Effects of Cry1Ab Toxin on *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) Through Its Prey, *Nilaparvata lugens* Stål (Homoptera: Delphacidae), Feeding on Transgenic *Bt* Rice

Y. Y. BAI,[1,2] M. X. JIANG,[1] J. A. CHENG,[1,3] AND D. WANG[1]

Environ. Entomol. 35(4): 1130–1136 (2006)

ABSTRACT Laboratory feeding experiments using rice plants containing a synthetic *cry1Ab* gene derived from *Bacillus thuringiensis* Berliner (*Bt*) were carried out to study the effects of *Bt* rice–fed prey on the predator *Propylea japonica* (Thunberg). Plants were obtained from two homozygous transgenic *Bt-cry1Ab* expressing rice lines, Kemingdao 1 (KMD1) and Kemingdao 2 (KMD2), and their untransformed parental variety Xiushui 11 (XS11). The herbivorous prey species tested was the brown planthopper, *Nilaparvata lugens* Stål, one of most serious insect pests of rice and not targeted by KMD1 or KMD2. The concentrations of Cry1Ab toxin expressed in KMD1 and KMD2 plants and that of the toxin transferred to *N. lugens* feeding on these plants were determined by enzyme immunosorbent assay technique. Development parameters of *P. japonica* reared on KMD1- or KMD2-fed *N. lugens* were assessed in the laboratory. The results showed that the concentration of Cry1Ab in rice leaves and stems significantly increased from the booting to grain filling stage and subsequently decreased as the plants matured. Cry1Ab could be detected in nymphs and adults of *N. lugens* feeding on the *Bt* rice plants. Development time, pupation, adult eclosion, pupal and adult weight, and male-adult locomotive activity of *P. japonica* that had preyed on KMD1- or KMD2-fed *N. lugens* nymphs as larvae were not significantly different from those that preyed on XS11-fed nymphs. In short, our results indicate that the nontarget insect *N. lugens* and its predator *P. japonica* are exposed to *Cry1Ab* toxin from transgenic *cry1Ab* rice, but development of this predator was not affected by the toxin through tritrophic interactions.

KEY WORDS *Bacillus thuringiensis*, predation, Cry1Ab, transgenic rice, tritrophic interactions

Transgenic *Bt* plants can express the *Bt* proteins in high doses and in most of their tissues throughout the season (Fearing et al. 1997, Wu et al. 2001), which provides the plants with season-long protection against target pests. Toxins from *Bt* are relatively host specific within certain insect orders (e.g., Lepidoptera), because only such insects that possess specific gut physiological conditions and receptor sites are susceptible to the toxins (Knowles 1994). However, insects outside of target groups also have been reported to be susceptible to the Cry 1Ab toxin from *Bt* corn (Event Bt11) expressing the *cry1Ab* gene from *Bt* subsp. *kurstaki* (Hilbeck et al. 1998b).

Bt insecticidal toxins may become available to natural enemies through nonsusceptible or sublethally affected herbivorous prey feeding on *Bt* plants (Schuler et al. 1999, Head et al. 2001, Jiang et al. 2004). To date, effects of *Bt* plants on predators have been studied in at least 11 studies in the context of tritrophic interactions among *Bt* plants, herbivores, and predators (Romeis et al. 2006). Predators with chewing mouthparts, such as lady beetles (Coccinellidae), are expected to ingest the toxin when preying on *Bt*-fed arthropods because they ingest the gut where most of the toxin is located. Previous studies showed that coccinellid predators suffered little risk from the Cry1Ab toxins expressed in *Bt* crops (Pilcher et al. 1997, Daly and Buntin 2005, Pilcher et al. 2005, Zhang et al. 2006). However, to clarify the conclusive impact of *Bt* plants on predaceous coccinellids, more extensive experiments are needed (Schoenly et al. 2003).

Transgenic *Bt* rice has the potential of eliminating yield losses caused by lepidopteran pests, which in Asia were estimated as 2–10% of the annual rice yield of 523 million tons (High et al. 2004). In China, the transgenic *cry1Ab* rice lines have proved to be highly resistant to eight lepidopteran pest species of rice under field conditions, including the striped stem borer, *Chilo suppressalis* (Walker), yellow stem borer *Scirpophaga incertulas* (Walker), and several defoliators (Shu et al. 2000, Ye et al. 2001, Wu et al. 2001). *Propylea japonica* (Thunberg) (Coleoptera: Coccinellidae) is a common predator in crop systems of China. Both the larva and adult of this insect are predaceous,

[1] Institute of Insect Sciences, Zhejiang University, Hangzhou 310029, China.
[2] Key Laboratory of Entomology and Pest Control Engineering, College of Plant Protection, Southwest University, Chongqing 400716, China.
[3] Corresponding author, e-mail: jacheng@zju.edu.cn.

