Eur. J. Entomol. **108**: 547–552, 2011
http://www.eje.cz/scripts/viewabstract.php?abstract=1651
ISSN 1210-5759 (print), 1802-8829 (online)

Effect of insect-resistant transgenic maize on growth and development, utilization of nutrients and in vivo activity of the detoxification enzymes of the Asian corn borer, *Ostrinia furnacalis* (Lepidoptera: Pyralidae)

MIN JUAN SHI, PEI LING LU, XIAO LI SHI and YI ZHONG YANG*

School of Horticulture and Plant Protection, Yangzhou University, Yangzhou 225009, China

Key words. Lepidoptera, Pyralidae, *Ostrinia furnacalis*, detoxification enzyme, growth and development, insect-resistant transgenic maize, nutritional indicators

Abstract. There is little systematic research on the effect of transgenic maize expressing *Bacillus thuringiensis* (Bt) toxins on insect survival and the response in vivo of their detoxification enzymes. Results of laboratory bioassays indicate that the whorl leaves of transgenic maize lines G03-2396 and G03-2739 significantly affected the survival of neonate and third instar larvae of the Asian corn borer, *Ostrinia furnacalis* (Güenée). Neonate mortality two days after being fed on G03-2739 was 72.5% and exceeded 92.5% four days later. The percentage mortality of third instar larvae six days after being fed whorl leaves from G03-2396 exceeded 65%. The resistance of whorl leaves to insect attack was greater than that of maize-ears, but less effective against attacks by third instar larvae. Neonate development was more prolonged when they were fed on whorl leaves of both the transgenic maize lines. In contrast, low pupation (<80% of that recorded in the controls), ecolosion and fecundity were recorded following ingestion of maize-ears, with pupal weights 10–14 mg lower than that of controls. The growth rates of third instar larvae recorded three and six days after feeding on whorl leaves were lower, but food utilization, conversion and relative metabolism were not significantly affected. Relative food ingestion, relative growth rate and other nutritional indicators of third instar larvae were significantly lower six days after the ingestion of ears and that of fifth instar larvae after three days. In vivo activity of carboxylesterase was decreased to a greater degree after three days when the larvae were fed on whorl leaves than ears, with no significant effect on fifth instar larvae. Furthermore, in vivo activity of glutathione S-transferase (GST) of third instar larvae was significantly affected following the ingestion of whorl leaves. These results are discussed in the context of the literature on the resistance of transgenic plants and of improving the resistance of plants to attack by the different larval stages of insects.

INTRODUCTION

The Asian corn borer (ACB), *Ostrinia furnacalis* (Güenée), is a major pest of maize, sorghum, millet and other cereal upland crops in China. Each year, Asian corn borers damage maize at the whorl and spike stages. Utilization of insect-resistant transgenic maize could potentially play an important role in controlling the level of damage to maize crops by ACB. The United States of America was one of the first countries in the world to plant transgenic maize crop expressing *Bacillus thuringiensis* (Bt) proteins in order to reduce the damage caused by maize pests (Jiao, 2005). In 1997, the Chinese government formally approved the planting of Bt transgenic maize event MON810 for "Restricted Field Testing and Enlarged Field Testing in China" and promoted research on transgenic maize in China.

In recent years, field trials in China of a number of domestic Bt maize events have resulted in higher yields, better quality and greater resistance to insect pests. In addition a series of maize lines that are more resistant to insect attack have been screened. For example, He et al. (2004) evaluated the resistance of transgenic maize to the Asian corn borer based on the survival of corn borer larvae, leaf consumption, stalk tunneling, tunnel length and the level of damage to maize-ears. Other researchers suggest that the utilization of food by insects, measured in terms of relative food ingestion, food utilization, food conversion and approximate digestive ability of the larvae (James, 2003) under different nutritional conditions may be used as indicators of host plant resistance.

