# Coleopteran-specific Cry3Bb Toxin from Transgenic Corn Pollen Does Not Affect the Fitness of a Nontarget Species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae)

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**ABSTRACT** *Coleomegilla maculata* DeGeer is a polyphagous predator that is important for suppressing pest populations in corn. We evaluated the impact of Cry3Bb-expressing transgenic corn pollen (event MON863) on *C. maculata* fitness parameters in the laboratory. *C. maculata* larvae were fed mixtures of pollen containing 0, 25, 50, 75, or 100% transgenic pollen, aphids, or were not fed; and the duration of each instar and pupal weight were compared among treatments. In a second trial, other *C. maculata* larvae were reared on one of the pollen mixtures or artificial diet; and the duration of larval and pupal stages, pupal weight, adult mobility, adult survivorship, and female fecundity were compared among treatments. There were no differences in any of the fitness parameters among *C. maculata* in the treatments fed different mixtures of pollen. Beetles in the pollen mixture treatments had faster larval development times, greater larval survivorship, and greater pupal weight than the beetles fed only aphids or an artificial diet. We conclude that we did not detect any effects on the fitness of *C. maculata* that ingested pollen from event MON863. However, these results do not necessarily apply to other transgenic crops expressing toxins specific to Coleoptera.

**KEY WORDS** *Bacillus thuringiensis*, beneficial insects, corn rootworm, Cry3Bb, transgenic.

WESTERN CORN ROOTWORM, *Diabrotica virgifera virgifera* Le Conte, and northern corn rootworm, *Diabrotica barberi* Smith &Lawrence, are dominant pests of corn in the United States, and successful management of these pests historically has involved a variety of pest management practices (Steffey et al. 1999). The seriousness of rootworms as pests, the independent development of resistance to crop rotations by several populations of rootworms (Krysan et al. 1984, Ostlie 1987, Sammons et al. 1997, O'Neal et al. 2001), and the difficulty of devising economically viable economic injury levels for rootworms (Foster et al. 1986) have prompted the development of transgenic corn that expresses a coleopteran-specific toxin (Cry3Bb) from the entomopathogen *Bacillus thuringiensis* (Bt). A major difference between Cry3Bb-expressing corn and varieties of Bt corn that target species of Lepidoptera is the degree of risk posed to agroecosystems. Many species of coleopterans are important predators that reduce or suppress pest populations within agroecosystems. By directly affecting predators that are facultatively phytophagous on transgenic plant tissue, the Cry3Bb-expressing varieties pose an additional ecological "pathway" for the toxin to affect nontarget species that the Lepidoptera-specific varieties do not.

Environmental risks of transgenic crops are evaluated in part by measuring the mortality in nontarget organisms resulting from acute exposure to the toxins expressed by transgenic plants. Appropriately, most species tested for these nontarget effects are insects (EPA 2000a, 2000b). Insects are a diverse group, and there is a wide range of susceptibility to toxins expressed in transgenic crops, even within the target order (Pilcher et al. 1997, Hilbeck et al. 1998, Jesse and Obrycki 2000, Wraight et al. 2000, Stanley-Horn et al. 2001; Hellmich et al. 2001). Differences in the susceptibility of insects and the large number of possible candidates necessitate the prioritization of key insect species found in agroecosystems as organisms to be tested for nontarget impacts associated with transgenic crops.

*Coleomegilla maculata* DeGeer is the most abundant chewing predator in Midwestern corn fields. *C. maculata* has been implicated in maintaining aphid populations below economic levels and is regarded as a consistent source of mortality to the European corn borer, *Ostrinia nubilalis* (Hübner) (Wright and Laing 1980, Andow and Risch 1985, Andow 1990, Phoofolo et al. 2001). In addition to prey, *C. maculata* relies on corn pollen as a component of its diet in the field (Cottrell and Yeargan 1998) and is capable of completing development on a diet consisting solely of corn pollen (Pilcher and Obrycki 1994). Ingesting prey that have

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fed on Coleoptera-specific toxins does not affect *C. maculata* in other systems (Riddick and Barbosa 1998). However, there is at least one instance where larval development rates of *C. maculata* were negatively impacted by ingesting a Coleoptera-specific Bt formulation that was offered directly in conjunction with pollen (Giroux et al. 1994).

