. In *C. praeclara*, penultimate instars were susceptible to Foray (by day 7), whereas last instars were not significantly affected. *C. sordida* (evaluated as midinstars) and *C. similis* W. H. Edwards (evaluated as late instars) apparently were not susceptible to Foray.

As was the case with the *Catocala*, intrageneric differences were recorded for the 3 *Lithophane* species evaluated (Table 4). Mortality was significant for antepenultimate instars of *L. grotei* and for 2nd instars of *L. petulca* (by day 7). However, penultimate instars of *L. unimoda* were unaffected. We also assayed *L. lemmeri* as second instars on *J. virginiana*. Mortality for this species differed between treatment replicates, precluding formal statistical analysis. However, 12 of 22 (54.5%) treated larvae did eclose as adults, including some from all replicates. This indicates that this

Table 2. Mortality in species subjected to foliage treated with Foray 48B

Species	Instar[a]	Control		Foray		P^b
		No. alive	No. dead	No. alive	No. dead	
Papilionidae						
Papilio glaucus[c]	1st–3rd	10	0	0	29	≤0.00001
Nymphalidae						
Speyeria diana[c]	2nd–3rd	10	0	1	15	≤0.00001
Limenitis arthemis astyanax[c]	n/n–1	10	0	0	20	<0.00001[d]
Astercampa clyton[a]	4th–5th	21	1	1	40	≤0.00001
Geometridae						
Alsophila pometaria	n	19	1	11	7	0.0164
Phiglia titea	n/n–1	20	0	43	7	0.1801
Euchlaena obtusaria	n–1	12	0	18	0	
Ennomos magnaria[c]	1st	22	1	0	66	≤0.00001
Ennomos magnaria	1st	17	14	0	27	≤0.00001
Lambdina fervidaria[c]	1st	17	1	10	26	≤0.00001
Eutrapela clemataria[c]	Hatchling	20	0	4	31	≤0.00001
Prochoerodes transversata	2nd	19	1	28	13	0.0237[d]
Lasiocampidae						
Malacosoma disstria	2nd	23	4	4[e]	26	≤0.00001
Malacosoma disstria[c]	n	20	0	1	44	≤0.00001
Saturniidae						
Hemileuca maia[c]	Hatchling	47	0	5	53	≤0.00001
Hemileuca maia	1st	70	1	48	312	≤0.001
Hemileuca maia[c]	1st	20	0	0	51	≤0.00001
Hemileuca maia[c]	2nd	109	1	0	111	≤0.00001
Antheraea polyphemus	1st	16	4	3[e]	57	≤0.00001
Actias luna	1st	26	14	0	96	≤0.00001
Lymantriidae						
Dasychira obliquata	4th	20	0	27	1	0.9999
Noctuidae						
Amphipyra pyramidoides	n–1	19	2	6[d]	24	≤0.00001
Amphipyra pyramidoides	n–1	20	0	11	37	0.0001
Xystopeplus rufago	1st–2nd	28	0	12	21	≤0.00001
Psaphida rolandi	n–1	19	1	18[e]	22	0.0001
Psaphida resumens[c]	1st–2nd	20	0	9[e]	41	≤0.00001
Egira alternans	1st	20	5	22	27	0.0059[d]
Egira alternans	2nd–3rd	18	0	35	2	1.0000
Zale aeruginosa	Hatchling	12	0	19	11	0.0175
Eupsilia vinulenta	n–1/n–2	20	0	19	1	0.9999
Eupsilia vinulenta	n–1/n–2	20	0	43	1	0.9999
Sericaglaea signata	4th	18	0	48	0	—
Metaxaglaea semitaria	n	20	0	51	1	0.9999
Chaetaglaea sericea	n–1	20	0	20	0	—
Chaetaglaea sericea	n–1	19	0	48	1	0.9999
Sunira bicolorago	n/n–1	20	0	45	3	0.5498
Sunira bicolorago	n	20	0	29	0	—
Xylotype capax	n–1	19	1	48	0	0.2941
Orthosia alurina	n–2	19	1	20	0	0.9999
Orthosia alurina	n–1	18	0	30	7	0.0823
Orthosia hibisci	n–1	20	0	39	0	—
Abagrotis alternata	n/n–1	29	0	50	0	—
Abagrotis alternata	n/n–1	18	0	13	0	—

See Table 4 for *Catocala* and *Lithophane* results. Counts of alive and dead larvae are at day 5 of the assays unless significant mortality did not occur until after day 6, than the counts given are at the time (usually day 7 or pupation) significant mortality occurred (see footnote[d]).

