ORIGINAL ARTICLE

Y. Y. Bai · M. X. Jiang · J. A. Cheng

Effects of transgenic *cry1Ab* rice pollen on fitness of *Propylea japonica* (Thunberg)

Abstract The transgenic rice lines Kemingdao 1 (KMD1) and Kemingdao 2 (KMD2) contain a synthetic *cry1Ab* gene derived from *Bacillus thuringiensis* Berliner and are highly resistant to rice stem borers and foliage-feeding lepidopterans. *Propylea japonica* (Coleoptera: Coccinellidae) is an important predator of rice insect pests; it also uses rice pollen as a food source under natural conditions. In the present study, the effects of KMD1 and KMD2 pollen expressing Cry1Ab protein on the fitness of *P. japonica* were assessed in the laboratory. *P. japonica* larvae and adults were provided with the following four diets: KMD1 pollen with the aphid *Myzus persicae*, KMD2 pollen with *M. persicae*, nontransgenic Xiushui 11 (parent variety of KMD1 and KMD2) pollen with *M. persicae*, and *M. persicae* only (KMD1–pollen, KMD2–pollen, XS11–pollen, and aphid treatments, respectively). The results showed that the longevity of female adults in the KMD1–pollen treatment was significantly lower than that in the XS11–pollen treatment, but was not significantly different from that in the KMD2–pollen and aphid treatments. Newly emerged males in the KMD2–pollen treatment were evidently less vital than those in the XS11–pollen treatment, but not significantly different from those in the KMD1–pollen and aphid treatments. The development, survival and reproduction indices for the three pollen treatments did not differ significantly from one another. In short, *Bt* toxin expressed in *Bt* rice pollen had no evident negative impacts on *P. japonica* fitness when the pollen was used as a food by this beetle.

Y. Y. Bai · M. X. Jiang · J. A. Cheng (✉)
Institute of Applied Entomology, Zhejiang University,
310029 Hangzhou, P.R. China
E-mail: jacheng@zju.edu.cn
Tel.: +86-571-86971212
Fax: +86-571-86971212

Y. Y. Bai
Plant Protection College, Southwest Agricultural University,
400716 Chongqing, P.R. China

Introduction

The striped stem borer (SSB), *Chilo suppressalis* (Walker) (Lepidoptera: Pyralidae), and the yellow stem borer (YSB), *Scirpophaga incertulas* (Walker), are two of the most serious pests of rice in temperate and subtropical Asia (Dale 1994). Over the past decade, several synthetic genes including *cry1Ab*, *cry1Ac* and *cry1B* derived from *Bacillus thuringiensis* Berliner (*Bt*) have been transferred into *indica* or *japonica* rice to confer resistance to SSB and YSB (Fujimoto et al. 1993; Wünn et al. 1996; Ghareyazie et al. 1997; Nayak et al. 1997; Wu et al. 1997; Cheng et al. 1998; Datta et al. 1998; Alam et al. 1999; Breitler et al. 2000; Khanna and Raina 2002). In China, the obtained transgenic *Bt* rice lines have been proven to be highly resistant to rice stem borers (including SSB and YSB) and foliage-feeding lepidopterans under field conditions (Shu et al. 2000; Ye et al. 2001).

Adverse effects of Cry1Ab-expressing *Bt* plants on arthropod predators may arise as a result of several factors, including high concentration of *Bt* protein in pollen (a food source for predators), modifications of the introduced *cry1Ab* gene, and expression of *Bt* protein throughout the plant (Jepson et al. 1994; Pilcher et al. 1997). Thus, potential risk exists for *Bt* plants to adversely affect the fitness of predators. In the case of *Bt* rice, Bernal et al. (2002) reported that both the mirid predator *Cyrtorhinus lividipennis* Reuter and its prey, the brown planthopper *Nilaparvata lugens* Stål, could be exposed to *Bt* toxins from *Bt* rice, but this exposure might not affect the fitness of *C. lividipennis*. Laboratory observation has indicated that *Bt* rice has no marked effect on the predation capacity of the wolf spider *Pirata subpiraticus* (Liu et al. 2003a). In rice fields, *Bt* rice also has no significant effect on the population dynamics of

dominant spider species, or the community of arthropod predators (Liu et al. 2002, 2003b). According to Schoenly et al. (2003), however, planting *Bt* rice appeared to alter the species richness of predators.

