Development and Behavior of Spodoptera exigua (Lepidoptera: Noctuidae) Larvae in Choice Tests with Food Substrates Containing Toxins of Bacillus thuringiensis

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Developmental time and behavior of Spodoptera exigua (Hübner) larvae and their pupal weight were investigated in dual-choice arenas containing Bacillus thuringiensis Berliner (Bt) toxins. In both artificial diet and cotton leaf-choice tests with Bt formulation MVP, the mean proportion of larvae on Bt-free diets was lower than on Bt-containing diets. Artificial diet tests further showed that larvae were more often found on diets where MVP was applied on the diet surface than on diets where the formulation was mixed in the diet. In leaf-choice tests with the Bt transgenic cotton line ‘C 1076’ and the nontransgenic ‘C 312’, more larvae were found on nontransgenic cotton leaves. Also, when feeding damage was measured in the leaf experiments, feeding damage was more frequently observed on Bt-free than on Bt-containing leaves in MVP and ‘C 1076’ choice tests. Leaf-choice tests with the Bt formulations Dipel ES (containing B. thuringiensis var. Kurstaki) and Xentari (containing B. thuringiensis var. Aizawai) and the transgenic Bt line ‘C 531’ showed patterns of larval behavior and feeding damage different than those obtained in the other leaf tests. The survival rate of larvae on MVP was comparable to the larval survival on control tests containing only Bt-free diets. However, lower pupal weight and longer developmental time were observed. Experiments with neonate S. exigua larvae on MVP-treated cotton plants demonstrated that the number of larvae remaining on the plant was negatively correlated with concentration and exposure time. The consequences and opportunities for behavior adaptation to Bt in pest management are discussed.

Key Words: Spodoptera exigua; Bacillus thuringiensis; behavior; pupal weight; developmental time; biological control.

INTRODUCTION

Control of pest insects with the bioinsecticide Bacillus thuringiensis Berliner (Bt) has increased in frequency in the last decade. Insecticide formulations with highly effective Bt strains are now available. Also, transgenic crop lines have recently been developed and introduced that produce toxic Bt proteins in plant tissue. When Bt is digested by a target insect, the reaction of Bt protoxins and insect midgut enzymes creates activated toxins that cause severe dysfunction of the midgut epithelium that ultimately leads to the insect’s death (Gill et al., 1992). Compared with conventional pesticides, Bt formulations are highly specific with low toxicity to nontarget organisms. Reports also indicate that Bt-infected insects remain suitable as reproductive and food resources for natural enemies and, in some cases, they are even more suitable since, due to Bt intoxication, an infected insect remains in a vulnerable stage for an extended time (Weseloh and Andreadis, 1982; Weseloh et al., 1983; Niwa et al., 1987; Johnson and Gould, 1992; Soares et al., 1994; Lewis, unpublished data). Therefore, the impact of Bt on the ecosystem is expected to be minimal compared with that of conventional pesticides.

However, as with conventional pesticides, pest insects have already shown resistance to Bt (McGaughey, 1985; Tabashnik et al., 1990; Gould et al., 1992; McGaughey and Whalon, 1992; Bauer, 1995). Although most studies investigate physiological resistance, it is suggested that a behavioral component can contribute to pesticide resistance as well (Sparks et al., 1989). For example, in the presence of pyrethroids, resistant strains of Heliothis virescens (F.) and Haemotobia irritans (L.) show different patterns of behavior than susceptible strains. The resistant strains are able to minimize contact with pyrethroids by reducing their movement. This typical behavior in a toxic environment is described as behavioral resistance (Lockwood et al., 1984). Other behavior studies do not compare susceptible and resistant strains, but merely investi-
gate the behavior of an insect species in the presence of toxins. The behavioral difference in a toxic and nontoxic environment should, therefore, be described as behavioral avoidance or behavior adaptation. Few studies have investigated behavioral avoidance of insects to Bt. Avoidance of Bt-contaminated laboratory diets was observed with H. virescens larvae in choice tests (Gould et al., 1991). Findings such as these pose serious problems when using Bt formulations for pest control. Under field conditions, a uniform coverage of pesticides on all plant parts is rarely achieved. Pest insects may be able to survive a Bt application not only as a result of physiological resistance but also by behavior adaptation, by temporarily avoiding Bt-contaminated plant parts and feeding mainly on the untreated parts. After biological activity of the Bt formulation has ceased, larvae may resume feeding on all plant parts.