0046-225X/06/1130–1136$04.00/0

with the larva being more voracious than the adult. In rice fields, it can prey on young nymphs of the brown planthopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae), which is one of the most serious pests of rice throughout temperate and subtropical Asia, often attacking rice in large numbers and causing hopperburn (Cheng 1996). *P. japonica* can also feed on aphids (Guo and Wan 2001) and pollens (Li et al. 1992). Our previous study indicated that development and reproduction of *P. japonica* was not negatively affected when feeding on *Bt* rice pollen compared with pollen of genetically untransformed rice (Bai et al. 2005).

In this study, development of immature stages and locomotive activity of adults were observed in this predator after being supplied as larvae with *N. lugens* feeding on *Bt* rice. The objective was to determine the prey-mediated effect of toxins in *Bt* rice on this predator.

Materials and Methods

Plant. The transgenic *Bt* lines Kemingdao 1 (KMD1) and Kemingdao 2 (KMD2) were derived from a Chinese commercial *japonica* rice variety Xiushui 11 (XS11), transformed by *Agrobacterium* infection. Both KMD1 and KMD2 were homozygous containing a synthetic *cry1Ab* gene derived from *B. thuringiensis* under the control of a maize ubiquitin promoter (Shu et al. 1998). They were highly resistant to eight lepidopteran pest species of rice under laboratory and field conditions (Cheng et al. 1998, Shu et al. 2000, Ye et al. 2001, Ye et al. 2003). Thirty-day-old plants of KMD1 and KMD2, and their parent nontransgenic XS11, were transplanted in a greenhouse on the Huajiachi campus of Zhejiang University in mid-June 2003. Plants at booting stage were transferred to the laboratory and served as hosts of *N. lugens* as described below.

Insect. *Propylea japonica* adults were collected from rice plants at flowering stage on the experimental farm of Zhejiang University during August 2003. Pairs were placed in glass tubes (1.4 cm diameter, 10 cm long) containing nymphs and adults of the green peach aphid, *Myzus persicae* (Sulzer), and maintained in a climatically controlled chamber at 28 ± 1°C and 60–80% RH with a photoperiod of 16:8 (L:D) h. *M. persicae* were supplied daily to the tubes. As egg deposition began, the female was transferred to clear glass tubes, and the tubes bearing eggs were maintained in the chamber until completion of hatch. A water-saturated cotton ball was placed in each glass tube to provide moisture. Egg hatch was checked daily, and newly hatched larvae were placed individually into tubes with a cotton stopper for subsequent use. The *M. persicae* used were previously reared on white cabbage *Brassica oleracea* L. variety *capitata* in the chamber.

Nilaparvata lugens adults were collected from insecticide-free rice fields located on farms of Zhejiang University. In the laboratory, adults were released in drum cages (20 cm diameter, 75 cm long) containing booting plants of KMD1, KMD2, or nontransgenic XS11, and maintained in the chamber as described above. After 15 d (when nymphs of the first generation had been produced and each instar could be found), *N. lugens* nymphs and adults were randomly sampled from each cage to determine their Cry1Ab concentration. As the second generation began, second- to third-instar nymphs were sampled from each cage and used as food for *P. japonica* in the experiments described below.

Treatments and Rearing of *P. japonica*. One newly hatched *P. japonica* larva and second- to third-instar nymphs of *N. lugens* were placed into glass tubes (1.4 cm diameter, 10 cm long) that contained one stem (≈5 cm long) of KMD1, KMD2, or nontransgenic XS11 plants. The stems for *N. lugens* nymph feeding were obtained by cutting plants near the ground at booting stage in the greenhouse and further cutting their stems into short pieces in the laboratory. The tubes were plugged with nonabsorbent cotton, and a piece of filter paper (4–5 cm long) was provided that had been moistened with distilled water containing 100 ppm benzimidazole to prevent fungal contamination of plant stems. A water-saturated cotton ball was placed in tubes to provide water for *P. japonica.* There were 40 such tubes for each of the three rice lines (KMD1, KMD2, and XS11), with each tube representing a replicate.

After these preparations, the tubes were maintained in the chamber described above. Rice stems and *N. lugens* nymphs in tubes were refreshed twice daily. As *P. japonica* grew, the number of *N. lugens* nymphs supplied in tubes was adjusted, with 5, 10, 30, and 50 nymphs supplied per day during the first, second, third, and fourth instar of *P. japonica*, respectively.

Observations on *P. japonica* Development. After rearing began, *P. japonica* larvae were checked every 3–4 h for cast skins and dead larvae to determine the developmental time of each instar. The developmental time of prepupae and pupae also was observed. Pupae and adults were weighed on an electronic balance to the nearest 0.001 g within 12 h after pupation and 6 h after emergence, respectively. Larvae in each treatment were divided randomly into three groups, with the percentages of larvae that pupated and pupae that eclosed being observed in each group and then averaged within the treatment.

