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Behavioral Response of *Helicoverpa armigera* (Lepidoptera: Noctuidae) to Cotton with and Without Expression of the CrylAc δ-Endotoxin Protein of *Bacillus thuringiensis* Berliner

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Behavioral responses of larvae and adults of cotton bollworm, Helicoverpa armigera *to cotton with (GK-12) and without (SI-3) expression of the CrylAc δ-endotoxin protein of* Bacillus thuringiensis *(Bt) Berliner were observed during 2001 and 2002. Our results showed that 8.3% individuals fed with flowers and bolls of GK-12 could develop from neonate to pupa; however, pupal weight decreased by 48.6% and duration of development was delayed by 7.6 days compared with those fed with flowers–bolls of SI-3. Deterrence index (DI) of larvae decreased in later instars, which indicated that the Bt toxin decreased with age. Feeding frequency of 4th-instar larvae on GK-12 leaves decreased by 38.8%, but movement frequency increased by 37.1%. Larvae moved at least one plant away by the age of 10 days in both pure and mixed plantings of SI-3 and GK-12 in the field. Adults preferred to lay eggs on SI-3. The total number of eggs deposited on SI-3 plants in 3 days were about 232 and 95% greater than that on GK-12 plants at bud–flower stage and flower–boll stage, respectively. Based on the behavior of larva and adults*

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in response to the transgenic cotton, the potential effect of refuge strategy in resistance management of H. armigera *is discussed.*

KEY WORDS: *Helicoverpa armigera*; *Bacillus thuringiensis*; behavior; resistance management.

INTRODUCTION

Transgenic cotton, *Gossypium hirsutum* (L.), expressing Cry 1Ac insecticidal δ-endotoxin protein of *Bacillus thuringiensis* Berliner, hereafter referred to as Bt cotton, has been commercially available in China since 1998 to control cotton bollworm, *Helicoverpa armigera* (Hiibner) (Guo, 1996; Jia *et al.*, 2001). Since then, Bt cotton has demonstrated significant control of cotton bollworms in China (Zhao *et al.*, 2000).

The greatest risk in the continued efficacy of Bt cotton against cotton bollworm is evolution of pest resistance. Although resistance of cotton bollworm to Bt cotton in field populations has not been well documented (Zhao *et al.*, 1997), it was reported that *H. armigera* can develop primary resistance to Bt protein (Shen, 1998). After 30 episodes of selection, a subset of *H. armigera* laboratory strain developed about 14.3-fold resistance to the Cry1 Ac toxin and the effectiveness of transgenic Bt cotton for bollworm control decreased significantly (Zhao *et al.*, 1998).

Resistance management strategies are oriented toward reducing selection pressure, currently practiced by the use of non-Bt refuge, an area consisting of plants free of *Bt* toxin that allows a part of pest population to survive and act as the reservoir of wild-type susceptible alleles. Mating of individuals from refuges with surviving individuals exposed to Bt toxin result in dilution of resistance alleles and reduction of intensity of selection pressure (Frutos *et al.*, 1999). The United States Environmental Protection Agency has mandated the use of refuges to delay resistance to *B. thuringiensis* in lepidopteran pests in cotton (U.S. Environmental Protection Agency, 1995). The development of insect resistance to chemicals largely depends on the behavioral responses of insects to the toxins (Frutos *et al.*, 1999; Gould, 1998; Peck *et al.*, 1999; Tabashnik, 1994a,b). Therefore, a comprehensive study on the feeding behavior and oviposition preference of *H. armigera* in transgenic Bt cotton is necessary to develop effective resistance management strategies for this pest.

The seed mixture planting was proposed as a strategy to delay development of resistance during the early 1990s because of its potential ease in implementation. This strategy insured the presence of a refuge, but had potential limitations influenced by larval behavior (Parker and Luttrell, 1999). Simulation models suggested that seed mixtures combined with an

external refuge might decrease the rate of resistance development by increasing survival of heterozygous individuals if the target insects move between transgenic and nontransgenic plants (Mallet and Porter, 1992). However, Tabashnik (1994b), using a computer simulation model similar to the one used by Mallet and Porter (1992), predicted that even with larval movement, seed mixtures were a more effective resistance management technique than pure plantings of transgenic cotton plants containing endotoxin in the absence of an external refuge. The relative merits of seed mixtures and refuge depend upon untested assumptions about pest movements, mating and inheritance of resistance (Tabashnik, 1994b). The solution will most likely be a case-by-case decision based on local conditions and on pest biology and mobility.

The objectives of this study were (1) to observe feeding behavior of *H. armigera* larvae fed with transgenic Bt cotton and nontransgenic cotton, (2) to evaluate oviposition preference of *H. armigera* adult in mixture of transgenic Bt cotton and nontransgenic cotton, and (3) to quantify movement of *H. armigera* larvae between plants in pure and mixed plantings of transgenic and nontransgenic cotton plants.

MATERIAL AND METHODS

Cotton

A 2-year study was conducted in Fugou County, Henan Province in 2001 and 2002. A conventional cotton cultivar, SI-3, was used in the study to compare with a transgenic Bt cotton cultivar, GK-12; the Bt cultivar contained a truncated gene for expression of the Cry 1Ac endotoxin of *Bacillus thuringiensis*. SI-3 and GK-12 cottons grow at similar rate. They were planted simultaneously in the same type soil and received the same agricultural practices.

Helicoverpa Armigera

Eggs and larvae of *H. armigera* from a laboratory colony maintained at the Insect Population Research Laboratory of Chinese Academy of Sciences were used in these studies. In all but one experiment, neonates as well as older instar larvae we used were placed in an environmentally controlled chamber maintained at $27 \pm 0.3^{\circ}$C, relative humidity of 75% and a photoperiod of 14:10 (L:D) h. Larvae were fed with artificial diet before use. Same size insects from the same colony were used in bioassays.

