Laboratory and Field Evaluations of Two *Bacillus thuringiensis* Formulations, Novodor and Raven, for Control of Cottonwood Leaf Beetle (Coleoptera: Chrysomelidae)

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ABSTRACT Laboratory and field experiments were conducted to determine the efficacy of two *Bacillus thuringiensis* Berliner formulations, Novodor and Raven, for controlling cottonwood leaf beetle, *Chrysomela scripta* F. (Coleoptera: Chrysomelidae) . In laboratory bioassays, larvae or adults were added to petri dishes containing *Populus* X *euramericana* Guinier 'Eugenei' foliage that had been treated with distilled water (control) or one of the commercial Bt formulations at either high or low label rates. Survival was recorded on a 24-h basis, and leaf area consumed was measured at the conclusion of all trials. Significant differences from the control in mortality and leaf area consumption resulted in the Novodor and Raven treatments for all life stages tested; however, adults were better able to withstand the effects of *B. thuringiensis* toxins than were the immatures. Early- and late instar C. *scripta* populations were monitored in the field (1998 and 1999) after treatment with either water or various concentrations of one of the commercial Bt formulations. Significant mortality resulted with all concentrations and for all life stages tested compared with the control (tap water). The commercial formulations also were tested under plantation conditions as part of a long-term defoliation study. Both Novodor and Raven reduced cottonwood leaf beetle defoliation damage after a single application, giving high efficacy for control of cottonwood leaf beetle under the conditions and concentrations evaluated.

KEY WORDS *Chrysomela scripta*, cottonwood leaf beetle, *Bacillus thuringiensis*, defoliation, mortality, short-rotation woody crops

As WE NEAR the 21st century, additional fuel and energy resources are needed to accommodate the world's growing population and industry. Short-rotation woody crop systems may provide a partial answer to these demands. Intensively managed monocultures of trees can produce large amounts of woody biomass for products or biofuels in a short time. *Populus* spp. show excellent potential for use in these systems because of their high biomass yield, regeneration ability, and well-developed agronomic techniques (Dickman and Stuart 1983, Zsuffa et al. 1996, Bauer 1997).

Cottonwood leaf beetle, *Chrysomela scripta* F. (Coleoptera: Chrysomelidae) is a major defoliator of young plantation *Populus* (Abrahamson et al. 1977, Head et al. 1977, Burkot and Benjamin 1979). Both larvae and adults prefer feeding on young, succulent leaf tissue (Bingaman and Hart 1992). A large proportion of young short-rotation woody crop *Populus* plantations is composed of trees with a higher percentage of preferred leaf tissue, and these trees are most susceptible to cottonwood leafbeetle damage during the first 3 yr of growth (Bingaman and Hart 1992, Augustin et al. 1997). Defoliation can result in reduced vigor and growth rate, increased susceptibility to insect and pathogen damage, or death of the individual terminal shoot or entire tree (Bassman et al. 1982).

Carbofuran (Abrahamson et al. 1977), chlorpyrifos (Page and Lyon 1976), and carbaryl (James et al. 1999) have been used effectively for controlling cottonwood leaf beetle. However, chemical control is costly, can damage the environment, and is often looked upon negatively by the consumer or general public. Biorational control methods are both more preferred and accepted.

Cottonwood leaf beetle has shown susceptibility to various *Bacillus thuringiensis* Berliner (Bt) δ-endotoxins (Bauer and Pankratz 1992, Frederici and Bauer 1998, James et al. 1999), but few commercial formulations have been tested according to label application rates and directions. From an economical standpoint, it is necessary to know whether the products available to the consumer will work under field conditions. Although a toxic Bt gene is currently -being evaluated in genetically engineered *Populus* (Bauer 1997), these transgenic trees may not be available for some time, and certainly should not be relied upon for all plantings. Plantation managers and growers need more immediate control measures for C. *scripta*. Therefore,

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our objectives were to test the efficacy of two commercially available Bt formulations, Novodor and Raven, for control of cottonwood leaf beetle. These formulations were evaluated according to label rates and directions.