Cry3Bb is the toxin incorporated into corn expressing event MON863 (Monsanto Corp., St. Louis), which is currently under evaluation by the U.S. Environmental Protection Agency (EPA) for registration as a control method for corn rootworms. Although numerical and fitness responses of nontarget species to the toxin have not been quantified, ELISA test strips specific for the Cry3Bb toxin detected the toxin in nitidulid beetles that occurred in research plots planted with MON863 (RNW, unpublished data). The objective of this research was to determine whether ingestion of genetically modified corn pollen (MON863) containing the Cry3Bb toxin affected the fitness of *C. maculata*, as assessed in the laboratory.

## Materials and Methods

**Insects.** The *C. maculata* colony originated from cultures maintained at the University of Minnesota and Iowa State University; voucher specimens were deposited in the Insect Museum at the Illinois Natural History Survey (Champaign). Beetles were reared at 25°C on the artificial diet #7 developed by Atallah and Newsom (1966), without tetracycline. Water was provided in 0.5-ml microcentrifuge tubes with a cotton wick. Males and females were maintained together in a 30 × 30 × 30 cm Plexiglas cage. Mated females were removed from the colony and reared individually in 60-mm diam petri dishes. Dishes were checked daily for eggs, and females and excess food were removed from dishes within 24-h of oviposition. Petri dishes were checked daily for newly hatched larvae, and larvae were removed and placed individually into 0.030-liter plastic cups with cardboard lids (Comet Products, Chelmsford, MA) after larvae had eaten the egg chorions. Corn leaf aphid [*Rhopalosiphum maidis* (Fitch)] and bird cherry-oat aphid [*Rhopalosiphum padi* (L.)] were obtained from the field and maintained on nontransgenic corn plants in greenhouse rooms that were isolated from rooms containing transgenic plants.

**Pollen collection.** Transgenic corn (event MON863) and nontransgenic corn (hybrid 34G81; Pioneer Hi-Bred International, Johnston, IA) were grown in 11.36-liter pots; transgenic and nontransgenic corn plants were grown in isolated greenhouse rooms. Tassels were contained in 2.27-kg paper bags that were stapled shut. After anthesis, pollen was sifted through No-see-um mesh (Balsam-Hercules Group, Pawtucket, RI), having a pore size of 160-$\mu$m, to remove contaminants. Pollen was placed in 35.57-ml sealed plastic vials (Bioquip Products, Gardena, CA) and kept at <4°C until use. Vials containing 100, 75, 50, 25, and 0% transgenic pollen were prepared by weighing the appropriate masses of greenhouse-collected transgenic and nontransgenic pollens with an electronic balance. The vials with the pollen mixtures were shaken by hand for 90 s to create a homogeneous pollen mixture. The presence of Cry3Bb toxin in each pollen mixture was tested with an ELISA strip specific for the Cry3Bb toxin (provided by Monsanto). Duan et al. (in press) found that a corn variety that expresses event MON863 had toxin levels that ranged between 37.4–101.0 $\mu$g/g fresh weight of pollen, with a mean concentration of 77.1-$\mu$g/g fresh weight of pollen; level of toxin in our pollen was unknown.

**Experiment 1: Larval Development and Pupal Weight.** Two hundred and fifty-two *C. maculata* first instars were randomly divided into seven groups (36 per group). Treatments assigned to the groups were unfed, fed only aphids, or fed one of the five pollen mixtures. For the aphid-only treatment, *C. maculata* larvae were fed three adult aphids daily. In the pollen treatments, first and second instars were reared with 0.015 g of pollen, third instars were reared with 0.021 g of pollen, and fourth instars were reared with 0.03 g of pollen. Pollen was weighed on an electronic balance. The plastic cups and pollen were replaced if fungal growth was detected. A single adult aphid was given to the *C. maculata* larvae at the beginning of the third and fourth instars to improve survivorship (Pilcher et al. 1997). Unfed larvae did not receive any food except for their chorions. Larvae in all treatments received water in the form of a saturated, 0.75-cm diam cotton ball. Pollen feeding was assured by dissecting a subset of larvae ($n = 5$) 3 h after feeding, and finding pollen in the gut.

Plastic cups were checked daily for dead larvae and cast skins. Pupae were weighed on an electronic balance to the nearest 0.001 g within 24 h of pupation. The durations of each instar and the total larval development times were compared among treatments with analysis of variance (ANOVA). ANOVA results with $P$ values <0.05 were subjected to the Tukey-Kramer means comparison to determine significant differences among the treatments. The unfed treatment was excluded from these analyses because only one larva completed the first instar in this treatment. Pupal weights were compared among treatments with ANOVA and the Tukey-Kramer means comparison test. Each larva was categorized as completing prepupal development or not, and the number of larvae in each category were compared among treatments with a Likelihood Ratio test to detect differences in larval mortality.

