

Laboratory Assessment of Flight Activity Displayed by Colorado Potato Beetles (Coleoptera: Chrysomelidae) Fed on Transgenic and Cry3a Toxin-Treated Potato Foliage

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ABSTRACT Adults of full-sib Colorado potato beetle families were separated into 3 groups fed on different diets: transgenic potato foliage, potato foliage treated with a foliar formulation of *B. thuringiensis* endotoxin (12.37 µg of Cry3A δ-endotoxin per 1 µl of mixture), and untreated potato foliage. After feeding for 3 h, all the beetles were placed on a computer-linked flight mill system. The number of beetles that flew, duration of each flight, and the number of flights for each beetle were recorded. Feeding on transgenic foliage had a strong negative effect on the proportion of beetles that flew, as well as the average number of flights per flying beetle. Mean flight duration was not influenced by the beetle diet, but interaction between family and diet was highly significant, with pronounced family effects observed for the beetles fed on standard and treated foliage. Beetles from families that performed the longest flights when fed on untreated foliage performed the shortest flights when fed on transgenic foliage. Suppression of beetle flight as a result of endotoxin ingestion could keep beetles within transgenic fields, thus increasing selection pressure toward development of physiological resistance. One possible way to reduce this pressure is to provide refugia for susceptible beetles in close association with fields planted to transgenic potato.

KEY WORDS *Leptinotarsa decemlineata*, *Bacillus thuringiensis*, flight behavior, transgenic potato plants

COLORADO POTATO BEETLE, *Leptinotarsa decemlineata* (Say), is the most damaging insect defoliator of potatoes (Weber and Ferro 1994). The diverse and flexible life history of this insect, together with a remarkable ability to develop insecticide resistance, make it a very difficult pest to control. Currently, field populations of the Colorado potato beetle are still susceptible to insecticides based on the *Bacillus thuringiensis* (Berliner) subsp. *tenebrionis* Cry3A δ-endotoxin. However, both theoretical models (Gould 1988, Ferro 1993) and laboratory selection experiments (Whalon et al. 1993, Rahardja and Whalon 1995) indicate that there is a potential threat of the failure of the *B. thuringiensis*-based materials, similar to the earlier failure of synthetic insecticides. With the widespread commercialization of transgenic potato plants that express persistently high doses of the Cry3A toxin in foliage, selection pressure toward resistance development in beetle populations increases dramatically (Ferro 1993). Therefore, development of a sound resistance management strategy is essential to extend the useful life of transgenic potatoes.

Movement, allowing for gene flow between genetically heterogeneous populations, is recognized as one

of the key factors influencing resistance evolution in insects (Comins 1977, Tabashnik and Croft 1982, Tabashnik 1986). In general, Colorado potato beetle is a highly mobile species, capable of moving both by flight and by walking (Weber and Ferro 1994). Under normal conditions, it can easily fly several kilometers at an average speed of ≈2 m/s (Weber 1992). Walking results in dispersal over much shorter distances, because beetles are able to walk only several hundred meters at a maximum speed of 1 cm/s, with vegetation strongly retarding beetle movement (Ng and Lashomb 1983). Insecticide exposure is known to alter behavior of arthropods, including their locomotory activity (Lockwood et al. 1984, Sparks et al. 1989, Hoy et al. 1998). In the Colorado potato beetle, larvae tended to move away from the leaves treated with high doses of the Cry3A toxin (Hoy and Head 1995), and there was a positive correlation between behavioral sensitivity and physiological resistance. Conversely, adult beetles decreased their movement in the cages containing plants treated with *B. thuringiensis* (Whalon et al. 1993). Currently, little is known about the possible effects of beetle exposure to the Cry3A toxin on beetle flight. Flight behavior of reproductive females will be of a particular interest in the system of transgenic crops because resistant females mated to resistant males will distribute homozygously resistant offspring within and between transgenic fields, and only resistant homozygotes are likely to survive high

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toxin concentrations expressed in transgenic foliage (Whalon and Ferro 1998).

Materials and Methods

Beetle Families and Rearing Conditions. During March 1996, ≈ 200 overwintering Colorado potato beetle adults were collected from soil in potato plots at the Ohio Agricultural Research and Development Center (OARDC) in Wooster, OH. Collected adults were brought to the greenhouse at OARDC, where they were held in moist, sterile sand at $\approx 22^\circ\text{C}$ and a photoperiod of 16:8 (L:D) h until diapause termination. The stems of freshly excised potato leaves were inserted into the moist sand to provide a food source for emerging beetles. After initiation of feeding, postdiapause adults were placed on potted potato plants in a rearing cage.