Mortality and Development of *H. armigera*

The experiment consisted of four treatments as follows: (1) neonate fed with leaves of SI-3, (2) neonate fed with leaves of GK-12, (3) neonate fed with flowers of SI-3 until the third instar, then fed with young bolls of SI-3, and (4) neonate fed with flowers of GK-12 until the third instar, then fed with young bolls of GK-12. Cotton leaves used in feeding bioassays were obtained from the third or fourth mainstem node from cotton terminal. Treatments consisting of reproductive parts included fresh bloom or young bolls (<2.5-cm diameter). Cottons used in the study did not receive insecticidal sprays. Plant structures (leaves, blooms, or bolls) were dipped in 0.4% javelle water (sodium hypochlorite) for 10 min, then rinsed with water before the feeding bioassay. Each treatment had four replicates and a total of 236 larvae were fed with leaves of SI-3 and 120 larvae were fed with flowers–bolls of SI-3; 408 larvae were fed with leaves of GK-12 and 200 larvae were fed with flowers–bolls of GK-12 (Table I). Each neonate was placed in an individual 8 cm × 2.5 cm glass tube maintained at 27 ± 0.3°C, relative humidity of 75% and a photoperiod of 14:10 (L:D) h until pupation. The numbers of live and dead individuals in each tube were checked daily. Larval mortality in all instars (1st–6th) was recorded and cumulative mortalities were calculated. Total numbers of days from neonate to pupation were recorded. Pupae were weighed using a digital microbalance.

Larval Foraging Behavior on Bt Cotton Leaves

Larvae in the 2nd, 3rd, 4th, and 5th instars were used in this experiment. Thirty larvae from each instar were placed individually in plastic petri dishes (12-cm diameter, 2-cm height). Freshly picked leaves from third or fourth nodes of the cotton terminal were dipped in 0.4% javelle water for 10 min, then rinsed with water before the bioassay. Leaves were weighed and placed in each test Petri dish for feeding. Petri dishes were placed in an environmentally controlled growth chamber maintained at 27 ± 0.3°C without illumination for 48 h. The remnants of the leaves in each dish were collected after 48 h. They were dried at 50°C for 48 h and weighed. Five fresh SI-3 and five GK-12 leaves were weighed individually then dried at 50°C for 48 h and reweighed. The rate of fresh weight to dry weight of the two cotton leaves was calculated. The initial fresh weights of leaves were transformed into dry weight before calculations. The deterrence index (DI) was calculated as [(weight of SI-3 leaves consumed by larvae)/(weight of GK-12 leaves consumed by larvae + weight of SI-3 leaves consumed by larvae)] (Zhou and Wang, 2000) to estimate the larval-feeding deterrence for transgenic Bt cotton.

Table I. Larval Survivorship, Developmental Duration, and Pupal Weight of *H. armigera* Fed with Leaves or Flowers–Bolls of SI-3 and GK-12 Cottons

Cultivar	Fed on	% Cumulative mortality ± SEM for each instar							Days to Pupation (d)	Pupal weight (g)
		n	1st	2nd	3rd	4th	5th	6th		
SI-3	Leaves	236	$0.0 \pm 0.0_a$	$11.7 \pm 5.3_c$	$17.5 \pm 5.5_b$	$28.8 \pm 6.0_c$	$40.0 \pm 5.6_c$	$49.6 \pm 2.1_b$	$18.9 \pm 0.2_b$	$0.200 \pm 0.80_b$
	Flowers–bolls	120	$0.0 \pm 0.0_a$	$0.0 \pm 0.0_d$	$8.8 \pm 3.8_b$	$19.3 \pm 10.7_c$	$34.3 \pm 10.0_d$	$34.3 \pm 10.0_c$	$15.9 \pm 0.3_c$	$0.228 \pm 0.013_a$
GK-12	Leaves	408	$1.3 \pm 1.9_a$	$24.8 \pm 4.8_b$	$58.3 \pm 10.8_a$	$81.8 \pm 5.4_a$	$100.0 \pm 0.0_a$	$100.0 \pm 0.0_a$	—	—
	Flowers–bolls	200	$0.0 \pm 0.0_a$	$47.5 \pm 8.3_a$	$55.0 \pm 4.3_a$	$65.0 \pm 7.9_b$	$78.3 \pm 3.3_b$	$91.7 \pm 4.3_a$	$23.5 \pm 0.2_a$	$0.140 \pm 0.040_c$

Note. Means ± SEM followed by the same subcript within a column did not differ significantly ($P < 0.05$). Percentage of cumulative mortality for Ist-instar, $F = 1.74$; df = 3.12; $P = 0.211$; for the 2nd-instar, $F = 55.25$; df = 3.12; $P = 0.000$; for 3rd-instar, $F = 57.50$; df = 3.12; $P = 0.000$; for 4th-instar, $F = 57.50$; df = 3.12; $P = 0.000$; for 5th-instar, $F = 70.92$; df = 3.12; $P = 0.000$; for 6th-instar, $F = 131$; df = 3.12; $P = 0.000$. Percentage for days to pupatation, $F = 1014.73$; df = 2.9; $P = 0.000$; for weight of pupa, $F = 44.06$; df = 2.9; $P = 0.000$.

In a nonchoice test, fourth instar larvae from a laboratory colony reared on artificial diet were placed individually in Petri dishes containing fresh leaves (GK-12 or SI-3) from third/fourth nodes below cotton terminals ($n = 20$ larvae for each cultivar). The leaves were dipped in 0.4% javelle water for 10 min, then rinsed with water before use. Feeding activity (contact of mouth with leaf with feeding action), movement (crawling, extending, or s GK-12 wing of body and chewing without contact of mouth with leaf), and resting (quiescent condition) were recorded every minute for 45 min from the initiation of the foraging bioassay.

Interplant Movement of Larvae in Cotton Seedling

This experiment was designed to determine if larvae preferentially moved from a Bt plant to a non-Bt plant placed adjacent to each other. GK-12 and SI-3 cottons were planted in pots (10 cm height × 12 cm diameter) and grown to 6–8 leaf stage. Each pot contained one cotton plant and was irrigated with 150 ml KNOP solution everyday. Three plants (pots) were placed 15 cm apart so that leaves from adjacent plants touched against each other. The treatments evaluated were (1) three adjacent SI-3 plants (CK-CK-CK), (2) three adjacent GK-12 plants (Bt-Bt-Bt), (3) two SI-3 plants on each side of a GK-12 plant (CK-Bt-CK), and (4) two GK-12 plants on each side of an SI-3 plant (Bt-CK-Bt). The experimental design was a randomized complete block with four treatments, with 20 (for neonates and 2nd instars) and 15 (for 3rd, 4th, and 5th instars) replicates per treatment. Larvae were placed individually on the terminal leaves of the middle plant. The numbers of live, dead, and missing larvae on each plant were recorded at 24 and 48 h after larvae were placed on the plant.