[a] n, last instar.

[b] No *P* value reported for species for which no mortality occurred.

[c] Highly sensitive to Foray 48B.

[d] Significant mortality between day 6 through pupation, but not by day 5.

[e] Although this species did not meet our highly sensitive criteria, >75% of day 5 survivors died before pupation.

rare species is insensitive or only moderately sensitive to *B. thuringiensis*. For all *Lithophane* species, most larvae alive on day 5 completed feeding and produced apparently normal adults.

Instar Difference. Mortality was significant following treatment for all of the 18 species evaluated in the 1st to 3rd instar with Foray (Table 1). Most species succumbed within the first 2–3 d on treated foliage; for *Prochoerodes transversata* (Drury) and *E. alternans*, mortality was not significant until after day 5 (Table 3). Of the 25 species evaluated in 4th to last instars with Foray, only 10 were significantly affected (Table 1).

Besides *C. ilia* and *C. praeclara*, 6 other species were assayed separately in more than 1 instar, and differences in instar sensitivity were noted in 2 of these species. In *Lithophane grotei*, 35% of the treated penultimates survived to adults but none of 38 antepen-

Table 3. Delayed mortality in 5 species subjected to foliage treated with Foray 48B

Species	Assayed instar[a]	% mortality			
		0–5 d after treatment		0 d through pupation	
		Control	Treated	Control	Treated
Limenitis arthemis astyanax	n/n–1	0	10	0	100
Procheorodes transversata	2nd	0	12	5	75
Catocala praeclara	n–1	0	12	0	68
Lithophane petulca	2nd	0	31	25	77
Egira alternans	1st	7	2	20	55

[a] n, last instar.

ultimates survived past day 4. Similarly in *C. vidua*, 10 of 51 (19.6%) Foray-treated late 2nd and early 3rd instars survived to produce large, apparently normal adults compared with only 1 of 20 first instars in 1992. All 20 larvae treated as 1st instars were dead by day 5 in 1993 (Table 4). *E. alternans* also showed slightly higher mortality by pupation for larvae assayed in the 1st instar than in the 2nd and 3rd instar (Tables 2 and 3). There were no apparent differences in instars for *H. maia* (1st versus 2nd instar), *Orthosia alurina* (antepenultimates versus penultimates), or *M. disstria* (2nd versus last instar) (Table 2).

Dipel 8AF Assays. In 8 of 14 (57%) species evaluated for susceptibility to Dipel 8AF, mortality was significant during the post-treatment period (Table 5); 7 of these species showed similar results with Foray. *Eupsilia vinulenta* was sensitive to Dipel only; although mortality was only 42% through adult eclosion for this species. All treated larvae of the 3 species evaluated in the early instars against Dipel succumbed following treatment. Five of 11 species tested as later instars were susceptible to Dipel (Table 5).

Discussion

Species Sensitivity. Thirteen species met the criteria for being highly sensitive to the *B. thuringiensis* formulation foray. There was at least 95% mortality in treated larvae and no more than 5% mortality in controls by days 5 or 7, or by pupation. These included *Papilio glaucus* (by day 5), *Speyeria diana* (day 5), *B. arthemis astyanax* (eclosion), *Astercampa clyton* (day 5), *Ennomos magnaria* (day 5), *Lambdina fervidaria* (Hübner) (the bivoltine-oak feeding species illustrated by Covell (1984, pl. 54: 15) as *L. f. athasaria*) (pupation), *E. clemataria* (pupation), *M. disstria* (last instar, day 5), *H. maia* (day 7), *C. ilia* (day 5), *C. coccinata* (pupation), *L. grotei* (antepenultimates only, day 5), and *Psaphida resumens* (day 7) (Tables 2, 4). *H. maia* was highly sensitive with 100% mortality by day 7 in 10 of 12 replicates, although when all data are pooled 9.0% (48 of 580) of treated larvae survived to pupation and adult eclosion. Despite the 2 treatment replicates with some survival, we still consider this species to be highly sensitive. Second-instar *M. disstria* did not meet the criteria of being highly sensitive because control larvae had greater than 5% mortality. However, we would consider 2nd-instar *M. disstria* to be highly sensitive because only 1 treated larvae survived to pupation and the treated last instars proved to be highly sensitive (Table 2).