Eggs laid by the postdiapause beetles were collected and reared to adults on potted potato plants in the same greenhouse. As the F_1 adult offspring of the field collected adults emerged from the soil, their sex was determined, and 53 beetle pairs were placed into individual cages for mating and oviposition (throughout this article, mating pairs and their progeny are called *families*). Eggs collected from 21 families were shipped to the University of Massachusetts. Upon arrival, eggs were incubated in an environmental chamber at 27°C and a 16:8 (L:D) h photoperiod until they hatched. Neonates from each family were placed on at least 3 separate caged potato plants in the greenhouse (25–30 larvae per plant) and allowed to complete development to adulthood at $24 \pm 2^\circ\text{C}$ and a photoperiod of 16:8 (L:D) h. Location of each plant within a greenhouse bench was selected at random.

Adults were collected daily as they emerged from the soil and placed in cages for another 7 d. The beetles were fed fresh potato foliage, allowing for development of flight muscles and the reproductive system. Males and females from the same family which emerged within 24 h were kept together (but separately from the beetles from other families, as well as from their siblings which emerged at a different time) and were allowed to mate. Separating beetles according to their emergence times ensured that all the beetles used in the current study were approximately of the same age. On the 8th d after emerging from the soil, female beetles were placed into individual Nalgene containers (12 by 7 by 5.5 cm) lined with moistened paper towels and fed freshly excised potato foliage. The foliage was kept fresh by inserting the stems into floral pics filled with water.

Experimental Protocol. On the 9th d after emergence from the soil, female beetles within each family were separated into 3 treatment groups. For the 1st treatment, beetles were fed for 3 h on transgenic foliage that expressed Cry3A toxin (NewLeaf 'Atlantic', NatureMark, Boise, ID). The level of toxin expression in such foliage is very high, killing $\approx 99\%$ of exposed adults within a 15-d period (Perlak et al. 1993). Females from the 2nd treatment were fed for 3 h on standard foliage treated with $12.37 \mu\text{g}$ toxin/ μl

solution of Cry3A δ -endotoxin (XTA-020, Novo Nordisk A/S, Bagsværd, Denmark). Toxin concentration used in the current study is known to kill 95–100% of 2nd-instar Colorado potato beetle larvae within 24 h after exposure (Ferro et al. 1997), but our observations showed this concentration to be sublethal for the adults. To obtain treated foliage, recently cut 'Atlantic' potato leaflets were dipped twice into a mixture of formulated product and water, and then allowed to air dry. Females from the control treatment were fed for 3 h on untreated foliage. For all treatments, foliage was provided in the form of disks freshly excised from potato leaflets. Four disks were placed into each container. Each disk had a uniform area of 2.58 cm^2 . To encourage feeding, food was withheld from the beetles for 4 h before providing them with leaf disks. Leaf area was measured after beetle feeding using a LiCor leaf area meter (model LI-3000, Lincoln, NE). Disks which were fed upon by the beetles from the same families were measured together.

All females were allowed to feed for the same 3-h time period. After that, they were placed on the flight mill for 1 h. The flight mill system used in the current study was very similar to the system described by Weber et al. (1993). The only major difference was that we used a PC-DIO-96 interface board (National Instruments, Austin, TX) instead of the self-made interface board used by Weber et al. (1993). Because flight initiation in the Colorado potato beetle is strongly related to the ambient air temperature (Caprio and Grafius 1990), the temperature inside the flight mill room was maintained at $25 \pm 2^\circ\text{C}$. The number of beetles that flew, duration of each flight, and the number of flights for each beetle were recorded. In total, 614 female beetles were tested during the experiment (206 beetles fed on transgenic foliage, 205 beetles fed on *B. thuringiensis*-treated foliage, and 203 beetles fed on untreated foliage). At least 22 females per family were tested, with at least 7 beetles per family exposed to each treatment (Table 1).

Statistical Analysis. Analysis of variance (ANOVA) (PROC GLM, SAS Institute 1988), chi-square tests, and Spearman rank correlations (PROC FREQ, SAS Institute 1988) were used for data analysis. In the ANOVA, rank transformations were applied to the data to equalize variances among the treatments and normalize the data distribution (Conover and Iman 1981). Means and standard errors were calculated from the nontransformed data only. Interaction terms were tested at $\alpha = 0.1$ because power, a measure of the probability of accepting a false null hypothesis, is of greatest concern in these tests. The level of significance for interaction terms was selected before the analysis was performed. When interaction terms were significant, we discuss family effects separately for each diet rather than averaged over the 3 diets. Non-flying beetles were excluded from the analysis of flight duration.