Percentages were transformed by arcsine square root transformation before analysis. Data were subjected to analysis of variance (ANOVA) and means were separated by Fisher's protected least significant difference (pLSD). Standard errors for percentage of surviving larvae on noninfested plants, which were used to calculate the 95% confidence interval (CI), were similar as standard errors for the percentage of surviving larvae on infested plants. We defined movement among plants to have occurred when the 95% CIs for percentages of surviving larvae found on adjacent plants did not include zeros (Parker and Luttrell, 1999).

Interplant Movement of Larvae in the Cotton Field

A field study was conducted to determine the movement of *H. armigera* larvae as affected by cotton genotypes in field conditions. Four treatments were as in the laboratory interplant movement study,

including (1) three adjacent SI-3 plants in a row (CK-CK-CK), (2) three adjacent GK-12 plants in a row (Bt-Bt-Bt), (3) one GK-12 plant in the center with SI-3 plants on each side (CK-Bt-CK), and (4) one SI-3 plant in the center with GK-12 plants on each side (Bt-CK-Bt). The experimental design was a randomized complete block with five treatments, with 40 (for neonate, 2nd and 3rd instars) and 20 (for 4th and 5th instars) replicates per treatment. Larger sample size was used to evaluate neonate and first two instars to accommodate for higher mortality in younger larvae.

Cottonseeds were sown by hand at field rates (nine plants per square meter) in May 2002. Plants in the flower–boll stage were used for the experiment. Larvae (neonate, 2nd, 3rd, 4th, and 5th instars) were individually placed on the middle plant of each treatment. The neonates were placed on the terminals, 2nd and 3rd instars inside the bracts of small buds 4–5 nodes down the main stem from the terminal, and 4th and 5th instars were placed inside the bracts of small bolls in the lower canopy (Guo, 1996). Locations of larvae on the plants were recorded 24 and 48 h after transfer. The numbers of live, dead, or missing larvae and whether the larvae were on an adjacent plant or on the original plant were recorded at 24 and 48 h. If a larva was found on a plant adjacent to the original plant, it was removed and no subsequent observation was made for that larva. We tried to equalize the number of surviving larvae in different treatments. Estimations of natural infestation were made by whole plant searches of 100 plants before placing larvae on plants. Average natural infestation for each genotype was calculated and subtracted from the experimental data. The percentage of surviving larvae on the infested plants was calculated as the ratio of number of larvae found to the number of larvae released minus the number of missing larvae. Data were reported as the percentage of the total number of larvae placed on the plants minus the number of missing larvae because of an unequal number of experimental units per replicate. Using percentage of total larvae minus missing larva removed the effect of mortality caused by the insecticidal activity of GK-12 cotton plants. Data were analyzed as in the terminal arena study. Cumulative movement of larvae was calculated using the results of the field study as [cumulative number of larvae moved/total number of initial larvae] $\times$ 100] (Parker and Luttrell, 1999).

Oviposition Preference of *H. armigera* Adults

SI-3 and GK-12 cotton seeds were sown randomly by hand at field rates (nine plants per meter) in May 2002. Plastic nets (4-m long, 3-m wide,

and 1.8-m high) were used to cage 108 plants per cage (experimental unit), with 3 cages each deployed in bud–flower stage and flower–boll stage per cultivar. Six pairs of moths, which were previously kept in smaller cages for three days for mating after eclosion, were released into each field cages. Each plant within each cage was inspected daily for 3 days and the number of eggs laid per cage was determined. Data were analyzed using ANOVA and means were separated using the Fisher's protected least significant difference (pLSD). Pairs of data were compared by *t*-test where necessary.

RESULTS

Mortality of *H. armigera*

Significant differences were observed among mortalities of *H. armigera* larvae fed with leaves and flowers–bolls from SI-3 and GK-12 (Table I). The endotoxin expressed in the GK-12 plants significantly increased the cumulative mortality of 2nd instar ($F = 55.25$; df = 3.12; $P = 0.000$), 3rd-instar ($F = 57.50$; df = 3.12; $P = 0.000$), 4th-instar ($F = 57.50$; df = 3.12; $P = 0.000$), 5th-instar ($F = 70.92$; df = 3.12; $P = 0.000$), and 6th instar larvae ($F = 131$; df = 3.12; $P = 0.000$). In general, mortality of larvae fed with leaves of both cotton genotypes was higher than that fed with flowers–bolls. Mortality of larvae fed with leaves of GK-12 was the highest and no individuals could develop to 5th instar. Average of 91.7% of larvae fed with flowers–bolls of GK-12 did not survive until pupal stage, and only 8.3% individuals pupated.

Duration of Development and Weight of *H. armigera*

The duration of development from neonate to pupa was significantly different among treatments ($F = 1014.73$; df = 2.9; $P = 0.000$) (Table I). It took about 23.5 days for neonates fed with flowers–bolls of GK-12 to develop to pupa, which was about 4.6 days and 7.6 days longer than those fed with leaves and flowers–bolls of SI-3, respectively. Body weights of pupae developed from neonates fed with flowers–bolls of GK-12 was lower ($F = 44.06$; df = 2.9; $P = 0.000$) compared with that of neonates fed with flowers-bolls and leaves of SI-3.

Larval Foraging Behavior on Bt Cotton Leaves

Deterrence index (DI) of Bt cotton decreased with larval development (Fig. 1). Significant differences in DI's were found between different stages

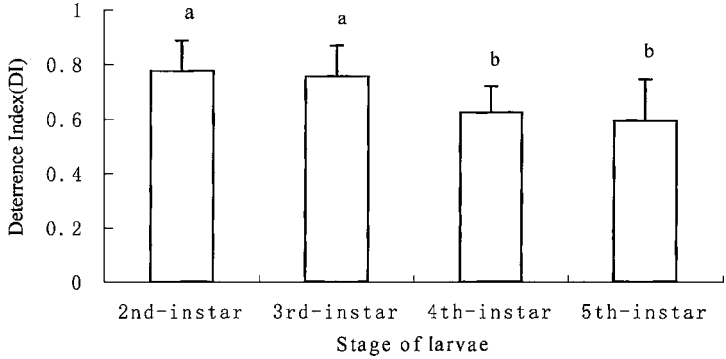

Fig. 1. Deterrence indices of *H. armigera* larvae.