Eight of these species were assayed as early instars and the other 6 were highly sensitive as mid-instars or late instars (*C. ilia* sensitive as early and late instars).

Table 4. Intrageneric comparisons of mortality in response to Foray 48B for *Catocala* and *Lithophane* (Noctuidae)

Species	Instar[a]	Control		Foray		*P*
		No. alive	No. dead	No. alive	No. dead	
Catocala obscura	1st	17	1	14	33	≤0.00001
C. vidua	1st	17	2	0	20	≤0.00001
C. vidua	1st	17	2	0	19	≤0.00001
C. vidua	2nd/3rd	16	0	24	27	≤0.00001
C. ilia	1st/2nd	10	0	0	40	≤0.00001
C. ilia[b]	3rd/4th	20	0	2	39	≤0.00001
C. sordida	3rd/4th	15	0	26	3	0.5401
C. coccinata[b]	n–1	18	0	5	45	≤0.00001
C. praeclara	n–1	16	0	5	11	≤0.00001[c]
C. praeclara	n	18	0	16	3	0.2299
C. similis	n–1/n–2	15	3	46	2	0.1203
C. lineella	1st/2nd	21	6	8	32	≤0.00001
Lithophane petulca	2nd	9	3	3	10	0.0169[c]
L. grotei[b]	n–2	20	0	0	38	≤0.00001
L. grotei	n–1	20	0	9	11	0.0001
L. unimoda	n–1	20	1	35	5	0.6531

Counts of alive and dead larvae are at day 5 of the assays unless significant mortality did not occur until after day 6, then the counts given are at the time (usually day 7 or pupation) significant mortality occurred (see footnote[c]).

[a] n, last instar.

[b] Highly sensitive to Foray 48B.

[c] Significant mortality occurred by day 7 but not by day 5.

Table 5. Susceptibility of 14 species of native Lepidoptera to Dipel 8AF

Species	Instar[a]	Control		Dipel		P^b
		No. alive	No. dead	No. alive	No. dead	
Geometridae						
Asterocampa clyton[c]	4th/5th	21	1	2	20	≤0.00001
Alsophila pometaria[c]	n	19	1	11	21	≤0.00001
Ennomos magnaria	1st	17	14	0	47	≤0.00001
Lasiocampidae						
Malacosoma disstria[c]	2nd	23	4	0	28	≤0.00001
Lymantriidae						
Daychira obliquata	4th	20	0	26	0	—
Noctuidae						
Catocala vidua[c]	1st	17	2	0	31	≤0.00001
Amphipyra pyramidoides[c]	n-1	19	2	3	35	≤0.00001
Lithophane grotei	n-1/n-2	20	0	22	28	≤0.00001
L. unimoda	n-1	19	1	38	9	0.1423
Eupsilia vinulenta	n-2	20	0	19	9	0.0063
Chaetaglaea sericea	n-1/n-2	20	0	30	0	—
Sunira bicolorago	n	20	0	41	0	—
Orthosia alurina	n-2	19	1	14	4	0.1698
Abagrotis alternata	n/n-1	18	0	31	1	0.9999

[a] n, last instar.
[b] No *P* value reported for species for which no mortality occurred in treated or control larvae.
[c] Highly sensitive to Dipel 8AF

The 6 species that were highly sensitive as mid-instars or late instars were *B. arthemis astyanax* (last instar), *A. clyton* (4th/5th instar), *M. disstria* (last instar), *C. ilia* (both 2nd and penultimate instar), *C. coccinata* (penultimate instar), and *L. grotei* (antepenultimate instar). It should be noted that for some of the highly sensitive species—*L. fervidaria* , *H. maia* , and *C. coccinata*—several of the treated larvae survived to produce apparently normal adults.

Of the 14 species that were evaluated against Dipel 8AF, 5 met our criteria for being highly sensitive to this *B. thuringiensis* product: *A. clyton* (by day 5), *A. pometaria* (pupation), *M. disstria* (day 5), *C. vidua* (day 5), and *A. pyramidoides* (day 7) (Table 5). *A. pometaria* was highly sensitive to Dipel but not as highly sensitive to Foray, whereas the other 4 species were highly sensitive to Foray as well.

Species that are highly sensitive by our definition probably are at greatest risk from the aerial application of *B. thuringiensis* because they can be expected to incur massive population crashes or local extirpations from even a single application. Such species could be eradicated from areas treated with *B. thuringiensis* if populations are sparse or small. These eradications could be permanent if species in localized colonies are sufficiently isolated that recolonization is impossible. The species in our assay that failed to meet the highly sensitive criteria had sufficient survivors on treated foliage (generally >20%) so that local eradication in the field would be unlikely from 1 application of *B. thuringiensis*. Nor would recolonization be necessary for recovery.

