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Survivorship and development of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), on conventional and transgenic maize cultivars expressing *Bacillus thuringiensis* Cry9C and Cry1A(b) endotoxins

(*Keywords:* biological control, *Spodoptera frugiperda*, survivorship, transgenic maize)

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Abstract. Growth and development of fall armyworm, *Spodoptera frugiperda* (J. E. Smith), were compared in two laboratory trials between individuals oered transgenic maize seedlings, conventional maize seedlings, or artificial diet. Transgenic maize seedlings in the first and second trials expressed Cry9C (Event CBH 351) and Cry1A(b) (Event MON 810) *Bacillus thuringiensis* (Berliner) (*Bt*) endotoxin genes, respectively. Significant dierences were observed in both trials between fall armyworm fed transgenic and conventional maize for larval and pupal survival, weight, and development time. Survivorship of fall armyworm larvae was 28–70% on both transgenic cultivars, compared to 62–97% recorded on both conventional cultivars and artificial diet. Fall armyworm fed Cry9C transgenic maize had lower larval and pupal weights relative to those fed conventional maize, but the dierence was significant only for pupae, whereas those fed Cry1A(b) transgenic maize had significantly lower larval weights, while pupal weights were similar. Developmental periods of larvae fed transgenic or conventional maize were similar in the trial involving Cry9C maize, and longer for larvae fed transgenic maize in the trial involving Cry1A(b) maize. Pupal developmental periods were longer for larvae fed either Cry9C or Cry1A(b) transgenic maize relative to conventional maize, but the dierence was significant only in the former case. Estimation of *Bt* endotoxin concentration in plant tissues via enzyme-linked immunosorbent assays revealed that endotoxin expression was ~10 times greater in Cry9C maize relative to Cry1A(b) maize. Results are discussed in reference to implications for biological control of pests, such as fall armyworm, that are not targets of *Bt* transgenic maize.

1. Introduction

In the US, the fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] (Lepidoptera: Noctuidae) commonly infests maize, cotton, Bermuda grass, peanuts, and sorghum (Sparks, 1979, Pitre and Hogg, 1983), but its host range includes plants from 68 genera (Tietz, 1972). In maize, the fall armyworm is a secondary pest after the European corn borer [*Ostrinia nubilalis* (Hübner)] and the Southwestern corn borer (*Diatraea grandiosella* Dyar). However, relatively high damage is occasionally reported (Porter *et al*., 2000). Fall armyworm larvae feed on young whorls, ears and tassels causing substantial damage to maize crops. Insecticide applications are at present the main control measure against fall armyworm, but several applications are required to be effective (Hruska and Gladstone, 1988) and development of resistance to selected insecticides has been reported (Pitre, 1986; Guillebau and All, 1991).

Recently, genetically engineered insect-resistant crops were developed as control tactics against various pests. Transgenic maize lines resistant to various insect pests were developed by incorporating genes from the bacterium *Bacillus thuringiensis* (Berliner) (hereafter *Bt*) (Koziel *et al*., 1993). Several transformation events of transgenic *Bt* maize have been developed leading to commercial cultivars expressing Cry9C (Event CBH 351), Cry1A(b) (Event 176, Event BT-11, Event MON 810) and Cry1A(c) (Event DBT 418) endotoxin genes (Andow, 2002). *Bt* endotoxins in Cry9C (Event CBH 351) and Cry1A(b) (Event MON 810) cultivars are expressed in both vegetative and reproductive structures and offer good protection against European and Southwestern corn borers (Armstrong *et al*., 1995). However, non-target insects feeding on these cultivars may be affected to different degrees. Risks associated with widespread deployment of *Bt* cultivars have been evaluated to different degrees. However, risk concerns focus largely on resistance development in pests and cultivar sustainability, and more recently on potential impacts on populations of non-target herbivores (Gould, 1998; Andow, 2002; Losey *et al*., 2002). The potential impact of these cultivars on the dynamics of non-target herbivore and natural enemy populations has been investigated in less detail, although they are increasingly attracting attention (Schuler *et al*., 1999; Hilbeck, 2002). Non-target herbivores and natural enemies may be affected directly by lethal and sublethal effects of *Bt* cultivars, while natural enemies may also be affected indirectly via host-or prey-mediated effects. Fall armyworm is not a target of *Bt* transgenic maize cultivars. However, recent studies show that fall armyworm feeding on *Bt* maize had reduced growth, longer times to pupation and adult eclosion, and reduced feeding activity and survival (All *et al*., 1996, Pilcher *et al*., 1997, Lynch *et al*., 1999, Adamczyk *et al*., 2001). These effects on fall armyworm are important in the context of biological control because populations of this pest support at least 53 parasitoid species from 43 genera and 10 families (Ashley, 1979). Moreover, fall armyworm is a secondary pest of maize and several other crops, and likely plays an important role in agroecosystems as a host or prey for generalist parasitoids that contribute to natural biological control of this and other pests within and without maize fields. Widespread deployment of *Bt* maize cultivars may reduce fall armyworm populations and negatively

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affect the quality of surviving individuals as hosts for parasitoids. The objective of this study was to compare survivorship and development of a non-target pest, fall armyworm, on *Bt* transgenic maize cultivars versus conventional cultivars and artificial diet in the laboratory. Additionally, host plant acceptance by larvae and *Bt*-endotoxin levels were compared between transgenic and conventional cultivars in order to identify likely causes of any differences detected in fall armyworm survivorship and development between these cultivars. Pursuing these objectives was necessary to obtain base-line data useful for planning future studies addressing the effects of *Bt* maize cultivars on parasitoids of fall armyworm.

