

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 (containing delta endotoxins var. Kurstaki), the mean proportion of larvae on *Bt*-free diets was higher 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. © 1998 Academic Press

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-

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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 1½- 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 aerial spray applicator, using CO₂ as an 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, reared 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 leaves (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

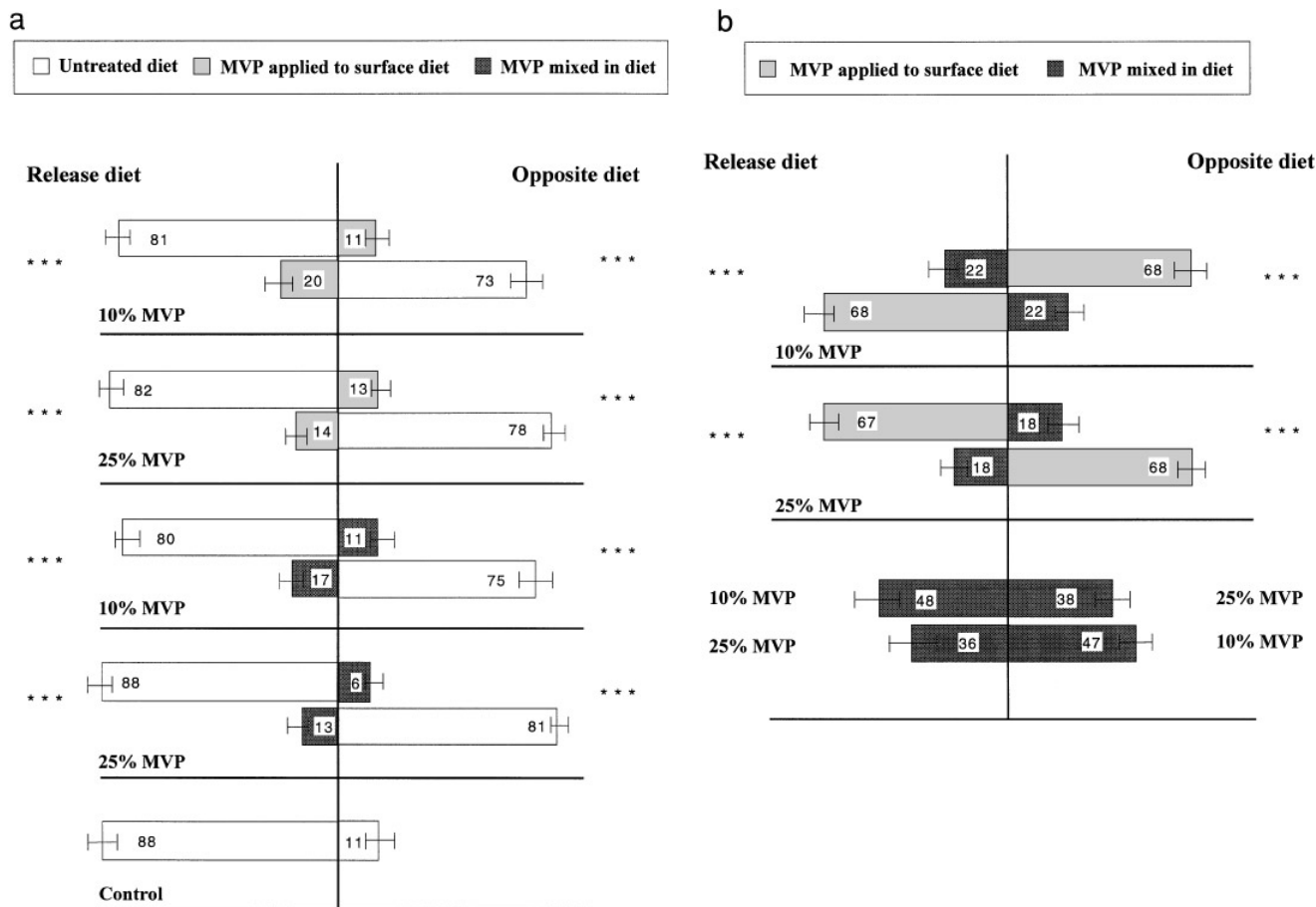


FIG. 1. (a) Mean proportion of release and opposite diets containing larvae of *S. exigua* in dual-choice test arenas where diets were untreated or treated with the *Bt* formulation MVP. *Bt* was either applied on the surface or mixed in the diet. Larvae were released on the diet indicated as “release diet” on the left side of the graph. Asterisks indicate significant difference between release diets and between opposite diets within treatments at levels $P = 0.05$ (*), $P = 0.01$ (**), or $P = 0.001$ (***). (b) Mean proportion of release and opposite diets containing larvae of *S. exigua* in dual-choice test arenas where diets were treated with the *Bt* formulation MVP. *Bt* was either applied on the surface or mixed in the diet. Larvae were released on the diet indicated as “release diet” on the left side of the graph. Asterisks indicate significant difference between release diets and between opposite diets within treatments at levels $P = 0.05$ (*), $P = 0.01$ (**), or $P = 0.001$ (***).

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

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

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

^a 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: $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***)).

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 2

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

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

^a 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: $P \leq 0.05$ (*), $P \leq 0.01$ (**), or $P \leq 0.001$ (***)).

