Response of the Cottonwood Leaf Beetle (Coleoptera: Chrysomelidae) to *Bacillus thuringiensis* var. *san diego*

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ABSTRACT A standardized laboratory bioassay was used to quantify the lethal and sublethal responses of larval and adult cottonwood leaf beetles, *Chrysomela scripta* F., to *Bacillus thuringiensis* var. *san diego*, formulated as M-One standard powder (Mycogen Corporation, San Diego). The median lethal concentration (LC_{50}) for second instars, after a 96-h exposure to *B. thuringiensis* var. *san diego*, was 21,000 Colorado potato beetle international units per milliliter water. The LC_{50} estimates for the third instars and adults were 20 and 40 times higher than for second instars, respectively. Larval LC_{50} estimates were halved when mortality for the entire larval period was included in the LC_{50} estimate. Adult mortality continued for approximately 14 d after initial exposure to *B. thuringiensis* var. *san diego*. The adult LC_{50} calculated at 14 d was half the 4-d estimate. Age of adults at treatment did not significantly affect the LC_{50}. Median lethal times (LT_{50}) were similar for larvae and adults, with overlapping confidence limits ranging from 2.1 to 3.5 d. Larvae surviving treatments as second and third instars showed a significant dose-dependent decrease in adult dry weight at eclosion and an increase in the larval developmental period.

KEY WORDS Insecta, *Chrysomela scripta*, *Bacillus thuringiensis*, *Populus*

THE COTTONWOOD LEAF BEETLE, *Chrysomela scripta* F. (Coleoptera: Chrysomelidae), is one of the most serious defoliators of cottonwood and poplar (*Populus* spp.) throughout the United States (Burkot & Benjamin 1979, Harrell et al. 1981). This multivoltine defoliator threatens the short-rotation, intensive culture of hybrid poplar throughout its range (Harrell et al. 1982). Larvae and adults consume the new, succulent stem and foliar growth, and severely deform nursery and plantation trees. Defoliation of poplars during the first and second year after planting also reduces growth rates and survival because of increased weed competition (Head et al. 1977). The potential for loss necessitates insecticide treatments at regular intervals (Page & Lyon 1976). The development of microbial-based insecticides for coleopteran control will provide tree growers with an alternative to synthetic insecticides that is safer and more compatible with other biotic control factors.

Bacillus thuringiensis, a soil-dwelling bacterium, produces an insecticidal protein crystal within the bacterial cell during sporulation. The crystal protein, known as δ-endotoxin, is the primary active ingredient of *B. thuringiensis* formulations. Ingestion of δ-endotoxin by susceptible insects results in gut paralysis and feeding inhibition, followed by disruption of midgut epithelial cells and, eventually, death (Fast 1981, Krieg et al. 1984).

Toxicity of *B. thuringiensis* is highly specific. Lepidopteran- and dipteran-specific isolates form the basis of several registered microbial insecticides. *B. thuringiensis* isolates that are toxic to coleopterans have been discovered (Krieg et al. 1983, Herrnstadt et al. 1986). *B. thuringiensis* var. *san diego* is the active ingredient of M-One (Mycogen Corporation, San Diego), a microbial insecticide registered for use against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say).

This paper describes a study to determine the *per os* toxicity of *B. thuringiensis* var. *san diego* in the cottonwood leaf beetle and to measure the sublethal effects on survivors.

Materials and Methods

Colony and Rearing Conditions. In 1987, cottonwood leaf beetle adults and eggs were collected from foliage of field-grown poplars in E. Lansing, Mich. These insects were used to establish a laboratory colony. Insects were reared in ventilated plastic crisper boxes (200 by 100 by 80 cm) at 24 ± 1°C with a 16:8 (L:D) photoperiod, and fresh foliage was provided every 2–3 d.