Materials and Methods

Insects. All laboratory experiments (with the exception of the egg mass studies) used beetles from a laboratory colony started from adults received from Leah S. Bauer (USDA Forest Service, East Lansing, MI). Beetles were reared in ventilated plastic crisper containers (27 by 19 by 9 cm) with a photoperiod of 16:8 (L:D) h at a 24:18°C temperature regime. Colonies were fed greenhouse-grown *Populus X euramericana* Guinier 'Eugenei' foliage. Foliage was replaced daily. Field experiments and egg mass studies used native Iowa cottonwood leaf beetle populations. Eugenei leaves with egg masses attached to them were picked in the field and brought immediately to the laboratory for treatment.

***Bacillus thuringiensis* Formulations.** Novodor (15,000 Leptinotarsa units [LTU] /g formulation, 3% [AI] *B. thuringiensis* subsp. *tenebrionis*, Cry3Aa toxin [Abbott Laboratories, North Chicago, IL]) was one commercial formulation used. This product contained both spores and toxin, and was registered for use on Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) and elm leaf beetle, *Pyrrhalta luteola* (Müller) (Coleoptera: Chrysomelidae). The label cited potatoes, tomatoes, and eggplant as the host range of *L. decemlineata*. The host range listed for *P. luteola* included shade trees and ornamentals.

Raven (1,300,000 LTU/g formulation, 10% [AI] *B. thuringiensis* subsp. *kurstaki*, Cry1Ac toxin [Ecogen, Langhorne, PA]) was the second formulation evaluated, and also contained both spores and toxin. This strain contained a coleopteran active toxin (8% [AI]) that worked synergistically with a lepidopteran active toxin (2% [AI]) . This product was registered for use on *L. decemlineata*, whose host range also was listed as potatoes, tomatoes, and eggplant.

Laboratory Trials. For all trials, six cottonwood leaf beetle life stages were tested: egg masses, neonate (<24 h posthatch), second instar (4 d old), third instar (6 d old), newly emerged adult (<24 h postemergence), and mature adult (10 d postemergence). Mature adults were reared using Eugenei leaves from pupal emergence until experimental trials. Whole Eugenei leaves of the same phenological development stage (e.g., leaf plastochron index [LPI] = 3, Larson and Isebrands 1971); were used for the larvae and adults because leaves at this stage are the most highly preferred for feeding (Bingaman and Hart 1992). Egg masses or leaves were treated with a hand-held Plant & Garden Sprayer (Sprayco, Detroit, MI) rather than dipped because a protocol simulating field-like application was desired. Novodor treatments included 1.25 and 5.00% solutions (125 and 500 mg Novodor, respectively, in 1 liter of distilled water). Distilled water served as the control. These solutions represented the range of recommended label application rates (1.25% = 2.34 liter/ha [1 qtlacre], 5.00% = 9.36 liter/ha [4 qt/acre]) for light or single-aged cottonwood leaf beetle infestations. Raven treatments included 0.625% (62.5 mg Raven in 1 liter of distilled water) and 3.75% (375 mg Raven in 1 liter of distilled water) solutions, and a distilled water control. These solutions also represented the range of recommended label application rates (0.625% = 1.17 liter/ha [0.5 qt/acre], 3.75% = 7.02 liter/ ha [3 qt/acre]) . One spray was applied per leaf side and the solution was allowed to air-dry for 15 min. Leaves, along with egg masses, larvae (five neonate or second instars, or three third instars), or three adults were placed in petri dishes with moistened Whatman #1 filter paper (Whatman, Hillsboro, OR). Filter paper was moistened daily with distilled water to prevent leaf and insect desiccation. Neonate, second, and third instars did not receive new leaves throughout the experiment. New, untreated leaves were added to petri dishes containing both freshly emerged and mature adults every 48 h if there were any surviving insects. New leaf tissue was added because the control insects had nearly run out of leaf material at this time. Fifteen replications were included for all treatments and the control, and were arranged in a randomized complete block design inside a growth chamber under a photoperiod of 16:8 (L:D) h and 24:18°C temperature regime.