Predaceous coccinellids play important roles in natural control of pest insects (Obrycki and Kring 1998). Previous studies demonstrated that *Bt* crop pollen expressing *Bt* proteins, such as Cry1Ab, Cry3A and Cry3Bb1, poses little risk to this group of predators (Pilcher et al. 1997; Riddick and Barbosa 1998; Duan et al. 2002). However, to clarify conclusively the impact of *Bt* plant pollen on predaceous coccinellids, more extensive experiments are needed (Schoenly et al. 2003).

Propylea japonica (Thunberg) (Coleoptera: Coccinellidae) is a very common and abundant predator in many crop systems throughout China including rice (Yang 1983; Ma and Liu 1985; Zou et al. 1986; Zhou and Xiang 1987; Cheng 1996). Both larval and adult *P. japonica* are predaceous, feeding predominantly on aphids (Song et al. 1988). When insect prey is scarce, they also use plant pollen as a supplemental food source (Li et al. 1992). In rice fields, *P. japonica* preys on the aphid *Macrosiphum avenae* (F.), young larvae of the rice leaffolder *Cnaphalocrocis medinalis* Guenée, young nymphs of *N. lugens*, and others (Song et al. 1988); it can also feed on rice pollen during both larval and adult stages (personal observation). In this study, development, survival, and reproduction of *P. japonica* feeding on *Bt* rice pollen were observed. The objectives were to determine effects of *Bt* rice pollen on the fitness of this beetle, and subsequently to broaden our knowledge about the ecological risk of transgenic *Bt* plants pending commercial release.

Materials and methods

Propylea japonica

Propylea japonica adults were collected from rice plants during the 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 the green peach aphid, *Myzus persicae* (Sulzer), and maintained in a climatically controlled chamber with photoperiod 16:8 h (L:D), $28 \pm 1°C$, and 60–80% RH. *M. persicae* was supplied daily to the tubes. As egg deposition occurred, adults were transferred to other clear glass tubes, and the tubes bearing eggs were maintained until egg hatch. The newly hatched larvae were subjected to experiments. A water-saturated cotton ball was placed in each glass tube to maintain moisture. The *M. persicae* had been raised on *Brassica oleracea* var. *capitata* L. in the climatically controlled chamber.

Rice pollen

Pollen was obtained from field-grown plants of the *Bt* rice lines Kemingdao 1 (KMD1) and Kemingdao 2 (KMD2), and from plants of the nontransgenic line, *japonica* rice cv. Xiushui 11 (XS11, parent variety of KMD1 and KMD2). Both KMD1 and KMD2 were homozygous containing a synthetic *cry1Ab* gene derived from *B. thuringiensis*. They were transformed by *Agrobacterium* infection (Cheng et al. 1998; Shu et al. 1998). Expression of the *cry1Ab* gene is driven by maize ubiquitin promoter (Cheng et al. 1998).

One day before anthesis, inflorescences were cut from plants. In the laboratory, anthers enclosed within glume were collected, lyophilized, ground, and sifted through a 1-mm sieve. The obtained anther powder (containing pollens) was placed in sealed plastic vials and kept at $-20°C$ until use. The concentration of Cry1Ab toxin in anther powder was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Envirologix).

Effects of *Bt* rice pollen on preimaginal fitness of *P. japonica*

Propylea japonica could not complete larval development solely on rice pollen (personal observation). Thus *M. persicae*, an optimal food source of *P. japonica* (Guo and Wan 2001), was supplemented in the three pollen treatments. The experimental treatments were KMD1 pollen with aphid, KMD2 pollen with aphid, and XS11 pollen with aphid (described hereafter as KMD1–pollen, KMD2–pollen, and XS11–pollen treatments, respectively). A control diet of only *M. persicae* (described hereafter as aphid treatment) was used to verify that experimental conditions used in this study were suitable for *P. japonica*. A total of 200 newly hatched *P. japonica* larvae from 20 female sources were used, with 50 larvae being subjected to each treatment. In addition, to determine average percentage of larvae that pupated and percentage of pupae that eclosed, the larvae in each treatment were divided into three subgroups.