Bioassay. A bioassay was designed to measure toxicity of *B. thuringiensis* var. *san diego* for second and third (last) instars and for adults collected 1 and 25 d after adult eclosion. The bioassay procedure used immature leaves of similar phenolog-

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Table 1. Maximum-likelihood 96-h estimates[a] of the median lethal concentrations (LC_{50}) for cottonwood leaf beetle exposed to *B. thuringiensis* var. *san diego* for 96 h at 24°C

Age	Assays[b]	LC_{50}[c]	95% Fiducial limits[c]	Slope ± SE	y	Initial fresh weight ± SE (mg fw)[d]	LC_{50}/mg fw[c]
2nd instar	3	21	14–37	1.76 ± 0.30	1,065	2.95 ± 0.13	7.2
3rd instar	5	410	345–523	1.90 ± 0.25	−330	10.76 ± 0.50	38.1
1-d adult	4	1,022	494–600,000	1.04 ± 0.36	780	28.87 ± 1.28	35.4
25-d adult	2	639	461–850,000	3.26 ± 0.97	−2,560	—	—

[a] LC_{50} estimates included larval or adult mortality occurring during the 96-h exposure period.
[b] Number of assays pooled. Each replicate assay included five M-One concentrations and a sterile distilled water control applied to hybrid poplar leaves (dipped and air dried) with 24 insects/concentration.
[c] LC_{50} unit = × 10^3 CPB IU/ml sterile distilled water.
[d] n = 24 insects/stage.

ical age selected by the leaf plasticron index (Larson & Isebrands 1971). Leaves were taken from freshly cut branches of field-grown hybrid poplars (*Populus* × *euramericana* 'Eugenii').

Bacillus thuringiensis var. *san diego* used for these bioassays was formulated as M-One technical powder (lot no. 5653) and contained 50,000 Colorado potato beetle international units (CPB IUs)/mg formulation (Ferro & Gelernter 1989). Five concentrations of M-One were prepared by serial dilutions from a stock solution. These concentrations ranged from 2.5 × 10^3 to 3.75 × 10^4 CPB IUs/ml of sterile distilled water for second instars and from 5.0 × 10^4 to 6.0 × 10^6 CPB IUs/ml for third instars and adults. Sterile distilled water served as a control.

Leaves were dipped into the M-One solutions or sterile distilled water, agitated slightly for 5 s, then air dried. All insect stages were treated in groups of six individuals per single treated leaf in a Petri dish (60 mm diameter) and were allowed to feed for 96 h on the treated leaves. Each concentration was replicated four times within each assay. Following exposure to M-One, survivors within a treatment were consolidated and placed on fresh foliage with no more than six insects per Petri dish.

Lethal and Sublethal Effects. Daily mortality and length of the larval period following the treatments (duration of the second and third stadia) were monitored. Insects surviving larval bioassays were maintained until adult eclosion, frozen (within 24 h), oven dried at 70°C to a constant weight, and weighed. Beetles surviving adult bioassays were maintained on foliage for 14 d and monitored daily for mortality. The maximum-likelihood estimates of the median lethal concentrations (LC_{50}'s) were calculated for second and third instars and for 1- and 25-d-old adults. Estimates of the median lethal times (LT_{50}'s) for concentrations at and above the LC_{50} and within each age class were not significantly different. Therefore, the reported LT_{50} estimates were the result of pooled concentrations. A sample of larvae and adults was weighed before treatment to determine if the mortality response was a function of insect weight.

Statistics. Means and standard errors of larval periods and adult dry weights were calculated for each treatment. Regression analyses were used to determine the relationship between concentration and sublethal response variables. Maximum-likelihood estimates of median lethal concentrations and times were calculated using probit analysis (Finney 1971). The Statistical Analysis System was used for all statistical analyses (SAS Institute 1982).