2. Materials and methods

2.1. Plants

Transgenic and conventional maize plants were grown in planters (65 × 30 × 25 cm) in the greenhouse. The transgenic cultivars were hybrids expressing Cry9C (Event CBH 351, Garst 8366 *Bt*) and Cry1A(b) (Event MON 810, Pioneer 35N05) endotoxin genes in both vegetative and reproductive structures. The corresponding conventional cultivars were hybrids lacking *Bt* endotoxin genes, Garst 8366 (a near-isogenic cultivar with Garst 8366 *Bt*) and Pioneer 34A55. Three to four rows of seeds were sown per planter in a 50:50 mixture of moist vermiculite and potting soil (SunGro Horticulture Inc., Canada). Plants were not fertilized and were watered daily. Ten to 14 day-old plants were used in the experiments by individually transplanting them in 148 ml plastic vials (49 × 85 mm) (BioQuip Products Inc., Gardena, CA, USA) perforated at the bottom for drainage.

2.2. Insects

Fall armyworm larvae were obtained from a stock culture maintained at 25 ± 2°C, 50–70% relative humidity, and a 14 h L:10 h D light regime for ca. 8 generations. Newly laid eggs were incubated to larval emergence in 1 l wide-mouth glass jars under the same environmental conditions. Neonate larvae were transferred to 40 mm (diam.) plastic cups containing ≈5 g of artificial diet (Martinez *et al.*, 1988), which were covered with waxed-paper lids.

2.3. Larval survival, development and host plant acceptance

Three treatments were compared in each of two separate trials. The treatments in the first trial were: (i) artificial diet; (ii) transgenic maize seedlings expressing Cry9C endotoxin (Event CBH 351) (Garst 8366 *Bt*) and (iii) conventional maize seedlings (GARST 8366). In the second trial, both the transgenic and conventional cultivars were changed so treatments were: (i) artificial diet; (ii) transgenic maize seedlings expressing Cry1A(b) endotoxin (Event MON 810) (Pioneer 35N05) and (iii) conventional maize seedlings (Pioneer 34A55). The experimental conditions for both trials were set at 30 ± 1°C, 50–70% relative humidity, and 14 h L:10 h D regime, and the same protocol (described below) was followed in each trial.

Neonate larvae were maintained from emergence through 5 days on artificial diet after which they were transferred to one of the maize treatments or kept on artificial diet. At 10 days of age, larvae were removed from plants or diet, weighed to the nearest 0.1 mg, and returned to their respective diets after weighing. A 1 cm-layer of white sand (Play Sand, Quikrete®, Atlanta, GA, USA) was spread over the soil surface surrounding the base of seedlings transplanted in plastic vials, which facilitated recovery of fall armyworm larvae or pupae. Seedlings were covered by small cages made of 148 ml (49 × 85 mm) plastic vials with their bottoms replaced with fine-mesh metal screen. Pots and cages were joined with their corresponding lids, which had a ∼40 mm (diam.) perforation to allow passage of the plant from the pot to the cage. Seedlings consumed by larvae were replaced with new seedlings as necessary. Fall armyworm larvae kept on artificial diet were maintained in their original diet cups covered with lids and placed in tray units.

Pupae were collected daily from diet cups or pots and immediately weighed and sexed. They were transferred to sterile plastic cups without diet, covered with a lid, and kept under the experimental conditions until adult emergence.

Host plant acceptance was assessed by scoring the amount of plant tissue consumed by individual larvae on the original seedling by the end of the first 4 days of feeding. Scores were assigned on a 1–5 scale where: 1 = rejection (no tissue consumed); 2 = slight acceptance (⩽ 1/3 of seedling consumed); 3 = moderate acceptance (⩽ 1/2 of seedling consumed); 4 = strong acceptance (⩽ 2/3 of seedling consumed), and; 5 = full acceptance (> 2/3 of seedling consumed).

Six replicates of 15 pots of each maize cultivar, and six trays of 15 diet cups each, i.e. 270 fall armyworm larvae, were used in the first trial. The same number of maize cultivar replications and four diet replications, i.e. 240 fall armyworm larvae, were used in the second trial.

2.4. Quantification of Bt-endotoxin levels in plant tissue

Presence and expression levels of *Bt* endotoxin in plant tissues were assessed in 10–14-day-old seedlings grown in a greenhouse as described above. Plant tissue was collected ≈30 min prior to assays and kept in plastic bags at ca. 5°C until used. Assays compared *Bt* endotoxin levels between corresponding pairs of transgenic and conventional cultivars; assay protocols were identical for each cultivar pair with the exceptions noted below. For assays, three replicates of four planters (= 12 planters) each were sown in a greenhouse for each transgenic and conventional cultivar pair. Each planter was sown with one row (10 seedlings) each of the transgenic and corresponding conventional cultivar. One transgenic maize seedling was taken from each of the four planters and one conventional seedling from each of two planters in each replicate; seedlings were of the same age (between 10–15 days old) in each case, and seedling roots were removed. Tissue from each seedling was homogenized (see below) and used at two dilutions (wt/vol) for each cultivar pair in the assays: 1:2,500 and 1:2,700 for Cry9C, and 1:300 and 1:500 for Cry1A(b). These dilution factors were chosen based on preliminary assays (unpublished data). Three measurements of *Bt*-endotoxin concentration were taken per each of the seedlings, yielding 36 and 18 observations per dilution for transgenic and conventional seedlings, respectively. Thus, the total numbers of observations were 72 and 36 for each of the transgenic and conventional cultivars, respectively.

A double-antibody sandwich enzyme-linked immunosorbent assay (DAS ELISA) was performed following established testing procedures, with some modifications (Voller *et al*., 1976; Clark and Adams, 1977). Specifically, detection and quantification of *Bt* endotoxin in plant tissue was accomplished using two different commercial 96-well quantification microtiter plates (Agdia Inc., Elkhart, IN, USA). Plant tissues were homogenized using an OMNI GLH, EZ Connect Homogenizer (Omni International Inc., Warrenton, VA, USA) and diluted to 1:10 in phosphate-buffered saline with 0.4 g of non-fat dried milk and 0.5 g of Tween 20 detergent (PBST) for each 100 ml of sample extract buffer needed. Further dilutions were made in 1:10 solution of conventional plant tissue in PBST. Test samples (transgenic and conventional) and purified truncated Cry1A(b) or Cry9C endotoxin standards were dispensed into wells coated with antibodies against *Bt* toxins. A solution of secondary antibody conjugated to alkaline phosphatase was then used to detect *Bt* endotoxins. A substrate detection and colour development reagent consisting of a 1:1 mixture of BP-A (Tetramethyl benzidine- TMB) and BP-B (Hydrogen peroxide in citric acid buffer) was added to each well, and the plate was incubated for 30 min out of direct light. Results were evaluated with an ELISA VERSAmax™ Tunable microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 630 nm SOFTmax Pro® software (Molecular Devices, Sunnyvale, CA, USA).