Larvae were reared individually in glass tubes (1.4 cm diameter, 10 cm long). In the aphid treatment, 10, 20, 40 and 60 *M. persicae* adults per day were provided to each larva during the first, second, third and fourth instars, respectively. In the pollen treatments, each larva was given 20 anthers together with 5, 10, 20 and 30 *M. persicae* adults per day during the first, second, third and fourth instars, respectively. To increase the probability that *P. japonica* would feed on pollen, aphids were not supplied during the first 12 h of each instar.

The experiment was conducted in the climatically controlled chamber described earlier. Water was provided to larvae by placing a water-saturated cotton ball in each tube. Anthers in tubes were refreshed twice daily. Larvae were checked every 3 h for ecdysis and mortality; development time and survival rate of each preimaginal stage were determined. Weight of pupae and adults was measured approximately 12 h after pupation and within 6 h after emergence, respectively.

Effects of *Bt* rice pollen on adult fitness of *P. japonica*

For each treatment, adults (only those obtained around emergence peak) were transferred to a Petri dish (10.0 cm diameter, 2.6 cm tall) after being weighed, and were given the same diet as during the fourth-instar stage. After 3 days, adults were paired and placed in glass tubes (1.4 cm diameter, 10 cm long) containing sufficient *M. persicae* but no rice pollens. Thereafter, oviposition and adult survival were monitored every 3 h. When egg clutches were observed, the *P. japonica* adults as well as the residual aphids were transferred to other clear glass tubes, and the number of egg clutches and number of eggs in each clutch were recorded. To determine hatch rates, 28–40 egg clutches in each treatment that were deposited around oviposition peak were gathered, divided randomly into three groups, and placed individually in glass tubes (1.4 cm diameter, 10 cm long) containing a wet cotton ball. The number of total and hatched eggs in each group of egg clutches was recorded. Adult longevity was also measured.

Flip time has been used to indicate adult fitness of coccinellids in earlier studies (Smith and Krischik 1999; Lundgren and Wiedenmann 2002). Such an indicator was also used in the present study. To obtain sufficient male adults for the test, another group of *P. japonica* was raised under each diet treatment described above. At 6 h after emergence, males were placed on their dorsa on a piece of filter paper (90 mm diameter), and the time until the beetle flipped over was measured with a stopwatch to the nearest 1 s. Timing was stopped at 60 s for beetles that did not flip. Flip time was divided into six grades, 1–5, 6–10, 11–15, 16–20, 21–25 and 26–60 s. Percentage of adults in each flip-time grade and average flip time of the adults flipping within 15 s (which accounted for 79.4–86.2% of the individuals observed) were determined.

Verification of pollen ingestion by *P. japonica* larvae and adults

For each pollen treatment, feces of *P. japonica* larvae during each instar stage and of adults within 3 days of emergence were treated with I_2–KI solution and then observed under a binocular microscope. If there were stained pollens present, it would prove that the pollen used in our experiments had been ingested by the insect.

Data analysis

Development, survival and reproduction data were analyzed using analysis of variance (ANOVA) (SPSS 2002). The χ^2 test was conducted to evaluate the relationship between adult flip time and diet treatment at the level $P = 0.05$. Before being subjected to ANOVA, data in percentage were transformed using arcsine square root, and the flip-time data of adults flipping within 15 s was transformed using square root. Means were compared among treatments using the Tukey HSD test at the level $P = 0.05$.

Results

Using ELISA, the concentration of *B. thuringiensis* protein (Cry1Ab) in the anthers (containing pollens) of transgenic rice lines KMD1 and KMD2 was determined as 13.1 ± 2.6 and 31.4 ± 4.3 µg g^{-1} lyophilized anther powder, respectively. In the pollen treatments, pollens were found in the feces of *P. japonica* at each feeding stage (i.e., during each larval instar and within 3 days after emergence), suggesting that pollen had been ingested by the insects.

ANOVA indicated that the type of diet (KMD1–pollen with aphid, KMD2–pollen with aphid, XS11–pollen with aphid, and aphids only) fed to *P. japonica* had significant effects on the development time of second, third, and fourth instar larval stages and on the entire larval stage, but not on the development time of first instar larvae, pre-pupae, or pupae, or on the percentage of larvae that pupated, percentage of pupae that eclosed, pupal or adult weight (Table 1). As compared with the aphid treatment, significantly prolonged development was observed during the second instar in the KMD1–pollen and KMD2–pollen treatments, during the third instar in the KMD1–pollen treatment, during the fourth instar in the KMD2–pollen and XS11–pollen treatments, and during the entire larval stage in each pollen treatment ($P < 0.05$). The development time of the third and fourth instars in the KMD1–pollen treatment was significantly longer than that in the XS11–pollen treatment ($P < 0.05$). However, when comparing the development time of the entire larval stage among the three pollen treatments, no significant difference was found ($P > 0.05$) (Table 1).