Phytophagous insects have evolved ways of overcoming various poisons, such as enzymes for the detoxification and elimination of toxicants (Yao et al., 2002; Zhang et al., 2004). For instance, carboxylesterase and glutathione S-transferase (GST) are important in vivo enzymes of pests, which play important roles in the detoxification and metabolism of exogenous compounds. Xu et al. (2006) studied the effect of Bt transgenic maize on the activity of phosphatase in corn borer larvae. However, at present there is little systematic research on the toxicity and nutritional quality of insect-resistant Bt transgenic maize to borer larvae, or on its effect on the in vivo activity of the detoxification enzymes of the larvae. Here we evaluate the effect of different plant tissues of two Bt transgenic maize lines (G03-2396 and G03-2739) on larval development and survival of ACB. The nutritional quality of these maize lines for the third and fifth instar larvae and their effect on the in vivo activity of detoxification enzymes were also examined. The results of this study is discussed in relation to the literature on plant-

* Corresponding author; e-mail: yzyang@yzu.edu.cn

insect interactions and their potential application in the development of new Bt crops for use in the control of insect pests.

MATERIAL AND METHODS

ABBREVIATIONS: ACB – Asian corn borer; BSA – bovine serum albumin; CDNB – 2, 4-dinitrochlorobenzene; GSH – glutathione; GST – glutathione S-transferase; L1 – ACB neonates; L3 – third instar ACB larvae; L5 – fifth instar ACB larvae; OD_{600} – optical density at 600 nm.

Test maize lines and planting methods

Two maize lines, G03-2396 and G03-2739, which contain *Cry1Ab* insecticidal genes, were provided by the China Agricultural University (Beijing, China). The conventional maize cultivar, Suyu 16, served as the control, and was provided by the Maize Breeding Group of Yangzhou University (Yangzhou, China). The three maize lines were all planted in an experimental plot in the agricultural pastures of Yangzhou University. Following the normal method of planting in the Yangtze River Valley of China, the maize was planted in both widely and narrowly spaced rows, that is, the rows were either 80 cm or 50 cm apart. The seeds were planted in rows at intervals of 37 cm in a planting area of 67 m^2 per line. Crops were subjected to routine management practices, with no chemical pesticides applied during the growing period.

Source of the insects tested

The ACB used in this study originated from overwintering larvae collected locally the previous year and fed on the artificial diet described by Zhou et al. (1980).

Effect of Bt maize on the growth and development of ACB

Samples of whorl leaves and ears were collected from the three maize lines at two different stages of plant development; the whorl leaf (or heart leaf) and earing (or female spike) stages of development and fed to corn borer larvae. At the whorl stage, leaves that had not yet expanded were removed, washed, dried and cut into pieces 7–8 cm long and 3–4 cm wide. These sections of leaf were kept moist by attaching moist cotton wool to each end. A total of 3 sections of leaf from each maize line were placed in a jar, 8cm in diameter by 15 cm high, and 30 newly hatched larvae were subsequently added to each jar. There were four replicates for each maize line. The opening of the jar was tightly covered with copper wire netting.

The survival and pupation of the larvae was recorded and the food (whorl leaves or ears) replaced every three days. After pupation, the pupae were weighed and adult emergence recorded. The percentage of pupae that gave rise to adults and their fecundity was statistically analyzed. The measure of fecundity used was the number of eggs laid per day. A pair of adults was placed in a cage (L × W × H: 30 × 30 × 40 cm) covered with gauze for 24 h after which the number of eggs laid was counted.

During the reproductive stage of the maize, the maize ears (2–3 cm in diameter) were cut into thin slices, 3–5 mm thick. A total of 2–3 maize-ear slices of each maize line were placed in a jar and larvae of different instars added (Yang et al., 1998). This experiment included two treatments. In the first, neonates were reared to pupation on specific maize tissues. In the second, third instar larvae, which had been fed on an artificial diet since hatching, were then reared to pupation on maize tissue as described in "Source of the insects tested".

Determination of nutritional indicators

Nutritional indicators used were those described by Waldbauer (1968). Newly moulted third instar larvae of ACB were kept without food for 10 h and then fed whorl leaves or ears after determining their fresh weight. The remnants of the whorl leaves and ears in the containers with viable insects were removed and weighed 48 h latter. The larvae were then kept without food for 6 h and weighed following defecation. Test insects, faeces and the remnants of whorl leaves and ears were dried at 80°C and their dry weight determined. The dry-wet ratio of whorl leaves, ears and larvae, were determined in order to estimate the dry weight of whorl leaves, maize-ears and test insects prior to rearing. Procedures for the experiments on newly moulted fifth instar larvae of ACB (which were fed an artificial diet prior to this moult) were the same as those used in the experiment on third instar larvae.