Mortality was recorded at 24, 48, 72, 96, and 168 h after application, or until 100% mortality occurred in the Bt treatments. Third-instar pupation and adult emergence were recorded and compared with that of the control. Total leaf area consumed (cm') was recorded at the conclusion of all larval and adult trials using a Delta T area meter (Decagon Devices, Pullman, WA).

1998 Field Trials. Field experiments were conducted near the Iowa State University Institute for Physical Research and Technology (IPRT) , Ames, IA, on a mixture of 1-yr-old hybrid poplar (*Populus* spp.) clones. Novodor treatments included 5.00% (500 mg Novodor in 1 liter of tap water) and 2.50% (250 mg Novodor in 1 liter of tap water) solutions and tap water alone (control). These Novodor solutions represented the range of recommended label application rates (2.50% = 4.68 liter/ha [2 qtlacre], 5.00% = 9.36 liter/ha [4 qt/acre]) for heavy or mixed (both larvae and adults present) populations of cottonwood leaf beetles. Novodor was applied with a backpack sprayer (Solo, Newport News, VA) at 40 psi over the entire tree to the point of wetting. Ten replications (one C *scripta* larval cohort each) were included for each treatment. Five of the 10 replications used first instar populations and the other five replications used second or third instar populations, or a combination of second and third instar populations.

In addition to the field efficacy trials, Novodor was evaluated under field conditions as part of an ongoing study designed to compare the effects of cottonwood leaf beetle defoliation on the long-term (6-8 yr) growth of four hybrid poplar clones. Using a split plot

Table 1. Larval hatch (mean ± SE) and mortality of cottonwood leaf beetle larvae 24 and 48 h after treatment (mean ± SE) in laboratory studies where egg masses were treated

Formulation	Conc, %	Larval hatch, %	Mortality at 24 h after treatment, %	Mortality at 48 h after treatment, %
Novodor	Control (distilled water)	85.20 ± 0.24	1.80 ± 0.01	13.29 ± 0.95
	1.25	82.35 ± 0.27	88.14 ± 0.45	100.0 ± 0.00
	5.00	85.28 ± 0.14	85.34 ± 0.62	100.0 ± 0.00
Raven	Control (distilled water)	83.64 ± 0.95	0.51 ± 0.00	6.56 ± 0.27
	0.625	79.88 ± 0.68	82.17 ± 1.35	100.0 ± 0.00
	3.75	80.06 ± 0.45	83.31 ± 1.35	100.0 ± 0.00

design, we sprayed half of each plot with Novodor to restrict defoliation and the other half was not sprayed. Each clone was planted in April 1998 in 32-tree plots (a 10-m grass strip and two rows of border trees separated the two halves of the plot) and replicated in five blocks. The day before the application of Novodor, all trees within both the control and treated treatments were rated for cottonwood leaf beetle damage. Damage ratings were 0, no detectable amount of defoliation on LPI 1-8; 1, >33% defoliation; 2, 33–50% defoliation; 3, 50–75% defoliation: 4, >75% defoliation and feeding damage to shoot (Fang 1997).

Damage ratings were nearly identical before treatment (mean ± SE treated plot was 0.58 ± 0.32 compared with an average rating of 0.62 ± 0.32 for the control). Approximately 40 trees that had larval populations present were flagged in both treatments to allow postapplication evaluation. Larval populations consisted of a mixture of early and late instars. Adult beetles and egg clutches also were observed on trees. A 1.50% Novodor solution (1.50% = 3.5 liter/ha [1.5 qt/acre]) was applied with a Solo backpack sprayer to all trees within the treated plots on 31 July 1998. Flagged trees were observed 72 and 96 h *after* application for larval survival, Damage ratings were again conducted on all trees on 6 August 1998.

1999 Field Trials. Protocol followed that used in 1998. Field experiments were conducted near the IPRT on a mixture of 2-yr-old hybrid *Populus* clones between 2-5 June 1999. Novodor treatments included 5.00% and 2.50% solutions and a tap water control. Raven treatments included 3.75 and 1.25% (375 and 125 mg Raven, respectively, in 1 liter of tap water) solutions and a tap water control. These Raven treatments represent the recommended label application rates for heavy infestations (1.25% = 2.34 liter/ ha [1 qt/acre], 3.75% = 7.02 liter/ha [3 qt/acre]). The Bt formulations were applied with a Solo backpack sprayer at 40 psi over the entire tree to the point of wetting. Five replications of both early (first) and late (second and third) instars were included for each treatment and control. Populations were checked for mortality at 24, 48, and 72 h after treatment.