Flip time has been used successfully as an indicator of fitness in adult coccinellids (Smith and Krischik 1999, Lundgren and Wiedenmann 2002, Lundgren and Wiedenmann 2005). In this study, to obtain adequate male adults of *P. japonica* for flip-time tests, another three groups of this insect were reared in tubes containing *N. lugens* nymphs and plant stems, using the methods described above. At 6 h after emergence, an individual male was placed on its dorsum on a piece of filter paper (90 mm diameter), and the time taken by the beetle to right itself was measured with a stopwatch to the nearest 0.01 s. Measurement was terminated at 60 s for beetles that did not flip within this time.

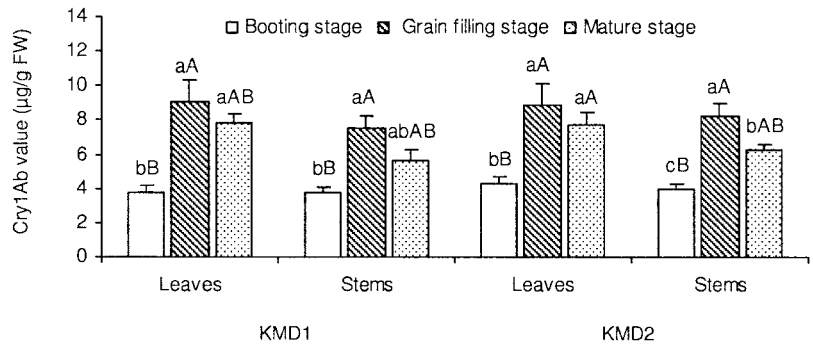

Fig. 1. Concentrations (mean ± SEM) of Cry1Ab toxin in leaves and stems of transgenic *Bt* rice KMD1 and KMD2 at different growth stages. Columns with lowercase and capital letters within a tissue are significantly different at $P \leq 0.05$ and 0.01, respectively. Tukey HSD test.

Determination of Cry1Ab Concentration in Plant Tissues and Insects. At 65, 105, and 120 d after transplanting, when plants were at booting, grain filling, and mature stages, respectively, stems and leaf blades of KMD1 and KMD2 were sampled. After the field-collected *N. lugens* had been feeding on KMD1 or KMD2 plants for 15 d, adults and nymphs of first, second to third, and fourth to fifth instars were sampled and killed immediately by freezing. These plant tissue and insect samples were stored in a deep freezer (−80°C) before Cry1Ab concentration was determined.

The Cry1Ab concentration in samples was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (EnviroLogix, Portland, ME) (Xie and Shu 2001). Before the assay, the frozen plant tissue (0.1–0.01 g) and insect samples (6–20 individual *N. lugens*) were thawed, weighed in a 1.5-ml centrifuge tube, added to milk extraction buffer (PBS/0.55% Tween-20/0.4% nonfat dry milk), and mixed. The samples were homogenized for ≈10 s at 3,000 rpm with a Wheaton overhead stirrer. The homogenized buffer solution was centrifuged at $10{,}000 \times g$ for 1 min, and the supernatant was analyzed using the quantitative Cry1Ab ELISA kit. A 96-well solid microplate (EnviroLogix) was coated with a Cry1Ab antibody. A three-point linear standard curve of 0.5, 2.5, and 5.0 ppb Cry1Ab calibrator (EnviroLogix) was added to the plate, along with Cry1Ab negative control (NC). Absorbance was measured at 450 nm with a MQX200 plate reader (Bio-Tek Instruments, Winooski, VT). Concentrations of Cry1Ab were determined in each sample by finding its optical density (OD_{450}) value and the corresponding concentration level on the graph of the standard linear curve of Cry1Ab. Dilution factors were calculated, and Cry1Ab results were reported as parts per billion fresh weight (FW).

Statistical Analysis. One-way analysis of variance (ANOVA) was used to analyze the relation of Cry1Ab concentration in leaves and stems to plant growth stage and the relation of *P. japonica* development to rice lines. Percentage data were arcsine square root transformed before being subjected to ANOVA. The Tukey honestly significant difference (HSD) test was used to compare means among treatments at the $P = 0.05$ level. The χ^2 test was used to compare the proportions of *P. japonica* adults falling in different flip-time periods among rice-line treatments at the $P = 0.05$ level. All statistical analyses were performed with SPSS Statistical Software (SPSS 2002).