Nutritional indicators were calculated using the following formulae; Relative food ingestion = I / (B × T), Relative growth rate = G / (B × T), Approximate digestibility (%) = (I – F) / I × 100, Food utilization (%) = G / I × 100, Food conversion (%) = G / (I – F) × 100, Relative metabolic rate = (I – F – G) / (B × T). In these formulae, G equals the weight increase of the larvae (i.e. G = post-rearing larval dry weight minus pre-rearing larval dry weight); I equals dry weight of food ingested by larvae (i.e. I = pre-rearing dry weight of food minus post-rearing dry weight of food); F equals faecal dry weight; B equals mean body weight of larvae during the experimental period [i.e. B = (pre-rearing larval dry weight + post-rearing larval dry weight) / 2]; T equals the number of days the experiment lasted.

Determination of the activity of detoxification enzymes

Test reagents

Two analytically pure carboxylesterase reagents, α-naphthol and α-naphthyl acetate (α-NA), and analytically pure sodium dodecyl sulphate were purchased from the Shanghai Chemical Reagent No. 1 Factory (Shanghai, China). Fast blue B salt was purchased from Fluka (Shanghai, China).

GST (glutathione S-transferase) reagent and reduced glutathione (GSH) were purchased from Sigma (Chengdu, China). The analytically pure GST reagent and 2, 4-dinitrochlorobenzene (CDNB) were obtained from the Shanghai Chemical Reagent No. 1 Factory.

Preparation of the enzyme solution

Third and fifth instar larvae were fed on young and tender whorl leaves and spike stalks of the three different maize lines for three days as described above in "Effect of Bt maize on the growth and development of ACB". Three days later living larvae were collected and stored in a 2 mL centrifuge tube at –30°C. The enzyme solution was prepared using the method described by Xu et al. (2006). In brief, 10 third instar larvae or five fifth instar larvae were placed into a pre-cooled glass homogenizer containing 3 mL 0.1 mol/L phosphate buffer (pH 7.0) and placed in an ice bath before homogenization. The homogenate was then centrifuged at 10,000 g for 10 min at 4°C, and the supernatant retained as the enzyme source. All the trials were replicated three times, following the same procedure as described for the third instar larvae and the optical density determined using a spectrophotometer (model 752 UV-Vis, Shanghai Precision Scientific Instrument Co., Ltd, Shanghai, China).

Determination of carboxylesterase activity

Carboxylesterase activity was determined using the method described by van Asperen (1962), using α-NA as the substrate. First, 5 mL of the substrate solution (α-NA in 0.03 mol/L acetone, diluted 100-fold with buffer) was placed in a cuvette and maintained for 5 min at 25°C. Subsequently, 1 mL enzyme solution was added to the cuvette, shaken at 25°C, incubated for 30

TABLE 1. The percentage (%) mortality of Asian corn borer neonates (L1) and third instar larvae (L3) that fed on whorl leaves and fresh ears of control (Suyu) and transgenic maize varieties. d = day.

Varieties	Total number of larvae	Whorl leaves						Ears					
		L1			L3			L1			L3		
		2d	4d	6d	2d	4d	6d	2d	6d	8d	2d	6d	8d
Suyu 16	120	12.5c	30.0b	33.0b	5.0b	5.0b	17.5c	7.5a	10.0b	17.5b	2.5a	7.5a	12.5b
G03-2739	120	72.5a	92.5a	97.0a	19.5a	20.0a	32.5b	5.0a	13.3b	20.0b	5.0a	7.5a	27.5a
G03-2396	120	42.5b	92.5a	100.0a	19.5a	25.0a	65.0a	9.7a	20.0a	25.0a	4.5a	7.5a	25.0a

Note: Values followed by different lower case letters, in the same column and across rows, are significantly different $P < 0.05$ levels.

min at the same temperature and then 1 mL of colourimetric solution (1% fast blue B salt solution and 5% sodium dodecyl sulfate, mixed at a ratio of 2 : 5) was added. When the colour stabilized after 30 min, the OD_{600} (optical density at 600 nm) value was determined using a spectrophotometer and the control enzyme solution replaced by 1 mL buffer (0.04 mol/L pH 7.0 phosphate buffer).