This study deals with the behavior of Spodoptera exigua (Hübner) larvae on Bt-containing and Bt-free food substrates. Relative to other lepidopteran species, S. exigua has shown high resistance levels to many insecticides including Bt. This study presented an opportunity to investigate behavioral changes of S. exigua larvae in the presence of Bt toxins. Resistance of S. exigua to Bt may be due, in part, to behavioral avoidance.

**MATERIALS AND METHODS**

**General.** In contrast to other Bt choice studies, experiments were performed with two food substrates: artificial pinto bean diet (Burton, 1969) and cotton leaves. This allowed us to assess any variability which may have been attributable to differences between cotton leaves. Furthermore, the food substrates in the choice-test arenas were spatially divided and therefore larvae had to cover some diet-free space before contacting the opposite substrate.

**Insects.** All experiments were conducted in a climate-controlled room at 25°C, 70% RH, and a photoperiod of 14:10 (L:D) h. The S. exigua larvae were obtained from a strain reared in the laboratory for 8 years. Newly molted third instars were used in the choice experiments. In this developmental stage S. exigua usually begins to disperse, which offered a higher mobility in the experiments. Newly hatched larvae were used in drop-off experiments.

**Plants.** The cotton plants were grown in a greenhouse at 25°C, 40-70% RH, and a photoperiod of 14:10 (L:D) h and were 1 1/2 to 2-months old. For the leaf tests, we used mature leaves (80-120 cm²) that were removed from the upper third of the plants as needed.

**Bt formulations and transgenic cotton plants.** In both artificial diet tests and cotton leaf tests the commercial Bt formulation MVP (Mycogen Corp., San Diego, CA) was used. This is an aqueous flowable formulation with 10% active ingredient containing delta endotoxins of B. thuringiensis var. Kurstaki encapsulated in killed Pseudomonas fluorescens (Migula). In the cotton leaf tests two additional Bt formulations were used: (1) Dipel ES (Abbott Laboratories, Chicago, IL), emulsifiable suspension with 3.5% active ingredient containing B. thuringiensis var. Kurstaki; and (2) Xentari (Abbott), a water dispersible granule with 10.3% active ingredient containing B. thuringiensis var. Aizawai. Two transgenic cotton lines with Bt genes also were tested: the transgenic lines ‘Coker 531’ (C 531) and ‘Coker 1076’ (C 1076) were both tested against a nontransgenic Coker line ‘C 312’.

**Artificial diet tests.** Plastic 30-ml cups containing 10 ml of pinto bean diet were used and a 0.5-ml solution of MVP (10% or 25% MVP) was either applied with a spray applicator (CO₂ as propellant) on the surface of the diet or mixed in the diet at the time of diet preparation. The tests were conducted by placing two diet cups end to end, held together with rubber bands. The two cups were placed horizontally with a distance of 70 mm between the diets. Larvae were individually released in the dual-choice test arena on the diet (release diet). The position of the larvae (release diet, opposite diet, or middle of the test arena) was recorded daily until pupation or death occurred. Pupae were weighed 24 h after the onset of pupation. Twenty or more individual larvae were observed per treatment and treatments were replicated at least two times (n = 40 or more).