($F = 16.57$; df $= 3.116$; $P = 0.000$). Larger larvae (4th and 5th instars) fed on greater amount of GK-12 leaves than smaller larvae (1st and 2nd instars). Fourth instar larvae fed on GK-12 leaves moved more ($p < 0.01$), but had a lower amount of feeding bouts ($p < 0.05$), than those fed SI-3 leaves. Compared to larvae fed SI-3 leaves, frequency of feeding of the 4th-instar larvae on GK-12 leaves decreased by 38.8%, while frequency of movement increased by 37.1% (Fig. 2).

Interplant Movement of Larvae in Cotton Seedling

We defined significant movement among plants when the 95% CI of the percentage of surviving larvae found on adjacent plants did not include

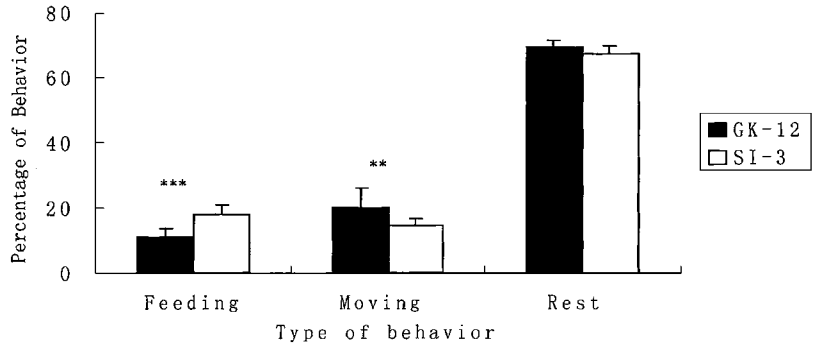

Fig. 2. Percentage of feeding, moving, and resting of fourth instar larvae on leaves of SI-3 and GK-12 (***$P < 0.01$, **$P < 0.05$; Paired *t*-test).

zero. Based on 95% CI of the percentage of larvae on adjacent plants, larvae in all treatments, except 4th instar larvae on BT-CK-BT after 24 h, on CK-CK-CK after 48 h and 5th instar larvae on BT-CK-BT after 48 h, moved between seedlings (Table II). Except for neonates ($F = 3.70$; df $= 3.12$; $P = 0.043$), there were no significant differences with respect to surviving larvae on adjacent plants between treatments in 24 h. However, the numbers of surviving larvae in the first ($F = 3.67$; df $= 3.12$; $p = 0.044$) and the fifth ($F = 6.427$; df $= 3.12$; $P = 0.008$) instars on adjacent plants differed significantly between treatments after 48 h. The numbers of neonates on adjacent plants in the BT-CK-BT treatment were significantly lower than in the CK-CK-CK, BT-CK-BT and BT-BT-BT treatments after both 24 and 48 h. Larvae of 2nd to 5th instars moved less in the BT-CK-BT treatment than in the other three treatments of 48 h. Cumulative movement of larvae from neonate to 5th instar indicated that more than 85% of the larvae moved at least one plant away from their original site by the age of 10 d in all treatments (Table II).

Interplant Movement of Larvae in the Field

Percentage survivorship of 2nd, 4th, and 5th instar larvae after 24 h did not significantly differ among treatments. The highest survivorship in the 1st and 3rd instars occurred in CK-CK-CK treatment while the lowest survivorship in those early instars was found in the BT-BT-BT treatment (Table III). Except for neonates and 2nd instars in the BT-CK-BT and 4th instar in the BT-BT-BT treatments, 95% CI of percentage of larvae on adjacent plants indicated that movements occurred between plants within each treatment. No significant differences were found in movements of 2nd and 5th instars between treatments. The lowest larval movement in 1st and 3rd instars was in the BT-CK-BT treatment while the greatest movement occurred in the BT-BT-BT treatment. Movement of the 4th instar larvae in the CK-BT-CK treatment was significantly higher than in the CK-CK-CK, BT-CK-BT and BT-BT-BT treatments.

Percentage survivorship of larvae differed between treatments after 48 h (except 2nd instar) (Table IV). The highest survival rates for almost all larvae occurred in the CK-CK-CK treatments, while the lowest survival rates were found in the CK-BT-CK treatment for neonates and in the BT-BT-BT treatment for 3rd, 4th, and 5th instar larvae. The 95% CI of percentage of larvae on adjacent plants indicated that the larvae other than neonates and 5th instars in the CK-CK-CK and BT-CK-BT and 4th instars in the BT-CK-BT treatments moved between plants. No significant

Table II. Movement of *H. armigera* Larvae on SI-3 and GK-12 Seedling Plants After Infestation in a Laboratory Experiment

