NOTE

Cry3A-Intoxicated *Leptinotarsa decemlineata* (Say) are Palatable Prey for *Lebia grandis* Hentz[1]

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Transgenic potatoes provide a direct delivery of Cry3A δ-endotoxin, derived from *Bacillus thuringiensis* Berliner subsp. *tenebrionis,* to the Colorado potato beetle, *Leptinotarsa decemlineata* (Say), causing temporary paralysis within 24 h to all instars and even adults (Perlak et al. 1993. Plant Molec. Biol. 22: 313-321). The effect of transgenic plants containing the Cry3A insecticidal protein on non-target organisms has become an area of study in recent years (Jepson et al. 1994. Molec. Ecol. 3: 81-89). Consuming the tissues of intoxicated *L. decemlineata* neonates (those that had fed on foliage of Cry3A-transgenic potato) may or may not affect the fitness of insect predators.

We designed an experiment to determine whether Cry3A-intoxicated *L. decemlineata* neonates were palatable prey for the carabid *Lebia grandis* Hentz. We previously observed that *L. grandis* adults were rarely captured in fields containing pure stands of transgenic potato plants or in seed-mixed fields (containing transgenic and nontransgenic plants) on 3 farms in Maryland (Riddick et al. 1998. Ann. Entomol. Soc. Am. 91: 647-653).

In late spring 1995, we collected *L. grandis* adults from the foliage and the soil surface in nontransgenic potato fields on the Southern Maryland Research farm in Upper Marlboro, University of Maryland. Adults were visible on potato foliage during the day and were captured by gently trapping each individual within a plastic shell vial on the foliage or on the ground. Captured beetles were held in the vials and returned to the laboratory and temporarily placed in a plastic shoebox at room temperature (20-24°C).

Russet Burbank transgenic (New Leaf®) and nontransgenic certified seed potatoes were obtained from Nature Mark™ (a unit of Monsanto Company, Boise, ID) and stored in a cold-room at 7°C until use. The Cry3A δ-endotoxin [USEPA Reg. No.

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524-474] is expressed in transgenic potato at a range of 0.01 to 0.2% leaf dry wt. Cry3A is expressed in all tissues at all times. Seed pieces were potted in a professional soil medium (Pro-Gro Products™, Inc., McCormick, SC) and grown at ambient temperature in a greenhouse. We planted one or two transgenic or nontransgenic seed pieces in each of 80 plastic pots (15 cm diam). These were used in the laboratory experiments after plants passed the tuber initiation phase (8 to 12 leaves).

Egg masses of *L. decemlineata* were obtained from a laboratory colony at the New Jersey department of Agriculture (Trenton, NJ). All egg masses (a total of 120 per shipment) which had been oviposited within 24 h prior to shipment, were placed into Petri dishes and held in a refrigerator at 7°C. Later, Petri dishes were removed from the refrigerator and placed in an incubator (Percival™) at 27°C. Eggs hatched within 5 d, and neonates were removed at random from the Petri dishes as needed for experimentation.

Two fully-open primary leaflets were excised from the leaf at the second, third, and fourth petiole from the growing tip of each sample plant. Two disks (10 mm diam) were removed from each leaflet (one from either side of the midrib) and placed within a Petri dish with the abaxial surface facing down. Neonates were randomly placed on top of the leaf disks within Petri dishes. The larvae remained undisturbed for 0.5 h. After this time each larva was examined to insure that it was on the foliage disk and had begun feeding on either transgenic or normal potato foliage. From 80 to 100% of all larvae within each Petri dish had begun feeding on foliage within 1 h from the time of placement on leaf disks. A simple toxicity test determined that transgenic foliage from potted plants was toxic to neonates, but 48 h were required for 98% of all individuals to become moribund or die (Riddick and Barbosa. 1998. Ann. Entomol. Soc. Am. 91: 303-307). Transgenic foliage was also highly toxic to neonates in the present study.