**Cotton leaf tests.** Solutions of the Bt formulations MVP (10 and 25%), Dipel ES (0.26%), or Xentari (0.13% by weight) were applied to leaves of cotton variety ‘DPL 5415’. Except for MVP, the concentrations tested were the rates recommended by the manufacturer for treatment against early instar S. exigua in cotton. Higher concentrations of MVP were used when preliminary tests showed that S. exigua was not affected by the recommended dose. The formulations were applied to leaves with a regulator-controlled aerosol spray applicator, using CO₂ as a propellant at a constant pressure of 70 kPa. One to 1.5 ml of Bt solution per leaf was applied on both leaf surfaces. Untreated leaves were sprayed with water. The leaves were air-dried before they were pinned in the test arena. As a dual-choice set-up for the experiments, two paraffin-filled petri dishes (diameter, 90 mm) separated by an acetate transparent cylinder (width, 70 mm) were used. In both petri dishes, cotton leaves were pinned to moist filter paper on the paraffin layer. The structure was held together with rubber bands and placed in a horizontal position (petri dishes in vertical position). For each choice-test treatment, third instar S. exigua larvae, rear on cotton leaves, were individually placed in a test arena: 20 were placed on Bt-treated leaves and 20 on untreated leaves. For the control treatments, in which leaves were all un-
treated or treated with Bt, 40 larvae were placed on the leaves in the arena on either side and the release side was marked. The cotton leaves were replaced every other day with fresh leaves, whereby the larvae were disturbed as little as possible. After the leaves were replaced, the test arenas were positioned vertically for a few minutes, with the side occupied by the larvae down, to allow the larva to settle on the leaf. The quantity of leaf material in each dish was sufficient for 48 h. During testing, the position of each larva (release leaf, opposite leaf, or middle of the test arena) and the occurrence of damage were recorded on a daily basis.

Neonate drop-off experiments. The cotton plants used in these experiments were 2 to 3 months old. The plants were sprayed with 10 ml of either water or MVP in two concentrations (10% or 25%) and allowed to dry. Egg masses containing 50–150 eggs were cut from paper where they had been oviposited by S. exigua moths. The number of eggs in each mass was counted. Just before hatch, the egg masses were randomly pinned on cotton leaves, five per plant. The number of neonates present on the leaves and the number of unhatched eggs were counted after 24 or 48 h.

Statistical analysis. When the Bt formulations were tested, the mean proportion of larvae on the release side was compared between treated and untreated substrates. Similarly, the mean proportion of larvae on the opposite side was compared between treated and untreated substrates. The same comparisons were made in the control treatments. The mean proportion of larvae on release side or opposite side was compared between substrates. When transgenic cotton leaves were tested, the mean proportion of larvae on the release side was compared between transgenic and nontransgenic leaves. Similarly, the mean proportion of larvae on the opposite side was compared between transgenic and nontransgenic leaves. In the leaf tests, the proportion of leaves receiving feeding damage was compared using a similar procedure. All above data were independent and comparisons were made with t tests. For evaluating pupal weight and developmental time within treatments in the artificial-diet experiments, t test was used. In the leaf experiments, pupal weight and developmental time within treatments were pooled, when no significant difference was found between the release sides. Pupal weight and developmental time in the leaf tests and results obtained from the neonate drop-off experiments were analyzed with an ANOVA, followed by Duncan multiple range test, when significance was demonstrated (P < 0.05; SAS, 1985).

RESULTS

Artificial diet tests. When the mean number of release or opposite diets occupied by larvae within a treatment are compared, significantly more untreated than treated diets contained larvae during the experiments. In choice tests, only a few diets were occupied by larvae when MVP was either mixed in the diet or sprayed on the surface of the diet at concentrations of 10 and 25% (Fig. 1a). Larvae in untreated control arenas tended to remain on the release side (88%). In arenas where the choices were MVP mixed versus MVP sprayed on the surface, the latter diet was more occupied by larvae during the experiments (Fig. 1b). For both concentrations (10 and 25%) the results were similar. However, no difference was observed when larvae had to choose between two MVP concentrations mixed in the diets (Fig. 1b).

Survival remained high in all treatments (85–100%, Table 1) except in arenas with only MVP-treated diets (Table 2). Except for larvae in arenas with surface applied MVP (25%) versus untreated, all other treatments showed that the developmental time of larvae released on the MVP-treated diets was significantly longer than the developmental time of larvae released on the untreated diets (Table 1). However, average pupal weight was not significantly different. The lowest survival and longest developmental time were observed in larvae that were offered MVP-treated diets only (Table 2).

