Impact of Cry3A-Intoxicated *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and Pollen on Consumption, Development, and Fecundity of *Coleomegilla maculata* (Coleoptera: Coccinellidae)

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ABSTRACT Predator consumption, development, and fecundity were assessed for the predator–prey interaction between *Coleomegilla maculata* (De Geer) and *Leptinotarsa decemlineata* (Say) feeding on Cry3A-transgenic or normal (nontransgenic) foliage of potato. The influence of bee pollen, a food supplement, on development and fecundity was also assessed. Neonates of *L. decemlineata* feeding on transgenic foliage lost considerable body weight, in comparison to those feeding on normal foliage. After differences in prey weight were considered, no significant difference was found between the proportion of transgenic-fed or normal-fed neonates consumed by *C. maculata*. There was no significant difference between the proportion of *C. maculata* developing into pupae and adults when reared on transgenic-fed or normal-fed prey; nor did the live weight of teneral adults differ. The proportion of *C. maculata* reaching maturation when reared on transgenic-fed prey with pollen was greater than those reared on pollen alone, which suggests the suitability of a diet containing animal and plant material. Predator fecundity, measured as egg deposition per day, was not significantly different between mated females provided with transgenic-fed prey with pollen or normal-fed prey with pollen. These data suggest that Cry3A-intoxicated *L. decemlineata* can be eaten by *C. maculata* without any observable adverse effects on their survival or predation potential.

KEY WORDS *Bacillus thuringiensis*, transgenic plants, endotoxin, predator, prey

THE CRY3A δ-ENDOTOXIN from *Bacillus thuringiensis* Berliner subsp. *tenebrionis* is toxic to select coleopterans, particularly chrysomelids (Krieg et al. 1983, Herrnstadt et al. 1986, Bauer 1990, MacIntosh et al. 1990, Eckberg and Cranshaw 1994); it is insecticidal to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Ferro and Gelernter 1989). The Cry3A endotoxin binds to receptors on the midgut epithelium, and causes cytolysis of the midgut cells, septicemia, and subsequent death (Slaney et al. 1992, Federici 1993). Cry3A-transgenic potato provides direct delivery of Cry3A δ-endotoxin to *L. decemlineata* and causes temporary paralysis within 24 h to all instars and even adults (Perlak et al. 1993).

The effect of transgenic plants containing the Cry3A insecticidal protein on nontarget organisms has become an area of concern in recent years (Jepson et al. 1994, Yu et al. 1997). Nontarget herbivores and even carnivores may be impacted by feeding directly on the Cry3A-transgenic plant (tissues and pollen) or by consuming the tissues of intoxicated target pests feeding on the plant. With the advent of genetically engineered potato, a new interaction between plant, pest, and nontarget organism may arise. It is conceivable that the toxic proteins in the plant could be transferred to beneficial insects, such as predators that consume *L. decemlineata*.

The impact of transgenic potato on predation by *Coleomegilla maculata* (De Geer) is unknown. However, a spray formulation of *B. thuringiensis* subsp. *san diego*, M-One (Mycogen, San Diego, CA), reduced its consumption of *L. decemlineata* eggs in comparison to unsprayed eggs (Giroux et al. 1994).

Coleomegilla maculata is a common, native entomophagous and pollenophagous species that inhabits managed and natural ecosystems in North America. In the potato ecosystem, *C. maculata* commonly attacks the eggs and 1st– 2nd instars of *L. decemlineata* (Groden et al. 1990, Hazzard et al. 1991, Hough-Goldstein et al. 1993, Giroux et al. 1995, Hilbreck and Kennedy 1996) and aphids (Smith 1965, Hazzard and Ferro 1991). Pollen of cultivated plants (corn, alfalfa, and others) is a dominant component in the diet of *C. maculata* in the field (Hodek 1993); bee pollen is used in laboratory rearing of this lady beetle (Smith 1960, 1965).