Results

ANOVA showed that, in both leaves and stems of each *Bt* rice line, the concentration of Cry1Ab was significantly related to plant growth stage (leaves of KMD1: $F_{2,6} = 12.58$, $P < 0.01$; stems of KMD1: $F_{2,6} = 7.12$, $P = 0.03$; leaves of KMD2: $F_{2,6} = 10.21$, $P = 0.01$; stems of KMD2: $F_{2,6} = 22.19$, $P < 0.01$). The Cry1Ab concentration increased significantly ($P < 0.05$) from booting to the grain-filling stage in both leaves and stems of each line (Fig. 1). The concentration decreased as plants developed from grain filling to the mature stage, but the change was significant only in the stems of KMD2 (Fig. 1).

Cry1Ab in first- to fifth-instar *N. lugens* nymphs feeding on plants of the *Bt* lines KMD1 or KMD2 was detectable using ELISA, but only as a trace (<0.025 or <0.05 ng/nymph). In adults of the insect, the Cry1Ab concentration was much higher, with 0.11–0.12 ng Cry1Ab detected per adult (Table 1). Thus, the Cry1Ab protein in both *Bt* lines can be transferred to *N. lugens* during its feeding on such plants.

The food supplied to *P. japonica* (i.e., *N. lugens* reared on KMD1, KMD2, or XS11) had no significant effects on the development time of first-, third-, and fourth-instar larvae, prepupae, and pupae, the percentage of larvae that pupated and pupae that eclosed, or the pupal and adult weight ($P > 0.05$; Table 2). Development time of second instars was significantly shorter on KMD-fed *N. lugens* than on XS11-fed *N. lugens*.

At 6 h after emergence, ≥80% of male adults from each source flipped within 12 s, with a large proportion (40–54%) flipping between 4 and 6 s (Fig. 2). The distribution of flip-time was not significantly different

Table 1. Concentrations of Cry1Ab toxin at different developmental stages of *N. lugens* feeding on *Bt* rice lines KMD1, KMD2, and the control rice line XS11

Rice line	Developmental stage of *N. lugens*	Testing unit (no. individuals)	Cry1Ab value (ng per individual, mean ± SEM)
KMD1	First-instar nymph	20	<0.025
	Second- to third-instar nymph	10	<0.05
	Fourth- to fifth-instar nymph	10	<0.05
	Adult	6	0.11 ± 0.02
KMD2	First-instar nymph	20	<0.025
	Second- to third-instar nymph	10	<0.05
	Fourth- to fifth-instar nymph	10	<0.05
	Adult	8	0.12 ± 0.05
XS11	Adult	10	0

The trace concentrations (<0.025 or <0.05 ng/nymph) of Cry1Ab meant that the optical density (OD_{450}) of corresponding samples was higher than that of the negative control but was lower than that of the low calibrator 0.5 ppb Cry1Ab determined by ELISA.

among the adult sources ($\chi^2 = 8.090$, df = 16, $P = 0.946$).

Discussion

Our results from this study show that Cry1Ab in *Bt* rice can be transferred to the phytophagous insect *N. lugens* and thus to its predator *P. japonica.* That is, this predator can be exposed to Cry1Ab toxin when it preys on the *N. lugens* nymphs fed on *Bt* rice. Similarly, Chen et al. (2005) found that Cry1Ab toxin in *Bt* plants could be passed to the wolf spider, *Pirata subpiraticus* (Boesenberg et Strand) (Araneae: Lycosidae) through tritrophic interactions. However, it is not clear if the Cry1Ab protein is bioaccumulated in *N. lugens* or its predators. Bernal et al. (2002) detected Cry1Ab toxin in honeydew from *N. lugens* that fed on transgenic rice lines and concluded that *N. lugens* and its predator *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae) could be exposed to *Bt* toxins from transgenic rice.

We found that the developmental time of preimaginal stages and male-adult locomotive activity of *P. japonica* were not negatively affected by Cry1Ab toxin, which was passed from *Bt* rice plants through *N. lugens.* It is possible that Cry1Ab is not toxic to *P. japonica* or that this predator was not exposed to sufficient concentrations to have a toxic effect. However, other studies involving lepidopteran-specific *Bt* cotton have shown that this predator can be significantly affected through trophic interactions. For example, larvae of the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), feeding on leaves of Cry1Ab-expressing *Bt* cotton had a significantly smaller body weight comparing to those feeding on leaves of nontransgenic cotton, and thus greater numbers were consumed by *P. japonica* (but it was not examined whether the mean total mass of prey consumed differed significantly between the two treatments) (Cui and Xia 1999). In addition, Zhang et al. (2006) found that Cry1Ab expressed in *Bt* cotton can be transmitted to *P. japonica* through a nontarget pest insect, the cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae), and alter the biology and behavior of this predator.