2.5. Data collected

Larval survivorship and weight at day 10, and survivorship and weight of newly formed pupae were recorded. Larval and pupal survivorship were calculated as number of pupae recovered/total number of larvae, and number of adults recovered/total number of larvae, respectively. The durations of the larval and pupal developmental periods were scored in days. Host plant acceptance values were scored according to the scale given above. Homogenate absorbance values at 630 nm were used to estimate *Bt* endotoxin concentrations for each of the transgenic and conventional cultivars.

2.6. Statistical analyses

Larval and pupal weights, and lengths of larval and pupal developmental periods were subjected to one-way ANOVA (Zar, 1999). Treatment means were separated as warranted using Student Newman-Keuls' (SNK) test (Zar, 1999). Homogeneity of variances test conducted on growth and development parameters of fall armyworm between the two artificial diet treatments from both trials indicated that variances were not homogenous. Thus, data corresponding to the artificial diet treatment in both trials were not pooled for statistical analyses. Proportions were arcsin $\sqrt{X}$-transformed prior to analysis and results are presented as back-transformed data. A LIFETEST procedure was used to test for homogeneity of the larval survivorship curves for the three diet treatments used in the two trials (SAS Institute, 1996). Larval and pupal survivorship on the different diets were separated by the Wilcoxon likelihood tests (SAS Institute, 1996). The distributions of host plant acceptance scores for transgenic and conventional maize cultivars in each trial, and for both transgenic cultivars were compared using contingency table log-likelihood ratio tests (Zar, 1999). Absorbance values were compared between cultivars, within trials, via one-way analysis of variance, and means corresponding to each of the conventional cultivars were compared against a hypothesized value of zero via one-sample *t*-tests (Zar, 1999). *Bt* endotoxin concentration values of transgenic maize cultivars were subjected to one-way analysis of variance (Zar, 1999).

3. Results

3.1. Fall armyworm growth and development

Larval and pupal weights of surviving larvae varied significantly with treatment for both the trial involving Cry9C and Cry1A(b) cultivars ($p < 0.0001$) (figure 1). Larvae fed artificial diet weighed significantly more than those fed plant tissue in both trials, while those fed conventional plant tissue weighed

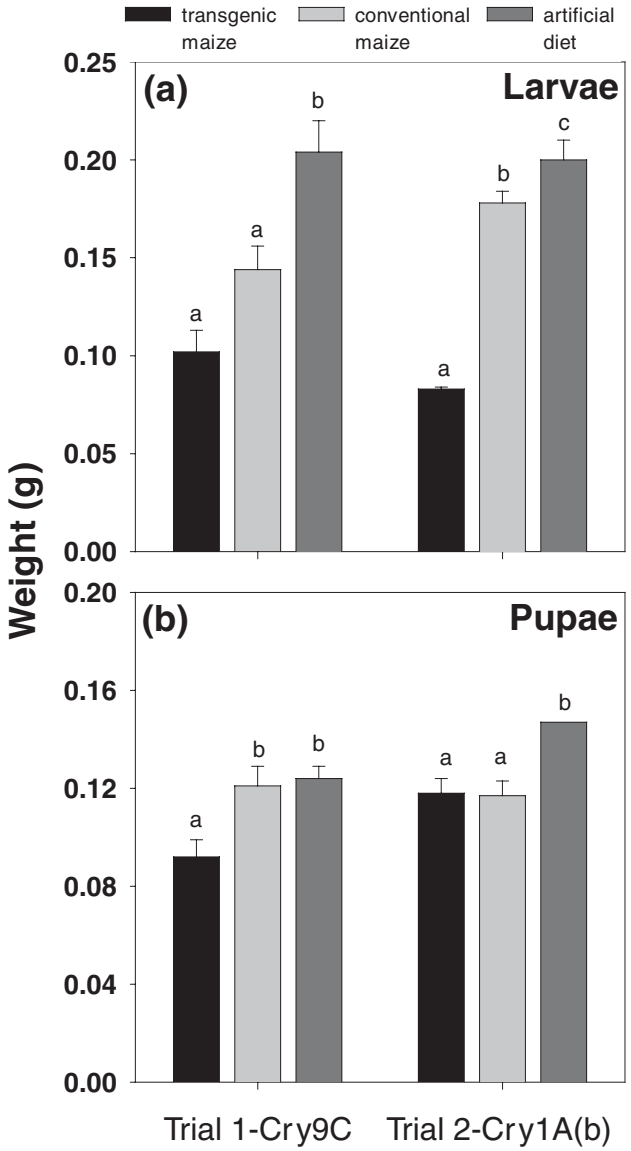

Figure 1. Weight (g) of Spodoptera frugiperda *(a) 10-day-old larvae, and (b) pupae reared on artificial diet, transgenic maize, or conventional maize. Within trials, columns sharing letters are not significantly different. Larvae: Trial 1, $F_{2,135} = 12.92$, $p < 0.0001$, and; Trial 2, $F_{2,214} = 66.97$, $p < 0.0001$. Pupae: Trial 1, $F_{2,116} = 7.26$, $p = 0.001$, and; Trial 2, $F_{2,188} = 4.25$, $p = 0.016$. Trial 1 included a cultivar expressing a Cry9C endotoxin gene (Event CBH 351), while Trial 2 included a cultivar expressing a Cry1A(b) endotoxin gene (Event MON 810).*

significantly more ($\approx 2\times$) than those fed transgenic tissue in the trial involving Cry1A(b) but not Cry9C plants. Similarly, pupae obtained from larvae fed artificial diet weighed more than those from Cry1A(b) and conventional plants in the second trial ($p = 0.016$) (figure 1b). In contrast, pupae obtained from artificial diet or conventional plants had similar weights, but weighed more ($\approx 1.3\times$) than those obtained from Cry9C plants in the first trial ($p = 0.001$) (figure 1b).