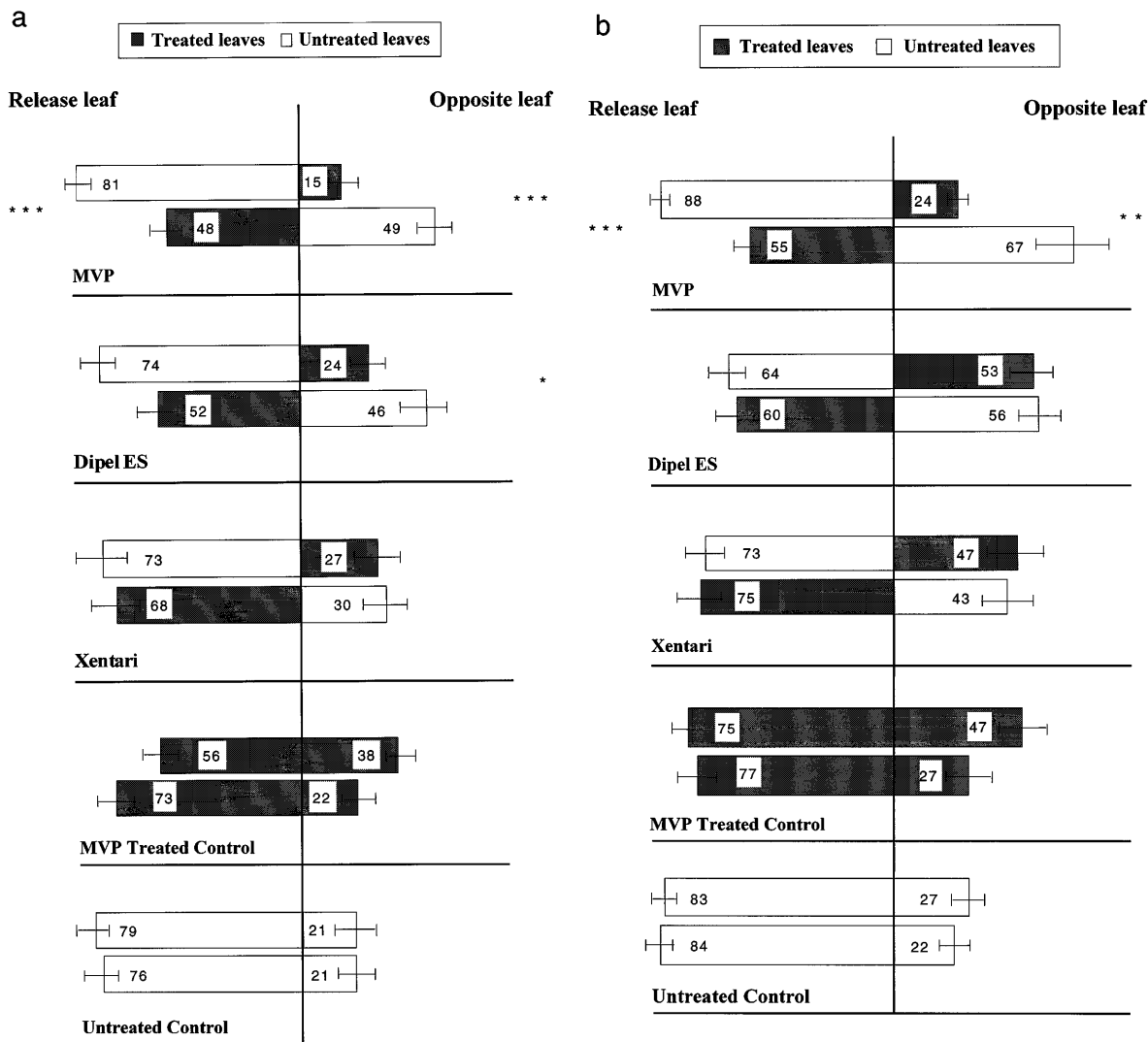


FIG. 2. (a) Mean proportion of release and opposite cotton leaves containing larvae of *S. exigua* in dual-choice test arenas where cotton leaves were either untreated or treated with three *Bt* formulations. Larvae were released on the leaves indicated as “release leaf” on the left side of the graph. 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 $P = 0.001$ (***).