Cotton leaf tests. In choice arenas with MVP, the mean proportion of larvae on treated release leaves was significantly lower (48%, Fig. 2a) than the proportion of larvae on untreated release leaves (81%). Also, the mean proportion of larvae on treated opposite leaves was significantly lower (15%, Fig. 2a) than the proportion of larvae on untreated opposite leaves (49%). When the proportion of leaves with feeding damage was compared, a similar trend was observed. More untreated (88%, Fig. 2b) than treated release leaves (55%) were damaged and also more untreated (67%) than treated opposite leaves (24%) were damaged. S. exigua larvae in untreated control arenas remained on the release leaves (76–79%, Fig. 2a) and these leaves were often damaged by feeding (83–84%, Fig. 2b). Therefore, no significant differences were found between release leaves or opposite leaves in control tests with untreated leaves. Control tests with only treated leaves showed trends similar to those seen for the control tests with only untreated larvae (Figs. 2a and 2b).

Choice tests conducted with Dipel ES demonstrated a significant difference in larval occurrence on untreated and treated opposite leaves (46 and 24%, respectively, Fig. 2a), but no difference between untreated and treated release leaves (74 and 52%, respectively) was found. Also, no differences in proportion of damaged leaves between untreated and treated leaves were observed on either release side or opposite side (Fig. 2b). Similarly, choice tests with Xentari showed no differences in either proportion of leaves containing...
larvae (Fig. 2a) or proportion of leaves damaged (Fig. 2b).

In choice tests with the two transgenic cotton lines, one line (C 1076) showed significant differences in larval occupancy on the release side (Fig. 3a) and proportion of damaged leaves between transgenic and nontransgenic leaves (Fig. 3b). Release leaves of nontransgenic cotton contained significantly more larvae (72%) than release leaves from transgenic C 1076 (46%) and more nontransgenic (81%) than transgenic (47%) opposite leaves were damaged. Similar results were obtained from the opposite side of the test arenas. Opposite nontransgenic leaves contained more larvae (54%) than opposite C 1076 leaves (28%), and more nontransgenic (71%) than transgenic (36%) opposite leaves were damaged. No differences in larval occupancy and proportion of damaged leaves were observed in transgenic line C 531.

Since average developmental time and pupal weight were not related to release side, as was observed in the artificial diet choice tests, data were grouped and compared per treatment. The average developmental time for larvae in tests with only MVP-treated leaves was significantly longer (16 days) than developmental time for larvae on the untreated control (9 days), while larvae in the choice tests showed an intermediate developmental time (11 days; Table 3). The average weight of pupae from larvae in MVP choice tests was similar to the average weight of pupae from the untreated control, but pupal weight from larvae in arenas with treated leaves only was significantly lower.

The survival rate of larvae in choice tests with Dipel ES and Xentari was relatively low and developmental time longer than that observed in MVP choice tests. However, the average weight of pupae from surviving larvae was comparable to the weight of pupa from
larvae in untreated control tests (Table 3). Survival, developmental time, and pupal weight were equal between choice tests with the two transgenic cotton lines (Table 4). These treatments were not compared with the other treatments because of possible cotton variety effects.

Neonate drop-off tests. The mean percentage of neonates found after 24 h was significantly lower on MVP-treated cotton plants than on control plants (Table 5). After 24 h, no significant difference was observed between the two MVP concentrations. However, after 48 h, significantly fewer larvae remained on the cotton plants that were treated with 25% MVP than the 10% MVP and control treatments.

**DISCUSSION**

Our study demonstrates that the Bt formulation MVP and one of the transgenic Bt cotton lines, C 1076, cause behavioral avoidance of Bt diets in larvae of *S. exigua* in situations where both Bt-containing and Bt-free diets are present. Even though developmental time is longer for larvae in MVP choice situations, weight of pupae is not different from the weight of control pupae. However, high mortality, low pupal weight, and longer developmental time were observed in no-choice situations with Bt-treated diets. Therefore, these results show that *S. exigua* is able to survive and complete larval development in a dual-choice toxic environment as a result of behavioral avoidance.