Determination of GST activity

Enzyme activity was determined using CDNB as the substrate, following the method described by Scharf et al. (1998). The reaction system included 30 µL enzyme solution, 3.22 mL CDNB analytical liquid (0.1 mol/L pH6.5 phosphate buffer containing 15 mmol/L GSH) and 150 µL CDNB storage fluid (63 mmol/L CDNB methanol solution), incubated at 25°C for 10 min. The OD_{600} value was determined at 344 nm using a spectrophotometer (the enzyme activity was measured in terms of ΔOD/mg.min).

Determination of soluble protein content

The Coomassie Brilliant Blue G-250 method (Bradford, 1976), with bovine serum albumin (BSA) as the protein standard, was used.

Statistical analysis

Data on growth and development of the Asian corn borer (ACB) and nutritional untilization of the food eaten by larvae fed on different tissues of the control and transgenic Bt maize lines were processed using Microsoft Excel 2003 software and analyzed using Data Processing System (DPS) v3.01 statistical software. The Least Significant Difference (LSD) method was used for multiple comparisons (Tang et al., 2002).

RESULTS

Effect of different maize lines on larval survival and development

Effect on larval survival

The growth of ACB larvae fed on transgenic maize was significantly reduced. The mortality of neonates (L1) that ingested maize tissue was high (Suyu 16, n = 120; 23.0%; G03-2739, n = 120; 97.0%; G03-2396, n = 120; 100.0%, 6d after feeding on leaves). However, the mortality of third instar larvae (L3) fed on the same tissue was lower (Suyu 16, n = 120; 17.5%; G03-2739, n = 120; 32.5%; G03-2396, n = 120; 65.0%, 6d after feeding on leaves). For larvae of the same instar the mortality was higher after feeding on whorl leaves (Suyu 16, n = 120; 12.5%; G03-2739, n = 120; 72.5%; G03-2396, n = 120; 42.5%, 2d after feeding on leaves) than on ears (Suyu 16, n = 120; 7.5%; G03-2739, n = 120; 5.0%; G03-2396, n = 120; 9.7%, 2d after feeding on ears). The longer the ACB larvae fed on maize the greater the mortality. The survival of the corn borer larvae fed on the different maize lines differed significantly (Table 1).

Effect on larval development, pupal weight and fecundity

ACB neonates did not complete their larval development when fed whorl leaves of transgenic maize. A few of the ACB larvae that were fed on maize-ears, however, pupated but the pupae were small and produced adults that had a low fecundity. However, pupal weight and the number pupating were less affected when third instar larvae were fed transgenic maize compared with the neonate (Table 2).

TABLE 2. Duration of development, pupal weight and fecundity of individuals of the Asian corn borer that were reared from the neonate (L1) and third instar larval (L3) stages on whorl leaves and ears of control (Suyu) and transgenic varieties of maize varieties. d = day.

Plant organs	Varieties	Total number of larvae	Larval period (d)	Third instar to pupation (d)	Mean pupal weight (mg)		Pupation (%)		Adult emergence (%)		Fecundity / eggs per female	
					L1	L3	L1	L3	L1	L3	L1	L3
Whorl leaves	G03-2396	120	—	18.0a	—	66.0b	—	2.5c	—	—	—	—
	G03-2739	120	—	19.6a	—	81.0a	—	12.5b	—	40.0a	—	391.5a
	Suyu 16	120	23.00a	17.7a	69.2	65.5b	20.0	37.5a	62.5	40.0a	310.7	356.5a
Ears	G03-2396	120	23.00a	13.9a	65.8b	72.6b	10.0b	75.0a	50.0b	73.3a	0.0b	274.0a
	G03-2739	120	21.00a	12.1a	62.8b	75.7b	6.7b	85.0a	75.0ab	73.5a	283.0a	249.8a
	Suyu 16	120	16.26b	10.2a	76.9a	103.4a	51.7a	87.5a	83.9a	85.7a	278.3a	234.0a

Note: Values followed by different lower case letters, in the same column and across rows, are significantly different $P < 0.05$ levels.

TABLE 3. Nutritional indicators calculated for third instar larvae of the Asian corn borer that were fed on whorl leaves and ears of control (Suyu) and three transgenic varieties of maize. ± = standard deviation values.