The research presented in this paper considers the interaction between *C. maculata* and neonates of *L. decemlineata* feeding on Cry3A-transgenic or nontransgenic foliage of potato. We report the results of

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experiments designed to answer the following 3 questions: (1) Do *C. maculata* consume intoxicated neonates of *L. decemlineata* as readily as healthy ones? (2) Can *C. maculata* complete development on a diet containing intoxicated neonates, or on a diet containing intoxicated neonates with pollen versus pollen alone? (3) Are *C. maculata* capable of producing as many offspring when provided a diet containing pollen with intoxicated or healthy neonates? The answers to these questions will help us predict the survival and predation potential of this lady beetle in fields containing Cry3A-transgenic potato.

Materials and Methods

Acquiring and Culturing *C. maculata*. Larvae (primarily 2nd instars) of the lady beetle, *C. maculata*, were shipped to us from the Mission Biological Control Laboratory, USDA-APHIS, Mission, TX. These larvae were from a lab colony that was 11–50 generations old and had no previous exposure to *B. thuringiensis*. Also, adults and larvae were normally fed commercial bee pollen and a semi-artificial diet consisting of lyophilized (freeze-dried) eggs of the Mexican fruit fly, *Anastrepha ludens* (Loew), plus fructose. This rearing medium was also used in our laboratory when *C. maculata* were not involved in experiments.

In our laboratory, each *C. maculata* larva or adult was held individually within a capped 5-dram, clear plastic shell vial. Bee pollen (Natural Brand, General Nutrition, Pittsburgh, PA) and semi-artificial diet obtained from the Mission Laboratory were added to each vial. A 2-cm long piece of a dental wick was positioned inside every vial, then moistened with distilled water. All filled vials were then placed within a walk-in growth chamber at 25–27°C, 12 h of scotophase, and 60–80% RH.

Culturing of Potato Plants. Russet Burbank transgenic (New Leaf) and nontransgenic (normal) certified seed potatoes, *Solanum tubersoum* L., were obtained from Nature Mark (Monsanto, Boise, ID), and stored in a cold-room at 7°C until use. The Cry3A δ-endotoxin (USEPA Reg. No. 524-474) is expressed in transgenic potato at a range of 0.01–0.2% leaf dry weight. It is expressed in all tissues at all times.

Seed pieces were potted in a professional soil medium (Pro-Gro, McCormick, SC) and grown at ambient temperature in a greenhouse. We planted 1 or 2 transgenic or normal (nontransgenic) seed pieces in each of 80 plastic pots (15 cm diameter); they were used in the laboratory experiments after plants passed the tuber initiation phase, with 8–12 leaves.

Culturing Colorado Potato Beetles and Exposing them to Potato Foliage. In total, 120 *L. decemlineata* egg masses, ≈4,800 individual eggs, were obtained every week (May to September) from a laboratory colony at the New Jersey Department of Agriculture (Trenton, NJ). Eggs, which had been laid within 24 h prior to shipment, were placed into a refrigerator at 7°C upon arrival. We staggered egg hatch dates by subdividing the 120 egg masses into 4 petri dishes. Each day, 1 petri dish was removed from the refrigerator and placed in an incubator (Percival) at 27°C. Eggs hatched within 5 d and larvae were removed at random from the dishes as needed for experiments.

Two fully open primary leaflets were excised from the leaf at the 2nd, 3rd or 4th petiole from the growing tip of each transgenic or normal plant. One disk (10 mm diameter) was removed from each leaflet (from either side of the midrib) with a cork borer, and placed within a petri dish. Thus, each petri dish contained two disks of transgenic or normal foliage. Neonates were placed randomly on the 2 leaf disks within each petri dish. The larvae were left undisturbed for 0.5 h, then each was examined to ensure that it was on a disk and had begun feeding. From 80–100% of all larvae had begun feeding on foliage within 1 h after placement within petri dishes.

Determining Toxicity of Transgenic Potato Plants to Neonates of *L. decemlineata*. A simple toxicity test was performed to determine whether Cry3A-transgenic foliage from the potted plants was toxic to neonates and whether intoxicated larvae would recover if removed from the transgenic foliage. Ten 1st instars were placed together in each of 11 petri dishes and provided with 2 leaf disks of transgenic foliage. The next day, the larvae still showing some movement were removed and cohorts were placed in each of 11 dishes containing 2 leaf disks of normal foliage. Each larva was probed with forceps and observed under a dissecting microscope to detect any visible movement. Then the percentage of survivors after 24 h and 48 h of exposure to normal foliage was assessed.

