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 (LC₉₀) 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 LC₉₀ estimates for the third instars and adults were 20 and 40 times higher than for second instars, respectively. Larval LC₉₀ estimates were halved when mortality for the entire larval period was included in the LC₉₀ estimate. Adult mortality continued for approximately 14 d after initial exposure to B. thuringiensis var. san diego. The adult LC₉₀ calculated at 14 d was half the 4-d estimate. Age of adults at treatment did not significantly affect the LC₉₀. Median lethal times (LTₕ) 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-
The bioassays were formulated as M-One technical powder (lot no. 5653) and contained 50,000 Colorado potato beetle international units (CPB) per mg. Freshly cut branches of field-grown hybrid poplars were dipped and 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 treatment was replicated four times within each assay. Foliar treatments (duration of the second and third stadia) were monitored. Insects surviving larval bioassays were 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 $x^2$ 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 $L_{C50}$ estimate for second instars after 96 h was 21,000 CPB IUs/ml water (Table 1). The $L_{C50}$ estimate included all mortality occurring during the larval period. Adult $L_{C50}$ estimates included mortality during a 14-d period after start of adult treatments.

### Table 1. Maximum-likelihood 96-h estimates\(^a\) of the median lethal concentrations ($L_{C50}$) for cottonwood leaf beetle exposed to *B. thuringiensis* var. *san diego* for 96 h at 24°C

<table>
<thead>
<tr>
<th>Age</th>
<th>Assays(^b)</th>
<th>$L_{C50}$(^c)</th>
<th>95% Fiducial limits(^c)</th>
<th>Slope ± SE</th>
<th>$y$</th>
<th>Initial fresh weight ± SE (mg fw)(^d)</th>
<th>$L_{C50}$/mg fw(^e)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd instar</td>
<td>3</td>
<td>21</td>
<td>14–37</td>
<td>1.76 ± 0.30</td>
<td>1.065</td>
<td>2.95 ± 0.13</td>
<td>7.2</td>
</tr>
<tr>
<td>3rd instar</td>
<td>5</td>
<td>410</td>
<td>345–523</td>
<td>1.90 ± 0.25</td>
<td>-530</td>
<td>10.76 ± 0.50</td>
<td>38.1</td>
</tr>
<tr>
<td>1-d adult</td>
<td>4</td>
<td>1,022</td>
<td>494–600,000</td>
<td>1.04 ± 0.36</td>
<td>780</td>
<td>28.87 ± 1.28</td>
<td>35.4</td>
</tr>
<tr>
<td>25-d adult</td>
<td>2</td>
<td>639</td>
<td>461–850,000</td>
<td>3.26 ± 0.97</td>
<td>-2,560</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

\(a\) $L_{C50}$ 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.

\(c\) $L_{C50}$ unit = $\times 10^3$ CPB IU/ml sterile distilled water.

\(d\) $n = 24$ insects/stage.

### Table 2. Maximum-likelihood estimates\(^a\) of the median lethal concentrations ($L_{C50}$) for cottonwood leaf beetle exposed to *B. thuringiensis* var. *san diego* for 96 h at 24°C

<table>
<thead>
<tr>
<th>Age</th>
<th>Assays(^b)</th>
<th>$L_{C50}$(^c)</th>
<th>95% Fiducial limits(^c)</th>
<th>Slope ± SE</th>
<th>$y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd instar</td>
<td>3</td>
<td>10</td>
<td>5–15</td>
<td>1.67 ± 0.26</td>
<td>1.415</td>
</tr>
<tr>
<td>3rd instar</td>
<td>5</td>
<td>220</td>
<td>198–244</td>
<td>2.80 ± 0.26</td>
<td>-1,220</td>
</tr>
<tr>
<td>1-d adult</td>
<td>4</td>
<td>320</td>
<td>271–400</td>
<td>1.72 ± 0.26</td>
<td>90</td>
</tr>
<tr>
<td>25-d adult</td>
<td>2</td>
<td>565</td>
<td>288–442</td>
<td>2.14 ± 0.50</td>
<td>-565</td>
</tr>
</tbody>
</table>

\(a\) Larval period $L_{C50}$ estimates included all mortality occurring during the larval period. Adult $L_{C50}$ 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.

\(c\) $L_{C50}$ unit = $\times 10^3$ CPB IU/ml sterile distilled water.

\(d\) $n = 24$ insects/stage.
Table 3. Maximum-likelihood estimates of the pooled median lethal times (LT50) for cottonwood leaf beetle exposed to B. thuringiensis var. san diego for 96 h at 24°C

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>LT50a estimate</th>
<th>95% Fiducial limit</th>
<th>Slope estimate ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd instar</td>
<td>49</td>
<td>2.29</td>
<td>2.06-2.50</td>
<td>5.28 ± 0.50</td>
</tr>
<tr>
<td>3rd instar</td>
<td>89</td>
<td>2.94</td>
<td>2.35-3.45</td>
<td>3.35 ± 0.36</td>
</tr>
<tr>
<td>1-d adult</td>
<td>98</td>
<td>2.54</td>
<td>2.66-3.00</td>
<td>5.50 ± 0.35</td>
</tr>
<tr>
<td>25-d adult</td>
<td>90</td>
<td>2.54</td>
<td>2.64-3.03</td>
<td>4.56 ± 0.29</td>
</tr>
</tbody>
</table>

a All concentrations at and above the LC50 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 ≤ 0.05) after the LC50 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 LC50 for 1-d-old adults was approximately twice the LC50 for the last (or third) instars. Adjustment of the adult LC50 by their initial fresh weight indicated a response similar to third instars, on a per milligram basis (Table 1). The LC50 for 25-d-old adults treated with M-One was similar to the LC50 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 LC50 calculations reduced the LC50 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 LC50 estimates were also reduced to half the 96-h estimates when mortality for this 14-d period was included.

The LT50'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 ≤ 0.0008) and negative for adult weight (R = 0.4, P ≤ 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 ≤ 0.05). Similar sublethal effects at higher concentrations were measured for insects surviving third-instar treatments.

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, Otiorrhyncus 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 LC50 estimate for second instar cottonwood leaf beetle (21 × 10^6 CPB IU/ml) is comparable to that reported for second-instar Colorado potato beetle (10 × 10^9 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-instar 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 x 10⁴ CPB IU/s/ha) in York, Pa., during the 1988 fall season (P. G. Bystrak, personal communication).

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