Plant organs	Number of days	Varieties	Total number of larvae	Relative food ingestion	Approximate digestibility (%)	Relative growth rate	Food utilization (%)	Food conversion (%)	Relative metabolic rate
Whorl leaves	3	G03-2396	60	3.19 ± 1.78a	51.24 ± 6.98a	0.09 ± 0.01a	2.78 ± 0.74a	6.03 ± 0.31a	2.67 ± 0.40a
		G03-2739	60	2.76 ± 1.05a	53.52 ± 7.24a	0.15 ± 0.06a	5.24 ± 0.29a	17.72 ± 8.80a	2.35 ± 0.67a
		Suyu 16	60	5.31 ± 1.87b	57.13 ± 4.51a	3.84 ± 1.24b	5.50 ± 0.89a	13.61 ± 3.09a	2.35 ± 0.30a
	6	G03-2396	60	2.68 ± 0.72a	72.08 ± 18.82a	0.05 ± 0.09a	2.71 ± 0.39a	5.24 ± 0.73a	1.96 ± 0.05a
		G03-2739	60	2.25 ± 0.81a	62.68 ± 13.03a	0.09 ± 0.05a	4.42 ± 0.33a	16.92 ± 1.98a	1.22 ± 0.39a
		Suyu 16	60	5.34 ± 1.00b	72.62 ± 13.67a	0.21 ± 0.03b	5.71 ± 0.68a	28.66 ± 2.78a	1.55 ± 0.82a
Corn-ears	3	G03-2396	60	1.98 ± 0.46a	83.37 ± 9.44a	0.19 ± 0.05a	10.00 ± 3.99a	12.09 ± 4.56a	1.45 ± 0.40a
		G03-2739	60	3.66 ± 0.07b	76.03 ± 5.41a	0.21 ± 0.01a	8.72 ± 0.64a	13.14 ± 2.41a	2.83 ± 0.30ab
		Suyu 16	60	4.88 ± 0.62b	78.78 ± 6.04a	0.44 ± 0.04b	8.96 ± 0.91a	11.53 ± 1.42a	3.41 ± 0.55b
	6	G03-2396	60	1.52 ± 0.24a	68.90 ± 8.42a	0.15 ± 0.03a	9.89 ± 3.04a	14.34 ± 3.69a	0.91 ± 0.22a
		G03-2739	60	2.40 ± 0.73b	75.67 ± 2.56a	0.15 ± 0.05a	7.02 ± 3.68a	9.22 ± 4.88a	1.66 ± 0.54b
		Suyu 16	60	2.57 ± 0.31b	69.82 ± 2.62a	0.26 ± 0.04b	16.88 ± 3.41b	24.27 ± 5.51b	1.84 ± 0.21b

Note: Values followed by different lower case letters, in the same column and across rows, are significantly different $P < 0.05$ levels.

The nutritional indicators of corn borer larvae fed on the different maize lines

Three and six days after the third instar larvae were first fed whorl leaves or maize-ears, their relative food ingestion and growth rates were both significantly lower than those of the control group (Suyu 16). Six days after third instar larvae were first fed maize-ears, the food utilization, food conversion and relative metabolic rate were significantly lower than those of the control group (Suyu 16). Three days after fifth instar larvae were first fed whorl leaves of the two transgenic maize lines the nutritional indicators did not differ from those of the control group (Suyu 16). Relative ingestion of food by fifth instar larvae fed on whorl leaves of G03-2396 line was significantly less than that of the control larvae (Suyu 16) (Table 3 and 4).

Effect of feeding on the different maize lines on the activity in vivo of larval detoxification enzymes

Effect on carboxylesterase activity

The activity in vivo of carboxylesterase in third instar larvae fed on the different transgenic maize lines for three days was significantly lower than in the control group (Suyu 16) (Table 5). In addition, the degree of inhibition after feeding on whorl leaves was greater than after feeding on maize-ears (G03-2396, G03-2739). No significant effect on the activity in vivo of carboxylesterase in fifth instar larvae was recorded (G03-2396, G03-2739, Suyu 16).

Effect on GST activity

Third instar corn borer larvae that fed on the whorl leaves of the two Bt lines showed a decrease in the activity in vivo of GST. However, there were no significant differences in GST activity between larvae fed on ears of transgenic maize lines and the control group (Suyu 16). The activity in vivo of GST in fifth instar larvae, after ingestion of whorl leaves and maize-ears of the two transgenic maize lines, did not differ significantly from that of the control group (Suyu 16).