Raven also was evaluated under field conditions in the long-term study planting. Protocol and area sprayed were identical to that in 1998. The area to receive the Raven treatment had a lower initial defoliation rating (1.55 ± 0.62) compared with the control area (1.99 ± 0.65). A 1.25% Raven solution (1.25% = 2.34 liter/ha [1 qt/acre]) was sprayed with a 113.6-liter (30-gal) sprayer (Fimco, Sioux City, IA) to all trees within the treated plots on 22 June 1999. Approximately 40 early-instar larval populations or egg masses were flagged to allow for posttreatment evaluation at 72 and 96 h after application. Damage ratings were again taken on all trees on 29 June 1999.

Statistical Analysis. Differences in mortality at 24, 48, 72, 96, and 168 h after treatment (when applicable) in laboratory and field trials were evaluated by a two-way analysis of variance. Means were separated using the Tukey honestly significant difference (HSD) test. Differences in mortality between Bt treatments and total leaf area consumed from the laboratory study were examined by Student t-test ($\alpha = 0.05$). Percentage of larval hatch was analyzed by visual comparison of means. Field damage ratings were analyzed by a split-plot analysis (SAS Institute 1998).

Results

Laboratory Trials. *Egg Masses.* Although percent larval hatch was not affected by the Bt treatments, 24 h after treatment neonate mortality was significant for both Novodor ($F = 131.73$; $df = 2, 14$; $P < 0.001$) and Raven ($F = 582.07$; $df = 2, 14$; $P < 0.001$). Larval mortality was >85% in Bt treatments compared with <15% in the control (Table 1). One hundred percent larval mortality occurred by 48 h after treatment for all Bt concentrations tested.

Mortality. Total larval and adult mortality was significantly higher for all Novodor and Raven treatments compared with controls (Table 2). Significant differences in mortality were found between high and low concentrations of Novodor at 24, 48, and 72 h after application in the second-instar trials, at 72 and 96 h after application in the third-instar trials, and at 72, 96, and 168 h after application in the mature adult trials ($t = 2.02$, $df = 39$, $P < 0.05$). Other Novodor treatments showed no significant differences between high and low concentrations at any time. Mortality differences in second instars exposed to high and low concentrations of Raven were also significant at 24 h after application ($t = 2.02$, $df = 39$, $P < 0.05$). All other Raven treatments showed no significant differences between concentrations at any time.

Pupation. No third instars successfully pupated in either Novodor treatment, whereas 39 (87%) pupated in the control, and all of these pupae survived to adult emergence. One successful pupation occurred in each

Table 2. Larval and adult cottonwood leaf beetle mortality under laboratory conditions