To date, there have been a number of studies involving effects of *Bt* rice, *Bt* corn, and *Bt* cotton on predators through herbivore prey. Bernal et al. (2002) reported that fitness of *C. lividipennis*, an important predator of *N. lugens*, was not negatively affected by

Table 2. Developmental parameters of *P. japonica* stages feeding on *N. lugens* reared on *Bt* rice lines KMD1 and KMD2, and on the control rice line XS11

Fitness parameters	Food of *P. japonica*			ANOVA		
	N. lugens reared with KMD1	*N. lugens* reared with KMD2	*N. lugens* reared with XS11	*F*	*P*	df
Developmental time (d)						
First-instar larva (*n*)	2.50 ± 0.10a (38)	2.57 ± 0.12a (38)	2.46 ± 0.10a (35)	0.25	0.78	2,108
Second-instar larva (*n*)	1.19 ± 0.07b (37)	1.07 ± 0.06b (36)	1.52 ± 0.08a (35)	9.86	<0.01	2,105
Third-instar larva (*n*)	1.38 ± 0.08a (37)	1.35 ± 0.07a (36)	1.51 ± 0.09a (35)	1.11	0.33	2,105
Fourth-instar larva (*n*)	2.56 ± 0.11a (37)	2.74 ± 0.14a (36)	2.49 ± 0.14a (35)	0.97	0.38	2,105
Whole larval stage (*n*)	7.57 ± 0.16a (37)	7.69 ± 0.18a (36)	7.90 ± 0.20a (35)	0.91	0.41	2,105
Prepupa (*n*)	0.59 ± 0.03a (35)	0.66 ± 0.03a (35)	0.59 ± 0.03a (34)	2.10	0.13	2,101
Pupa (*n*)	2.60 ± 0.07a (31)	2.53 ± 0.05a (31)	2.65 ± 0.05a (34)	1.62	0.21	2,93
Percent larvae that pupated (*n*)	77.10 ± 5.78a (40)	78.10 ± 5.00a (40)	84.01 ± 4.67a (40)	0.76	0.51	2,6
Percent pupae that eclosed (*n*)	100.0 ± 0.0 (31)	100.0 ± 0.0 (31)	100.0 ± 0.0 (34)			
Pupal wt (mg) (*n*)	4.53 ± 0.23a (25)	4.91 ± 0.22a (25)	4.89 ± 0.21a (24)	0.79	0.46	2,71
Adult wt (mg) (*n*)	3.72 ± 0.20a (25)	3.87 ± 0.14a (25)	3.92 ± 0.14a (24)	0.40	0.67	2,71

Experiments were conducted using a photoperiod of 16:8 (L:D) h and temperature of 28 ± 1°C. Within a row, values (mean ± SEM) followed by the same letter are not significantly different from each other ($P > 0.05$; Tukey HSD test). *n*, the no. of individuals measured.

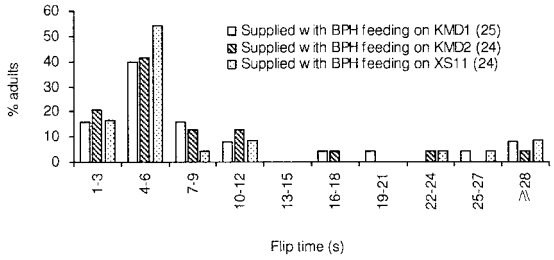

Fig. 2. The percentages of *P. japonica* male adults (6 h old) that flipped within different periods of time. The insects were supplied at the larval stage with nymphs of *N. lugens* (brown planthopper) that fed on transgenic *Bt* rice KMD1 or KMD2, or nontransgenic rice XS11. The value in parentheses indicates the number of adults observed.

preying on *N. lugens* fed on *Bt* rice containing Cry1Ab toxin. In the predator *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) larvae, some studies reported that transgenic *Bt* corn expressing the *cry1Ab* gene caused negative effects on this predator (Hilbeck et al. 1998a, b, 1999, Dutton et al. 2002), whereas others did not find such an effect (Romeis et al. 2004). The effects of *Bt* corn on *C. carnea* larvae were prey quality–mediated rather than being caused by direct effects of the Cry1Ab toxin, and predator larvae were unlikely to detect Cry1Ab when feeding on herbivore prey containing the toxin (Dutton et al. 2003a, b). The midgut of *C. carnea* larvae lacks specific receptors for Cry1Ab or Cry1Ac; therefore, the Cry toxins from transgenic crops have no detrimental effect on this predator when ingested either directly or through the prey (Rodrigo-Simón et al. 2006). The mortality of *Orius majusculus* (Reuter) (Heteroptera: Anthocoridae) from hatch to adult eclosion was not significantly different when nymphs were reared on *Bt*-fed or *Bt*-free prey, *Anaphothrips obscurus* (Müller) (Thysanoptera: Thripidae), a pest of corn without sensitivity to Cry1Ab toxin (Zwahlen et al. 2000). Al-Deeb et al. (2001) found no effect in the predator *Orius insidiosus* (Say) when preying on *Bt* corn–fed *Ostrina nubilalis* (Hübner) (Lepidoptera: Pyralidae). Ponsard et al. (2002) found that, when supplied with *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) that were reared on *Bt* cotton, the heteropteran predators *Orius tristicolor* (White) and *Geocoris punctipes* (Say) had significantly decreased longevity, whereas such an effect did not occur in two other heteropteran predators, *Zelus renardii* (Kolenati) and *Nabis* sp.