The lengths of the larval developmental periods varied significantly with treatment in both trials ($p < 0.0001$) (figure 2a). Larvae fed Cry9C or conventional plant tissue had similar developmental periods, both longer than that of larvae fed artificial diet (figure 2a). In contrast, those fed Cry1A(b) plant tissue had significantly longer ($\approx 1.2\times$) developmental periods than those developing on either conventional plant tissue or artificial diet, which had similar developmental periods (figure 2a). The lengths of the pupal developmental periods varied significantly with treatment in both trials ($p \leqslant 0.01$) (figure 2b). Larvae fed Cry9C plant tissue had pupal periods significantly longer ($\approx 1.7\times$) than those of larvae fed conventional plant tissue, while those of larvae fed artificial diet were intermediate in length (figure 2b). In contrast, pupal developmental periods were shorter on conventional plant tissue relative to artificial diet, and intermediate on Cry1A(b) plant tissue (figure 2b).

3.2. *Fall armyworm survivorship*

Survivorship of *S. frugiperda* larvae varied significantly with treatments in both the first and second trials (figure 3). Larval survivorship was lowest on both Cry9C ($\approx 0.43\times$ relative to the conventional cultivar) and Cry1A(b) ($\approx 0.73\times$) plants relative to the corresponding conventional plants and artificial diet ($p < 0.001$ in both cases) (figure 3a). Similarly, pupal survivorship was lowest on both Cry9C ($\approx 0.46\times$) and Cry1A(b) ($\approx 0.64\times$) plants relative to the corresponding conventional plants and artificial diet ($p < 0.001$ in both cases) (figure 3b). Larval and pupal survivorship were similar on conventional plants and artificial diet in both trials (figure 3a, b).

3.3. *Host plant acceptance by fall armyworm*

The distribution of host plant acceptance scores varied significantly with maize cultivar in both trials ($p < 0.001$) (figure 4). Approximately 80% of conventional plants were moderately to fully accepted, receiving acceptance scores of 3 or above, while $> 80\%$ of corresponding transgenic plants were either moderately to slightly accepted or rejected, receiving acceptance scores of 3 or below. In addition, Cry1A(b) plants were moderately to fully accepted with greater frequency than Cry9C plants ($G = 25.13$, df = 4, $p < 0.001$).

3.4. Bt *endotoxin levels in plants*

Absorbance values were near nil in both conventional cultivars, and were significantly lower than in the corresponding transgenic cultivars ($p < 0.0001$); non-zero mean values corresponding to the conventional cultivars represent background absorbance by plant tissue homogenates at that wavelength (table 1). Moreover, the means corresponding to the conventional cultivars do not differ significantly from zero ($p < 0.0001$)

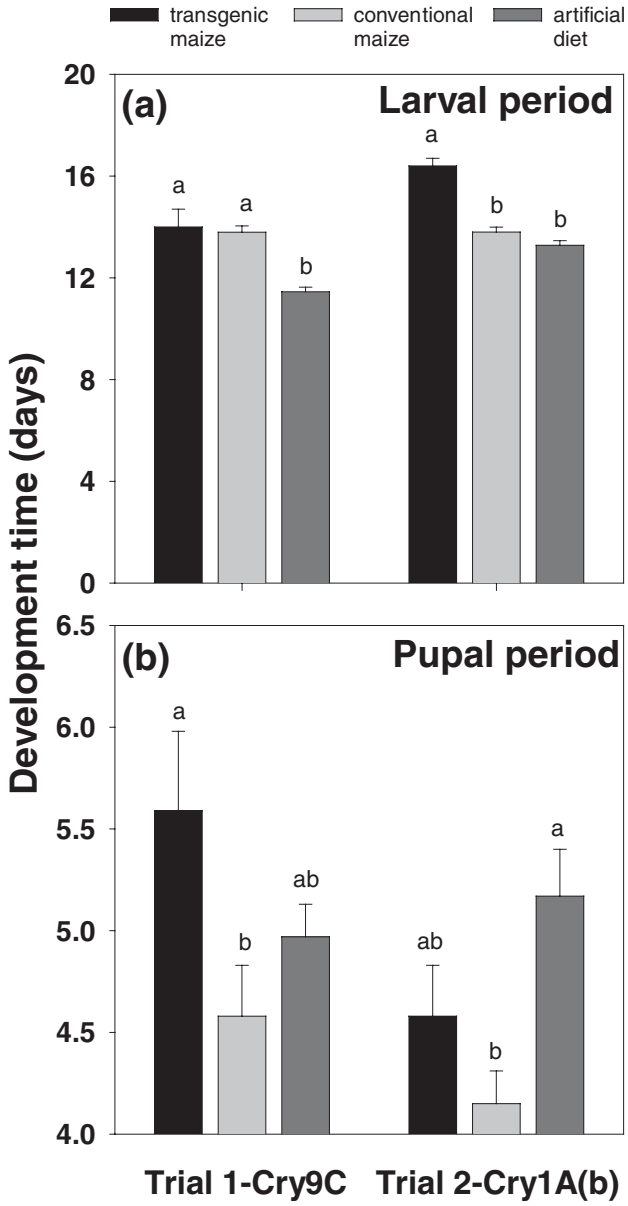

Figure 2. (a) Larval and (b) pupal developmental periods (days) of Spodoptera frugiperda *reared on artificial diet, transgenic maize, or conventional maize. Within trials, columns sharing letters are not significantly different. Larval period: Trial 1, $F_{2,120} = 18.12$,* p *< 0.0001, and; Trial 2, $F_{2,203} = 49.27$,* p *< 0.0001. Pupal period: Trial 1, $F_{2,98} = 4.44$,* p *= 0.014, and; Trial 2, $F_{2,178} = 6.01$,* p *= 0.003. Trial 1 included a cultivar expressing a Cry9C endotoxin gene (Event CBH 351), while Trial 2 included a cultivar expressing a Cry1A(b) endotoxin gene (Event MON 810).*