**Experiment 2: Adult Fitness and Larval Development.** Three hundred and fifty-two (58–60 per treatment) first instar *C. maculata* were divided among six treatments. The five pollen-fed treatments were the same as in the first experiment, but the unfed treatment was omitted in experiment 2. The aphid treatment from experiment 1 was replaced with an artificial diet treatment because larval development is faster and survivorship higher when larvae are fed artificial diet than when larvae are fed aphids alone (JGL, unpublished data). Larvae in the diet treatment received 0.015 g of diet for the duration of each instar,

and diet was replaced when it began to desiccate. Larvae in the pollen treatments were reared with a maximum of 0.015 g of the pollen mixture, regardless of larval stage. In all treatments, each larva was fed an adult aphid every 2–4 d and received water in the form of a saturated, 0.75-cm diam cotton ball. Plastic cups and pollen were changed if fungal growth was observed. Adult beetles produced from this experiment were maintained on artificial diet and water wicks in 60-mm diam petri dishes. Thus, beetles were exposed to the Cry3Bb toxin only as larvae.

The time until pupation and pupal duration was monitored for each beetle as in the first experiment because the rearing methods for the larvae had changed in the second experiment. Pupal weight was measured on an electronic balance to the nearest 0.001 g within 24 h of pupation. Mean larval development times, pupal weights, and pupal duration were compared among treatments with ANOVA and Tukey-Kramer means comparisons. Larvae were categorized as surviving 30 d past adult emergence or not, and the proportion in each category was compared among treatments with a Likelihood Ratio test.

Adults were sexed 3 to 4 d after emergence. Males were taken out of the laboratory colony and placed 3/100-mm diam petri dish. These untreated males were maintained on artificial diet and a water wick. Individual females from the experiment were placed in the petri dishes with the untreated males for 24-h to allow mating. After 24-h, the females were removed from the mating arena and returned to the 60-mm diam rearing dish and monitored. Times from mating to first and second oviposition were recorded for each female, as were the sizes of the first two clutches. Females that did not oviposit within 27 d of emergence were scored as having zero eggs, and these females that did not lay eggs were factored into the analyses. The mean time until first and second ovipositions and the mean clutch sizes were compared among treatments with ANOVA. The proportion of females that laid two clutches within 27 d of emergence was compared among treatments with a Likelihood Ratio test.

Analyses of walking speed and flip time have been used successfully as indicators of fitness in other research (Smith and Krischik 1999). We implemented these tests by placing each male on its dorsum on a piece of 90-mm diam Whatman filter paper. The time until the beetle flipped over was measured with a stopwatch to the nearest 0.01 s. Timing was stopped at 30 s for beetles that did not flip. The males were placed on a 21.6 × 27.9 cm white laser printer paper to measure walking speed. The walking path of the beetle was followed with a pencil for a maximum of 30 s that was timed to the nearest 0.01 s. Beetles that did not move in 30 s were scored as zero, and these beetles that did not walk were factored into the analysis. A piece of string was placed over the pencil line and measured to the nearest 0.1 cm. The distance was divided by the amount of time walking, resulting in walking speed (cm/s). Males were subjected to four repetitions of the flip time and walking speed experiments; these repetitions occurred sequentially without intermission, and all walking speeds were taken before the flip times for each individual. Mean flip time and walking speed were compared among treatments and experiment repetitions with repeated-measures ANOVA. After the fitness tests, five adult males from each treatment were randomly selected to be tested for the presence of the toxin. Beetles were ground in 1.5-ml microcentrifuge tubes with abrasive silicon sand and a pestle in a 10 mM phosphate buffered saline solution (Sigma, St. Louis). These solutions were tested with ELISA test strips specific for Cry3Bb.

**Calculation of Detectable Effects.** To determine whether our analyses were strong enough to avoid falsely rejecting a true null hypothesis (type II error), we calculated the detectable effects for the ANOVA statistics that we ran using a conservative $\beta$ level. The effect (d) is defined by Stiedl et al. (1997) as the change in the parameter due to application of a treatment. To calculate detectable effects in our analyses, we used a variation of an equation from Ott (1993, p. 228):

$$d^2 = \frac{\sigma^2(F_{\alpha/2} + F_{\beta})^2}{n}$$

Where d is the effect, $\sigma$ is the root mean square error, $n$ is the sample size in each treatment, and $\alpha$ and $\beta = 0.05$. The effect, therefore, gives the value for the difference in the parameter below which there is a chance for type I and type II errors.

