Bacteriological Insecticide M-One Effects on Predation Efficiency and Mortality of Adult *Coleomegilla maculata lengi* (Coleoptera: Coccinellidae)

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ABSTRACT Laboratory experiments were done to determine whether the adult *Coleomegilla maculata lengi* Timberlake was affected by ingesting M-One, a commercial formulation of *Bacillus thuringiensis* var. *san diego* used to control the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Over a period of 10 consecutive days, consumption of pollen contaminated with 200 ml M-One per liter (5.6×10^9 CPBIU per liter) had no lethal effects on adult *C. maculata*. The level of predation by adult *C. maculata* on Colorado potato beetle eggs was reduced by M-One. At the manufacturer's recommended field rate of 20 ml M-One per liter (i.e., 5.6×10^8 CPBIU per liter), the number of eggs attacked was 37.0 and 14.5% lower than the untreated eggs, respectively, 3 and 24 h after the beginning of the test. The feeding behavior and results of a water versus 200 ml M-One per liter two-choice test suggest that the decrease in food intake caused by M-One cannot be explained by a δ-endotoxin-induced paralysis of the mouth parts or midgut.

KEY WORDS Coccinellidae, *Coleomegilla maculata*, *Bacillus thuringiensis*

THE COLORADO POTATO BEETLE, *Leptinotarsa decemlineata* (Say), is the agricultural insect pest requiring the largest amount of insecticide for its control in the northeastern United States (Forgash 1985) and in Quebec (Chagnon et al. 1990). The intensive use of broad-spectrum, synthetic insecticides has led to the development of several Colorado potato beetle populations resistant to arsenicals, chlorinated hydrocarbons, carbamates, organophosphates, and pyrethroids (Forgash 1985, Martel 1987). In addition, most chemical insecticides used to control the Colorado potato beetle also have contributed to the demise of its natural enemies. Therefore, alternatives to chemical control of Colorado potato beetle populations are needed.

Bacillus thuringiensis is a gram-positive bacteria which, upon sporulation, produces a protein inclusion called the crystal, which is toxic to some insects. When the crystal is ingested by a susceptible insect, a paralysis of the insect's mouth parts and gut occurs within minutes, followed by a drop in the pH of the midgut. The insect stops feeding, and, if it does not recover, dies (Andrews et al. 1987, Gill et al. 1992). *Bacillus thuringiensis* var. *tenebrionis* (Krieg et al. 1983) and var. *san diego* (Herrnstadt et al. 1986) have proven effective in controlling Colorado potato beetle larvae (Ferro & Gelernter 1989, Zehnder & Gelernter 1989). These two strains later were found to be identical using serotype testing (de Barjac & Frachon 1990) and in more than 20 biochemical tests (Krieg et al. 1987).

Coleomegilla maculata lengi Timberlake is a polyphagous predator (Hodek 1973) commonly found in potato fields in Canada (Boiteau 1987), Rhode Island, and Michigan (Groden et al. 1990). *C. maculata* may feed on pollen (Hodek et al. 1978); aphids (Coderre et al. 1987); eggs of lepidopterans such as the fall webworm, *Hyphantria cunea* (Drury) (Warren & Tadic 1967), and the European corn borer, *Ostrinia nubilalis* (Hübner) (Hudon 1986); or eggs and larvae of *L. decemlineata* (Groden et al. 1990). Laboratory studies have shown that a single adult *C. maculata* can attack 20 eggs in 48 h (Hazzard & Ferro 1991) and eats an average of 11.2 first-instar Colorado potato beetles in 24 h (Groden et al. 1990). In the field, *C. maculata* can contribute significantly to reductions of Colorado potato beetle eggs and small larvae (Groden et al. 1990). However, many studies have shown susceptibility of *C. maculata* to chemical insecticides such as malathion, cypermethrin, carbaryl, benomyl, methyl parathion, and methomyl (Johnson 1974, Coats et al. 1979, Lecrone & Smilowitz 1980, Scott et al. 1983, Roger et al. 1991). Consequently, efforts to preserve this natural enemy

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could help reduce the use of chemical pesticides (Hazzard et al. 1991).