(table 1). Differences in absorbance values among tissue from four plants of each transgenic cultivar were not significant ($p \geqslant 0.5$) (table 2), suggesting that *Bt* endotoxin levels in transgenic plants were similar. *Bt* endotoxin concentration was ≈ 10 times greater in the Cry9C cultivar, 7908.4 $\pm$ 197.0 ng/ml, relative to the Cry1A(b) cultivar, 774.3 $\pm$ 25.2 ng/ml ($F = 1273.0$, df = 1, 142, $p < 0.0001$).

4. Discussion

Transgenic maize cultivars affected fall armyworm in several ways. Larval and pupal weights were reduced while developmental times were prolonged on both Cry9C and Cry1A(b)

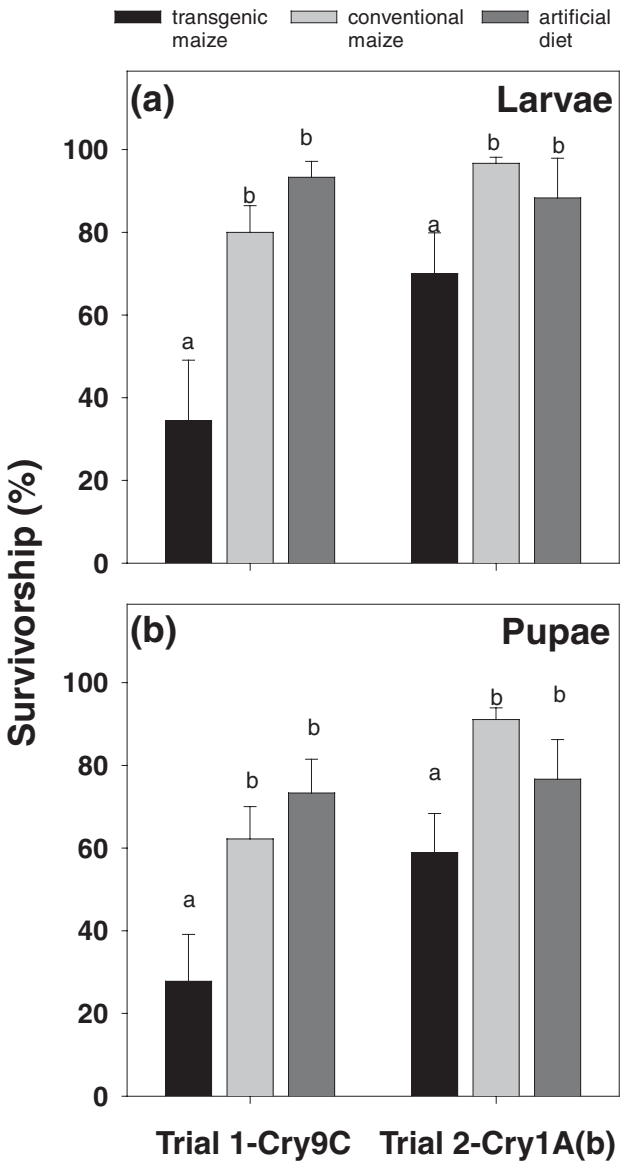

Figure 3. (a) Larval and (b) pupal survivorship of Spodoptera frugiperda *reared on artificial diet, transgenic maize, or conventional maize. Within trials, columns sharing letters are not significantly different. Larval: Trial 1,Wilcoxon* $\chi^2 = 67.82$, $df = 2$, $p < 0.001$, *and; Trial 2, Wilcoxon* $\chi^2 = 32.96$, $df = 2$, $p < 0.001$. *Pupal: Trial 1, Wilcoxon* $\chi^2 = 72.19$, $df = 2$, $p < 0.001$, *and; Trial 2, Wilcoxon* $\chi^2 = 28.39$, $df = 2$, $p < 0.001$. *Trial 1 included a cultivar expressing a Cry9C endotoxin gene (Event CBH 351), while Trial 2 included a cultivar expressing a Cry1A(b) endotoxin gene (Event MON 810).*