## Results

All of the pollen mixtures with transgenic pollen tested positive for the Cry3Bb toxin. Toxin was not detected in beetles that were treated as larvae but tested as adults.

Larval development times and pupal weights were similar among the beetles fed the pollen mixtures (Table 1). No significant differences in the first- and second-instar development times were observed among the treatments ($F_{5, 179} = 1.32$, $P = 0.25$; $F_{5, 168} = 2.20$, $P = 0.057$; respectively). Development times of third and fourth instars and total larval development time significantly differed among treatments ($F_{5, 164} = 19.29$, $P < 0.0001$; $F_{5, 145} = 102.45$, $P < 0.0001$; $F_{5,135} = 125.87$, $P < 0.0001$; respectively). Larvae fed aphids had significantly longer development times than larvae in the pollen treatments, which all had similar development times (Table 1). Pupal weight in the aphid treatment was significantly different than pupal weights in the pollen treatments ($F_{5, 147} = 20.16$, $P < 0.0001$), and pupal weights did not vary among the pollen treatments (Table 1). None of the unfed larvae survived for longer than 6 d, and only one larva completed the first instar. The proportion of larvae reaching pupation was similar among treatments ($\chi^2 = 7.90$, df = 212, $P = 0.25$; note that the unfed treatment is excluded from this analysis).

Mean larval durations and mean pupal weights were significantly different in the artificial diet treatment than in the pollen treatments ($F_{5, 307} = 10.62$, $P <$

**Table 1. Effects of ingesting transgenic corn pollen containing Cry3Bb toxin on *C. maculata* fitness—Experiment 1**

| Treatment | Mean 1st instar duration, days (SEM) | Mean 2nd instar duration, days (SEM) | Mean 3rd instar duration, days (SEM) | Mean 4th instar duration, days (SEM) | Mean larval duration, days (SEM) | Mean pupal mass, mg (SEM) | Proportion of larvae that pupated, % |
|---|---|---|---|---|---|---|---|
| 0% Transgenic pollen | 3.29 (0.13) | 2.59 (0.16) | 3.51 (0.12)a | 4.91 (0.17)a | 14.48 (0.34)a | 13.07 (0.67)a | 69.44 |
| | $n = 31$ | $n = 29$ | $n = 27$ | $n = 22$ | $n = 21$ | $n = 23$ | $n = 36$ |
| 25% Transgenic pollen | 3.15 (0.12) | 2.58 (0.12) | 3.55 (0.14)a | 5.04 (0.16)a | 14.24 (0.27)a | 12.34 (0.42)a | 75.00 |
| | $n = 33$ | $n = 33$ | $n = 31$ | $n = 25$ | $n = 25$ | $n = 26$ | $n = 36$ |
| 50% Transgenic pollen | 3.32 (0.12) | 2.69 (0.13) | 3.34 (0.11)a | 4.93 (0.18)a | 14.38 (0.33)a | 12.66 (0.56)a | 84.38 |
| | $n = 31$ | $n = 29$ | $n = 29$ | $n = 27$ | $n = 26$ | $n = 27$ | $n = 32$ |
| 75% Transgenic pollen | 3.17 (0.17) | 2.93 (0.13) | 3.21 (0.10)a | 5.16 (0.24)a | 14.64 (0.32)a | 13.46 (0.48)a | 85.29 |
| | $n = 29$ | $n = 27$ | $n = 29$ | $n = 25$ | $n = 22$ | $n = 25$ | $n = 34$ |
| 100% Transgenic pollen | 3.27 (0.12) | 2.59 (0.16) | 3.30 (0.13)a | 4.70 (0.12)a | 14.26 (0.25)a | 13.19 (0.42)a | 83.78 |
| | $n = 33$ | $n = 29$ | $n = 33$ | $n = 30$ | $n = 29$ | $n = 30$ | $n = 37$ |
| Aphid | 2.86 (0.14) | 2.25 (0.26) | 5.52 (0.46)b | 13.77 (0.83)b | 24.78 (0.58)b | 7.32 (0.30)b | 72.97 |
| | $n = 29$ | $n = 24$ | $n = 21$ | $n = 22$ | $n = 18$ | $n = 22$ | $n = 37$ |

Means within columns were compared with ANOVA and the Tukey-Kramer means comparisons when applicable, or the Likelihood Ratio test for proportional data. Significant differences among means ($P < 0.05$) within a column are represented as different capital letters. Letters were omitted from columns if means were not significantly different.