B. thuringiensis var. *san diego* has a potential use in biological control programs against first- and second-instar Colorado potato beetle (Zehnder & Gelernter 1989). However, its effect on *C. maculata* populations is unknown. Coleopteran susceptibility to *Bacillus thuringiensis* var. *san diego* varies considerably according to species and developmental stage. Unlike the Colorado potato beetle, third- and fourth-instars and, in some cases, adults of other chrysomelid beetles such as the spotted cucumber beetle, *Diabrotica undecimpunctata howardi* Barber, and the elm leaf beetle, *Pyrrhalta luteola* (Müller), are susceptible to this bacterium (Herrnstadt et al. 1986, 1987). Larvae and adults of the boll weevil, *Anthonomus grandis grandis* Boheman, are also susceptible to *Bacillus thuringiensis* var. *san diego* (Herrnstadt et al. 1987, 1987). In Bulgaria, 30% mortality of adult *Coccinella septempunctata* L. (Coleoptera: Coccinellidae) occurred in a potato field 5 d after a treatment with Novodor (Novo Nordisk, Denmark) (*B. thuringiensis* var. *tenebrionis*) (Mateeva et al. 1991).

The two objectives of this study were to determine whether M-One, *B. thuringiensis* var. *san diego*: (1) would kill adult *C. maculata* and (2) would affect its predation efficiency on Colorado potato beetle eggs.

Materials and Methods

Insects. Adult *C. maculata* were collected in early May 1991 from hibernation sites close to cornfields in Saint-Hyacinthe (72° 56′ W, 45° 39′ N), Quebec, Canada. They were kept in an incubator on a wild flower pollen diet at 25°C, 70% RH, and a photoperiod of 16:8 (L:D) h. These conditions were maintained throughout the experimental period. Before each experiment, the *C. maculata* were placed individually in a glass petri dish and starved for 24 h.

Eggs and larvae of the Colorado potato beetle were obtained from a stock of individuals collected at Trois-Pistoles (69° 10′ W, 48° 07′ N), Quebec, Canada, in June 1990 and reared on 'Kennebec' potato plants.

Biological Insecticide. M-One (lot number 8278560, Mycogen, San Diego, CA), a commercial formulation of *Bacillus thuringiensis* var. *san diego*, was used for all experiments. It consists of a 12.5% water-dispersible suspension containing 2.8×10^{10} Colorado potato beetle international units (CPBIU) per liter. Three dilutions were prepared containing 0.2 ml of M-One per liter (5.6×10^6 CPBIU per liter), 20 ml of M-One per liter (5.6×10^8 CPBIU per liter), and 200 ml of M-One per liter (5.6×10^9 CPBIU per liter). These represented, respectively, 0.01, 1, and 10 times the manufacturer's recommended field concentration, hereafter referred to as 0.01X, 1X, and 10X.

Pollen. Wild flower pollen was irradiated with gamma rays to kill any microorganisms. It was ground finely, and 25 g of pollen was mixed with 25 ml of M-One dilution for 2 min with a magnetic stirrer. The mixture was filtered under vacuum on Whatman paper #4 and dried under vacuum for 3 h at 60°C. The treated pollen was ground further for 90 sec. This procedure was repeated for the four treatments (the three M-One concentrations and a control containing water only).

Assessment of M-One Toxicity against Colorado Potato Beetle Larvae. Fifty early first-instar Colorado potato beetles were weighed and placed individually on a potato leaf that previously had been dipped in the recommended concentration of M-One (20 ml per liter) and air-dried for 20 min. This procedure was repeated with water for the control. The weight of the young instars used in the control and in the M-One treatment was 0.7 ± 1.1 mg and 0.7 ± 1.0 mg (mean ± SD), respectively. Mortality was recorded after 72 h. Differences in the proportion of dead larvae between the control and the M-One treatment were determined with a χ^2 test done with the software Statview (version 1.03 for Macintosh) (Abacus Concepts 1988).

Level of Predation by Adult *C. maculata* on Colorado Potato Beetle Eggs Treated with M-One. Colorado potato beetle egg masses were taken from potato plants 24 h after oviposition. Leaf sections containing 15 eggs each were dipped for 2 sec in the appropriate solution and dried in still air for 20 min.

For each treatment, 50 adult *C. maculata* of unknown sex, starved for 24 h, were presented individually with a mass of 15 treated eggs. Attacked eggs were counted after 1, 2, 3, 4, 5, 6, 7, and 24 h. The differences in the number of eggs attacked (as determined by broken chorion) between the control and the three M-One concentrations at each observation time were determined by a Fisher's protected least significant difference (LSD) test, done with the software SuperAnova (version 1.1 for Macintosh) (Abacus Concepts 1989).

Mortality of Adult *C. maculata*. After 24 h, Colorado potato beetle eggs were removed and replaced with 100 mg of pollen treated with the corresponding M-One treatment. Because adult *C. maculata* consume mostly the noncontaminated material inside the eggs, treated pollen was presented to enhance ingestion of M-One. Mortality was noted every 24 h over 10 d. A χ^2 test was conducted with the software Statview (version 1.03 for Macintosh computers) (Abacus Concepts 1988) to determine if the proportion of dead *C. maculata* differed significantly from the control and each of the three treatments.