Estimating Consumption of *L. decemlineata* by *C. maculata*. The *L. decemlineata* neonates in a given petri dish (9 by 1 cm, diameter) were weighed immediately after being allowed to feed on foliage disks for 24 h and just before exposure to *C. maculata*. Each predator was added to a dish and allowed to feed undisturbed on the prey for ≈24 h. Afterward, the uneaten prey or remnants of prey in each dish was removed and weighed. Then the remains were air-dried in a Radiant Heat Oven at 60°C for 4 d and reweighed to the nearest 0.01 mg using a Mettler (M3) microbalance. The dry weight amount of prey tissues consumed per dish was estimated from a dry weight regression model generated from a sample of 11 replicate dishes, each containing 10 live, healthy *L. decemlineata* neonates exposed to normal foliage for 24 h.

This procedure was used during 4 consumption experiments. In 3 of the experiments, 10 prey larvae per dish were exposed to transgenic foliage, and 10 were similarly exposed to normal foliage for 24 h. Predators were adult *C. maculata* in experiment I, primarily 4th instars in experiment II, 3rd instars in experiment III, and 4th instars in experiment IV. In experiment IV, we manipulated the number of prey neonates to account for the greater body weight of the healthy neonates compared with the intoxicated neonates; each replicate dish contained either 8 larvae exposed to normal foliage or 12 *L. decemlineata* larvae exposed to transgenic foliage. The mean ± SEM dry weight of prey, after feeding on foliage for 24 h and just before availability to predators, was estimated based on the pre-

viously described regression model and compared for transgenic foliage versus normal foliage. The mean ± SEM dry mass of prey and the proportion of prey mass consumed in 24 h by *C. maculata* was determined and compared for prey feeding upon transgenic foliage versus normal foliage.

Estimating Development Time of *C. maculata*. The same environmental conditions used in the consumption experiments were used in the development experiments. The test involved the rearing of primarily 2nd-instar *C. maculata* on 2 diets to ascertain whether intoxicated *L. decemlineata* prey altered the developmental time of immature predators. In the 1st experiment, the 2 treatment diets were intoxicated prey versus healthy prey. The previously described procedures for intoxicating prey were used, except complete leaflets rather than leaf disks were presented to neonates. One prey neonate was added to the appropriate treatment as needed, which was every day if the predator had consumed the prey, or every other day, if the prey had not been consumed. The proportion of *C. maculata* reaching the pupal and adult stages on each treatment diet was tabulated. This experiment was terminated after 20 d, at which time all *C. maculata* larvae had reached maturation or had died. Also, the mean ± SEM live weight of teneral adults was determined and compared between treatments.

In the 2nd experiment, the treatment diets were pollen with intoxicated prey versus pollen alone. The proportion of *C. maculata* reaching the pupal and adult stages was tabulated. This experiment was terminated after 21 d, at which time all predator larvae in the pollen with intoxicated prey treatment had reached maturation or had died. The mean ± SEM live weight of teneral adults was determined and compared between treatments.

Estimating Fecundity of *C. maculata*. Fecundity, as measured by daily egg deposition, of *C. maculata* was determined over an 11-d period. Emerged adults, which were reared to the adult stage in a vial containing the previously described bee pollen and semi-artificial diet, were placed in a laboratory cage (46 by 46 by 46 cm) containing the same food source for ≈10 d to allow for mating. Pairs of *C. maculata*, in copula, were observed and then gently removed. Each pair was isolated inside a petri dish with moistened filter paper in the bottom, and provided with bee pollen and semi-artificial diet. After 24–48 h, the male was taken out of the dish, the food was removed, and the female was provided with a treatment diet, either intoxicated prey with pollen or healthy prey with pollen. Each day, all dishes were examined for the presence of *C. maculata* eggs which were counted and removed at once. The mean ± SEM number of eggs laid per female per day was determined and then compared between treatments.