0.0001; $F_{5, 304} = 19.16$, $P < 0.0001$; respectively) (Table 2). Pupal duration did not differ among the treatments ($F_{5, 289} = 1.52$, $P = 0.18$). The proportion of larvae that pupated and the proportion of larvae surviving 30 d past adult emergence were similar among treatments ($\chi^2 = 10.78$, df = 350, $P = 0.06$; $\chi^2 = 8.48$, df = 317, $P = 0.13$; respectively).

The number of days between mating and the first and second ovipositions were similar among treatments in experiment 2 ($F_{5, 110} = 1.28$, $P = 0.28$; $F_{5, 109} = 0.81$, $P = 0.54$; respectively). In addition, there were no differences in the sizes of the first and second clutches among the treatments ($F_{5, 109} = 0.63$, $P = 0.68$; $F_{5, 109} = 1.72$, $P = 0.14$; respectively). Finally, there was no difference in the reproductive capacity of females among the different treatments as measured by the number of females that laid two clutches in 27 d ($\chi^2 = 8.36$, df = 116, $P = 0.14$). There were no differences in the mean flip times or mean walking speeds among the treatments ($F_{5, 98} = 1.06$, $P = 0.39$; $F_{5, 90} = 1.68$, $P = 0.15$; respectively) (Table 3). The detectable effects for thirteen analyses are listed in Table 4.

## Discussion

These experiments suggest that there were no adverse effects on *C. maculata* fitness from the ingestion of corn pollen expressing event MON863 and the

**Table 2. Effects of ingesting transgenic corn pollen containing Cry3Bb toxin on *C. maculata* fitness—Experiment 2**

| Treatment | Larval duration, d (SEM) | Pupal duration, d (SEM) | Pupal mass, mg (SEM) | Proportion that survived 30 d post-eclosion | Proportion of females that laid 2 clutches | Days between mating and 1st oviposition (SEM) | No. eggs in the 1st clutch (SEM) | Days between mating and 2nd oviposition (SEM) | No. eggs in the 2nd clutch (SEM) |
|---|---|---|---|---|---|---|---|---|---|
| Artificial diet | 15.53 (0.23)a | 3.41 (0.08) | 11.17 (0.31)a | 0.52 | 0.75 | 15.19 (2.26) | 4.94 (1.00) | 17.94 (1.80) | 4.63 (1.23) |
| | $n = 49$ | $n = 44$ | $n = 49$ | $n = 52$ | $n = 16$ | | | | |
| 0% Transgenic pollen | 13.94 (0.20)b | 3.26 (0.09) | 13.85 (0.32)b | 0.47 | 0.95 | 10.40 (1.06) | 6.25 (1.50) | 14.65 (1.61) | 5.15 (0.86) |
| | $n = 47$ | $n = 43$ | $n = 45$ | $n = 53$ | $n = 20$ | | | | |
| 25% Transgenic pollen | 13.83 (0.18)b | 3.22 (0.07) | 14.84 (0.28)b | 0.70 | 0.96 | 13.50 (1.11) | 7.17 (1.17) | 15.54 (1.47) | 8.25 (1.23) |
| | $n = 53$ | $n = 49$ | $n = 52$ | $n = 54$ | $n = 24$ | | | | |
| 50% Transgenic pollen | 14.00 (0.21)b | 3.19 (0.08) | 14.01 (0.27)b | 0.55 | 0.88 | 13.53 (1.98) | 4.88 (1.02) | 16.35 (1.75) | 4.71 (1.35) |
| | $n = 53$ | $n = 54$ | $n = 55$ | $n = 51$ | $n = 17$ | | | | |
| 75% Transgenic pollen | 14.02 (0.14)b | 3.17 (0.07) | 13.96 (0.27)b | 0.59 | 0.91 | 11.24 (1.37) | 7.48 (1.82) | 14.52 (1.57) | 7.33 (1.31) |
| | $n = 52$ | $n = 48$ | $n = 52$ | $n = 52$ | $n = 21$ | | | | |
| 100% Transgenic pollen | 14.42 (0.19)b | 3.14 (0.06) | 13.75 (0.24)b | 0.66 | 1.00 | 11.83 (1.57) | 5.67 (1.36) | 13.50 (1.70) | 8.61 (2.10) |
| | $n = 57$ | $n = 57$ | $n = 57$ | $n = 53$ | $n = 18$ | | | | |

Larval development and pupal mass were analyzed separately from Experiment 1 (Table 1) because the rearing methods were different in the second experiment. Means within columns were compared with ANOVA and the Tukey-Kramer means comparisons when applicable, or the Likelihood Ratio Test for proportional data. Significant differences among means ($P < 0.05$) within columns are represented as different capital letters. Letters were omitted from columns in which means were not significantly different.