A number of field studies showed that transgenic *Bt* corn did not have adverse effects on populations of nontarget predaceous arthropods in the corn system (Daly and Buntin 2005, Pilcher et al. 2005) and that transgenic *Bt* corn is less toxic to the most abundant predators [*Coleomegilla maculate* (DeGeer), *Harmonia axyridis* (Pallas), and *O. insidiosus*] in corn fields than the pyrethroid, lambda cyhalothrin (Musser and Shelton 2003). Pilcher et al. (1997) reported no detrimental effects in the abundance of *Ostrinia nubilalis* (Hübner) predators (coccinellids, anthocorids, chrysopids) in transgenic *cry1Ab* corn compared with nontransgenic corn during 2 yr of field evaluations. However, Gullu et al. (2004) found that the predator *C. carnea* was significantly more abundant in *Bt* corn plots than in non-*Bt* hybrids. In cotton, Naranjo (2005) reported recently that *Bt* cotton essentially had no effects on the function of natural enemies and that the minor reductions in densities of several predator taxa in *Bt* cotton might have little ecological meaning relative to the natural enemy impact on key pests. In the rice system, *Bt* rice has no significant effects on the population dynamics of dominant spider species or the community of arthropod predators (Liu et al. 2002, 2003). However, according to Schoenly et al. (2003), planting *Bt* rice seemed to alter species richness of predators.

In conclusion, our results indicate that the nontarget insect *N. lugens* and its predator *P. japonica* are exposed to Cry1Ab toxin from transgenic *cry1Ab* rice, but development of this predator is not affected by the toxin through tritrophic interactions.

Acknowledgments

We thank Q. Y. Shu (Institute of Nuclear Agricultural Sciences, Zhejiang University) for revising the manuscript and providing rice seeds; I. Altosaar and M. A. Zaidi (Department of Biochemistry, Microbiology & Immunology, Ottawa University) for reviewing the manuscript and contributing helpful information to this article; and H. M. Shen (College of Plant Protection, Nanjing Agricultural University), X. J. Gu, and L. S. Xia (Department of Plant Protection, Zhejiang University) for collecting data. We also thank J. J. Wang (College of Plant Protection, Southwest University) for revising the manuscript. This study was financially supported by the National Science Foundation of China under Agreement No. 30070500 awarded to J. A. Cheng.

References Cited

Al-Deeb, M. A., G. E. Wilde, and R. A. Higgins. 2001. No effect of *Bacillus thuringiensis* corn and *Bacillus thuringiensis* on the predator *Orius insidiosus* (Hemiptera: Anthocoridae). Environ. Entomol. 30: 625–629.

Bai, Y. Y., M. X. Jiang, and J. A. Cheng. 2005. Effects of transgenic *cry1Ab* rice pollen on fitness of *Propylea japonica* (Thunberg). J. Pest Sci. 78: 123–128.

Bernal, C. C., R. M. Aguda, and M. B. Cohen. 2002. Effect of rice lines transformed with *Bacillus thuringiensis* toxin genes on the brown planthopper and its predator *Cyrtorhinus lividipennis.* Entomol. Exp. Appl. 102: 21–28.

Chen, M., G. Y. Ye, X. M. Liu, C. Hu, Y. F., Peng, Q. Y. Shu, and I. Altosaar. 2005. Biotransfer and bioaccumulation of Cry1Ab insecticidal protein in rice plant-brown planthopper-wolf spider food chain. Entomol. Sin. 48: 208–213.

Cheng, J. A. 1996. Rice insect pests. Agricultural Press, Beijing, China.

Cheng, X. Y., R. Sardana, H. Kaplan, and I. Altosaar. 1998. *Agrobacterium*-transformed rice plants expressing synthetic *cry1A(b)* and *cry1A(c)* genes are highly toxic to striped stem borer and yellow stem borer. Proc. Natl. Acad. Sci. U.S.A. 95: 2767–2772.

Cui, J. J., and J. Y. Xia. 1999. Effects of transgenic *Bt* cotton on the population dynamic of natural enemies. Acta Gossypii Sin. 11: 84–91.

Daly, T., and G. D. Buntin. 2005. Effect of *Bacillus thuringiensis* transgenic corn for lepidopteran control on nontarget arthropods. Environ. Entomol. 34: 1292–1301.

Dutton, A., H. Klein, J. Romeis, and F. Bigler. 2002. Uptake of *Bt*-toxin by herbivores on transgenic maize and consequences for the predator *Chrysoperla carnea.* Ecol. Entomol. 27: 441–447.