Stage of larvae	Hours after infestation treatment[a]	% Surviving larvae on noninfested plants[b]	CI[c]	% Cumulative movement
Neonate	24CK-CK-CK	$33.8 \pm 4.79_{a}$	26.1–41.4	33.8
	BT-CK-BT	$25.0 + 4.1_{b}$	18.5–31.5	25.0
	-BT-CK	$35.0 + 7.1_{a}$	23.8–46.3	35.0
	BT-BT-BT	$35.0 + 4.1_{a}$	28.5–51.5	35.0
	48CK-CK-CK	$17.8 + 4.5_{a}$	10.7–25.0	45.6
	BT-CK-BT	$14.9 \pm 2.6_{b}$	10.8–19.0	36.2
	-BT-CK	$33.8 + 11.2_{a}$	15.6–51.9	56.9
	BT-BT-BT	$28.9 + 14.8_{a}$	5.27–52.4	53.8
2nd-instar	24CK-CK-CK	$36.7 + 11.6_{a}$	18.3–55.0	65.5
	BT-CK-BT	$33.3 + 7.7_{a}$	21.1–45.6	57.5
	-BT-CK	$38.3 + 8.4_{a}$	25.0–71.7	73.4
	BT-BT-BT	$33.3 + 5.4_{a}$	24.7–42.0	69.2
	48CK-CK-CK	$32.3 \pm 10.5_{a}$	15.62–49.0	76.7
	BT-CK-BT	$17.9 \pm 6.2_{b}$	8.1–27.8	65.1
	-BT-CK	$27.5 \pm 7.3_{ab}$	15.8–39.1	80.7
	BT-BT-BT	$18.9 \pm 7.1_{b}$	7.6–30.2	75.0
3rd-instar	24CK-CK-CK	$31.7 \pm 11.4_{a}$	13.5–49.8	84.0
	BT-CK-BT	$26.7 \pm 12.2_{a}$	7.3–46.0	74.4
	-BT-CK	$40.0 + 12.2_{a}$	20.6–59.4	88.4
	BT-BT-BT	$35.0 + 10.0_{a}$	19.1–50.9	83.7
	48CK-CK-CK	$18.4 + 10.1_{a}$	2.4–34.3	87.0
	BT-CK-BT	$15.0 + 8.4_{a}$	1.6–28.4	78.2
	-BT-CK	$26.9 \pm 10.2_{a}$	10.7–43.0	91.5
	BT-BT-BT	$21.7 \pm 8.4_{a}$	8.3–35.0	87.3
4th-instar	24CK-CK-CK	$21.7 \pm 11\ A_{a}$	3.6–39.8	89.8
	BT-CK-BT	$13.3 + 9.4_{a}$	−1.7–28.3	78.2
	-BT-CK	$21.7 + 6.4_{a}$	11.5–31.8	93.4
	BT-BT-BT	$25.0 \pm 6.4_{a}$	14.8–35.2	90.4
	48CK-CK-CK	$15.9 + 18.7_{a}$	−13.9–45.7	89.8
	BT-CK-BT	$11.5 + 4.8_{a}$	3.9–19.1	80.7
	-BT-CK	$24.1 \pm 11.2_{a}$	5.0–43.1	95.0
	BT-BT-BT	$24.0 + 8.6_{a}$	10.3–37.8	92.7
5th-instar	24CK-CK-CK	$35.0 + 10.0_{a}$	19.1–50.9	93.4
	BT-CK-BT	$31.7 \pm 3.3_{a}$	26.4–37.0	86.8
	-BT-CK	$43.3 + 8.6_{a}$	29.6–57.0	97.2
	BT-BT-BT	$31.7 \pm 8.4_{a}$	18.3–45.0	95.0
	48CK-CK-CK	$14.8 + 5.6_{b}$	5.9–23.7	94.3
	BT-CK-BT	$14.3 + 11.9_{b}$	−4.6–33.2	86.8
	-BT-CK	$45.0 + 13.4_{a}$	23.7–66.2	98.4
	BT-BT-BT	$37.1 + 10.6_{a}$	20.3–53.9	96.9

Note. Means within a column for an individual stage followed by the same subscript did not differ significantly ($P > 0.05$, Fisher protected LSD test). Data were transformed by arcsince square-root transformation before analysis; untransformed means and standard errors are reported.

[a]Bt = GK-12 cotton, and CK = SI-3 cotton.

[b]Percentage survivorship on noninfested plants at 24h for neonate, $F = 3.70$; df = 3.12; $P = 0.043$; for 2nd-instar, $F = 0.29$; df = 3.12; $P = 0.833$; for 3rd-instar, $F = 1.01$; df = 3.12; $P = 0.421$; for 4th-instar, $P = 1.42$; df = 3.12; $P = 0.347$; and for 5th-instar, $F = 1.78$; df = 3.12; $P = 0.204$, at 48 h for neonate, $F = 3.67$; df = 3.12; $p = 0.044$, for 2nd-instar, $F = 2.93$; df = 3.12; $P = 0.077$; for 3rd-instar, $F = 1.18$; df = 3.13; $P = 0.358$; for 4th-instar, $F = 1.14$; df = 3.12; $P = 0.374$; for 5th-instar, $F = 6.427$; df = 3.12; $P = 0.008$.

[c]Movement occurred because none of the 95% CI of the mean percentage of surviving larvae on noninfested seedling cotton included zero.

Table III. Mean ± SEM Percentage of Larvae Surviving on the Infested (Center) Plant and on Adjacent (Noninfested) Plants 24 h After Infestation in Field Plantings of Cotton

Age of larvae	Treatment[a]	% Survival[b]	% On the center plant[b]	% On the adjacent plant[b]	95% CI[c]
Neonate	CK-CK-CK	$70.5 \pm 6.9_{a}$	$63.3 \pm 6.9_{a}$	$7.2 \pm 2.8_{ab}$	3.7–10.7
	BT-CK-BT	$66.0 \pm 8.4_{ab}$	$60.6 \pm 8.4_{a}$	$5.4 \pm 4.4_{b}$	−0.01–10.8
	CK-BT-CK	$60.5 \pm 5.7_{b}$	$47.5 \pm 5.7_{b}$	$12.0 \pm 2.6_{a}$	9.8–16.2
	BT-BT-BT	$59.5 \pm 7.2_{b}$	$46.9 \pm 7.1_{b}$	$12.6 \pm 4.0_{a}$	7.6–17.6
2nd-instar	CK-CK-CK	$71.0 \pm 6.8_{a}$	$53.8 \pm 6.8_{a}$	$17.2 \pm 9.2_{a}$	5.8–28.6
	BT-CK-BT	$71.5 \pm 6.5_{a}$	$57.7 \pm 6.5_{a}$	$13.8 \pm 12.6_{a}$	−1.90–30.0
	CK-BT-CK	$70.0 \pm 7.9_{a}$	$56.3 \pm 7.9_{a}$	$13.8 \pm 7.3_{a}$	4.8–22.8
	BT-BT-BT	$63.0 \pm 10.8_{a}$	$45.7 \pm 10.8_{a}$	$17.3 \pm 5.7_{a}$	10.2–24.4
3rd-instar	CK-CK-CK	$88.5 \pm 4.2_{a}$	$81.6 \pm 4.2_{a}$	$6.9 \pm 5.0_{ab}$	0.7–13.1
	BT-CK-BT	$84.5 \pm 8.9_{ab}$	$78.4 \pm 8.9_{ab}$	$6.1 \pm 3.9_{b}$	1.3–11.0
	CK-BT-CK	$82.0 \pm 9.1_{ab}$	$72.0 \pm 9.1_{ab}$	$10.0 \pm 3.5_{ab}$	5.7–14.4
	BT-BT-BT	$77.0 \pm 8.2_{b}$	$64.3 \pm 8.2_{b}$	$12.7 \pm 6.7_{a}$	4.4–21.0
4th-instar	CK-CK-CK	$90.0 \pm 7.9_{a}$	$82.0 \pm 7.91_{a}$	$8.0 \pm 5.1_{b}$	1.6–14.4
	BT-CK-BT	$88.0 \pm 7.6_{a}$	$78.9 \pm 7.6_{a}$	$9.1 \pm 3.1_{b}$	5.3–12.9
	CK-BT-CK	$82.0 \pm 5.7_{a}$	$67.3 \pm 5.7_{a}$	$14.7 \pm 7.2_{a}$	5.7–23.6
	BT-BT-BT	$82.0 \pm 4.5_{a}$	$71.2 \pm 4.5_{a}$	$10.9 \pm 9.3_{b}$	−0.64–22.4
5th-instar	CK-CK-CK	$92.0 \pm 7.6_{a}$	$85.4 \pm 7.6_{a}$	$6.6 \pm 4.4_{a}$	1.2–12.0
	BT-CK-BT	$87.0 \pm 4.5_{a}$	$81.4 \pm 4.5_{a}$	$5.6 \pm 3.8_{a}$	1.0–10.3
	CK-BT-CK	$90.0 \pm 7.9_{a}$	$79.9 \pm 7.9_{a}$	$10.1 \pm 4.8_{a}$	4.2–16.0
	BT-BT-BT	$90.0 \pm 6.1_{a}$	$79.8 \pm 6.1_{a}$	$10.2 \pm 6.4_{a}$	2.3–18.1