Two-Choice Tests. After a 24-h starvation period, adult *C. maculata* were presented individually with two masses of potato beetle eggs; one was treated with either 1X or 10X M-One concentration, and the other was not treated. The number of eggs attacked per egg mass was noted after 1, 2, 3, 4, 5, 6, 7, and 24 h. The experiment was conducted using 35 adults for the 1X-control choice test and 40 adults for the 10X-control choice test. A Fisher's protected LSD test was carried out with the software SuperAnova (version 1.1 for Macintosh) (Abacus Concepts 1989) to determine if there were significant differences between the number of eggs attacked in the treated and untreated groups.

Results and Discussion

Assessment of M-One Toxicity Against Colorado Potato Beetle Larvae. The bioassays conducted with Colorado potato beetle larvae confirmed the toxicity of the M-One sample. After 72 h at 25°C, the percentage mortality of early first instars fed on potato foliage treated with M-One at the recommended field concentration (20 ml per liter) was 100%. This differed significantly from the 4% mortality obtained in the control (χ^2 = 92.31, df = 3, P = 0.0001). With 0.62 ml per liter, Zehnder & Gelernter (1989) obtained, at 27°C, after the same period of time, 32% mortality with second instars.

Level of Predation by Adult *C. maculata* on Colorado Potato Beetle Eggs Treated with M-One. There was no significant reduction in the level of predation at the 0.01X concentration compared with the control (Fisher's protected LSD test; $P > 0.05$) (Fig. 1). Three hours after the beginning of the bioassays and onward, significantly fewer eggs treated with a 1X concentration were attacked than eggs in the control (Fisher's protected LSD test; $P < 0.05$). For instance, after 3 h and 24 h, egg predation decreased by 37.0 and 14.5%, respectively. With a 10X concentration, the mean number of attacked eggs was significantly lower than in the control for all observation times (Fisher's protected LSD test; P = 0.0001). For instance, after 3 h and 24 h, predation decreased by 61.1 and 47.9%, respectively. The number of *C. maculata* that did not attack any eggs during the 24-h period increased significantly as the M-One concentrations increased (Table 1). On the contrary, the number of *C. maculata* that attacked all eggs decreased as the M-One concentrations increased (Table 1). In all treatments, *C. maculata* seemed to waste food by going from one egg to the other without emptying any of the eggs.

Mortality of Adult *C. maculata*. The mortality of adult *C. maculata* after 10 d of feeding on pollen treated with any M-One concentration never exceeded 6%. Percentage mortality at any concentration did not differ significantly from

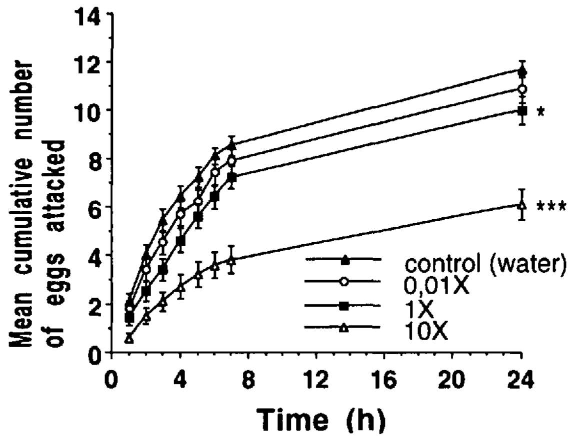

Fig. 1. Predation efficiency of adult *C. maculata lengi* on 15 *L. decemlineata* eggs treated with M-One. 1X corresponds to the manufacturer's recommended field concentration of 20 ml per liter (i.e., 5.6 × 10^8 CPBIU per liter). Vertical bars indicate standard errors; an asterisk (*) indicates 1X treatment mean is significantly different from that of the control at 3, 4, 5, 6, 7, and 24 h (Fisher's protected LSD test, P = 0.05). Three asterisks (***) indicate 10X treatment mean is significantly different from the control mean at all observation times (Fisher's protected LSD test, P = 0.001). n = 50 adult *C. maculata* per treatment.

that of the control (χ^2, df = 3, $P > 0.05$). Although some coleopteran adults, unlike the Colorado potato beetle, are susceptible to *B. thuringiensis* var. *san diego* (Herrnstadt et al. 1986, 1987), M-One does not cause adult *C. maculata* mortality, even at 10 times the manufacturer's recommended concentration.