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-

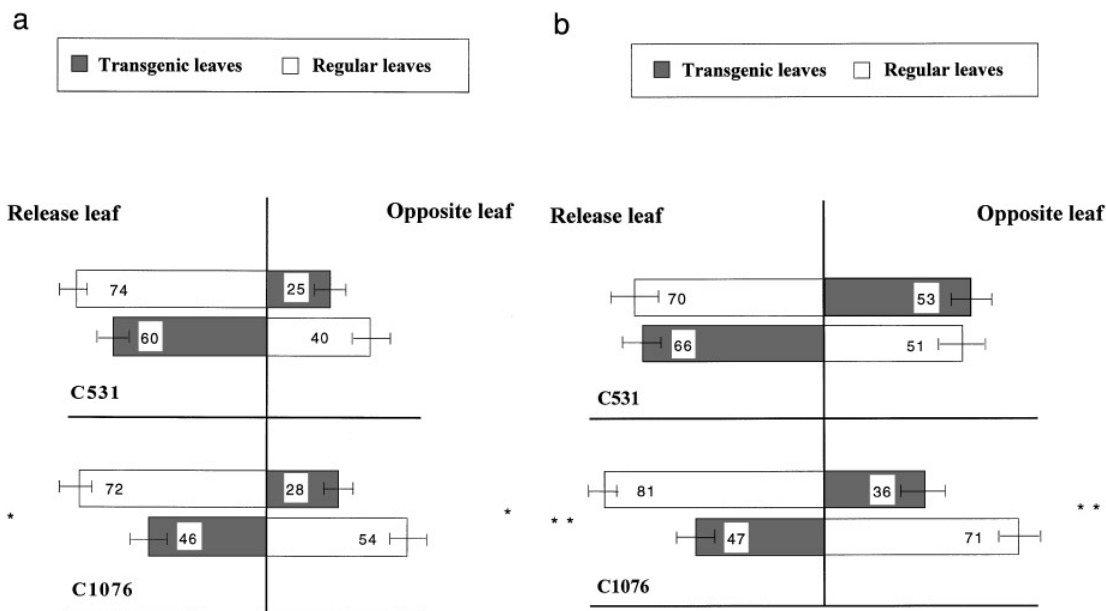


FIG. 3. (a) Mean proportion of release and opposite cotton leaves containing larvae of *S. exigua* in dual-choice test arenas with cotton leaves from two transgenic *Bt* cotton lines (C 531 and C 1076) and a nontransgenic line (C 312). Larvae were released on the leaves indicated as “release leaf” on 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 $P = 0.001$ (***).