Results

Concentration–Mortality Response. The χ^2 test for goodness of fit showed no evidence of heterogeneity within each replicate assay. The results of replicate assays within each stage were similar ($P \leq 0.05$); therefore, those data were pooled. The LC_{50} estimate for second instars after 96 h was 21,000 CPB IUs/ml water (Table 1). The LC_{50} estimate

Table 2. Maximum-likelihood estimates[a] of the median lethal concentrations (LC_{50}) for cottonwood leaf beetle exposed to *B. thuringiensis* var. *san diego* for 96 h at 24°C

Age	Assays[b]	LC_{50}[c]	95% Fiducial limits[c]	Slope ± SE	y
2nd instar	3	10	3–15	1.67 ± 0.26	1,415
3rd instar	5	220	198–244	2.80 ± 0.26	−1,220
1-d adult	4	320	271–400	1.72 ± 0.26	90
25-d adult	2	365	298–442	2.14 ± 0.50	−565

[a] Larval period LC_{50} estimates included all mortality occurring during the larval period. Adult LC_{50} estimates included mortality during a 14-d period after start of adult treatments.
[b] Number of assays pooled. Each replicate assay included five concentrations of M-One and a sterile distilled water control applied to hybrid poplar leaves (dipped and air dried) with 24 insects/concentration.
[c] LC_{50} unit = × 10^3 CPB IU/ml sterile distilled water.
[d] n = 24 insects/stage.

Table 3. Maximum-likelihood estimates of the pooled median lethal times (LT_{50}) for cottonwood leaf beetle exposed to *B. thuringiensis* var. *san diego* for 96 h at 24°C

Age	*n*	LT_{50}[a] estimate	95% Fiducial limit	Slope estimate ± SE
2nd instar	49	2.29	2.06–2.50	5.28 ± 0.50
3rd instar	89	2.94	2.35–3.45	3.35 ± 0.36
1-d adult	98	2.84	2.66–3.00	5.50 ± 0.35
25-d adult	90	2.84	2.64–3.03	4.56 ± 0.29

[a] All concentrations at and above the LC_{50} were not significantly different and were pooled within each age class.

for the third instar was approximately 20 times higher than for the second instar (Table 1). Decreased susceptibility to M-One with larval development remained significant ($P \leq 0.05$) after the LC_{50} estimates were corrected for initial larval weight (Table 1).

Adults were also susceptible to mortality after consumption of treated foliage (Table 1). The 96-h LC_{50} for 1-d-old adults was approximately twice the LC_{50} for the last (or third) instars. Adjustment of the adult LC_{50} by their initial fresh weight indicated a response similar to third instars, on a per milligram basis (Table 1). The LC_{50} for 25-d-old adults treated with M-One was similar to the LC_{50} for newly emerged adults. Differences among slopes were not significant.

Larvae continued to die after the initial 96-h exposure to M-One. Including mortality for the entire larval period in the LC_{50} calculations reduced the LC_{50} estimates for both larval stages to half of the 96-h estimates (Table 2). Similarly, adult mortality continued for approximately 14 d after exposure (Table 2). Adult LC_{50} estimates were also reduced to half the 96-h estimates when mortality for this 14-d period was included.

The LT_{50}'s were similar for larvae and adults, with overlapping confidence limits ranging from 2.1 to 3.5 (Table 3).

Sublethal Effects. The length of the larval period and adult weight of individuals surviving M-One treatments as second instars were significantly correlated with concentration of M-One (Fig. 1). This relation was positive for larval period ($R = 0.3$, $P \leq 0.0008$) and negative for adult weight ($R = 0.4$, $P \leq 0.0002$). The low coefficients of determination result from the high variability measured for larval period and adult weight within this species. No significant lack-of-fit was determined for these models ($P \leq 0.05$). Similar sublethal effects at higher concentrations were measured for insects surviving third-instar treatments.