All of the 4 butterflies we evaluated were highly sensitive versus 10 of 38 species of the moth species. In tabulating results for a number of butterflies and several *B. thuringiensis* varieties, Krieg and Langenbruch (1981) found that, by their definition, most butterfly species were highly sensitive. Some of the Nymphalidae listed by Krieg and Langenbruch appeared to be only moderately sensitive, but delayed mortality (as in our *Limenitis* assay) might have been overlooked in other studies.

Speyeria diana, one of the butterfly species shown to be highly sensitive to *B. thuringiensis* in our studies, is found as early instars at the time of gypsy moth spraying and is of particular concern in forestry applications of *B. thuringiensis* for gypsy moth suppression in Appalachia. This species was a candidate for listing under the United States Endangered Species Act until the candidate list was abandoned in 1995. Also, 2 noctuid species assayed, *L. lemmeri* and *Xylotype capax*, are listed as rare by Natural Heritage Programs in several states. *X. capax* proved insensitive to *B. thuringiensis* and 12 of 22 treated *L. lemmeri* survived to adulthood. Most of the other species assayed occur in relatively high numbers over large areas, and, therefore, would be expected to recolonize quickly any *B. thuringiensis*-sprayed area even if they were eradicated within it.

Only 11 of 42 species evaluated in our assays met the criteria for being insensitive to Foray 48B or Dipel 8AF or both. These included a geometrid, *Euchlaena obtusaria; Dasychira obliquata*, the only native lymantriid assayed; and the following Noctuidae: *E. vinulenta, Sericaglaea signata* (French), *Metaxaglaea semitaria* Franclemont, *Chaetaglaea sericea* (Morrison), *Sunira bicolorago, X. capax, Orthosia alurina* (J. B. Smith), *Orthosia hibisci* (Guenée), and *A. alternata*. Of these species, *D. obliquata, C. sericea, S. bicolorago*, and *A.alternata* also were insensitive to Dipel. All of the insensitive species were assayed as mid- or late instars. This is consistent with the generally accepted belief that reduced sensitivity to *B. thuringiensis* often is related to larval instar, with older larvae generally less sensitive to both Foray 48B and Dipel 8AF.

Delayed Mortality. For most of the species, mortality was significant by day 5. However, day 5 mor-

tality often will substantially underestimate the true impact from *B. thuringiensis*. For 5 species, nearly all of the mortality occurred after day 5 (Table 3). Of particular note was the nymphalid, *L. arthemis astyanax*, for which no mortality was observed in larvae that fed on treated foliage, yet all the prepupae and pupae died before eclosion. All of the *L. a. astyanax* larvae fed untreated foliage completed development and produced normal adults. For 7 highly sensitive species and 4 other species, 75–100% of the day 5 survivors died before pupation, usually during days 6–10 (Table 2). For example, the 9 *P. resumens* that survived to day 5 were dead by day 7 and only 4 of the 18 *P. rolandi* surviving to day 5 lived long enough to pupate. Our data suggest that one cannot conclude that a species is unaffected or only moderately affected by *B. thuringiensis* solely on the basis of low mortality during the first 5 d or so after consuming treated foliage. We suggest the *B. thuringiensis* assays should be monitored for at least 7 d, and preferably to pupation.

Sublethal Effects and Pupal Viability. We attempted to investigate sublethal effects despite having few survivors of *B. thuringiensis*-sensitive species; in certain assays this often precluded rigorous statistical analysis. Larvae developed more slowly than their control counterparts for most species evaluated from day 7 to pupation. This was especially true for *L. lemmeri* treatment larvae that finished feeding ≈11 d after control larvae. The 4 surviving *P. rolandi* were delayed ≈8 d. For *H. maia*, treatment larvae took nearly 3 wk longer than controls to complete larval development, although this delay did not result in lower pupal weights (male: 1.1 versus 1.0 g; female: 1.4 versus 1.5 g) or reduced survival of pupae. Many other species, including all sensitive species and some insensitive ones, showed some apparent delay in development to pupation, typically about 5 d. Again, small sample sizes (especially among surviving larvae from treated foliage) precluded statistical analysis of this effect. Although the larvae of all these species later produced apparently normal pupae and adults, delayed development could have some biological consequences in the field, including increased parasitism (Ticehurst et al. 1982, Wallner et al. 1983, Weseloh 1984) and reduced weight and fecundity (Schweitzer 1979, Schneider 1980).