Statistical Analysis. Consumption data were square-root transformed before analysis. The *t* -test was used to compare the dry weight of prey fed on transgenic or normal foliage that were available to predators, as well as dry mass of such prey consumed by predators. The *z* test, which included the Yates corrrection for continuity (see Glantz 1992), was used to compare the proportion of prey mass consumed by predators and also to compare the proportion of pupae and adults reared successfully on treatment diets. The *t* -test was used again to compare the live weight of teneral adults, as well as the fecundity (daily egg deposition) data. The Mann–Whitney test, which is a nonparametric test analogous to the parametric *t* -test, was used when the assumptions of normality and equal variances were not met (see Sokal and Rohlf 1981). All data analyses were performed with Sigma Stat software (Sigma Stat 1994).

Results

Toxicity of Transgenic Foliage from Potato Plants to *L. decemlineata*. After exposure to Cry3A-transgenic potato foliage for 24 h, 84% (average) of 1st-instar *L. decemlineata* larvae were alive. But by 48 h (even though larvae had been placed onto normal foliage after 24 h), only 2% of the larvae exhibited any visible movement. Therefore, 48 h were required for 98% of the larvae to become moribund or to die.

Consumption of *L. decemlineata* by *C. maculata*. In experiment I, prey weight available to *C. maculata* adults and the prey mass consumed by *C. maculata* were significantly lower for *L. decemlineata* neonates that fed on transgenic foliage than neonates that fed on normal foliage (Table 1). However, the actual proportion of transgenic-fed prey versus normal-fed prey consumed was not significantly different. The same patterns were evident for experiments II and III, with *C. maculata* 4th instars and 3rd instars, respectively (Table 1).

In experiment IV, the weight of transgenic-fed prey available to *C. maculata* 4th instars was not significantly different from the weight of normal-fed prey (Table 1); the mass of transgenic-fed prey consumed by *C. maculata* also was not significantly different from that consumed when normal-fed prey were offered. The proportion of transgenic-fed versus normal-fed prey consumed was not significantly different.

Development Time of *C. maculata*. No significant differences were detected in the proportion of *C. maculata* developing into pupae or adults within 20 d when provided a diet of transgenic-fed prey or normal-fed prey (Table 2). The live weight of teneral adults also was not significantly different between the treatments. The proportion of *C. maculata* developing into pupae within 21 d did not differ significantly when these predators were provided a diet of transgenic-fed prey with pollen versus pollen alone. However, a greater proportion of *C. maculata* adults emerged successfully from the pupal stage after consuming transgenic-fed prey with pollen versus pollen alone. The live weight of teneral adults was not significantly different between the treatments.

Fecundity of *C. maculata*. Within an 11-d period, *C. maculata* deposited an average of 9.62 ± 0.45 (SEM) eggs per day when subjected to a treatment diet of transgenic-fed prey with pollen ($n = 4$); it deposited 8.48 ± 0.87 (mean ± SEM) eggs per day when exposed to normal-fed prey with pollen ($n = 5$). Egg deposition

Table 1. Mean ± SEM dry weight of *L. decemlineata* (prey) neonates per petri dish, *n*, and dry mass and proportion of prey consumed by *C. maculata* in 24 h

Experiment[a]	*n*	Prey wt, mg Transgenic-fed prey[b] Normal-fed prey[c]	Mass consumed, mg	Proportion consumed
I	6	1.39 ± 0.02	0.97 ± 0.06	0.69
	6	2.18 ± 0.07	1.52 ± 0.10	0.69
		$t = -10.9$, df = 10	$t = -4.56$, df = 10	$z = -0.62$
		$P < 0.0001$	$P = 0.001$	$P = 0.53$
II	10	1.23 ± 0.04	0.75 ± 0.14	0.61
	10	2.40 ± 0.06	1.64 ± 0.14	0.68
		$t = -1.64$, df = 18	$t = -4.09$, df = 18	$z = -0.12$
		$P < 0.0001$	$P = 0.0007$	$P = 0.90$
III	15	1.27 ± 0.03	0.49 ± 0.04	0.38
	14	2.28 ± 0.05	0.76 ± 0.08	0.33
		$t = -18.9$, df = 27	$T = 277.0$	$z = -0.09$
		$P < 0.0001$	$P = 0.004$	$P = 0.93$
IV[d]	14	1.74 ± 0.03	1.34 ± 0.05	0.77
	15	1.58 ± 0.09	1.20 ± 0.10	0.76
		$T = 248.5$	$T = 236.5$	$z = -0.37$
		$P = 0.10$	$P = 0.26$	$P = 0.71$