The type of diet had significant effects on female longevity, but not on preovipositional period, ovipositional period, number of eggs per egg clutch, fecundity, male longevity, or egg hatch rate (Table 2). Female longevity in the KMD1–pollen treatment was significantly shorter than that in the XS11–pollen treatment ($P < 0.05$), but not than that in the aphid treatment ($P > 0.05$). The χ^2 test showed that flip time of young (6-h-old) male adults (Fig. 1a) was not significantly related to the type of diet that had been fed during the larval stage (df = 15, $\chi^2 = 20.60 < \chi^2_{0.05, 15}$). For adults flipping within 15 s, flip time of the individuals given KMD1–pollen or KMD2–pollen did not differ significantly from that of the individuals given XS11–pollen, or from that of the individuals given aphids only ($P > 0.05$) (Fig. 1b).

Discussion

The Cry1Ab protein expressed in *Bt* rice is lepidopteran-specific with high activity against *C. suppressalis*,

Table 1 Fitness of the preimaginal coccinellid *Propylea japonica* fed different diets during larval stages

Fitness indicators	Diet[a]				ANOVA		
	KMD1 pollen with aphid	KMD2 pollen with aphid	XS11 pollen with aphid	Aphids only	df	F	p
Developmental time (days)							
First-instar larva	2.3 ± 0.1 (46)	2.1 ± 0.2 (43)	2.3 ± 0.1 (44)	2.1 ± 0.1 (46)	3	2.54	0.058
Second-instar larva	1.4 ± 0.1 (46) a	1.4 ± 0.1 (42) a	1.3 ± 0.1 (43) ab	1.2 ± 0.0 (45) b	3	3.27	0.025
Third-instar larva	1.5 ± 0.1 (46) a	1.4 ± 0.1 (40) ab	1.3 ± 0.1 (42) b	1.3 ± 0.0 (43) b	3	3.05	0.033
Fourth-instar larva	2.1 ± 0.1 (46) bc	2.3 ± 0.1 (36) ab	2.4 ± 0.1 (42) a	2.0 ± 0.1 (43) c	3	3.68	0.015
Entire larval stage	7.5 ± 0.1 (46) a	7.3 ± 0.2 (36) a	7.4 ± 0.1 (42) a	6.6 ± 0.11 (42) b	3	6.04	< 0.01
Pre-pupa	0.6 ± 0.0 (46)	0.6 ± 0.0 (36)	0.6 ± 0.0 (42)	0.6 ± 0.0 (42)	3	1.25	0.29
Pupa	2.4 ± 0.0 (41)	2.4 ± 0.0 (35)	2.5 ± 0.0 (40)	2.5 ± 0.1 (41)	3	0.42	0.74
Larvae that pupated (%)	81.0 ± 1.5 (50)	71.5 ± 2.1 (50)	79.0 ± 2.3 (50)	82.0 ± 2.1 (50)	3	3.08	0.057
Pupae that eclosed (%)	100.0 ± 0.0 (41)	100.0 ± 0.0 (35)	100.0 ± 0.0 (40)	100.0 ± 0.0 (41)	3	0.26	0.85
Pupal weight (mg)	5.8 ± 0.3 (36)	5.8 ± 0.2 (32)	5.8 ± 0.2 (36)	5.6 ± 0.2 (40)	3	0.15	0.93
Adult weight (mg)	4.6 ± 0.2 (36)	4.7 ± 0.2 (32)	4.8 ± 0.1 (36)	4.8 ± 0.1 (40)	3	0.15	0.93

In a row, data (mean ± SE) followed by the *same letter* are not significantly different from each other, Tukey HSD test, P > 0.05. *Values in parentheses* indicate the number of samples tested