**Table 3. Mean flip times and walking speeds of adult *C. maculata* males**

| Treatment | No. | Flip time, s (SEM) | Walking speed, cm/s (SEM) |
|---|---|---|---|
| Diet | 15 | 3.38 (0.57) | 3.71 (0.02) |
| 0% Transgenic pollen | 11 | 2.22 (0.15) | 4.50 (0.26) |
| 25% Transgenic pollen | 20 | 2.22 (0.46) | 4.13 (0.16) |
| 50% Transgenic pollen | 18 | 3.70 (0.13) | 4.06 (0.04) |
| 75% Transgenic pollen | 17 | 1.97 (0.19) | 4.17 (0.13) |
| 100% Transgenic pollen | 23 | 2.01 (0.34) | 4.52 (0.08) |

Treatment means were generated by calculating a mean flip time and walking speed for each individual from the four measurements taken per individual. These individual means were then compiled into a treatment mean. A comparison with a repeated-measures ANOVA revealed no significant differences among the treatments ($\alpha = 0.05$).

Cry3Bb protein. Beetles that received no food were dead within three days of hatching, which indicates that the beetles in the pollen treatments fed on the pollen; dissections of *C. maculata* midguts validated the presence of the pollen. In the 100% transgenic pollen treatment, ingesting the toxin was unavoidable for the larvae, yet the toxin was undetectable in the adult beetles. Furthermore, preliminary tests conducted in our laboratory showed that ELISA tests did not detect Cry3Bb toxin in *C. maculata* larvae fed transgenic pollen or aphids that were reared on transgenic plants (J.G.L., unpublished data). If the toxin is not activated in or does not bind to the gut in nonsusceptible insects, there may be a short gut-retention period for the toxin in these insects, or the toxin may be altered in the guts of these insects so such that the ELISA test does not register its presence.

We believe that our assay procedure was sensitive enough to detect biologically relevant differences in the development times and pupal weights of treated larvae. Table 4 lists detectable effects for all of the ANOVA tests that we conducted. Differences among the treatment means that are smaller than these values cannot be reliably detected by our statistical tests when $\alpha$ and $\beta = 0.05$. We were able to detect differences of 10% in total larval development time, and differences of ≈16% in pupal weight. The high variance in the clutch sizes and the times to first and second ovipositions within treatments decreased the sensitivity of our analyses when $\beta$ is set at a level of 0.05. In fact, the variance is so high in these analyses that increasing $\beta$ to 0.25 has little effect at reducing the detectable effect.

**Table 4. The detectable effects for various fitness parameters of treated *C. maculata***

| Analysis | df | $\alpha$ | Effect (units) |
|---|---|---|---|
| *Experiment 1* | | | |
| 1st Instar development | 5, 179 | 0.735 | 0.838 (d) |
| 2nd Instar development | 5, 168 | 0.823 | 0.758 (d) |
| 3rd Instar development | 5, 164 | 0.961 | 0.901 (d) |
| 4th Instar development | 5, 145 | 1.690 | 1.676 (d) |
| Larval development | 5, 135 | 1.642 | 1.662 (d) |
| Pupal weight | 5, 147 | 0.002 | 1.984 (mg) |
| *Experiment 2* | | | |
| Larval development | 5, 307 | 1.385 | 0.918 (d) |
| Pupal duration | 5, 289 | 0.536 | 0.366 (d) |
| Pupal weight | 5, 304 | 0.002 | 1.326 (mg) |
| Time to 1st oviposition | 5, 110 | 6.705 | 7.630 (d) |
| Time to 2nd oviposition | 5, 109 | 7.214 | 8.001 (d) |
| Size of 1st clutch | 5, 110 | 6.098 | 6.939 (eggs) |
| Size of 2nd clutch | 5, 109 | 6.055 | 6.890 (eggs) |

Treatment effects below the listed values cannot reliably be detected when $\alpha$ and $\beta = 0.05$.