Correlations for mean flight duration for the beetles within each family fed on different diets were calculated as the correlations of family means:

Table 1. Family means for number and duration of flights performed by beetles fed on different diets

Family	Diet								
	Transgenic foliage			Treated foliage			Standard foliage		
	<i>n</i> ^a	Mean no. flights (SE)	Mean flight duration, 5 (SE)	<i>n</i>	Mean no. flights ^b (SE)	Mean flight duration, 5 ^c (SE)	<i>n</i>	Mean no. flights (SE)	Mean flight duration, 5 ^d (SE)
1	8	0.13 (0.13)	233.10 (N/A) ^e	7	0.00 (0.00)	— —	7	0.71 (0.29)	2,023.64 (742.25)
2	10	0.80 (0.51)	511.65 (184.33)	10	0.40 (0.22)	674.42 (455.69)	10	1.20 (0.61)	1,333.19 (525.57)
3	8	0.63 (0.50)	1,728.80 (1,216.20)	8	0.63 (0.38)	1,564.42 (670.94)	8	0.88 (0.74)	1,782.40 (659.00)
4	10	1.20 (0.57)	376.70 (149.33)	10	0.80 (0.25)	545.09 (473.22)	10	1.30 (0.34)	1,694.22 (918.53)
5	8	0.38 (0.26)	1,855.18 (400.68)	8	0.50 (0.38)	1,794.92 (22.57)	8	0.88 (0.30)	1,080.23 (671.93)
6	10	0.50 (0.50)	1,162.30 (N/A)	10	1.10 (0.38)	1,241.08 (393.80)	10	0.70 (0.26)	1,709.38 (344.72)
7	10	0.40 (0.31)	1,842.13 (722.03)	10	1.60 (0.85)	606.14 (214.31)	10	0.20 (0.20)	2,695.40 (N/A)
8	11	1.09 (0.74)	1,393.40 (1,076.75)	10	0.40 (0.22)	853.80 (776.88)	11	0.45 (0.16)	567.22 (190.34)
9	10	0.70 (0.42)	325.08 (169.91)	10	0.80 (0.42)	1,567.20 (565.36)	10	1.20 (0.51)	1,851.06 (532.31)
10	11	0.55 (0.25)	337.94 (241.68)	11	0.55 (0.21)	590.55 (409.03)	11	1.73 (0.41)	880.78 (274.60)
11	10	0.10 (0.10)	2,058.25 (N/A)	10	1.40 (0.75)	879.79 (532.61)	10	1.10 (0.23)	1,506.93 (379.64)
12	10	1.00 (0.37)	1,012.41 (490.75)	11	0.36 (0.28)	263.43 (187.48)	10	0.60 (0.22)	886.29 (331.83)
13	10	1.80 (0.90)	893.42 (372.13)	10	1.30 (0.79)	1,294.54 (514.11)	10	0.70 (0.50)	1,866.73 (833.97)
14	10	0.60 (0.40)	1,331.07 (335.39)	10	2.70 (1.62)	1,184.26 (319.26)	10	0.80 (0.42)	963.91 (698.60)
15	10	2.30 (1.19)	458.78 (200.50)	10	0.80 (0.33)	947.04 (426.72)	10	1.30 (0.47)	437.91 (216.20)
16	10	1.00 (0.60)	1,897.93 (614.53)	10	2.20 (0.53)	942.75 (286.01)	10	0.80 (0.25)	1,570.64 (510.71)
17	10	1.60 (0.78)	512.39 (245.95)	10	0.50 (0.31)	489.42 (289.90)	10	0.80 (0.25)	762.08 (296.37)
18	10	0.00 (0.00)	— —	10	0.20 (0.13)	28.55 (26.05)	9	2.11 (1.09)	869.73 (259.64)
19	10	1.40 (0.72)	755.26 (315.80)	10	1.00 (0.68)	898.84 (700.10)	10	1.00 (0.54)	2,355.65 (628.71)
20	10	0.30 (0.15)	1,092.03 (546.47)	10	0.60 (0.31)	1,237.76 (512.46)	10	0.50 (0.22)	404.83 (120.34)
21	10	0.20 (0.13)	1,696.45 (600.05)	10	0.50 (0.22)	1,311.98 (522.58)	10	0.70 (0.21)	168.91 (71.08)
Treatment mean (SE)		0.81 (0.12)	981.47 (110.83)		0.89 (0.13)	962.91 (91.23)		0.94 (0.10)	1,163.18 (109.78)

^a Number of females tested within a family-treatment combination.