DISCUSSION

The whorl leaves of both transgenic maize lines G03-2396 and G03-2739 are resistant to attack by newly hatched neonates and third instar larvae. In contrast, the resistance of the ears was relatively low particularly against third instar larvae and did not differ from the control. The study of Xu et al. (2007) suggests that the difference in the resistance of different plant tissues to corn borers is related to their toxic protein content. Bt-maize has a sustained insecticidal effect on Asian corn borer larvae (Lou et al., 2007). Our results show that Bt toxin

TABLE 4. Nutritional indicators calculated for fifth instar larvae (L5) of the Asian corn borer that were fed for three days on the whorl leaves and ears of control (Suyu) and three varieties of transgenic maize. ± = standard deviation values.

Plant organ	Varieties	Total number of larvae	Relative food ingestion	Approximate digestibility (%)	Relative growth velocity	Food utilization (%)	Food conversion (%)	Relative metabolic rate
Whorl leaves	G03-2396	60	0.74 ± 0.30a	70.34 ± 5.07a	0.05 ± 0.02a	6.74 ± 0.49a	8.41 ± 0.57a	0.54 ± 0.22a
	G03-2739	60	1.24 ± 0.22ab	49.28 ± 1.32a	0.05 ± 0.01a	3.98 ± 1.14a	9.40 ± 0.24a	0.57 ± 0.26a
	Suyu 16	60	1.85 ± 0.63b	78.93 ± 4.32a	0.09 ± 0.07a	3.13 ± 0.67a	8.04 ± 0.67a	1.22 ± 0.45a
Corn-ears	G03-2396	60	1.09 ± 0.34a	61.70 ± 10.31ab	0.20 ± 0.03a	19.51 ± 7.43a	32.93 ± 15.67a	0.47 ± 0.23ab
	G03-2739	60	1.12 ± 0.41a	51.82 ± 10.00a	0.18 ± 0.09a	16.67 ± 7.42a	31.89 ± 12.12a	0.39 ± 0.17a
	Suyu 16	60	1.60 ± 0.35b	65.76 ± 3.65b	0.36 ± 0.03b	23.71 ± 7.74a	36.42 ± 13.83a	0.70 ± 0.27b

Note: Values followed by different lower case letters, in the same column and across rows, are significantly different $P < 0.05$ levels.

TABLE 5. Effect of feeding on control (Suyu) and three transgenic varieties of maize on the in vivo activity of carboxylesterase in third (L3) and fifth (L5) instar larvae of the Asian corn borer (total number of larvae, L3 = 30, L5 = 15).

Varieties	Whorl leaves				Ears			
	Enzyme activity (nmol/mg, Pr/min)		Ratio		Enzyme activity (nmol/mg, Pr/min)		Ratio	
	L3	L5	L3	L5	L3	L5	L3	L5
G03-2396	0.09 ± 0.00b	0.09 ± 0.01a	0.70	1.00	0.15 ± 0.01b	0.12 ± 0.05a	0.71	1.36
G03-2739	0.05 ± 0.03b	0.11 ± 0.01a	0.42	1.21	0.16 ± 0.00b	0.10 ± 0.01a	0.76	1.14
Suyu 16	0.12 ± 0.05a	0.09 ± 0.00a	1.00	1.00	0.21 ± 0.01a	0.09 ± 0.00a	1.00	1.00

Note: Values followed by different lower case letters, in the same column and across rows, are significantly different $P < 0.05$ levels.

not only had a strong toxic effect on newly hatched neonate larvae and third instar larvae of corn borer, but also affected subsequent instars resulting in the production of a few small pupae that produced adults that had a low fecundity, which indicates that the insecticidal protein had a cumulative effect on Asian corn borers. However, the different transgenic maize lines were resistant to ACB for different periods, so it is necessary to select and breed lines that are resistant to ACB throughout the entire maize growing season, like the hybrid-NX4777 of Bt11 event (He et al., 2004).

The nutritional quality of plants is extremely important for insects. The fitness of insects is affected by their ability to digest and convert plant tissues into insect tissues and these indicators are commonly used as measures of their ability to utilize plants (Qin, 1987). These nutritional indices indicate that both whorl leaves and the ears of the two transgenic maize varieties inhibited the growth of third and fifth instar larvae. Food intake and the relative growth rate of the ACB larvae fed on transgenic maize were both lower than that of the larvae fed on Suyu 16 maize, the control. Zhang et al. (2004) also record that transgenic Bt cotton significantly inhibits the growth of cotton bollworm larvae and significantly affects their nutritional utilization of these plants. Wang et al. (2005) suggest that the avoidance or rejection of the Bt toxin by pests would lead to a decrease in the intake of insecticidal protein by target pests, consequently reducing the ultimate effectiveness of these toxins. But Yang et al. (2008) indicate that the ability of neonates to find more suitable tissues on GM plants may have contributed to the continuing and occasional pest status of Helicoverpa on GM cotton. In this study the relative consumption and growth rate of third instar larvae of ACB fed on Bt maize were significantly lower than that of the controls. This indicates that the insecticidal properties of *Cry1Ab* insecticidal proteins were stronger and there was no obvious dose effect.