[a]KMD1 and KMD2 were transgenic *Bt* rice lines expressing Cry1Ab toxin in pollen; XS11 was a nontransgenic rice line (parent variety of KMD1 and KMD2). The species of aphids was *Myzus persicae*

S. incertulas and many other lepidopteran pests (Shu et al. 2000; Wu et al. 2001; Ye et al. 2001). Although this would suggest that *Bt* rice would not have any negative effect on predators, suitability of rice pollen as a food source for predators may have been altered due to the introduction of *cry1Ab* gene. Such a potential effect requires proper assessment of the ecological risks posed by *Bt* rice, by carrying out a combination of laboratory tests, field experiments and longer-term monitoring, as suggested by Jepson et al. (1994). As a case study, our observations indicated that *Bt* rice pollen has no adverse effect on *P. japonica* when used as a supplemental food. This result agrees with the studies of Pilcher et al. (1997) and Sims (1995), who found that feeding *Bt* corn pollen that expresses Cry1Ab or feeding a diet of Cry1Ac protein (also lepidopteran-specific) at 20 μg/ml (> 100 times the amount of Cry1Ac protein present in pollen and nectar of transgenic cotton) posed little risk to the

coccinellids *Coleomegilla maculata lengi* Timberlake, and *Hippodamia convergens* Guerin.

The aphid *M. persicae* is a suitable prey for *P. japonica* (Wei and Ran 1983), but the amount of this aphid consumed by *P. japonica* during larval and adult stages is not clear. According to Song et al. (1988), when provided with suitable aphid prey, such as *Aphis citricidus* (Kirkaldy), *A. gossypii* Glover, *Macrosiphum avenae* (Fabricius), and *A. glycines* Matsumura, a *P. japonica* larva can consume on average 10, 20, 36 and 47 aphids per day during first, second, third and fourth instar, respectively. In the present study, nearly all of the aphids provided were consumed in the aphids-only treatment, when as many as 10, 20, 40 and 60 aphids per day were provided during the four respective *P. japonica* instars (personal observation). Overall, such an aphid-consumption capacity is in accordance with that reported by Song et al. (1988). Based on this, we speculated that in

Table 2 Fitness of the adult *P. japonica* fed different diets during larval and early adult (3 days after emergence) stages

Fitness indicators	Diet[a]				ANOVA		
	KMD1 pollen with aphid	KMD2 pollen with aphid	XS11 pollen with aphid	Aphids only	df	F	p
Preoviposit. period (days)	12.1 ± 0.5 (12)	13.4 ± 0.4 (13)	13.1 ± 0.4 (13)	13.3 ± 0.3 (15)	3	0.30	0.83
Oviposit. period (days)	31.8 ± 3.5 (11)	33.4 ± 2.5 (13)	41.4 ± 2.3 (13)	36.33 ± 2.5 (15)	3	1.43	0.25
Eggs/egg clutch	4.5 ± 0.4 (69)	4.8 ± 0.3 (67)	4.2 ± 0.2 (104)	4.2 ± 0.2 (81)	3	1.24	0.30
Fecundity (eggs/female)	308.8 ± 45.2 (11)	321.8 ± 19.4 (13)	436.3 ± 30.8 (13)	338.6 ± 36.0 (15)	3	1.75	0.18
Female longevity (days)	42.2 ± 3.2 (11) b	44.50 ± 2.8 (13) ab	53.9 ± 2.7 (13) a	49.2 ± 2.1 (15) ab	3	2.13	0.012
Male longevity (days)	40.0 ± 0.8 (6)	38. 7 ± 1.2 (7)	47.4 ± 3.0 (9)	43.0 ± 4.2 (7)	3	0.73	0.55
Egg hatch rate (%)	84.7 ± 5.3 (381)	77.3 ± 5.7 (371)	85.0 ± 4.3 (357)	69.7 ± 4.6 (293)	3	2.10	0.13

In a row, data (mean ± SE) followed by the *same letter* are not significantly different from each other, Tukey HSD test, P > 0.05. *Values in parentheses* indicate the number of samples tested