Not all Bt formulations and Bt-transgenic cotton leaves caused a similar change in larval behavior. A higher mortality rate, as observed in Dipel ES and Xentari-choice tests, may have influenced the results. Especially for Xentari, no change in behavior was observed because the dosage used may have been too high. As a result, the few surviving larvae apparently became immobile as a result of intoxication. Also, no

**TABLE 1**

Survival Rate, Pupal Weight, and Developmental Time of *S. exigua* Larvae Released Individually in Dual-Choice Arenas with Bt-Treated and Untreated Artificial Diets

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>Release side</th>
<th>n</th>
<th>Percentage survival of larvae b</th>
<th>Mean pupal weight (mg) ± SE</th>
<th>Mean developmental time c (days) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface applied (10%) vs untreated</td>
<td>Surface</td>
<td>29</td>
<td>100</td>
<td>123.3 ± 4.3 d</td>
<td>9.5 ± 0.4** d</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>30</td>
<td>86.7</td>
<td>121.5 ± 5.3</td>
<td>8.0 ± 0.3</td>
</tr>
<tr>
<td>Surface applied (25%) vs untreated</td>
<td>Surface</td>
<td>30</td>
<td>96.4</td>
<td>103.2 ± 5.9</td>
<td>8.3 ± 0.4**</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>30</td>
<td>92.6</td>
<td>115.9 ± 5.0</td>
<td>10.3 ± 0.4</td>
</tr>
<tr>
<td>Mixed (10%) vs untreated</td>
<td>Mixed</td>
<td>20</td>
<td>90.0</td>
<td>108.6 ± 6.8</td>
<td>10.6 ± 0.9*</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>20</td>
<td>90.0</td>
<td>111.8 ± 7.2</td>
<td>8.1 ± 1.7</td>
</tr>
<tr>
<td>Mixed (25%) vs untreated</td>
<td>Mixed</td>
<td>18</td>
<td>100</td>
<td>114.3 ± 6.3</td>
<td>10.1 ± 0.4**</td>
</tr>
<tr>
<td></td>
<td>Untreated</td>
<td>20</td>
<td>90</td>
<td>128.8 ± 5.3</td>
<td>8.4 ± 0.4</td>
</tr>
<tr>
<td>Untreated control</td>
<td>Untreated</td>
<td>19</td>
<td>94.7</td>
<td>145.2 ± 4.3</td>
<td>6.6 ± 0.2</td>
</tr>
</tbody>
</table>

† The Bt formulation MVP was either applied on the surface of the diet or mixed in the diet.

b Larvae that were able to pupate.

c From third instar to pupa.

d Asterisks indicate significant difference within a treatment (t tests: P ≤ 0.05 (*), P ≤ 0.01 (**), or P ≤ 0.001 (***)).

**TABLE 2**

Survival Rate, Pupal Weight, and Developmental Time of *S. exigua* Larvae Released Individually in Dual-Choice Arenas with Bt-Treated Artificial Diets

<table>
<thead>
<tr>
<th>Treatment†</th>
<th>Release side</th>
<th>n</th>
<th>Percentage survival of larvae b</th>
<th>Mean pupal weight (mg) ± SE</th>
<th>Mean developmental time c (days) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface applied vs mixed (10%)</td>
<td>Surface</td>
<td>18</td>
<td>55.6</td>
<td>101.1 ± 6.8 d</td>
<td>14.6 ± 1.2 d</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>19</td>
<td>52.6</td>
<td>92.7 ± 4.3</td>
<td>15.7 ± 0.7</td>
</tr>
<tr>
<td>Surface applied vs mixed (25%)</td>
<td>Surface</td>
<td>20</td>
<td>55</td>
<td>79.4 ± 4.9</td>
<td>15.8 ± 0.7</td>
</tr>
<tr>
<td></td>
<td>Mixed</td>
<td>19</td>
<td>52.6</td>
<td>86.4 ± 6.7</td>
<td>17.3 ± 1.3</td>
</tr>
<tr>
<td>Mixed (10%) vs mixed (25%)</td>
<td>Mixed 10%</td>
<td>16</td>
<td>12.5</td>
<td>58.8 ± 5.8</td>
<td>18.0 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>Mixed 25%</td>
<td>20</td>
<td>20</td>
<td>69.1 ± 6.4</td>
<td>16.3 ± 1.7</td>
</tr>
</tbody>
</table>