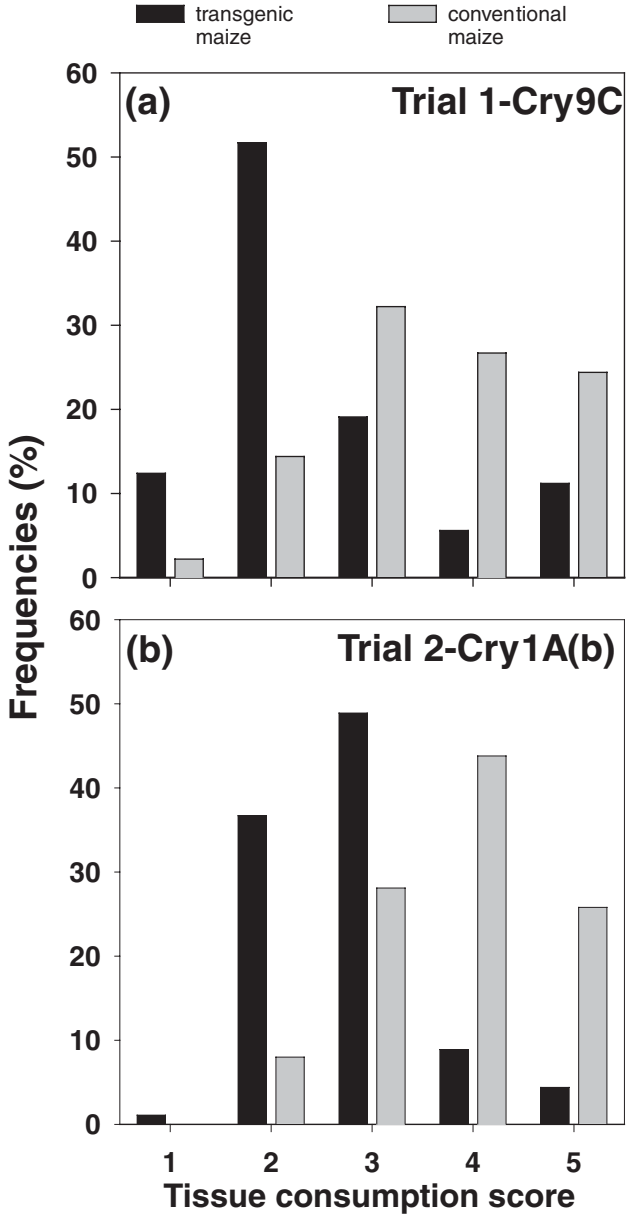

Figure 4. Distribution of host plant acceptance scores for transgenic and conventional maize seedlings offered to Spodoptera frugiperda *larvae for 4 days. (a) Trial 1, which included a transgenic maize cultivar expressing a Cry9C endotoxin gene (Event CBH 351) (*$G = 44.96$, $df = 4$, $p < 0.001$*). (b) Trial 2, which included a transgenic cultivar expressing a Cry1A(b) endotoxin gene (Event MON 810) (*$G = 126.23$, $df = 4$, $p < 0.001$*). Acceptance scores vary between 1 for rejection (no tissue consumed) and 5 for full acceptance (> 2/3 of seedling consumed); see text for detailed explanation of scores.*

cultivars in comparison to conventional cultivars. Similar effects of transgenic cultivars were previously reported for *Pseudaletia unipuncta* (Haworth) (Pilcher *et al*., 1997) and *Helicoverpa zea* (Boddie) on maize (Adamczyk *et al*., 1998, Lynch *et al*., 1999, Moore *et al*., 1999, Wiseman *et al*., 1999, Porter *et al*., 2000). Moreover, survivorship of fall armyworm fed transgenic maize was significantly lower than those fed conventional maize, and differences were more marked during early larval instars when high mortality rates were recorded on both transgenic cultivars (data not shown). Larval survivorship recorded in this study was lower than that reported by Lynch *et al*. (1999), who recorded up to 97–100% for fall armyworm larvae fed transgenic plants from day 6 of age. The low acceptance scores of fall armyworm on transgenic versus conventional maize seedlings recorded in this study show that the presence of *Bt* endotoxins substantially reduced feeding, as reported by earlier authors (Adamczyk *et al*., 1998, Lynch *et al*., 1999, Wiseman *et al*., 1999).

A substantial percentage of larvae reached the pupal stage on the transgenic maize cultivars although survivorship was significantly lower on these cultivars relative to the corresponding conventional cultivars and artificial diet. The suitability of these surviving larvae and resulting pupae as prey/hosts for natural enemies remains to be examined, but is likely compromised. For example, fall armyworm larvae and pupae weighed less and had longer larval periods on transgenic relative to conventional maize. Thus, transgenic maize affected survivorship as well as

Table 1. Absorbance values at 630 nm per sample unit (mean ± SE) of transgenic and conventional maize cultivars. Transgenic cultivars express Bacillus thuringiensis *(Berliner) endotoxins*

	Transgenic	Conventional	*F*	df	*p*
Trial 1: Cry9C	0.298 ± 0.039	0.007 ± 0.002[a]	41.4	1.107	< 0.001
Trial 2: Cry1A(b)	0.335 ± 0.032	0.003 ± 0.001[b]	27.7	1.107	< 0.001

[a]Not significantly different from zero: one-sample *t* test, $t = 4.03$, df = 35, $p < 0.0001$.
[b]Not significantly different from zero: one-sample *t* test, $t = 4.38$, df = 35, $p < 0.0001$.

Table 2. Absorbance values at 630 nm per sample unit (mean ± SE) of four transgenic and conventional maize seedlings. Transgenic cultivars express Bacillus thuringiensis *(Berliner) endotoxins*

	Trial 1: Cry9C	Trial 2: Cry1A(b)
Plant 1	0.327 ± 0.087	0.343 ± 0.066
Plant 2	0.304 ± 0.082	0.358 ± 0.072
Plant 3	0.285 ± 0.072	0.354 ± 0.073
Plant 4	0.276 ± 0.076	0.286 ± 0.046
F	0.08	0.73
df	3.69	3.69
p	0.97	0.54

growth and development of fall armyworm. Similar results were obtained in other studies involving fall armyworm and *Bt* transgenic maize and cotton (Jenkins *et al*., 1997, Adamczyk *et al*., 1998, Lynch *et al*., 1999, Wiseman *et al*., 1999). Moreover, truncated *Bt* endotoxins are present in early-instar fall armyworm fed Cry1A(b) transgenic maize at levels (37.2 ± 5.4 ng/ml) that may affect natural enemies feeding on these larvae (Bokonon-Ganta and Bernal, unpubl.). Bernal *et al*. (2002) showed that immature-stage mortality in the ectoparasitoid *Parallorhogas pyralophagus* (Marsh) was greater on hosts (*Eoreuma loftini* Dyar) fed *Bt* maize relative to those fed conventional maize, which suggested that hosts fed transgenic maize were toxic to developing parasitoids. Thus, fall armyworm surviving on a diet of *Bt* maize likely represent poor quality hosts for parasitoids because they are smaller, have longer developmental periods, and contain truncated *Bt* endotoxins, and natural enemies feeding on these larvae may be lethally or sublethally affected. Such effects on fall armyworm and associated natural enemies will likely affect natural enemy population dynamics and biological control of fall armyworm.