It is possible that there may have been adverse effects of ingesting the Cry3Bb toxin on fitness that were not detected in this study. For example, it is likely that the larvae in our experiment were stressed as indicated by the larval mortality that was observed (Tables 1 and 2). This stress may have depressed the fitness of the population to the point that any effects of the toxin would have been masked. Although the survival and developmental rates of larvae in our experiment were consistent or higher than previous research on pollen-reared *C. maculata* (Pilcher and Obrycki 1994, Todorova et al. 1996, Pilcher et al. 1997, Riddick and Barbosa 1998), improvements in rearing procedures and subsequent reductions in ancillary larval mortality could increase the sensitivity of assays like the one used in our study. Also, development rates and size have been shown to be sex specific in *C. maculata*, and future tests should include sex as a covariate in the comparisons of fitness parameters.

The results of our study are consistent with the literature pertaining to fitness costs resulting from ingestion of commercially available, coleopteran-specific, Bt toxins on *C. maculata;* and the results from our study are very similar to results from a similar study using the same transgenic toxin, but different rearing methods (Duan et al. in press). The only published report we found of adverse impacts of a coleopteran-specific Bt toxin on *C. maculata* was published by Giroux et al. (1994). Their study found longer development times in the *C. maculata* larvae that ingested (nontransgenic) wild-flower pollen mixed with a formulation of *B. thuringiensis san diego* relative to a control that was fed only pollen. Although we found no effects of the Cry3Bb toxin on *C. maculata* fitness, it is important to note that our results only apply to this particular corn hybrid and event; other hybrids that express coleopteran-specific Bt proteins need to be evaluated independently. Furthermore, increasing the level of expression of the Cry3Bb toxin may result in deleterious effects on *C. maculata* fitness that were not observed in this study.

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## References Cited

**Andow, D. A. 1990.** Characterization of predation on egg masses of *Ostrinia nubilalis* (Lepidoptera: Pyralidae). Ann. Entomol. Soc. Am. 83: 482–486.

**Andow, D. A., and S. J. Risch. 1985.** Predation in diversified agroecosystems: relations between a coccinellid predator *Coleomegilla maculata* and its foods. J. Appl. Entomol. 22: 357–372.

**Atallah, Y., and L. D. Newsom. 1966.** Ecological and nutritional studies on *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). I. The development of an artificial diet and a laboratory rearing technique. J. Econ. Entomol. 59: 1173–1179.

**Cottrell, T. E., and K. V. Yeargan. 1998.** Effect of pollen on *Coleomegilla maculata* (Coleoptera: Coccinellidae) population density, predation, and cannibalism in sweet corn. Environ. Entomol. 27: 1402–1410.

**Duan, J. J., G. Head, M. McKee, T. Nickson, J. W. Martin, and F. S. Sayegh.** In press. Evaluation of dietary effects transgenic corn pollen expressing Cry3Bb1 protein on a nontarget ladybird beetle, *Coleomegilla maculata.* Entomol. Exp. Appl.

**EPA (U.S. Environmental Protection Agency). 2000a.** Biopesticide fact sheet: *Bacillus thuringiensis* Cry1Ab delta-endotoxin and the genetic material necessary for its production in corn [MON810]. http://www.epa.gov/pesticides/biopesticides/factsheets/fs006430t.htm

**EPA (U.S. Environmental Protection Agency). 2000b.** Biopesticide fact sheet: *Bacillus thuringiensis* CryIII(A) delta endotoxin and the genetic material necessary for its production in potato. http://www.epa.gov/pesticides/biopesticides/factsheets/fs006432t.htm.

**Foster, R. E., J. J. Tollefson, J. P. Nyrop, and G. L. Hein. 1986.** Value of adult corn rootworm (Coleoptera: Chrysomelidae) population estimates in pest management decision making. J. Econ. Entomol. 79: 303–310.

**Giroux, S., D. Coderre, C. Vincent, and J. C. Cote. 1994.** Effects of *Bacillus thuringiensis* var. *san diego* on predation effectiveness, development and mortality of *Coleomegilla maculata legni* (Col.: Coccinellidae) larvae. Entomophaga 39: 61–69.