^b Means are significantly different among the families (ANOVA, *F* = 1.71; *df* = 20, 184; *P* = 0.0344).

^c Means are significantly different among the families (ANOVA, *F* = 2.00; *df* = 19, 162; *P* = 0.0109).

^d Means are significantly different among the families (ANOVA, *F* = 1.71; *df* = 20, 169; *P* = 0.0354).

^e Only one flight was observed within a family-treatment combination.

$$r_m = \frac{\text{Cov}(\bar{X}, \bar{Y})}{[\text{Var}(\bar{X}) \text{Var}(\bar{Y})]^{1/2}}$$

where \bar{X} and \bar{Y} represent mean durations of flights performed by the beetles belonging to the same family, but fed on different diets (Zangerl and Berenbaum 1990). To reduce possible bias in the population estimates and test for nonzero parameters, jackknife procedures were performed using an inverted tangent transformation of correlation coefficients (Sokal and Rohlf 1981). Reported estimates were back transformed to the original scale.

Results

Beetles from all 3 treatments flew after being placed on the flight mill (32.04% of the beetles fed on transgenic foliage, 42.44% of the beetles fed on treated foliage, and 51.72% of the beetles fed on standard foliage). The percentage of beetles flying was significantly different between treatments (chi-square test, $\chi^2 = 8.21$, *df* = 2, *P* = 0.0165) (Fig. 1), but not between families ($\chi^2 = 62.22$, *df* = 60, *P* = 0.3970). Beetle diet had a highly significant effect on the number of flights performed by the beetles (ANOVA, *F* = 6.52; *df* = 2, 613; *P* = 0.0016). Tukey pairwise com-

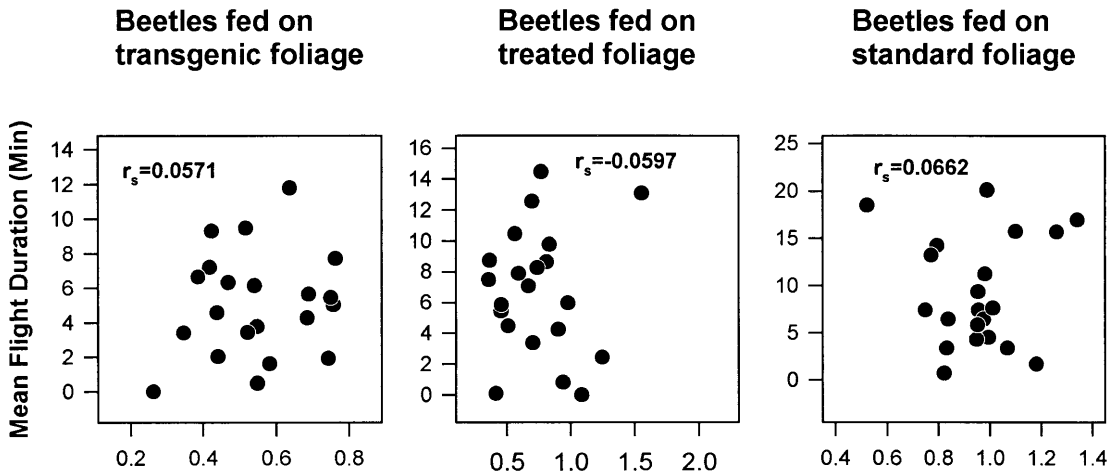


Fig. 1. Correlations between mean foliage consumption and mean flight duration of Colorado potato beetle females fed on different diets.

parisons showed that the beetles fed on transgenic foliage engaged in fewer flights than the beetles fed on standard foliage ($P < 0.05$), whereas there were no differences between other treatments. Flight numbers varied among beetle families, but the difference was only marginally significant at $\alpha = 0.05$ (ANOVA, $F = 1.57$; $df = 20, 613$; $P = 0.0553$). The interaction between family and diet was statistically significant at $\alpha = 0.1$ (ANOVA, $F = 1.39$; $df = 40, 613$; $P = 0.0583$). When analyzed separately within each treatment, family effects were meaningful only for the beetles fed on treated foliage (Table 1). There was no correlation between the numbers of flights performed by the beetles fed on different diets (beetles fed on transgenic foliage \times beetles fed on standard foliage, $r_m = 0.0751$, $P = 0.6790$; beetles fed on transgenic foliage \times beetles fed on treated foliage, $r_m = 0.0640$, $P = 0.8321$; beetles fed on standard foliage \times beetles fed on treated foliage, $r_m = 0.2479$, $P = 0.1702$).