Dutton, A., H. Klein, J. Romeis, and F. Bigler. 2003a. Prey-mediated effects of *Bacillus thuringiensis* spray on the predator *Chrysoperla carnea* in maize. Biol. Control. 26: 209–215.

Dutton, A., J. Romeis, and F. Bigler. 2003b. Assessing the risks of insect resistant transgenic plants on entomophagous arthropods: *Bt*-maize expressing Cry1Ab as a case study. BioControl. 48: 611–636.

Fearing, P. L., D. Brown, D. Vlachos, M. Meghji, and L. Privalle. 1997. Quantitative analysis of CryI(A)b expression in *Bt* maize plants, tissue, and silage and stability of expression over successive generations. Mol. Breed. 3: 169–176.

Gullu, M., F. Tatli, A. D. Kanat, and M. Islamoglu. 2004. Population development of some predatory insects on *Bt* and non-*Bt* maize hybrids in Turkey. Bull. OILB/SROP. 27: 85–91.

Guo, J. Y., and F. H. Wan. 2001. Effect of three diets on development and fecundity of the ladybeetles *Harmonia axyridis* and *Propylaea japonica.* Chin. J. Biol. Control. 17: 116–120.

Head, G., C. R. Brown, M. E. Groth, and J. J. Duan. 2001. Cry1Ab protein levels in phytophagous insects feeding on transgenic corn: implications for secondary exposure risk assessment. Entomol. Exp. Appl. 99: 37–45.

High, S. M., M. B. Cohen, Q. Y. Shu, and I. Altosaar. 2004. Achieving successful deployment of *Bt* rice. Trends Plant Sci. 9: 286–292.

Hilbeck, A., M. Baumgartner, P. M. Fried, and F. Bigler. 1998a. Effects of transgenic *Bacillus thuringiensis* corn-fed prey on mortality and development time of immature *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 27: 480–487.

Hilbeck, A., W. J. Moar, M. Pusztai-Carey, A. Filippini, and F. Bigler. 1998b. Toxicity of *Bacillus thuringiensis* Cry1Ab toxin to the pradator *Chrysoperla carnea* (Neuroptera: Chrysopidae). Environ. Entomol. 27: 1255–1263.

Hilbeck, A., W. J. Moar, M. Pusztai-Carey, A. Filippini, and F. Bigler. 1999. Prey-mediated effects of Cry1Ab toxin and protoxin and Cry2A protoxin on the predator *Chrysoperla carnea.* Entomol. Exp. Appl. 91: 305–316.

Jiang, Y. H., Q. Fu, J. A. Cheng, Z. R. Zhu, M. X. Jiang, G. Y. Ye, and Z. T. Zhang. 2004. Dynamics of Cry1Ab protein from transgenic *Bt* rice in herbivores and their predators. Acta Entomol. Sin. 47: 454–460.

Knowles, B. H. 1994. Mechanisms of action of *Bacillus thuringiensis* insecticidal δ-endotoxins. Adv. Insect Physiol. 24: 275–308.

Li, K. S., X. D. Chen, and H. Z. Wang. 1992. New discovery of feeding habitats of some ladybirds. Shaanxi Forest Sci. Tech. 2: 84–86.

Liu, Z. C., G. Y. Ye, C. Hu, and S. K. Datta. 2002. Effects of *Bt* transgenic rice on population dynamics of main nontarget insect pests and dominant spider species in rice paddies. Acta Phytophyl. Sin. 29: 138–144.

Liu, Z. C., G. Y. Ye, C. Hu, and S. K. Datta. 2003. Impact of transgenic indica rice with a fused gene of *cry1Ab/cry1Ac* on the rice paddy arthropod community. Acta Entomol. Sin. 46: 454–465.

Lundgren, J. G., and R. N. Wiedenmann. 2002. Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a non-target species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). Environ. Entomol. 31: 1213–1218.

Lundgren, J. G., and R. N. Wiedenmann. 2005. Tritrophic interactions among *Bt* (Cry3Bb1) corn, aphid prey, and the predator *Coleomegilla maculate* (Coleoptera: Coccinellidae). Environ. Entomol. 34: 1621–1625.

Musser, F. R., and A. M. Shelton. 2003. *Bt* sweet corn and selective insecticides: impacts on pests and predators. J. Econ. Entomol. 96: 71–80.

Naranjo, S. E. 2005. Long-term assessment of the effects of transgenic *Bt* cotton on the function of the natural enemy community. Environ. Entomol. 34: 1211–1223.

Pilcher, C. D., J. J. Obrycki, M. E. Rice, and L. C. Lewis. 1997. Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environ. Entomol. 26: 446–454.