Formulation	Treatment (% formulation)	Lifestage	Total mortality, %				
			Hours after treatment				
			24	48	72	96	168
Novodor	Control	Neonate	4.0a	6.7a	NA	NA	NA
		Second instar	0.0a	0.0a	1.3a	1.3a	NA
		Third instar	0.0a	0.0a	2.2a	4.4a	13.3a
		Teneral adult	0.0a	0.0a	0.0a	0.0a	0.0a
		Mature adult	0.0a	0.0a	0.0a	0.0a	0.0a
	1.25	Neonate	97.3b	100.0b	NA	NA	NA
		Second instar	34.61,	74.6b	89.3b	100.0b	NA
		Third instar	2.2a	55.6b	75.61,	77.8b	100.0b
		Teneral adult	0.0a	2.2a	24.4b	57.8b	68.9b
		Mature adult	0.0a	2.2a	4.4a	6.7a	13.3a
	5.00	Neonate	100.0b	100.0b	NA	NA	NA
		Second instar	74.6c	100.0c	100.0c	100.0b	
		Third instar	6.7a	75.61,	91.1c	95.6c	1 %
		Teneral adult	0.0a	4.4a	24.4b	57.8b	71.1b
		Mature adult	0.0a	8.9a	44.4b	48.8b	53.3b
Raven	Control	Neonate	2.7a	NA	NA	NA	NA
		Second instar	0.0a	2.7a	NA	NA	NA
		Third instar	0.0a	0.0a	2.2a	NA	NA
		Teneral adult	0.0a	0.0a	0.0a	0.0a	0.0
		Mature adult	0.0a	0.0a	0.0a	2.2a	4.4a
	0.625	Neonate	100.0b	NA	NA	NA	NA
		Second instar	86.7b	100.0b	NA	NA	NA
		Third instar	13.3ab	91.1b	97.8b	NA	NA
		Teneral adult	2.2ab	86.7b	97.8b	100.0b	NA
		Mature adult	0.0a	28.9b	57.8b	68.9b	88.9b
	3.75	Neonate	100.0b	NA	NA	NA	NA
		Second instar	96.0c	100.0b	NA	NA	NA
		Third instar	17.8b	95.61,	97.8b	NA	NA
		Teneral adult	13.3b	86.71,	100.0b	100.0b	NA
		Mature adult	0.0a	40.0b	66.7b	77.8b	88.9b

Percentages within a formulation X treatment X lifestage followed by the same letter are not significantly different ($P \leq 0.05$, Tukey HSD). Neonate and second instar trials used 75 insects per treatment (15 repetitions, 5 insects each); third-instar and adult trials used 45 insects per treatment (15 repetitions, 3 insects each). Experiments were terminated when all insects exposed to one of the Bt formulations were dead.

of the Raven treatments compared with 44 (98%) in the control. One adult cottonwood leaf beetle emerged in the high Raven concentration, but died within 24 h. The pupa in the low Raven concentration did not emerge. Of the 44 successful pupations in the control, 42 (95%) emerged.

Leaf Significant differences in leaf area consumed occurred in all larval and adult trials ($P < 0.05$). Reductions in mean leaf area consumed per larva ranged from 94 to 99%, and reductions in leaf area consumed by immature or mature adults ranged from 42 to 82% for both Novodor and Raven treatments (Tables 3 and 4).

1998 Field Trials. Significantly higher mortality occurred in Novodor treatments compared with the control (Table 5). Mean mortality of early instars was significantly higher in Novodor treatments than in the control ($F = 12.99$; df $= 2, 4$; $P < 0.004$) at 24 h after treatment. Late instar mortality was significantly higher in the 2.50% Novodor treatment compared with the control or the 5.00% Novodor treatment ($F = 5.93$; df $= 2, 4$; $P < 0.025$). At 48 h after treatment, both Novodor treatments showed significantly higher mortality than the control ($F = 18.57$; df $= 2, 4$; $P < 0.001$). Larvae exposed to the Novodor treatments stopped feeding within 24 h and wan-

Table 3. Mean leaf area consumed (cm^2) (*SE) per larval or adult cottonwood leaf beetle for all Novodor treatments in the laboratory study

Lifestage	*n*	C. *scripta*/dish	Trial length, h	Mean leaf area consumed per insect		
				Control (distilled water)	Novodor concn	
					0.0125	0.05
Neonate	15	5	48	1.96 ± 3.08a	0.04 ± 0.01b	0.01 ± 0.01b
2nd instar	15	5	96	7.34 ± 7.36a	0.18 ± 0.02b	0.11 ± 0.02b
3rd instar	15	3	168	6.35 ± 5.70a	0.33 ± 0.08b	0.27 ± 0.14b
Teneral adult	15	3	168	13.64 ± 9.38a	2.91 ± 2.72b	2.35 ± 1.38b
Mature adult	15	3	168	13.61 ± 10.56a	7.90 ± 5.13ab	3.91 ± 1.26b

Values within a row followed by the same letter are not significantly different ($P < 0.05$, Tukey HSD)

Table. 4. Mean leaf area consumed (cm*) (±SE) per larval or adult cottonwood leaf beetle for all Raven treatments in the laboratory study