[a] For experiment I, *C. maculata* (predators) were adult males; experiment II, primarily 4th instars; experiment III, primarily 3rd instars; and experiment IV, primarily 4th instars.
[b] Ten 1st instars per petri dish isolated for 24 h on Cry3A-transgenic potato foliage.
[c] Ten 1st instars per petri dish isolated for 24 h on normal potato foliage.
[d] Prey weight per dish was manipulated (see text).

per day did not differ significantly between treatments ($t = 1.10$, df = 7, $P = 0.31$). The sample of females used in this experiment was small due to the difficulty of rearing larvae through to adults.

Discussion

The observation of weight loss in *L. decemlineata*, when fed transgenic foliage, has been reported previously (see Wierenga et al. 1996). A consequence is that predators given transgenic-fed prey have less prey mass to eat in comparison to those given normal-fed prey. This can give the false impression that the mean mass of prey eaten by predators differs significantly between these treatments. By examining the proportion of total prey mass consumed by *C. maculata*, we found no significant differences between the two treatments. Furthermore, by manipulating the prey weight per dish, we demonstrated that no real differences existed. Thus, intoxicated prey were palatable to *C. maculata* in these laboratory experiments.

Our observation that *C. maculata* was capable of completing its development on transgenic-fed prey strongly suggests that the uptake of Cry3A toxins from prey tissues will have no chronic effects on *C. maculata*. Note that Cry1Ab-transgenic corn pollen did not significantly affect the preimaginal development or survival of *C. maculata* (Pilcher et al. 1997). A diet of transgenic-fed prey with pollen resulted in a significantly greater proportion of *C. maculata* metamorphosing into adults than when reared on a diet of pollen alone. This suggests that a diet containing animal and plant material is more suitable for *C. maculata* development.

The observation that transgenic-fed prey with pollen had no negative effect on the number of eggs deposited by *C. maculata*, when compared to the number deposited when feeding on normal-fed prey with pollen, further indicates that Cry3A-intoxicated *L. decemlineata* will probably have no chronic effect on *C. maculata*. In a related study, putative Cry3A-containing aphids [*Myzus persicae* (Sulzer)] were fed to the convergent lady beetle, *Hippodamia convergens* Guérin-Méneville, with no decrease in reproduction of this predator (Dogan et al. 1996).

In conclusion, the lack of any significant impact of Cry3A-intoxicated *L. decemlineata* on the consumption, development, and fecundity of C. *maculata* suggests that *C. maculata* will not be deterred from feeding on *L. decemlineata* in fields of transgenic potato.

Table 2. Proportion of 2nd-instar *C. maculata* metamorphosing to pupae and adults and mean ± SEM live weight of teneral adults

Treatment	*n*	Pupae	Adults	Adult wt, mg
Experiment I[a]				
Transgenic-fed prey[b]	40	0.27	0.17	8.35 ± 0.50
Normal-fed prey[c]	40	0.42	0.32	8.42 ± 0.42
		$z = 1.17$	$z = 1.29$	$t = -0.08$, df = 18
		$P = 0.24$	$P = 0.20$	$P = 0.93$
Experiment II[d]				
Pollen + transgenic-fed prey	50	0.32	0.26	8.49 ± 0.43
Pollen only	50	0.20	0.08	6.98 ± 0.70
		$z = 1.14$	$z = 2.13$	$t = 1.80$, df = 15
		$P = 0.25$	$P = 0.03$	$P = 0.09$

[a] Terminated after 20 d.
[b] *L. decemlineata* neonates isolated for 24 h on Cry3A-transgenic potato foliage.
[c] *L. decemlineata* neonates isolated for 24 h on normal potato foliage.
[d] Terminated after 21 d.

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