Two-Choice Tests. A choice test was carried out to study feeding behavior and choosing process of *C. maculata*. The results suggest there was no habituation mechanism involved during the 24-h time period because the insects continued to visit both treated and untreated egg masses equally. During the 24 h, in the 1X versus control choice test, the *C. maculata* moved from one egg mass to the other on an average of at least 1.9 times, compared with an average of at least 0.9 times in the 10X versus control choice test.

Table 1. Percentage (n = 50) of adult *C. maculata* that attacked none or all of the 15 eggs treated over a 24-h period

	Control (water)	M-One concentrations[a]		
		0.01X	1X	10X
No eggs attacked	0	2ns[b]	6*	20*
All eggs attacked	16	22ns	10ns	2*

*, Significantly different from the control (χ^2, df = 3, P < 0.05).
[a] 1X corresponds to the manufacturer's recommended field concentration, i.e., 20 ml/liter.
[b] ns, not significantly different from the control.

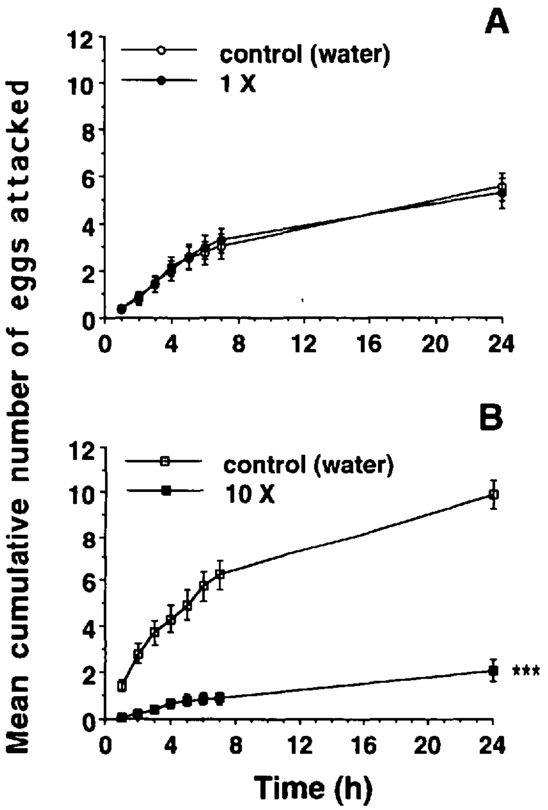

Fig. 2. Number of *Leptinotarsa decemlineata* eggs attacked by adult *Coleomegilla maculata lengi* Timberlake in a two-choice test. (A) 1X, corresponds to the manufacturer's recommended field concentration of 20 ml of M-One per liter (i.e., 5.6×10^8 CPBIU per liter), $n = 35$ adult *C. maculata*. (B) 10X, corresponds to 10 times the manufacturer's recommended field concentration of 200 ml of M-One per liter (i.e., 5.6×10^9 CPBIU per liter), $n = 40$ adult *C. maculata*. Vertical bars indicate standard errors. Three asterisks (***) indicate 10X treatment mean is significantly different from the control mean at all observation times (Fisher's protected LSD test, $P = 0.001$).

The eight *C. maculata* that attacked at least two eggs treated with a 10X concentration during the first 4 h attacked an average of 3.0 eggs (either treated or untreated) in the three subsequent hours. The 32 other *C. maculata*, who attacked fewer than two eggs treated with the 10X concentration during the first 4 h, attacked an average of 1.7 eggs (either treated or untreated) in the three subsequent hours. Because the *C. maculata* that attacked at least two eggs treated with a 10X concentration did not stop feeding and did not attack fewer eggs (either treated or untreated) during the three subsequent hours, we conclude that no δ-endotoxin-induced paralysis of the mouth parts or midgut occurred.

There was no significant preference between the eggs treated with a 1X concentration and the untreated eggs (Fisher's protected LSD test; $P > 0.05$) (Fig. 2a). However, the mean number of attacked eggs treated with a 10X concentration was significantly lower than in the control at all observation times (Fisher's protected LSD test; $P = 0.0001$) (Fig. 2b).

The decrease in the number of eggs of *L. decemlineata* attacked as a result of M-One use indicates that there might be negative effects on the rate of predation by *C. maculata* in treated potato fields. This may be a problem especially if *L. decemlineata* develops resistance to *B. thuringiensis* var. *san diego,* which has been observed in *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) populations in Hawaii with *Bacillus thuringiensis* var. *kurstaki* (Tabashnik et al. 1990), when users were forced to increase concentrations progressively. Field trials studying the effects of M-One on *C. maculata* populations and predation efficiency should be conducted to substantiate our laboratory assays.

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