Adult weights of species with significant larval mortality were similar for control and surviving treated *Xystopeplus rufago* (Hübner) (control versus treated: 0.045 versus 0.046 g), *C. vidua* (0.273 versus 0.253 g), *C. praeclara* (0.142 versus 0.148 g), and *C. lineella* (0.035 versus 0.037 g). This also was true for adult weights obtained for some species that were not susceptible to *B. thuringiensis*. These included the geometrid *E. obtusaria* (control versus treated: 0.050 versus 0.053 g) and the noctuids *S. signata* (0.079 versus 0.117 g), *C. sericea* (0.074 versus 0.087 g), *S. bicolorago* (0.019 versus 0.018 g), *X. capax* (0.063 versus 0.056 g), *O. alurina* (0.066 versus 0.059 g), *O. hibisci* (0.07 versus 0.07 g), and *A. alternata* (0.055 versus 0.058 g). For these species, it is apparent that *B. thuringiensis* had little lasting ill effect on surviving larvae, even though mortality in treated larvae ranged from 0 to 95% among these same species.

On the basis of the available data for the more *B. thuringiensis*-sensitive species, we conclude that most larvae that survived the consumption of treated foliage and reached pupation later produced normal adults. For example, 3 *H. maia* females that survived treatment as larvae laid 2 normal egg rings each. Viable eggs also were obtained from treated survivors of *A. pometaria*, *C. sericea*, and *D. obliquata*, although fecundity was very low in the former species. Nevertheless, our data do not allow a conclusion that all survivors of *B. thuringiensis*-sensitive species were completely unaffected.

Taxonomic Patterns. Despite the observed intrafamilial variation in sensitivity to *B. thuringiensis*, some taxonomic patterns were apparent from this study. Penultimate and last instars of at least 7 of 8 species of xylenini noctuids were insensitive to *B. thuringiensis*. The 8 late instars we assayed included 2 species of *Lithophane*, and 1 each of *Eupsilia*, *Chaetaglaea*, *Sericaglaea*, *Metaxaglaea*, *Xylotype*, and *Sunira*, but only *L. grotei* incurred significant mortality. Sensitivity among early- to mid-instar Xylenini (*L. grotei*, *L. lemmeri*, *L. petulca*, *X. rufago*) varied widely, but was significant for 3 species.

Both species of the noctuid genus *Orthosia* were little affected by *B. thuringiensis*, which agrees with reports by Krieg and Langenbruch (1981). These authors also reported the response of additional (principally European) species of *Alsophila*, *Ennomos*, *Lambdina*, *Malacosoma*, *Dasychira*, and *Papilio* to several varieties and formulations of *B. thuringiensis*; their findings largely agree with ours. When our results are pooled with those of Krieg and Langenbruch, *Catocala* and *Lithophane* remain the only genera for which comparison of effects of *B. thuringiensis* on more than 3 congenerics is possible, and in both genera these effects (based entirely on our results) varied significantly among the species tested.

It is important to note the *B. thuringiensis* was applied to treated foliage under ideal conditions in this study. The amount applied (equivalent of 89 BIU/ha for Foray and 99 BIU/ha for Dipel) is the maximum allowed in gypsy moth aerial application programs. Further, Foray and Dipel were applied to foliage in a laboratory spray tower, which resulted in nearly uniform coverage. Such uniformity is seldom achieved under field conditions. Thus, the laboratory mortality recorded for certain species under these ideal conditions might be greater than that which would occur in the field.

It is difficult to generalize when predicting the susceptibility of native Lepidoptera to *B. thuringiensis*, especially for mid- or late instars. For taxa such as butterflies, many species appear to be highly sensitive. Conversely, xylenine noctuids apparently are mostly insensitive, especially as late instars. But for many taxa the issue of susceptibility must be dealt with on a species-by-species basis, with special attention given to the phenology of species at the time of application

of *B. thuringiensis*. Our findings will complicate management decisions concerning the use of *B. thuringiensis*, particularly in habitats containing threatened or endangered species of Lepidoptera. We were unable to evaluate threatened or endangered species in our assays, though we did evaluate 3 species of conservation concern (1 was highly sensitive, 1 was insensitive, and the 3rd was moderately sensitive).

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