Additionally, the results of this study suggested that Cry9C maize was more toxic to fall armyworm than Cry1A(b) maize, though a statistical comparison between the two cultivars was not appropriate. This suggestion was supported by the results showing that Cry9C plants were more acceptable than Cr1A(b) plants to fall armyworm larvae, and *Bt* endotoxin levels were $\approx 10\times$ greater in the former versus the latter cultivar. Similar observations were made by Giles *et al*. (2000), who found that *Bt* endotoxin levels in Cry1A(b) maize kernels were nearly 20-fold lower than levels in kernels of Cry9C maize.

Overall, our results show that fall armyworm, a non-target, secondary pest is affected by *Bt* maize cultivars, and suggest that these effects will likely impact its biological control. This raises concerns over the compatibility of transgenic crops with other IPM tactics, and suggests that closer examination of this technology is warranted. In particular, transgenic cultivars should not interfere with biological control of both target and non-target pests within and outside the crop. A number of studies have evaluated the effects of transgenic plants on non-target organisms, including parasitoids (Fitt *et al*., 1994, Flint *et al*., 1995, Johnson *et al*., 1997, Schuler *et al*., 1999, Hilbeck *et al*., 2000). So far, results have been highly variable, suggesting interactions between transgenic crops and natural enemies from synergism to antagonism, while in some cases an interaction was not evident (Schuler *et al*., 1999, Hilbeck, 2002). Despite extensive studies on the effects of transgenic crops on target and non-target herbivores, the potential effects on parasitoids have not been adequately addressed. One factor partially responsible for this has been the difficulty of conducting such studies given the high mortality rates evident in herbivores that are fed *Bt* transgenic plant tissue. The results of this study suggest that studies on the direct and indirect effects of *Bt* transgenic plants on parasitoids of non-target herbivores such as fall armyworm can be conducted given that survivorship rates are sufficient to permit adequate replication. For example, *Cotesia marginiventris* (Cresson) is a common parasitoid of fall armyworm and other lepidopteran pests, and studies on the potential effects of *Bt* plants on this parasitoid would shed light on plausible spill-over effects of widespread planting of transgenic crops on biological control in transgenic and surrounding crops. Such studies are in the planning stage as part of more comprehensive research in our laboratory aiming to evaluate potential effects of transgenic crops on natural enemies and biological control.

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References

ADAMCZYK, J. J., HOLLOWAY, J. W., CHURCH, G. E., LEONARD, B. R. and GRAVES, J. B., 1998. Larval survival and development of the fall armyworm (Lepidoptera: Noctuidae) on normal and transgenic cotton expressing the *Bacillus thuringiensis* CrylA(c)-endotoxin. *Journal of Economic Entomology*, **91,** 539 – 545.

ADAMCZYK JR, J. J., HARDEE, D. D., ADAMS, L. C. and SUMMERFORD, D. V., 2001. Correlating differences in larval survival and development of Bollworm (Lepidoptera: Noctuidae) and Fall Armyworm (Lepidoptera: Noctuidae) to differential expression of Cry1A(c) δ-endotoxin in various plant parts among commercial cultivars of transgenic *Bacillus thuringiensis* Cotton. *Journal of Economic Entomology*, **94,** 284 – 290.

ALL, J. N., STANCIL, J. D., JOHNSON, T. B. and GOUGER, R., 1996. Controlling fall Armyworm (Lepidoptera: Noctuidae) infestations in whorl stage corn with genetically modified *Bacillus thuringiens* formulations. *Florida Entomologist*, **79,** 311 – 317.

ANDOW, D. A., 2002. Resisting resistance to Bt-corn. In Letourneau, D. K. and Burrows, B. E. (eds) *Genetically engineered organisms: assessing environmental and human health effects* (CRC Press LLC, Boca Raton), pp. 99 – 124.

ARMSTRONG, C. L., PARKER, G. B., PERSHING BROWN, S. M., SANDERS, P. R., DUNCAN, D. R., STONE, T., DEAN, D. A., DEBOER, D. L. and HART, J., 1995. Field evaluation of European Corn Borer control in progeny of 173 transgenic corn events expressing an insecticidal protein from *Bacillus thuringiensis*. *Crop Science*, **35,** 550 – 557.

ASHLEY, T. R., 1979. Classification and distribution of fall armyworm parasites. *Florida Entomologist*, **62,** 114 – 123.

BERNAL, J. S., GRISET, J. and GILLOGLY, P. O., 2002. Impacts of developing on *Bt* maize-intoxicated hosts on fitness parameters of a stem borer parasitoid. *Journal of Entomological Science*, **37,** 27 – 40.

CLARK, M. F. and ADAMS, A. N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. *Journal of General Virology*, **34,** 475 – 483.

FITT, G. P., MARES, C. L. and LLEWELLYN, D. J., 1994. Field evaluation and potential ecological impact of transgenic cottons (*Gossypium hirsutum*) in Australia. *Biocontrol Science and Technology*, **4,** 535 – 548.

FLINT, H. M., HENNEBERRY, T. J., WILSON, F. D., HOLGUIN, E., PARKS, N. and BUEHLER, R. E., 1995. The effects of transgenic cotton (*Gossypium hirsutum*), containing *Bacillus thuringiensis* toxin genes for the control of the pink bollworm *Pectinophora gossypiella* (Saunders) and other arthropods. *Southwestern Entomologist*, **20,** 281 – 292.

GILES, K. L., HELLMICH, R. L., IVERSON, C. T. and LEWIS, L. C., 2000. Effects of transgenic *Bacillus thuringiensis* maize grain on *Bacillus thuringiensis* – susceptible *Plodia interpunctella* (Lepidoptera: Pyralidae). *Journal of Economic Entomology*, **93,** 1011 – 1016.