There are no significant differences in the performance of first to third instar larvae of the Chinese tussah silkworm *Antherea pernyi* (Lepidoptera: Saturniidae) fed on diets containing pollen grains of GM or conventional cotton, which suggests that the widespread planting GM cotton is unlikely to have an adverse effect on this moth (Li et al., 2003). In addition the utilization by *O. furnacalis* and *Helicoverpa armigera* of phytase transgenic maize is also not affected (Zhang et al., 2010) Thus, the effects of transgenic crops on the development and nutritional utilization of crop plants by insects should be assessed on a case by case basis.

The midgut proteinase of Lepidoptera larvae is widely studied because of its important role in digestion (Li et al., 2005). Three days after ingesting tissue of whorl leaves or ears of two transgenic maize lines, the *in vivo* activity of carboxylesterase in the first three larval instars of ACB was significantly decreased, which is consistent with the results obtained by Xu et al. (2006). Furthermore, the level of inhibition after ingestion of whorl leaves was greater than after ingestion of ears. This suppression of enzyme activity indicates that the metabolism of insects is affected, which may be one of the most important mechanism by which the Bt toxins determine the resistance of maize to corn borers. Our research also indicates that the carboxylesterase in fifth instar larvae of the borer is better at degrading the maize toxic protein than that in third instar larvae. Hence, the developmental stage of the target insect should be considered when studying insect resistance to transgenic plant lines. Our

TABLE 6. Effect of feeding on control (Suyu) and three transgenic varieties of maize on the in vivo activity of the GST activity in third and fifth instar larvae of the Asian corn borer (total number of larvae, L3 = 30, L5 = 15).

Varieties	Whorl leaves				Ears			
	Enzyme activity (nmol/mg, Pr/min)		Ratio		Enzyme activity (nmol/mg, Pr/min)		Ratio	
	L3	L5	L3	L5	L3	L5	L3	L5
G03-2396	0.16 ± 0.01a	0.13 ± 0.15a	0.70	0.80	0.18 ± 0.08a	0.14 ± 0.08a	0.75	0.81
G03-2739	0.15 ± 0.00a	0.18 ± 0.00a	0.65	1.13	0.19 ± 0.06a	0.16 ± 0.03a	0.79	0.89
Suyu 16	0.23 ± 0.04b	0.16 ± 0.01a	1.00	1.00	0.24 ± 0.04a	0.18 ± 0.06a	1.00	1.00

Note: Values followed by different lower case letters, in the same column and across rows, are significantly different $P < 0.05$ levels.

results indicate that after feeding on whorl leaves of transgenic maize the activity of GST in third instar larvae was significantly reduced. In the current study, the effect of transgenic maize on the activity of just two of the detoxification enzymes of corn borer larvae was evaluated, however the metabolism of insects is also affected by the interaction of a range of in vivo enzymes, including the mid-gut protective protease enzymes (Candas et al., 2003; Li et al., 1994).

ACKNOWLEDGEMENT. This study was funded by a grant from the Key Project for Breeding Genetically Modified Organisms (2011ZX08012-004).

REFERENCES

van Asperen K. 1962: A study of housefly esterase by means of a sensitive colorimetric method. *J. Insect Physiol.* **8**: 401–416.

Bradford M.M. 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. *Analyt. Biochem.* **72**: 248–254.

Candas M., Loseva O., Oppert B., Kosaraju P. & Bulla L.A. 2003: Insect resistance to Bacillus thuringiensis: Alteration in the indianmeal moth larval gut proteome. *Mol. Cell. Proteom.* **2**: 19–28.

He K.L., Wang Z.Y., Wen L.P., Bai Z.X., Zhou D.R. 2004: Transgenic maize evaluated for resistance to the Asian Corn Borer (Lepidoptera: Pyralidae). *Chin. Agric. Sci. Bull.* **20**: 240–246.