Lifestage	*n*	*C. scripta*/dish	Trial length, h	Mean leaf area consumed per insect		
				Control (distilled water)	Raven concn 0.00625	Raven concn 0.0375
Neonate	15	5	24	0.88 ± 0.62"	0.01 ± 0.01b	0.01 ± 0.01b
2nd instar	15	5	48	2.75 ± 2.09a	1.12 ± 0.43b	1.01 ± 0.47b
3rd instar	15	3	72	4.96 ± 2.84a	1.53 ± 0.68b	1.04 ± 0.44b
Teneral adult	15	3	96	11.36 ± 7.51"	0.71 ± 1.06b	0.82 ± 0.39b
Mature adult	15	3	168	23.88 ± 10.28a	4.27 ± 3.98b	2.32 ± 1.91b

Values within a row followed by the same letter are not significantly different ($P < 0.05$, Tukey HSD).

dered considerably more than control larvae. Larvae in the Novodor treatments also turned black within 24 h after treatment and did not molt to the next instar.

The application of Novodor in the defoliation study greatly reduced cottonwood leaf beetle defoliation damage. The damage rating (mean ± SE) of trees in the treated plots was 0.41 ± 0.36 compared with 1.74 ± 0.52 for the control treatment. Defoliation in the Novodor treatment decreased slightly after 1 wk, but was not significantly different from the initial rating. Defoliation ratings on the untreated trees were significantly higher after 1 wk ($F = 27.16$; df = 1, 4; $P < 0.001$). Defoliation on trees that had been sprayed with Novodor was probably caused by a combination of adult feeding, a limited number of late instars that survived the spray treatment, and larvae that hatched after the treatment was initiated. Based on observations of trees that had been flagged, if larvae were still alive 72 h after application, they typically were wandering, not feeding, and black.

1999 Field Trials. Both Novodor and Raven showed excellent control of cottonwood leaf beetle larvae (Table 6). Significant differences in early-instar mortality occurred 24 h after treatment in both the Novodor ($F = 24.12$; df = 2, 4; $P < 0.001$) and Raven ($F = 48.43$; df = 2, 4; $P < 0.001$) treatments. Nearly 100% mortality occurred in all Novodor or Raven treatments after 48 h. For tests using late instars, significant differences in mortality resulted 24 h after treatment among each concentration tested in all Novodor ($F = 29.47$; df = 2, 4; $P < 0.001$) and Raven ($F = 7.11$; df = 2, 4; $P < 0.015$) treatments. As in 1998, mortality differences were significant between treated beetles and control beetles in both the Novodor ($F = 63.88$; df = 2, 4; $P < 0.001$) and Raven ($F = 66.28$; df = 2, 4; $P < 0.001$) treatments by 48 h after application.

Raven greatly reduced cottonwood leafbeetle damage in the long-term field study. Trees in the Raven treatment had a significantly lower damage rating (1 ± 0.23) at 1 wk posttreatment compared with the pretreatment defoliation ratings ($F = 29.1$; df = 1, 4; $P < 0.001$). Furthermore, damage ratings in the control plots increased significantly to 3.13 ± 0.89 without the Raven application ($F = 26.95$; df = 1, 4; $P < 0.001$). Any surviving larvae in the treated plots after 72 h were typically black, not eating, and had an unhealthy appearance.

Additional Observations. Laboratory observations indicated that Novodor may have a phytotoxic effect on leaves at solutions ≥ 50.0% (e.g., leaf margins turned brown after 24 h), and Raven solutions of ≥ 3.75% may have this effect on small leaves ($<25\ cm^2$). Larvae in Novodor and Raven treatments wandered considerably more than those in the control in both laboratory and field studies. Initial feeding seemed to have stopped after 24 h in all trials, and larvae never resumed feeding. Only after additional untreated leaves were added did feeding resume for the adults in the laboratory studies.