**Hellmich, R. L., B. D. Siegfried, M. K. Sears, D. E. Stanley-Horn, M. J. Daniels, H. R. Mattila, T. Spencer, K. D. Bidne, and L. C. Lewis. 2001.** Monarch larvae sensitivity to *Bacillus thuringiensis*-purified proteins and pollen. Proc. Nat. Acad. Sci. U.S.A. 98: 11925–11930.

**Hilbeck, A., M. Baumgartner, P. M. Fried, and F. Bigler. 1998.** Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 27: 480–487.

**Jesse, L.C.H., and J. J. Obrycki. 2000.** Field deposition of *Bt* transgenic corn pollen: lethal effects on the monarch butterfly. Oecologia 125: 241–248.

**Krysan, J. L., J. J. Jackson, and A. C. Lew. 1984.** Field termination of egg diapause in *Diabrotica* with new evidence of extended diapause in *D. barberi* (Coleoptera: Chrysomelidae). Environ. Entomol. 13: 1237–1240.

**O'Neal, M. E., M. E. Gray, S. Ratcliffe, and K. L. Steffey. 2001.** Predicting western corn rootworm (Coleoptera: Chrysomelidae) larval injury to rotated corn with Pherocon AM traps in soybeans. J. Econ. Entomol. 94: 98–105.

**Ostlie, K. R. 1987.** Extended diapause: northern corn rootworm adapts to crop rotation. Crops and Soil Mag. 39: 23–25.

**Ott, R. L. 1993.** An introduction to statistical methods and data analysis, 4th ed. Duxbury Press, Belmont, CA.

**Phoofolo, M. W., J. J. Obrycki, and L. C. Lewis. 2001.** Quantitative assessment of biotic mortality factors of the Eur. corn borer (Lepidoptera: Crambidae) in field corn. J. Econ. Entomol. 94: 617–622.

**Pilcher, C. D., and J. J. Obrycki. 1994.** Feeding development and survival of the lady beetle *Coleomegilla maculata* (Coleoptera: Coccinellidae) on transgenic *B. thuringiensis* corn pollen, pp. 261–266. *In* Proceedings, the Integrated Crop Management Conference, 30 Nov–1 Dec 1994: Iowa State Extension, Iowa State University, Ames.

**Pilcher, C. D., J. J. Obrycki, M. E. Rice, and L. C. Lewis. 1997.** Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environ. Entomol. 26: 446–454.

**Riddick, E. W., and P. Barbosa. 1998.** Impact of Cry3A-intoxicated *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and pollen on consumption, development, and fecundity of *Coleomegilla maculata* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 91: 303–307.

**Sammons, A. E., C. R. Edwards, L. W. Bledsoe, P. J. Boeve, and J. J. Stewart. 1997.** Behavioral and feeding assays reveal a western corn rootworm (Coleoptera: Chrysomelidae) variant that is attracted to soybean. Environ. Entomol. 26: 1336–1342.

**Smith, S. F., and V. A. Krischik. 1999.** Effects of systemic Imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae). Environ. Entomol. 28: 1189–1195.

**Stanley-Horn, D. E., G. P. Dively, R. L. Hellmich, H. R. Mattila, M. K. Sears, R. Rose, L.C.H. Jesse, J. E. Losey, J. J. Obrycki, and L. Lewis. 2001.** Assessing the impact of Cry1Ab-expressing corn pollen on monarch butterfly larvae in field studies. Proc. Nat. Acad. Sci. U.S.A. 98: 11931–11936.

**Steffey K., M. Rice, J. All, D. Andow, M. Gray, and J. Van Duyn, eds. 1999.** Handbook of corn insects. Entomological Society of America, Lanham, MD.

**Stiedl, R. J., J. P. Hayes, and E. Schauber. 1997.** Statistical power analysis in wildlife research. J. Wildl. Manag. 61: 270–279.

**Todorova, S. I., J. C. Cote and D. Coderre. 1996.** Evaluation of the effects of two *Beauveria bassiana* (Balsamo) Vuillemin strains on the development of *Coleomegilla maculata legni* Timberlake (Col., Coccinellidae). J. Appl. Entomol. 120: 159–163.

**Wraight, C. L., A. R. Zangerl, M. J. Carroll, and M. R. Berenbaum. 2000.** Absence of toxicity of *Bacillus thuringiensis* pollen to black swallowtails under field conditions. Proc. Nat. Acad. Sci. U.S.A. 97: 7700–7703.

**Wright, E. J., and J. E. Laing. 1980.** Numerical response of coccinellids to aphids in corn in southern Ontario. Can. Entomol. 112: 977–988.

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