[a]KMD1 and KMD2 were transgenic *Bt* rice lines expressing Cry1Ab toxin in pollen; XS11 was a nontransgenic rice line (parent variety of KMD1 and KMD2). The species of aphids was *Myzus persicae*
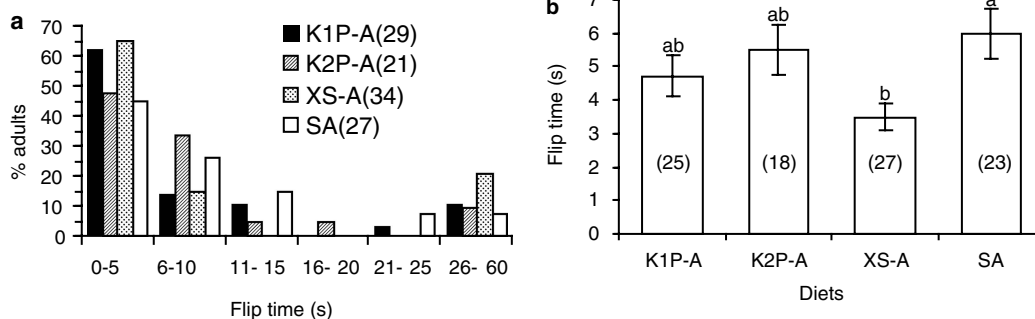
Fig. 1a, b Flip time of male *P. japonica* adults (6 h old, taking no food after emergence) that emerged from individuals fed different diets during the larval stage. *K1P-A* KMD1 pollen with the aphid *M. persicae*, *K2P-A* KMD2 pollen with aphid, *XS-A* XS11 with aphid, *SA* aphids only. **a** Percentage of adults in different flip-time grades. **b** Flip time (mean ± SE) of adults flipping within 15 s. *Bars with the same letter are not significantly different from each other,* Tukey HSD test, $P > 0.05$. *Values in parentheses* indicate the number of adults observed

the aphid + pollen treatments, during which only 5, 10, 20 and 30 aphids per day were provided during the four respective *P. japonica* instars, pollen had been used as food source for normal development. In addition, the presence of pollen granules in larval feces verifies the ingestion of pollen. Methodology should be developed to quantify rice pollen consumed by *P. japonica*, although such a trial appears to be difficult.

When provided with *A. gossypii*, *M. avenae* or *A. glycines*, a *P. japonica* adult can predate over 80 aphids during each of the 3 days after emergence; when receiving no food after emergence, adults die within 2 days (Lu et al. 1983; Yang 1983). In the present study, to determine the effect of pollen on *P. japonica* adult fitness, we provided only 30 aphids (together with pollen) per day to *P. japonica* adults during the 3 days after emergence. This suggests that the amount of *Bt* pollen ingested by young *P. japonica* adults, which was not measured, was probably great, as normal reproductive development occurred. The occurrence of normal reproductive development also supported our belief that the consumption of transgenic KMD1 and KMD2 rice pollen by larval or adult *P. japonica* has little adverse impact on fitness.

The lengthened development time of *P. japonica* larvae in pollen treatments compared to the aphids-only treatment could have been the result of insufficient nutrition. *M. persicae* has been reported as an optimal diet of *P. japonica* (Guo and Wan 2001), but apparently both *Bt* and non-*Bt* rice pollen are less suitable than *M. persicae* as a diet of *P. japonica* larvae. On the other hand, the *Bt* rice (KMD1 or KMD2) pollen, although posing no evident adverse effects on *P. japonica* fitness overall, appeared to be less suitable than the non-*Bt* rice pollen, as indicated by the increased time for young male adults to flip and the decreased female longevity in the KMD2–pollen and KMD1–pollen treatments, respectively.

Other studies have shown that lepidopteran-specific *Bt* crops could have significant effects on *P. japonica* through other ecological pathways than pollen. For example, larvae of the cotton bollworm, *Helicoverpa armigera* (Hübner) feeding on leaves of Cry1Ab-expressing *Bt* cotton had a significantly lower body weight than those feeding on leaves of normal cotton, and thus were predated in reater numbers by *P. japonica* (the study did not examine whether the mean mass of prey consumed differed significantly between the two treatments) (Cui and Xia 1999). Potential effects of *Bt* rice on *P. japonica* via trophic interactions other than consumption of pollen are to be evaluated in the future.