James C. 2003: Global review of commercialized transgenic crops. *Curr. Sci.* **84**: 303–309.

Jiao C.H. 2005: Production dynamic of transgenic crops in America. *World Agric.* **11**: 49–51.

Li H.R., Oppert B., Higgins R.A., Huang F.N., Buschman L.L., Gao J.R. & Zhu K.Y. 2005: Characterization of cDNAs encoding three trypsin-like proteinases and mRNA quantitative analysis in Bt-resistant and -susceptible strains of Ostrinia nubilalis. *Insect Biochem. Mol. Biol.* **35**: 847–860.

Li W.D., Wu K.M., Wang X.Q. & Guo Y.Y. 2003: Evaluation of impact of pollen grains of Cry1Ac- and Cry1A + CpTI-transgenic on the growth and development of Chinese tussah silkworm (Antherea pernyi). *J. Agric. Biotechnol.* **11**: 488–493.

Li Z.Z., Shen H.J., Jiang Q.G. & Ji B.Z. 1994: A study on the activities of endogenous enzymes of protective system in some insects. *Acta Entomol. Sin.* **37**: 399–403.

Luo M.H., Liu J.B., Fu G.C., Guo X.R. & Ma B.L. 2007: Resistance of Bt transgenic maize to Ostrinia furnacalis. *J. Henan Agric. Univ.* **41**: 77–80.

Qin J.D. 1987: *The Relationship between Insects and Plants – Comments on the Interaction between Insects and Plants and its Evaluation.* Science Press, Beijing, pp. 133–149.

Scharf M.E., Neal J.J., Marcus C.B. & Bennett G.W. 1998: Cytochrome P450 purification and immunological detection in an insecticide resistant strain of german cockroach (Blattella germanica L.). *Insect Biochem. Mol. Biol.* **28**: 1–9.

Tang Q.Y. & Feng M.G. 2002: *Practical Statistical Analysis and its DPS Data Processing System.* Science Press, Beijing, pp. 43–80.

Waldbauer G.P. 1968: The consumption and utilization of food by insects. *Adv. Insect Physiol.* **5**: 229–288.

Wang D.Y., Wang Z.Y., He K.L. & Cong B. 2005: Feeding behavior of Ostrinia furnacalis (Lepidoptera: Pyralidae) larvae on transgenic Bt corn expressing CryIAb toxin. *Chin. Bull. Entomol.* **42**: 258–262.

Xu Y.L., Wang Z.Y., He K.L. & Bai S.X. 2006: Effects of transgenic Bt corn expressing Cry1Ab toxin on activities of some enzymes in larvae of the Asian corn borer, Ostrinia furnacalis (Güenée) (Lepidoptera: Pyralidae). *Acta Entomol. Sin.* **49**: 562–567.

Xu Y.L., Wang Z.Y., He K.L. & Bai S.X. 2007: Tissue distribution and content of Cry1Ab insecticidal protein in the Bacillus thuringiensis resistant and susceptible Asian corn borer larvae fed on Bt-transgenic corn. *Acta Entomol. Sin.* **50**: 957–961.

Yang Y.Z., Johson M.L., Zalucki M.P. 2008: Effect of GMO cotton on behaviors and habits of early instar larvae of Helicoverpa armigera (Hübner) (Lepidoptera: Noctuidae). *Austr. J. Entomol*. **47**: 137–141.

Yao H.W., Ye G.Y. & Cheng J.A. 2002: Advances in the studies on the effects of host plants on insect susceptibility to insecticides. *Acta Entomol. Sin.* **45**: 253–264.

Zhang S., Zeng X.N. & Luo Y. 2004: Relationship between host plant and insecticide-resistance of isects. *Guangxi Agric. Sci.* **35**: 213–215.

Zhang Y., Liu C.X., Li Y.H. & Wu K.M. 2010: Phytase transgenic maize does not affect the development and nutrition utilization of Ostrinia furnacalis and Helicoverpa armigera. *Environ. Entomol*. **39**: 1051–1057.

Zhou D.R., Wang Y.Y., Liu B.L. & Ju Z.L. 1980: Studies on the mass rearing of corn borer. *Acta Phytopath. Sin*. **7**: 113–122.

Received November 8, 2010; revised and accepted June 14, 2011