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-

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

Treatment	<i>n</i>	Percentage larval survival ^a	Mean pupal weight (mg) ± SE	Mean developmental time ^b (days) ± SE
Untreated vs MVP	40	80.0	82.1 ± 3.2 a ^c	11.0 ± 0.4 a ^c
Untreated vs Dipel ES	40	50.0	91.1 ± 4.4 ab	13.3 ± 0.6 b
Untreated vs Xentari	40	37.5	87.9 ± 7.2 ab	12.2 ± 0.9 ab
MVP vs MVP	40	17.5	56.3 ± 8.7 c	16.0 ± 1.7 c
Untreated vs untreated (control)	40	82.5	90.9 ± 2.6 ab	8.9 ± 0.1 d

^a Larvae that were able to pupate.

^b From third instar to pupa.

^c 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

Treatment	<i>n</i>	Percentage larval survival ^a	Mean pupal weight (mg) ± SE	Mean developmental time ^b (days) ± SE
C-C 531	40	75.0	97.6 ± 2.6 a ^c	10.6 ± 0.3 a ^c
C-C 1076	40	77.5	92.3 ± 3.0 a	10.8 ± 0.4 a

^a Larvae that were able to pupate.

^b From third instar to pupa.

^c Means followed by the same letter within a column are not significantly different ($P \leq 0.05$; Duncan multiple range test).

TABLE 5

Proportion (%) of Neonate *S. exigua* Larvae Remaining on *Bt*-Treated Cotton Leaves 24 and 48 h after Eggs Hatched

<i>Bt</i> concentration ^a	Proportion			
	After 24 h		After 48 h	
	(egg masses)	Mean percentage neonates ± SE	(egg masses)	Mean percentage neonates ± SE
0%	28	63.6 ± 3.5 a ^b	27	45.8 ± 3.4 a ^b
10%	28	40.0 ± 3.7 b	29	34.3 ± 3.3 b
25%	27	36.2 ± 3.2 b	26	23.0 ± 3.5 c

^a The cotton leaves were treated with two concentrations of MVP and water was used as a control.

^b Means followed by the same letter within a column are not significantly different ($P \leq 0.05$; Duncan multiple range test).

thier (1980). He observed in choice tests that two polyphagous lepidopteran species avoided feeding on a previously preferred food plant which had caused acute illness. In our study, it is possible that taste or smell of the *Bt* formulation acted as a feeding deterrent or repellent to *S. exigua* larvae after their *Bt* intoxication experience. However, in preliminary choice experiments using M-Trak (Mycogen Corp., San Diego, CA), a *Bt* formulation effective against Colorado potato beetle but harmless to *S. exigua*, we found that larvae which had experienced MVP intoxication were not deterred or repelled by cotton leaves treated with M-Trak (J. O. Stapel, unpublished). Except for the active ingredient, M-Trak has the same chemical composition as MVP and thus probably has a similar smell and taste.

These findings suggest that acute illness caused by *Bt* was the only factor stimulating the larvae to move. Encounters with untreated diet in the choice test lacked this stimulus and the larvae remained on the untreated diet. Intoxication by high doses of Dipel ES and Xentari may have caused such severe weakness that larval mobility in the tests was impaired. The artificial diet tests did not show a relationship between MVP concentration and avoidance level. It is possible that dose/avoidance relationships will be observed when lower concentrations are used. Apparently "random sample" feeding is the primary strategy used by *S. exigua* to determine which plant is a suitable food source. Combined with the insect's broad physiological resistance to toxicants (Ruberson *et al.*, 1994), this feeding behavior may also play an important role in avoiding plant tissues contaminated with pesticides.

The application of *Bt* may have a larger impact on the survival of young *S. exigua* instars. Neonates are not very mobile and tend to stay aggregated until the third developmental stage is reached. However, on *Bt*-treated plants neonate larvae dropped off or spun down

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 also is 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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