† The Bt formulation MVP was either applied on the surface of the diet or mixed in the diet.

b Larvae that were able to pupate.

c From third instar to pupa.

d Asterisks indicate significant difference within a treatment (t tests: P ≤ 0.05 (*), P ≤ 0.01 (**), or P ≤ 0.001 (***)).
change in behavior was observed with the Bt-transgenic C 531. Bt toxin expression in the leaves was probably not as high as it was in transgenic C 1076 and, therefore, insufficient to alter the behavior of S. exigua larvae. This assumption is supported by behavioral studies dealing with H. virescens on a Bt-transgenic cotton line. Significant change in behavior was observed only after Bt-toxin expression in plant tissues was improved (Benedict et al., 1992, 1993).

The artificial diet tests demonstrate that larvae released on MVP-incorporated diets moved to diets where MVP had been applied to the surface. Larvae may have eaten through the thin MVP layer and, thereby, have been exposed to the Bt formulation for a relatively short time. These larvae were able to recover by feeding on Bt-free diet located below the MVP layer. It is unlikely that this behavior was caused by degradation of surface-applied MVP during the tests, because of the special attributes of this formulation. The encapsulation of the active ingredient in killed Pseudomonas cells delays its degradation considerably. Furthermore, the combination of high humidity and low UV exposure in the test arenas may also have prevented rapid degradation.

The behavioral responses of S. exigua larvae in the Bt choice tests showed strong similarities to stimulus-

**FIG. 2.** (a) Mean proportion of release and opposite cotton leaves containing larvae of S. exigua in dual-choice test arenas where cotton leaves were either untreated or treated with three Bt formulations. Larvae were released on the leaves indicated as “release leaf” on the left side of the graph. Asterisks indicate significant difference between release leaves and between opposite leaves within treatments at levels $P = 0.05 (*)$, $P = 0.01 (**)$, or $P = 0.001 (***)$. (b) Mean proportion of release and opposite leaves damaged by S. exigua larvae in dual-choice test arenas where cotton leaves were either untreated or treated with three different Bt formulations. Larvae were released on the leaves indicated as “release leaf” on the left side of the graph. Asterisks indicate significant difference between release leaves and between opposite leaves within treatments at levels $P = 0.05 (*)$, $P = 0.01 (**)$, or $P = 0.001 (***)$. 

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dependent behavioral avoidance as described by Georghiou (1972). Increased irritancy and illness caused by the effects of Bt ingestion may have stimulated the S. exigua larvae to abandon Bt-treated diets. A decline of the illness, probably related to continued feeding on untreated diets, made complete recovery of the intoxicated larvae possible. In the untreated control tests, movement between the diets, although limited, was also observed, particularly in the later stages of larval development. This may explain the occasional spontaneous movement in the choice tests of larvae from untreated to treated diets. These larvae usually returned to the untreated diets.

There are no clear indications from our study that behavioral avoidance of S. exigua to Bt includes an aversion-learning component, as demonstrated by De-

![FIG. 3.](image)

(a) Mean proportion of release and opposite cotton leaves containing larvae of S. exigua in dual-choice test arenas with cotton leaves from two transgenic Bt cotton lines (C 531 and C 1076) and a nontransgenic line (C 312). Larvae were released on the leaves indicated as “release leaf” on the left side of the graph. Asterisks indicate significant difference between release leaves and between opposite leaves within treatments at levels $P = 0.05$ (*), $P = 0.01$ (**), or $P = 0.001$ (**). (b) Mean proportion of release and opposite leaves damaged by S. exigua larvae in dual-choice test arenas with cotton leaves from two transgenic Bt cotton lines (C 513 and C 1076) and a nontransgenic line (C 312). Larvae were released on the leaves indicated as “release leaf” on the left side of the graph. Asterisks indicate significant difference between release leaves and between opposite leaves within treatments at levels $P = 0.05$ (*), $P = 0.01$ (**), or $P = 0.001$ (**).