Pilcher, C. D., M. E. Rice, and J. J. Obrycki. 2005. Impact of transgenic *Bacillus thuringiensis* corn and crop phenology on five non-target arthropods. Environ. Entomol. 34: 1302–1316.

Ponsard, S., A. P. Gutierrez, and N. J. Mills. 2002. Effect of *Bt*-toxin (Cry1Ac) in transgenic cotton on the adult longevity of four heteropteran predators. Environ. Entomol. 31: 1197–1205.

Rodrigo-Simón, A., R. A. de Maagd, C. Avilla, P. L. Bakker, J. Molthoff, J. E. González-Zamora, and J. Ferré. 2006. Lack of detrimental effects of *Bacillus thuringiensis* Cry toxins on the insect predator *Chrysoperla carnea:* a toxicological, histopathological, and biochemical analysis. Appl. Environ. Microbiol. 72: 1595–1603.

Romeis, J., A. Dutton, and F. Bigler. 2004. *Bacillus thuringiensis* toxin (Cry1Ab) has no direct effect on larvae of the green lacewing *Chrysoptera carnea* (Stephens) (Neuroptera: Chrysopidae). J. Insect Physiol. 50: 175–183.

Romeis, J., M. Meissle, and F. Bigler. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nat. Biotech. 24: 63–71.

Schoenly, K. G., M. B. Cohen, A. T. Barrion, W. J. Zhang, B. Gaolach, and V. D. Viajante. 2003. Effects of *Bacillus thuringiensis* on non-target herbivore and natural enemy assemblages in tropical irrigated rice. Environ. Biosaf. Res. 2: 181–206.

Schuler, T. H., G. M. Poppy, B. R. Kerry, and L. Denholm. 1999. Potential side effects of insect-resistant transgenic plants on arthropod natural enemies. TIBTECH. 17: 210–216.

Shu, Q. Y., G. Y. Ye, H. R. Cui, X. Y. Cheng, Y. B. Xiang, D. X. Wu, M. W. Gao, Y. W. Xia, C. Hu, R. Sardana, and I. Altosaar. 2000. Transgenic rice plants with a synthetic *cry1Ab* gene from *Bacillus thuringiensis* were highly resistant to eight lepidopteran rice pest species. Mol. Breed. 6: 433–439.

Shu, Q. Y., G. Y. Ye, H. R. Cui, Y. B. Xiang, and M. W. Gao. 1998. Development of transgenic *Bacillus thuringiensis* rice resistant to rice stem borers and leaf folders. J. Zhejiang. Agri. Univ. 24: 579–580.

Smith, S. F., and V. A. Krischik. 1999. Effects of systemic imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae). Environ. Entomol. 28: 1189–1195.

SPSS. 2002. SPSS 11.5 for windows. SPSS, Chicago, IL.

Wu, G., H. R. Cui, Q. Y. Shu, G. Y. Ye, X. B. Xie, Y. W. Xia, and M. W. Gao. 2001. Expression patterns of *cry1Ab* gene in progenies of "Kemingdao" and the resistance to striped stem borer. Sci. Agric. Sin. 34: 496–501.

Xie, X. B., and Q. Y. Shu. 2001. Studies on rapid quantitative analysis of *Bt* toxin by using Envirologix kits in transgenic rice. Sci. Agric. Sin. 34: 465–468.

Ye, G. Y., Q. Y. Shu, H. W. Yao, H. R. Cui, X. Y. Cheng, C. Hu, Y. W. Xia, M. W. Gao, and I. Altosaar. 2001. Field evaluation of resistance of transgenic rice containing a synthetic *cry1Ab* gene from *Bacillus thuringiensis* Berliner to two stem borers. J. Econ. Entomol. 94: 271–276.

Ye, G. Y., H. W. Yao, Q. Y. Shu, X. Cheng, C. Hu, Y. W. Xia, M. W. Gao, and I. Altosaar. 2003. High levels of stable resistance in transgenic rice with a *cry1Ab* gene from *Bacillus thuringiensis* Berliner to rice leaffolder, *Cnaphalocrocis medinalis* (Guenee) under field conditions. Crop Prot. 22: 171–178.

Zhang, G. F., F. H. Wan, G. L. Lövei, W. X. Liu, and J. Y. Guo. 2006. Transmission of *Bt* toxin to the predator *Propylaea japonica* (Coleoptera: Coccinellidae) through its aphid prey feeding on transgenic *Bt* cotton. Environ. Entomol. 35: 143–150.

Zwahlen, C., W. Nentwig, F. Bigler, and A. Hilbeck. 2000. Tritrophic interactions of transgenic *Bacillus thuringiensis* corn, *Anaphothrips obscurus* (Thysanoptera: Thripidae), and the predator *Orius majusculus* (Heteroptera: Anthocoridae). Environ. Entomol. 29: 846–850.

Received for publication 31 December 2005; accepted 12 May 2006.