Note. Means within a column for an individual age group followed by the same subscript did not differ significantly ($P > 0.05$). Movement data were transformed by arcsine square-root transformation before analysis. Untransformed mean and SEM are reported.

[a]Bt = GK-12 cotton, and CK = SI-3 cotton.

[b]Percentage of survival for neonates, $F = 2.72$; df = 316; $P = 0.079$, for 2nd-instar, $F = 1.089$; df = 316; $P = 0.382$, for 3rd-instar, $F = 1.89$; df = 3.16; $P = 0.173$, for 4th-instar, $F = 1.71$; df = 3.16; $P = 0.206$, and for 5th-instar, $F = 0.55$; df = 3.16; $P = 0.655$. Percentage on the center plant for neonates, $F = 5.04$; df = 3.16; $P = 0.012$, for 2nd-instar, $F = 0.805$; df = 3.16; $P = 0.509$, for 3rd-instar, $F = 2.29$; df = 3.16; $P = 0.118$, for 4th-instar, $F = 2.13$; df = 3.16; $P = 0.137$, and for 5th-instar, $F = 0.37$; df = 3.16; $P = 0.778$. Percentage on the adjacent plant for neonates, $F = 4.77$; df = 3.16; $P = 0.015$, for 2nd-instar, $F = 0.426$; df = 3.16; $P = 0.737$, for 3rd-instar, $F = 1.96$; df = 3.16; $P = 0.160$, for 4th-instar, $F = 0.83$; df = 3.16; $P = 0.478$, and for 5th-instar, $F = 0.36$; df = 3.16; $P = 0.782$.

[c]Movement occurred because none of the 95% CI of the mean percentage of surviving larvae on noninfested seeding cotton included zero.

differences in movements of the 2nd and 5th instar larvae were observed between treatments. In general, neonate, 3rd and 4th instar larvae in the CK-BT-CK and BT-BT-BT moved more than in the CK-CK-CK and BT-CK-BT treatments.

Cumulative movement of larvae up to 10-day old larva indicated that greater numbers of larvae moved when placed on Bt plants (Table V). On average, 49.5% of the larvae in the CK-CK-CK, 23.5% in the BT-CK-BT, 73.5% in the CK-BT-CK and 70.5% of the larvae in the BT-BT-BT treatments had moved at least one plant away by the age of 10 days. Only a few

Table IV. Mean ± SEM Percentage of *H. armigera* Larvae Surviving on the Infested (Center) Plant and on Adjacent (Noninfested) Plants 48 h After Infestation in Field Plantings of Cotton

Age of larvae	Treatment[a]	% Survival[b]	% On the center plant[b]	% On the adjacent plant[b]	95% CI[c]
Neonate	CK-CK-CK	$62.0 \pm 9.5_{a}$	$57.1 \pm 9.5_{a}$	$4.9 \pm 5.0_{b}$	−1.3–11.0
	BT-CK-BT	$60.2 \pm 12.1_{a}$	$56.5 \pm 12.1_{a}$	$3.7 \pm 3.6_{b}$	−0.7–8.1
	CK-BT-CK	$48.0 \pm 9.5_{b}$	$32.3 \pm 9.5_{b}$	$15.7 \pm 10.7_{ab}$	2.4–29.0
	BT-BT-BT	$52.2 \pm 8.1_{ab}$	$35.4 \pm 8.1_{a}$	$16.8 \pm 8.4_{a}$	6.4–27.2
2nd-instar	CK-CK-CK	$60.1 \pm 9.1_{a}$	$51.3 \pm 9.1_{a}$	$8.8 \pm 5.7_{a}$	1.7–15.9
	BT-CK-BT	$55.5 \pm 9.0_{a}$	$48.2 \pm 9.0_{ab}$	$7.4 \pm 5.1_{a}$	1.1–13.7
	CK-BT-CK	$53.1 \pm 8.8_{a}$	$35.7 \pm 8.8_{b}$	$17.4 \pm 6.3_{a}$	9.6–25.3
	BT-BT-BT	$49.8 \pm 8.2_{a}$	$34.2 \pm 8.2_{b}$	$15.6 \pm 10.2_{a}$	2.2–28.2
3rd-instar	CK-CK-CK	$72.8 \pm 12.2_{a}$	$64.2 \pm 12.2_{a}$	$8.6 \pm 5.3_{b}$	2.0–15.2
	BT-CK-BT	$73.6 \pm 10.1_{a}$	$67.4 \pm 10.1_{a}$	$6.1 \pm 3.8_{b}$	1.4–10.8
	CK-BT-CK	$71.4 \pm 12.4_{a}$	$54.4 \pm 12.4_{ab}$	$17.0 \pm 3.9_{a}$	12.2–21.8
	BT-BT-BT	$54.5 \pm 9.7_{b}$	$43.3 \pm 9.7_{b}$	$11.2 \pm 3.4_{ab}$	6.9–15.4
4th-instar	CK-CK-CK	$84.9 \pm 9.7_{a}$	$80.4 \pm 9.7_{a}$	$4.5 \pm 4.1_{b}$	−0.7–9.6
	BT-CK-BT	$80.1 \pm 7.1_{a}$	$73.6 \pm 7.1_{ab}$	$6.5 \pm 3.8_{ab}$	1.8–11.2
	CK-BT-CK	$81.6 \pm 2.4_{a}$	$64.3 \pm 2.4_{ab}$	$17.2 \pm 8.0_{a}$	7.3–27.1
	BT-BT-BT	$73.0 \pm 6.9_{b}$	$59.7 \pm 6.9_{b}$	$13.3 \pm 8.9_{ab}$	2.3–24.3
5th-instar	CK-CK-CK	$86.6 \pm 8.1_{a}$	$80.7 \pm 8.1_{a}$	$5.9 \pm 6.1_{a}$	−1.7–13.4
	BT-CK-BT	$81.7 \pm 9.0_{ab}$	$79.0 \pm 9.0_{a}$	$2.7 \pm 3.7_{a}$	−1.9–7.2
	CK-BT-CK	$78.4 \pm 7.2_{ab}$	$67.5 \pm 7.2_{a}$	$10.9 \pm 8.7_{a}$	0.1–21.7
	BT-BT-BT	$76.8 \pm 4.0_{b}$	$68.9 \pm 4.0_{a}$	$7.9 \pm 6.0_{a}$	0.5–15.3