The *L. decemlineata* neonates in a given Petri dish (9 cm diam × 1 cm depth) were weighed after being allowed to feed on foliage disks for 24 h, and just prior to exposure to *L. grandis*. An adult *L. grandis,* irrespective of sex, was added to each Petri dish and allowed to feed undisturbed on the prey for approximately 24 h in a walk-in growth chamber (25 to 27°C, 12 h scotophase, 60 to 80% RH). Afterwards, the uneaten prey or remnants of prey in each dish were removed and weighed. Then the remains were air-dried in a Radiant Heat Oven (Lab-Line Instruments™, Inc.) at 60°C for 4 d, then re-weighed to the nearest 0.01 mg, using a Mettler (M3) microbalance. The dry weight (or mass) of prey tissues consumed per dish was estimated from a fresh-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 repeated for 3 consecutive days. Each day, 10 prey larvae per dish were exposed to transgenic foliage, and 10 were similarly exposed to nontransgenic foliage for 24 h. (Prey exposed to transgenic foliage will be referred to as T-fed prey, and those exposed to nontransgenic foliage as N-fed prey). We used 8 to 10 replicate Petri dishes per treatment per day. The same predators were presented with the same treatments for consecutive days.

The mean (±SEM) dry weight (or mass) of prey, after feeding on foliage for 24 h and just before being made available to predators, was estimated with the regression model (above) and compared for transgenic foliage versus nontransgenic foliage. The dry mass of prey and the proportion of the total prey mass consumed per 24 h by *L.*

grandis were determined and compared for prey feeding upon transgenic versus nontransgenic foliage.

All prey weight and mass consumption data were square-root transformed prior to analysis. Statistical analyses involved the use of the Student's *t*-test to compare the dry weight of prey (T-fed or N-fed) made available to predators. The Mann-Whitney rank-sum test, with statistic *T,* was used to compare the amount of prey dry mass consumed by predators because these data did not meet assumptions of normality or equal variance (Glantz. 1992. Primer of biostatistics, 3rd ed., McGraw-Hill Inc., NY). A Spearman Rank Correlation test, with coefficient r_s, was used to relate prey weight (or mass) available to prey mass consumed by *L. grandis.* Scatterplots of the untransformed data were generated. The Mann-Whitney rank-sum test also was used to compare the proportion of available prey eaten (determined as the dry mass consumed per dry mass available), after arcsine transformation, between the 2 treatments. All statistical analyses were performed with Sigma Stat® software (Jandel Scientific Software. 1994. San Rafael, CA).

The results indicated that the T-fed prey were significantly lighter (in total body mass) than their N-fed cohorts (Table 1). Consequently, *L. grandis* appeared to consume significantly less of the T-fed prey than the N-fed prey. Because the T-fed prey weighed less before being presented to predators, the proportion of available T-fed prey eaten by *L. grandis* did not differ significantly from the proportion of available N-fed prey eaten. There was a significant correlation between total prey mass

Table 1. Mean ± SEM dry weight of *L. decemlineata* (prey) neonates, mean ± SEM dry mass consumed, and proportion of the total dry mass consumed by *L. grandis* per day in each treatment

Day	n		Prey wt, mg	Mass consumed, mg	Proportion consumed
1	10	T-fed[a]	1.22 ± 0.03	1.03 ± 0.10	0.84 ± 0.08
	10	N-fed[b]	2.15 ± 0.08	1.99 ± 0.09	0.92 ± 0.02
			$t = -12.0$, $df = 18$	T[c] = 55.0	$T = 93.0$
			$P < 0.0001$	$P < 0.0001$	$P = 0.38$
2	8	T-fed	1.19 ± 0.02	1.09 ± 0.08	0.91 ± 0.06
	10	N-fed	2.35 ± 0.07	2.33 ± 0.07	0.99 ± 0.01
			$t = -16.1$, $df = 16$	$T = 36.0$	$T = 74.5$
			$P < 0.0001$	$P < 0.0001$	$P = 0.93$
3	8	T-fed	1.21 ± 0.05	1.18 ± 0.05	0.98 ± 0.02
	10	N-fed	2.42 ± 0.06	2.40 ± 0.06	0.99 ± 0.01
			$t = -16.1$, $df = 16$	$T = 36.0$	$T = 74.0$
			$P < 0.0001$	$P < 0.0001$	$P = 0.89$

[a] Ten first instars per Petri dish isolated for 24 h on Cry3A-transgenic potato foliage.
[b] Ten first instars per Petri dish isolated for 24 h on nontransgenic potato foliage.
[c] *T,* statistic for Mann-Whitney rank-sum test.