Mean flight duration differed significantly among beetle families (ANOVA, $F = 1.77$; $df = 20, 477$; $P = 0.0211$), but not among treatments (ANOVA, $F = 1.77$; $df = 2, 477$; $P = 0.1234$). Interaction between family and diet was highly significant (ANOVA, $F = 1.74$; $df = 40, 477$; $P = 0.0048$), with pronounced family effects observed for the beetles fed on standard and treated foliage (Table 1). There was a significant negative correlation between the mean flight durations of the beetles fed on transgenic foliage and the beetles fed on standard foliage ($r_m = -0.3731$, $P = 0.020$). In other words, the beetles with greater familiar predisposition to a higher flight activity were more strongly affected by ingestion of the Cry3A toxin. There was no correlation between mean flight duration and mean foliage consumption within beetle families (Spearman rank correlations, $P > 0.05$) (Fig. 1).

Discussion

Ingestion of *B. thuringiensis* Cry3A toxin had a detrimental effect on Colorado potato beetle flight behavior in laboratory assay, reducing both the number of beetle flights and the proportion of beetles that flew. Different beetle families had different responses to feeding on toxin-treated foliage, suggesting the presence of genetic and/or maternal effects. However, high toxin concentrations expressed in transgenic potatoes were detrimental to all the beetles regardless of their genetic composition. Our findings agree with the results of Whalon et al. (1993), who observed decreased beetle movement on caged potato plants treated with Cry3A toxin. Similar effects of insecticide exposure also were recorded for several other insect species. Shanks and Chapman (1965) reported that green peach aphids, *Myzus persicae* (Sulzer), remained longer on cucumber plants treated with parathion and phorate than on untreated cucumber plants. In the experiment of Boiteau et al. (1985), aldicarb reduced flying ability of the green peach aphid and the potato aphid, *Macrosiphum euphorbiae* (Thomas). Similarly, European earwigs, *Forficula auricularia* L., remained close to the place of their release when exposed to diflubenzuron, but tended to move away from the untreated control sites (Sauphanor et al. 1993). A lack of escape response in the Colorado potato beetle is consistent with the earlier findings that this species is incapable of gustatory detection of the *B. thuringiensis* Cry3A toxin (Hoy and Hall 1993; Hoy and Head 1995), and that feeding on suboptimal host plants, which causes lower survival and fecundity, does not increase the beetle's flight activity (Weber and Ferro 1996).

The effect of the toxin on mean flight duration proved to be somewhat more complicated than its effect on the number of flights and the proportion of

flying beetles. Although ingestion of toxin did not make any difference by itself, there was a significant interaction between diet and familial factors. Significant variation among the families fed on standard and treated foliage suggests the presence of genetic and/or maternal effects on flight duration, which are suppressed by feeding on transgenic potatoes. Furthermore, incorporation of the *B. thuringiensis* Cry3A toxin into the diet seemed to reverse the inherent tendency to engage in long flights, and the families that performed the longest flights under normal conditions performed the shortest flights when fed on transgenic foliage.

Limited dispersal in Colorado potato beetle facilitates development of resistance in individual populations (Grafius 1995). The current study was restricted to a laboratory assessment of a limited number of flight parameters, and a certain caution should be exercised when discussing its implications for resistance management in commercial potato fields. Nevertheless, Colorado potato beetle flight on a flight mill provides a good match to its flight under field conditions (Weber et al. 1993). Therefore, we believe that our findings could be useful in predicting possible developments within fields planted to transgenic potatoes. Suppression of beetle flight as a result of endotoxin ingestion could keep the beetles within transgenic fields, increasing selection pressure toward development of physiological resistance. However, our results also indicate that even high endotoxin doses expressed in transgenic potato plants allow for a fair amount of short-distance movement. Therefore, if a sufficiently large number of susceptible beetles are maintained in refugia adjacent to a transgenic crop, there could be significant gene flow between resistant and susceptible populations. Resistant and susceptible beetles mating with each other will leave offspring which are heterozygous at resistance loci. Because resistance to *B. thuringiensis* in the Colorado potato beetle is incompletely dominant (Rahardja and Whalon 1995), heterozygous individuals are unlikely to survive the high concentration of the Cry3A toxin expressed in transgenic potatoes.

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