Discussion

The cottonwood leafbeetle is susceptible to various strains of Bt (Frederici and Bauer 1998, James et al. 1999) and can be controlled effectively with Novodor

Table 5. Cottonwood leaf beetle early-instar mortality (mean percentage ±SE) resulting from 1998 Novodor and 1999 Novodor and Raven field evaluations

Treatment	Formulation, %	Time (h) after treatment 24	48	72
1998-Novodor	control	2.7 ± 0.00	19.1 ± 0.10	23.7 ± 0.09
	2.50	42.1 ± 0.04	99.0 ± 0.00	99.0 ± 0.00
	5.00	71.6 ± 0.36	100.0 ± 0.00	100.0 ± 0.00
1999-Novodor	Control	5.9 ± 0.05	12.3 ± 0.04	21.6 ± 0.07
	2.50	62.7 ± 0.01	98.5 ± 0.60	100.0 ± 0.06
	5.00	81.2 ± 1.44	106.0 ± 0.00	100.0 ± 0.00
1999-Raven	Control	7.6 ± 0.05	11.0 ± 0.04	23.6 ± 0.07
	1.25	69.9 ± 0.12	99.0 ± 0.00	100.0 ± 0.00
	3.75	88.1 ± 0.04	100.0 ± 0.00	100.0 ± 0.00

Table 6. Cottonwood leaf beetle late-instar mortality (mean percentage ±SE) resulting from 1998 Novodor and 1999 Novodor and Raven field evaluations

Treatment	Formulation, %	Time (h) after treatment 24	48	72
1998-Novodor	Control	10.6 ± 0.03	39.7 ± 0.21	57.1 ± 0.31
	2.50	40.2 ± 0.23	94.5 ± 0.01	96.9 ± 0.01
	5.00	15.2 ± 0.08	94.4 ± 0.03	98.0 ± 0.01
1999-Novodor	Control	14.0 ± 0.10	31.1 ± 0.12	44.4 ± 0.14
	2.50	56.7 ± 0.16	96.9 ± 0.01	98.5 ± 0.00
	5.00	76.4 ± 0.02	99.3 ± 0.00	99.3 ± 0.00
1999-Raven	Control	20.2 ± 0.29	28.2 ± 0.25	41.2 ± 0.41
	1.25	51.1 ± 0.18	92.5 ± 0.03	94.5 ± 0.01
	3.75	78.9 ± 0.25	98.0 ± 0.00	99.2 ± 0.00

and Raven. However, visual analyses of mean leaf area consumed and time to significant mortality suggest that Raven may be 'more effective and quicker in controlling cottonwood leafbeetle than Novodor. The two formulations were applied at the range of recommended label rates; these rates differed for the two formulations. The percentage of coleopteran-active toxin present within the formulations was much higher in Raven than in Novodor. Efficacy differences in our study were not great between formulation concentrations, yet the concentrations applied were not low. These recommended label rates may contain more toxin than necessary to give significant mortality. Significant differences in mortality have been gained with lower concentrations of Bt formulation than recommended on the label (Zehnder and Celernter 1989, Bauer 1990). Differences between treatments in mortality and time to significant mortality may be magnified when extremely low amounts of formulations are applied.

Novodor contains *B. t. tenebrionis*, a coleopteran-specific toxin, whereas Raven contains *t. kurstaki*, a lepidopteran-specific toxin. The Raven label states that this particular strain contains both coleopteran- and lepidopteran-specific toxins, and that they act synergistically in herbivore control. The additional toxin present in Raven may account for the apparent increase in efficacy of this formulation. Furthermore, a higher percentage of Raven is active toxin; this too may account for some of the differences observed.

As reported in Bauer (1990) and James et al. (1999), adult cottonwood leaf beetles were less susceptible than larvae in all trials. Presumably, this relates to the Bt mode of action. During Bt sporulation, a toxic crystal protein is produaed (Bauer and Pankratz 1992, Bauer 1997). Once ingested, this toxin is activated and binds to epithelial cells in the gut, causing cell swelling and rupture, and eventually starvation (Aronson et al. 1986, Knowles 1994). Larger (and often older) insects have guts that contain more epithelial cells than the guts of smaller, often younger insects; thus, more toxin is needed to cause sufficient cell lysis that will lead to cessation of feeding and ultimately death. Although not applicable in all insects, especially Lepidopterans (Rock and Monroe 1983, Fast and Dimond 1984, James et al. 1993, Li et al. 1995, Liu et al. 1995), it seems that this may be the *case* with C. *scripta* because we found significant differences in mature adult mortality within the low treatment of Novodor. Beginning at 72 h after application, a 1.25% Novodor solution gave significantly lower mortality than did the 5.00% Novodor solution. Only mortality in the 5.00% Novodor solution was significantly higher than in the control. However, not all Bt strains elicit this mortality pattern in *C. scripta* (James et al. 1999). Our findings may have resulted from other factors such as formulation composition.