GOULD, F., 1998. Sustainability of transgenic insecticidal cultivars: integrating pest genetics and ecology. *Annual Review of Entomology*, **43,** 701 – 726.

GUILLEBEAU, L. P. and ALL, J. N., 1991. Use of pyrethroids, methomyl, and chlorpyrifos to control fall armyworm (Lepidoptera: Noctuidae) in whorl stage field corn, sweet corn and sorghum. *Florida Entomologist*, **74,** 261 – 270.

HILBECK, A., 2002. Transgenic host plant resistance and non-target effects. In Letourneau, D. K. and Burrows, B. E. (eds) *Genetically engineered organisms: assessing environmental and human health effects* (CRC Press LLC, Boca Raton), pp. 167 – 185.

HILBECK, A., MEIER, M. S. and RAPS, A., 2000. Review on non-target organisms and Bt-plants. Report to Greenpeace International, Amsterdam. Ecostrat GmbH, Zurich. .

HRUSKA, A. J. and GLADSTONE, S. M., 1988. Effect of period and level infestation of the fall armyworm, *Spodoptera frugiperda*, on irrigated maize yield. *Florida Entomologist*, **71,** 249 – 254.

JENKINS, J. N., MCCARTY JR, J. C., BUEHLER, R. E., KISER, J., WILLIAMS, C. and WOFFORD, T., 1997. Resistance of cotton with δ-endotoxin genes from *Bacillus thuringiensis* var. Kurstaki on selected lepidopteran insects. *Agronomy Journal*, **89,** 768 – 780.

JOHNSON, M. T., GOULD, F. and KENNEDY, G. G., 1997. Effects of natural enemies on relative fitness of *Heliothis virescens* genotypes adapted and not adapted to resistant host plants. *Entomologia Experimentalis et Applicata*, **82,** 219 – 230.

KOZIEL, M. G., BELAND, G. L., BOWMAN, C., CAROZZI, N. B., CRENSHAW, R., CROSSLAND, L., DAWSON, J., DESAI, N., HILL, N., KADWELL, S., LAUNIS, K., LEWIS, K., MADDOX, D., MCPHERSON, K., MEGHJI, M. R., MERLIN, E., RHODES, R., WARREN, G. W., WRIGHT, M. and EVOLA, S. V., 1993. Field performance of elite transgenic maize plants expressing an insecticidal protein derived from *Bacillus thuringiensis*. *Biotechnology*, **11,** 194 – 200.

LOSEY, J. E., OBRYCKI, J. J. and HUFBAUER, R. A., 2002. Impacts of genetically engineered crops on non-target herbivores: *Bt*-corn and Monarch butterflies as a case study. In Letourneau, D. K. and Burrows, B. E. (eds) *Genetically engineered organisms: assessing environmental and human health effects.* (CRC Press LLC, Boca Raton), pp. 143 – 165.

LYNCH, R. E., WISEMAN, B. R., PLAISTED, D. and WARNICK, D., 1999. Evaluation of transgenic sweet corn hybrids expressing Cry1A(b) toxin for resistance to corn earworm and fall armyworm (Lepidoptera: Noctuidae) on insect-resistant soybean genotypes. *Journal of Economic Entomology*, **92,** 246 – 252.

MARTINEZ, A. J., BARD, J. and HOLLER, T. A., 1988. Mass rearing sugarcane borer and Mexican rice borer for production of parasites *Allorhogas pyralophagus* and *Rhaconotus rosilensis*. USDA-APHIS-PPQ, APHIS 83 – 1.

MOORE, C. C., BENEDICT, J. H., FUCHS, T. W., FRIESEN, R. D. and PAYNE, C., 1999. *Bt* cotton technology in Texas: a practical view. *Texas Agricultural Extension Service* L-5169.

PILCHER, C. D., RICE, M. E., OBRYCKI, J. J. and LEWIS, L. C., 1997. Field and laboratory evaluations of transgenic *Bacillus thuringiensis* corn on secondary lepidopteran pests (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, **90,** 669 – 678.

PITRE, H. N., 1986. Chemical control of the fall armyworm (Lepidoptera: Noctuidae): an update. *Florida Entomologist*, **69,** 570 – 578.

PITRE, H. N. and HOGG, D. B., 1983. Development of the fall armyworm on cotton, soybean and corn. *Journal of Georgia Entomological Society*, **18,** 182 – 187.

PORTER, P., SCHUSTER, G., MORRISON, P., TROXCLAIR, N., CRONHOLM, G., PATRICK, C. and PARKER, R., 2000. *Bt* corn technology in Texas: a practical view. B-6090. *Texas Agricultural Extension Service).*

SAS INSTITUTE, 1996. SAS user's guide, statistics. SAS Institute, Cary, N.C. .

SCHULER, T. H., POPPY, G. M., KERRY, B. R. and DENHOLM, I., 1999. Potential side effects of insect-resistant transgenic plants on arthropods natural enemies. *Trends in Biotechnology*, **17,** 210 – 216.

SPARKS, A. N., 1979. A review of the biology of the fall armyworm. *Florida entomologist*, **62,** 82 – 87.

TIETZ, H. M., 1972. An index to the described life histories, early stages and host of the Microlepidoptera of the Continental United States and Canada. Vol I, II. (Allyn Museum of Entomology, Sarasota, Florida).

VOLLER, A., BARTLETT, A., BIDWELL, D. E., CLARK, M. F. and ADAMS, A. N., 1976. The detection of viruses by enzyme-linked immunosorbent assay (ELISA). *Journal of General Virology*, **33,** 165 – 167.

WISEMAN, B. R., LYNCH, R. E., PLAISTED, D. and WARNICK, D., 1999. Evaluation of *Bt* transgenic sweet corn hybrids for resistance to corn earworm and fall armyworm (Lepidoptera: Noctuidae) using meridic diet bioassay. *Journal of Entomological Science*, **34,** 415 – 425.

ZAR, J. H., 1999. Biostatistical analysis. 662pp (+ Apps. Upper Saddle River, New Jersey).