Note. Means within a column for an individual age group followed by the same subscript did not differ significantly ($P > 0.05$). Movement data were transformed by arcsine square-root transformation before analysis. Untransformed mean and SEM are reported.

[a]Bt = GK-12 cotton, and CK = SI-3 cotton.

[b]Percentage of survival for neonates, $F = 2.20$; df = 3.16; $P = 0.126$, for 2nd-instar, $F = 1.27$; df = 3.16; $P = 0.318$, for 3rd-instar, $F = 3.27$; df = 3.16; $P = 0.049$, for 4th-instar, $F = 1.96$; df = 3.16; $P = 0.161$, and for 5th-instar, $P = 1.78$; df = 3.16; $P = 0.191$. Percentage on the center plant for neonates, $F = 5.86$; df = 3.16; $P = 0.007$, for 2nd-instar, $F = 3.20$; df = 3.16; $P = 0.052$, for 3rd-instar, $F = 3.93$; df = 3.16; $P = 0.028$, for 4th-instar, $F = 2.66$; df = 3.16; $P = 0.083$, and for 5th-instar, $F = 2.05$; df = 3.16; $P = 0.147$. Percentage on the adjacent plant for neonates, $F = 3.98$; df = 3.16; $P = 0.041$, for 2nd-instar, $F = 1.41$; df = 3.16; $P = 0.277$, for 3rd-instar, $F = 3.34$; df = 3.16; $P = 0.046$, for 4th-instar, $F = 2.78$; df = 3.16; $P = 0.075$, and for 5th-instar, $F = 1.23$; df = 3.16; $P = 0.330$.

[c]Movement occurred because none of the 95% CI of the mean percentage of surviving larvae on noninfested seeding cotton included zero.

larvae moved before they were 4-day old in the BT-CK-BT treatment; but the 4-day old larvae moved between plants in all treatments.

Oviposition Preference of *H. armigera* Adult

The number of eggs laid by female moths was significantly higher on SI-3 plants compared with that on GK-12 plants at both the bud–flower stage and the flower–boll stage in all three days of oviposition inspection

Table V. Cumulative Movement Estimates Derived from 24- and 48-h Observations of *H. armigera* Larvae in a Cotton Field

Treatment[a]	Lever age (days)									
	1	2	3	4	5	6	7	8	9	10
CK-CK-CK										
% Larvae not moving[b]	100.0	92.8	92.8	76.8	70.0	65.2	59.6	59.6	55.7	52.0
% Observed movement[c]	7.2	0.0	17.2	8.8	6.9	8.6	8.0	0.0	6.6	0.0
% Culculative movement[d]	5.0	5.0	17.0	21.5	26.5	31.5	39.5	39.5	45.5	49.5
BT-CK-BT										
% Larvae not moving[b]	100.0	100.0	100.0	100.0	92.6	87.0	81.7	74.3	69.2	65.3
% Observed movement[c]	0.0	0.0	0.0	7.4	6.1	6.1	9.1	6.5	5.6	0.0
% Culculative movement[d]	0.0	0.0	0.0	2.5	7.5	10.5	12.5	16.5	21.5	23.5
CK-BT-CK										
% Larvae not moving[b]	100.0	88.0	74.2	64.0	52.9	47.6	39.5	33.7	27.9	25.1
% Observed movement[c]	12.0	15.7	13.8	17.4	10.0	17.0	14.7	17.2	10.1	10.9
% Culculative movement[d]	8.0	13.5	23.0	28.5	36.5	45.5	47.5	57.5	66.5	73.5
BT-BT-BT										
% Larvae not moving[b]	100.0	87.4	72.7	60.1	50.7	44.3	39.3	39.3	34.1	21.6
% Observed movement[c]	12.6	16.8	17.3	15.6	12.7	11.2	0.0	13.3	10.2	7.9
% Culculative movement[d]	7.5	12.0	23.0	27.0	36.5	40.5	49.5	56.5	65.5	70.5

[a]Bt = GK-12 cotton, and CK = SI-3 cotton.
[b]Number of larvae not moving at time t = number of larvae not moving at time $t - 10$ minus (number of larvae not moving at time $t - 1 \times$ [(percentage of observed movement at time $t - 10/1000$)].
[c]Observed movement obtained from the field movement study for a larval age. The 24- through 48-h observations for neonates were used to estimate movement for days 1–2. The 24- through 48-h observations of 2nd-instar larvae were used to estimate movement for days 3–4. The 24- through 48-h observations of 3rd-instar larvae were used to estimate movement for days 5–6. The 24- through 48-h observations of 4th-instar larvae were used to estimate movement for days 7–8. The 24- through 48-h observations of 5th-instar larvae were used to estimate movement for days 9–10.
[d]Cumulative movement is the summation of observed movement over time.