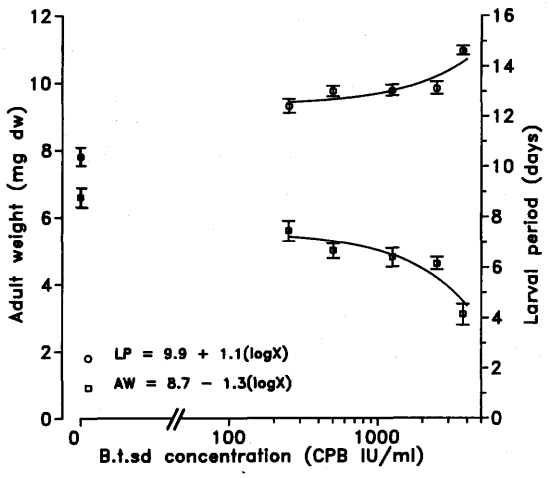

Fig. 1. Relationship of adult dry weight (mg dw) and larval period (days) after treatment of second instars with concentrations of *B. thuringiensis* var. *san diego*. Circles and squares with error bars denote $\bar{x} \pm$ SE for larval period and adult dry weight, respectively. Control data are denoted by the solid circle and square and were not included in the regression analyses.

Discussion

The discovery of *B. thuringiensis* isolates with toxic activity against coleopterans (Krieg et al. 1983, Herrnstadt et al. 1986) has stimulated much interest in identifying susceptible pest species. Several species of coleopterans have different degrees of sensitivity to *B. thuringiensis* var. *san diego*, including the Colorado potato beetle, elm leaf beetle, *Pyrrhalta luteola* (Müller), boll weevil, *Anthonomus grandis grandis* Boheman, yellow mealworm, *Tenebrio molitor* L., and the black vine weevil, *Otiorhynchus sulcatus* (F.) (Herrnstadt et al. 1986). This study reports the toxicity of *B. thuringiensis* var. *san diego* to cottonwood leaf beetle larvae and adults. The results show that the LC_{50} estimate for second instar cottonwood leaf beetle (21×10^3 CPB IU/ml) is comparable to that reported for second-instar Colorado potato beetle (10×10^3 CPB IU/ml [Ferro & Gelernter 1989]).

Decreasing susceptibility of the cottonwood leaf beetle to *B. thuringiensis* var. *san diego* with increasing larval age is similar to responses reported for many other insect species inoculated with *B. thuringiensis* and other pathogenic microorganisms. Changes in the concentration–mortality response of cottonwood leaf beetle per unit dry weight indicate that developmental factors, such as increased gut size, are responsible for age-correlated tolerance. The mode of action of *B. thuringiensis* var. *san diego* and changes associated with larval maturation need to be investigated further.

Unlike lepidopterans and dipterans treated with *B. thuringiensis*, adult and larval leaf-feeding chrysomelids occupy a similar niche. The number of cottonwood leaf beetle generations ranges from three to five in the northern United States (Burkot & Benjamin 1979) and from six to seven in the south (Head & Neel 1973). Generations overlap, and the long-lived cottonwood leaf beetle adults of previous generations feed alongside larvae on emergent *Populus* leaves and shoots. Therefore, a

foliar treatment of *B. thuringiensis* that is toxic to larvae and adults is highly desirable.

Unlike synthetic insecticides, the selective nature of *B. thuringiensis* allows insect parasitoids and predators to be retained in the environment. I observed that cottonwood leaf beetle adults and larvae placed on treated foliage ceased to feed and began to wander. This may disrupt the gregarious feeding that occurs during the first- and second-larval stadia, creating a greater opportunity for predators and parasitoids to exert control. In addition, the prolonged larval development times and lower adult weights found in survivors may allow more indirect mortality and lower fecundity. Such indirect mortality factors after applications of *B. thuringiensis* var. *san diego* in the field may explain the successful suppression of cottonwood leaf beetle populations during aerial treatment of 2 ha of *Populus* at a rate of 9.3 liter M-One/ha (2.1×10^{11} CPB IUs/ha) in York, Pa., during the 1988 field season (P. G. Bystrak, personal communication).

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