### TABLE 3
Survival Rate, Developmental Time, and Weight of Pupae from Larvae of S. exigua in Dual-Choice Test Arenas with Untreated Cotton Leaves and Leaves Treated with Three Bt Formulations

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Percentage larval survival</th>
<th>Mean pupal weight (mg) ± SE</th>
<th>Mean developmental time (days) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated vs MVP</td>
<td>40</td>
<td>80.0</td>
<td>82.1 ± 3.2 a&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11.0 ± 0.4 a&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Untreated vs Dipel ES</td>
<td>40</td>
<td>80.0</td>
<td>91.1 ± 4.4 ab</td>
<td>13.3 ± 0.6 b</td>
</tr>
<tr>
<td>Untreated vs Xentari</td>
<td>40</td>
<td>80.0</td>
<td>87.9 ± 7.2 ab</td>
<td>12.2 ± 0.9 ab</td>
</tr>
<tr>
<td>MVP vs MVP</td>
<td>40</td>
<td>80.0</td>
<td>56.3 ± 8.7 c</td>
<td>16.0 ± 1.7 c</td>
</tr>
<tr>
<td>Untreated vs untreated (control)</td>
<td>40</td>
<td>80.0</td>
<td>90.9 ± 2.6 ab</td>
<td>8.9 ± 0.1 d</td>
</tr>
</tbody>
</table>

<sup>a</sup> Larvae that were able to pupate.
<sup>b</sup> From third instar to pupa.
<sup>c</sup> Means followed by the same letter within a column are not significantly different ($P \leq 0.05$; Duncan multiple range test).

### TABLE 4
Survival Rate, Developmental Time, and Weight of Pupae from Larvae of S. exigua in Dual-Choice Test Arenas with Cotton Leaves from a Nontransgenic Cotton Variety C 312 and Two Transgenic Bt Cotton Lines C 531 and C 1076

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Percentage larval survival</th>
<th>Mean pupal weight (mg) ± SE</th>
<th>Mean developmental time (days) ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-C 531</td>
<td>40</td>
<td>75.0</td>
<td>97.6 ± 2.6 a&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10.6 ± 0.3 a&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C-C 1076</td>
<td>40</td>
<td>77.5</td>
<td>92.3 ± 3.0 a</td>
<td>10.8 ± 0.4 a</td>
</tr>
</tbody>
</table>

<sup>a</sup> Larvae that were able to pupate.
<sup>b</sup> From third instar to pupa.
<sup>c</sup> Means followed by the same letter within a column are not significantly different ($P \leq 0.05$; Duncan multiple range test).
after egg hatch and their fate could not be determined. A higher concentration of MVP made this response even more dramatic. Neonates may survive by dropping or spinning down on untreated leaves in the lower canopy and may recover from intoxication. It is also likely that these larvae become more susceptible to predation or parasitization as a result of delayed development by Bt intoxication and increased exposure to natural enemies.

Results presented in this study and other field data (Jyoti et al., 1996) pose interesting questions considering the current approaches of pest-resistance management. Bt formulations may change the feeding behavior of larval plant pests when applied under field conditions, although the effects of behavioral avoidance may be limited because of rapid degradation of the active ingredient. However, transgenic Bt crops may offer better opportunities as the toxins are always present. The use of conventional and Bt-transgenic seed mixtures or the development of crop lines with tissue-specific Bt expression is suggested as a means to control Bt resistance (Gould and Anderson, 1991). In an environment with both Bt-free and Bt-expressing tissues, pest insects may be stimulated to feed predominantly on Bt-free plant tissues. Although occasional feeding on Bt-expressing tissues will still occur, Bt intoxication may extend the pest insect’s vulnerable stage for natural enemies. This pest management approach is based on both implementation of behavioral avoidance and preservation of pest insects as food or host resources for natural enemies. More investigation is needed to determine whether susceptible pest strains show a behavioral trend similar to resistant strains in dual-choice tests with Bt-expressing plant tissue. It still remains unclear whether susceptible strains are able to survive and reproduce in an environment with mixtures of Bt-containing and Bt-free plant tissues and whether their fitness is comparable with the fitness of resistant strains.

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REFERENCES