(bud–flower stage, day 1: $F = 20.19$; df = 1.4; $P = 0.011$; day 2: $F = 108.16$; df = 1.4; $P = 0.000$; day 3: $F = 61.84$; df = 1.4; $P = 0.001$; flower–boll stage, day 1: $F = 23.88$; df = 1.4; $P = 0.008$; day 2: $F = 49.41$; df = 1.4; $P = 0.002$; day 3: $F = 65.34$; df = 1.4; $P = 0.001$) (Table VI). Number of eggs on SI-3 plants at bud–flower stage were 3.25, 3.54 and 3.14 times higher than

Table VI. Oviposition of *H. armigera* Adult on GK-12 and SI-3 Cottons During Bud–Flower Stage and Flower–Boll Stage of Crop

Stage of cotton	Cotton variety[a]	Day			Cumulative oviposition
		First[b]	Second[b]	Third[b]	
Bud–flower	CK	$6.73 \pm 1.35_a$	$8.04 \pm 0.61_a$	$5.80 \pm 0.71_a$	20.54
	BT	$2.07 \pm 0.57_b$	$2.27 \pm 0.50_b$	$1.84 \pm 0.08_b$	6.18
Flower–boll	CK	$8.01 \pm 1.17_a$	$11.22 \pm 0.95_a$	$8.42 \pm 0.65_a$	27.65
	BT	$3.91 \pm 0.22_b$	$6.16 \pm 0.36_b$	$4.14 \pm 0.37_b$	14.21

Note. Within a column, means, for the same stage of cotton, followed by different subscript are significantly different at $p > 0.05$.

[a]Bt = GK-12 cotton, and CK = SI-3 cotton.

[b]Number of eggs at bud–flower stage on day 1, $F = 20.19$; df = 1.4; $P = 0.011$; day 2, $F = 108.16$; df = 1.4; $P = 0.000$; and day 3, $F = 61.84$; df = 1.4; $P = 0.001$; at the flower–boll stage on day 1, $F = 23.88$; df = 1.4; $P = 0.008$; day 2, $F = 49.41$; df = 1.4; $P = 0.002$; and day 3, $F = 65.34$; df = 1.4; $P = 0.001$.

on GK-12 on the 1st, 2nd, and 3rd day of oviposition, respectively. Similar results were observed at flower–boll stage, with 2.05, 1.82, and 2.03 times higher oviposition on SI-3 plants than on GK-12 plants on the 1st, 2nd, and 3rd day of oviposition, respectively.

DISCUSSION

Our study showed that 8.3% of *H. armigera* individuals fed flowers–bolls of GK-12 could develop to pupa, indicating that CrylAc toxin expressed in GK-12 did not provide 100% control. Considering the CrylAc level expressed in the transgenic Bt cotton declines as plant senesces (Fitt *et al.*, 1994), more individuals could survive in GK-12 fields. Therefore, the high dose strategy, which is based on developing Bt plants that express very high levels of Bt endotoxin to insure that all nonresistant insect pests and even individuals which are heterozygous for resistance will be killed, may not be valid for the resistance management for *H. armigera*. This suggests that refuge area should be increased to delay development of resistance (Jeffey, 1996).

Sufficient mating between susceptible adults from refuges and resistant insects surviving on Bt crops is required for durable use of crops. It took about 7.6 days longer for neonates fed with flowers–bolls of GK-12 to develop to pupa than those fed with flowers–bolls of SI-3. Most matings in *H. armigera* occur between the third and the fifth days after eclosion (Wang *et al.*, 1999). If the neonates on SI-3 and GK-12 started to develop at the same time, few adults from SI-3 could mate with those from GK-12, which could accelerate the selection of individuals, which would accelerate

the development of resistance. It has been reported that flight capacity of *H. armigera* is positively correlated with nutrient status in larvae (Wu and Guo, 1997). Adults feeding on Bt cotton have small sizes, which suggests that they could have weaker flight capacities. The duration of ovipositon of *H. armigera* is more than 10 days (Wang *et al.*, 1999), which may lead to overlap of generations of *H. armigera* in fields and increase the chance of adults in GK-12 field to mate with those in SI-3.

Compared to frequency of feeding on SI-3, frequency of feeding of 4th instar larvae on GK-12 leaves decreased by 38.8%, while frequency of movement increased by 37.1%. The sensitivity level of *H. armigera* larvae to the toxin expressed in the GK-12 decreased with development time. Larger larvae (4th and 5th instars) consumed greater amount of GK-12 leaves than smaller larvae (1st and 2nd instars). Larvae exposed to *B. thuringiensis* toxin moved more but fed less than larvae not exposed to the toxin. This behavioral variation seems to explain why more movements of larvae occurred on GK-12 cottons than on SI-3 cottons in our field experiments. This result indicates that movement of *H. armigera* is not independent of plant genotypes, as assumed by Mallet and Porter (1992), Parker and Luttrell (1999), and Benedict *et al.* (1992) in studying movement of *H. virescens* larvae. Our data clearly demonstrated that larvae move between plants in pure plantings of SI-3 or GK-12 or mixed plantings of GK-12 and SI-3. In northern China, there are several host crops such as corn, soybean, and peanut that can serve as refuges for *H. armigera* (Tan *et al.*, 2001), but the effectiveness of these host crops as potential refuges have not been investigated.

Many studies have reported that lepidopteran pests do not generally display ovipositional preference for nontransgenic plants over transgenic Bt plants (Parker and Luttrell, 1998, Ramachandran *et al.*, 1998, Tang *et al.*, 1999). However, our observations indicated that *H. armigera* preferred to oviposit on SI-3 plants in mixed plantings of SI-3 and GK-12. This preference could be attributed to a potential difference in kairomone profile between the two cultivars (Schultz, 1998; Renwick, 1989). Yan *et al.* (2002 reported that the ratios of α-pinene and β-pinene were much higher in the volatiles of GK-12 than in those of SI-3, and two volatile compounds in GK-12 are absent in SI-3). Causal mechanism underlying oviposition preference of *H. armigera* for SI-3 over GK-12 should be further investigated. If adults prefer to lay eggs in refuges, the resistance alleles could be transferred to refuge area more quickly, thereby increasing the frequency of heterozygous individuals in the nonselected populations and potentially accelerate the evolution of resistance (Caprio and Tabashnik, 1992).

In conclusion, seed mixture is not a suitable strategy for managing resistance of *H. armigera* to transgenic Bt cotton in China. To postpone the development of the resistance, external refuge should be employed;

however, the rate of refuge ought to be increased considering the effect of transgenic Bt cotton to development of *H. armigera*.

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