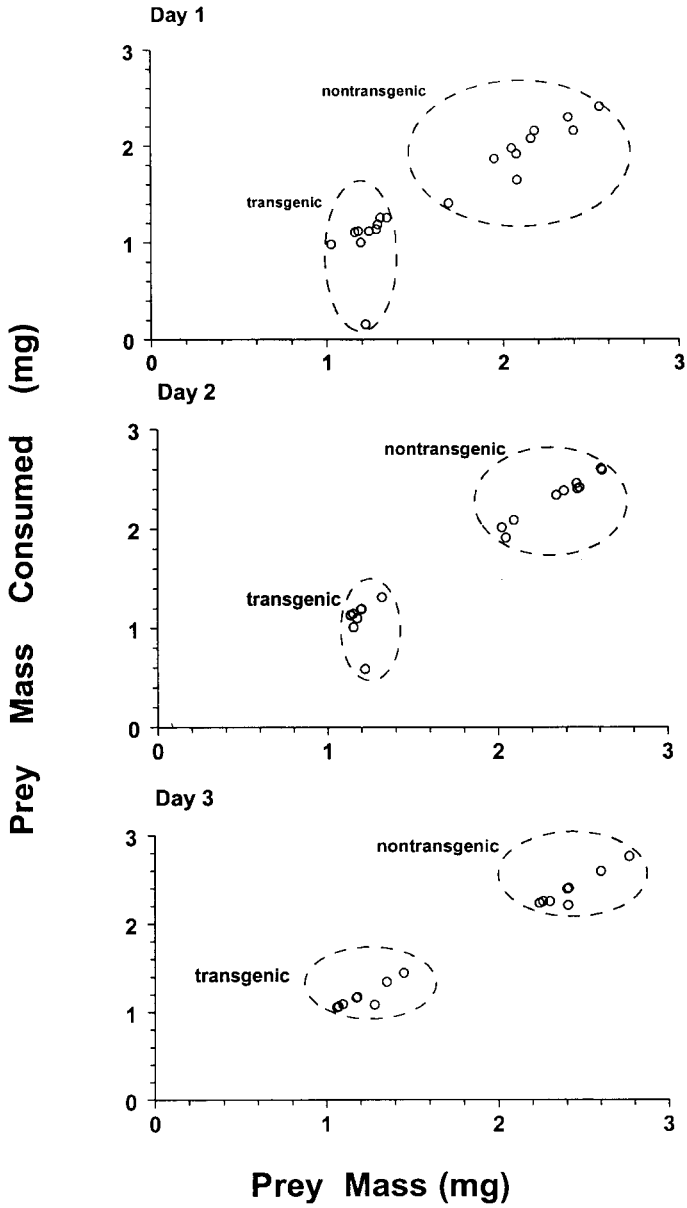

Fig. 1. Correlation analyses of total prey mass (mg, dry wt) and prey mass consumed by *L. grandis* on consecutive days. Transgenic and nontransgenic treatment data points are enclosed within stippled circles.

per Petri dish and prey mass consumed by *L. grandis* per Petri dish (Fig. 1); an increase in prey mass was strongly related to an increase in prey mass consumed (Day 1, $r_s = 0.96$, $P < 0.001$, n = 20; Day 2, $r_s = 0.93$, $P < 0.001$, n = 18; Day 3, $r_s = 0.96$, $P < 0.001$, n = 18). Notice that the data-points representative of the transgenic treatment are disparate from those representative of the nontransgenic treatment on each day.

In conclusion, we have demonstrated in this limited study that *L. decemlineata* neonates were palatable prey of *L. grandis* adults. The searching behavior of individual *L. grandis* adults was not closely monitored on transgenic plants in the field. In the laboratory, *L. grandis* males and females readily located, then consumed, eggs and neonates of *L. decemlineata* on potted transgenic plants (EWR, unpub. data).

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