Although not sign&ant, mortality was also higher in teneral than in mature adults. Mature adults in our study were fed untreated Eugenei leaves for 10 d before treatment. This feeding may have changed the insect's gut chemistry or pH, which could be a factor in the apparent increased tolerance to the Novodor or Raven commercial formulations. Mature adults, on average, consumed much more leaf area per beetle (both Bt treated and untreated) than did teneral adults.

Chrysomela scripta late instar mortality in the field was higher than that in the laboratory or other instars in the field. The primary reason for this is the tendency for late instars to wander as they search for a place to pupate. A number of late instars in field treatments disappeared; we attributed this disappearance to mortality. However, the fact remains that some larvae may have wondered off to pupate (or, if they can detect *B. thuringiensis* on the leaf, to find another food source) before they consumed enough treated foliage to induce mortality.

We were attempting to simulate foliar applications of commercially available Bt products in this study, whereas James et al. (1999) was trying to simulate the effects of transgenic plants that contain Bt toxin. Unlike in James et al. (1999), adult beetles in our laboratory bioassays were not continually exposed to leaves treated with or containing a Bt toxin. Furthermore, continued exposure to uniformly treated leaves rarely occurs in the field. Continued exposure would imply that a field application of pesticide covers all leaves on both sides equally. Few application machines or methods are capable of this complete coverage. We accomplished complete coverage with a backpack sprayer; however, it was exceedingly time-consuming and labor-intensive.

Our study found both commercial formulations to be highly efficacious in controlling C. *scripta*. Similar results using the toxin present in Novodor (Cry3A) were attained by James et al. (1999). However, the Cry1Ac toxin present in Raven performed better in this study than in James et al. (1999). As previously mentioned, this may have resulted from inert ingredients in the Raven formulation. Possibly, the relatively high concentration (10%) of active ingredients in Raven was suitable to provide significant C. *scripta* control. Nevertheless, this study showed effective control of *C. scripta* using two commercially available *Bacillus thuringiensis* formulations.

Timing of spray application is important, because younger C. *scripta* larvae are more susceptible to Bt toxins than are older larvae or adults (Bauer 1990, James et al. 1999). Our results showed high susceptibility of early instars to both commercial Bt formulations. Mortality in egg mass studies probably resulted from neonates consuming *B. thuringiensis* on the egg surface (Ghidiu et al. 1994), whereas first instars consumed Bt formulations on the leaf surface. Thus, maximum control should be gained when sprays are applied at a time when a high proportion of cottonwood leafbeetles present are in either the egg or early-instar stages. Also, initial defoliation ratings differed between the large-scale Novodor and Raven field studies. The Raven study was performed ≈1 mo later in the growing season than was the Novodor study. This time frame may have allowed the cottonwood leaf beetle

populations to increase more before the Raven study, causing the higher initial damage ratings.

There are many benefits to using Bt formulations as a pest control method, including high specificity, rapid degradation, and little or no harm to beneficial insects, nontarget invertebrates, or vertebrates (Croft 1990, Tabashnik 1994, Bauer 1995). However, resistance has been shown to develop in C. *scripta* (Bauer et al. 1994) and other chrysomelids, such as *Leptinotarsa decemlineata* (Say) (Whalon et al. 1993). Resistance should be managed with integrated pest management strategies that incorporate a. full suite of control methods (McGaughey and Whalon 1994; Tabashnik 1994; Alstad and Andow 1995; Bauer 1995, 1997; James et al. 1998). Field studies exploring the ability of *C. scripta* to develop resistance to *B. thuringiensis* are needed and will provide valuable information to foresters and short-rotation woody crop producers alike.

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