References

Alam MF, Datta K, Abrigo E, Oliva N, Tu J, Virmani SS, Datta SK (1999) Transgenic insect-resistant maintainer line (IR68899B) for improvement of hybrid rice. Plant Cell Rep 18:572–575

Bernal CC, Aguda RM, Cohen MB (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

Breitler JC, Marfa V, Royer M, Meynard D, Vassal JM, Vercambre B, Frutos R, Messeguer J, Gabarra R, Guiderdoni E (2000) Expression of a *Bacillus thuringiensiscry1B* synthetic gene protects Mediterranean rice against the striped stem borer. Plant Cell Rep 19:1195–1202

Cheng JA (1996) Rice insect pests. China Agricultural Press, Beijing

Cheng XY, Sardana R, Kaplan H, Altosaar I (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 USA 95:2767–2772

Cui JJ, Xia JY (1999) Effect of transgenic *Bt* cotton on the population dynamics of natural enemies. Acta Gossypii Sinica 11:84–91

Dale D (1994) Insect pests of the rice plant—their biology and ecology: stem borers. In: Heinrichs EA (ed) Biology and management of rice insects. Wily Eastern, New Delhi, pp 388–408

Datta K, Vasquez A, Tu J, Torrizo L, Alam MF, Oliva N, Abrigo E, Khush GS, Datta SK (1998) Constitutive and tissue-specific differential expression of the *cry1A(b)* gene in transgenic rice plants conferring resistance to rice insect pest. Theor Appl Genet 97:20–30

Duan JJ, Head G, McKee MJ, Nickson TE, Martin JW, Sayegh FS (2002) Evaluation of dietary effects of transgenic corn pollen expressing Cry3Bb1 protein on a non-target ladybird beetle, *Coleomegilla maculata*. Entomol Exp Appl 104:271–280

Fujimoto H, Itoh K, Yamamoto M, Kyozuka J, Shimamoto K (1993) Insect resistant rice generated by introduction of a modified delta-endotoxin gene of *Bacillus thuringiensis*. Biotechnology 11:1151–1155

Ghareyazie B, Alinia F, Menguito CA, Rubia LG, Palma JM de, Liwanag EA, Cohen MB, Khush GS, Bennett J (1997) Enhanced resistance to two stem borers in an aromatic rice containing a synthetic *cryIA(b)* gene. Mol Breeding 3:401–414

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

Jepson PC, Croft BA, Pratt GE (1994) Test systems to determine the ecological risks posed by toxin release from *Bacillus thuringiensis* genes in crop plants. Mol Ecol 3:81–89

Khanna HK, Raina SK (2002) Elite *indica* transgenic rice plants expressing modified Cry1Ac endotoxin of *Bacillus thuringiensis* show enhanced resistance to yellow stem borer (*Scirpophaga incertulas*). Transgenic Res 11:411–423

Li KS, Chen XD, Wang HZ (1992) New discovery of feeding habitats of some ladybirds. Shanxi Forest Sci Techn 2:84–86

Liu ZC, Ye GY, Hu C, Datta SK (2002) Effects of *Bt* transgenic rice on population dynamics of main non-target insect pests and dominant spider species in rice paddies. Acta Phytophylacica Sinica 29:138–144

Liu ZC, Ye GY, Fu Q, Zhang ZT, Hu C (2003a) Indirect impact assessment of transgenic rice with *cry1Ab* gene on predations by the wolf spider, *Pirata subpiraticus*. Chin J Rice Sci 17:175–178

Liu ZC, Ye GY, Hu C, Datta SK (2003b) Impact of transgenic *indica* rice with a fused gene of *cry1Ab/cry1Ac* on the rice paddy arthropod community. Acta Entomol Sin 46:454–465

Lu YX, Zhang WJ, Pei Q, Liao YZ (1983) On the biology and rearing of *Propylaea japonica* (Thunb.). Nat Enemies Insects 5(1):33–38

Lundgren JG, Wiedenmann RN (2002) Coleopteran-specific Cry3Bb toxin from transgenic corn pollen does not affect the fitness of a nontarget species, *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae). Env Entomol 31:1213–1218

Ma XY, Liu Z (1985) *Propylaea japonica* (Thumberg) and its seasonal fluctuation in cotton fields. Plant Protect 11(2):12–13

Nayak P, Basu D, Das S, Basu A, Ghosh D, Ramakrishnan NA, Ghosh M, Sen SK (1997) Transgenic elite *indica* rice plants expressing CryIAc delta-endotoxin of *Bacillus thuringiensis* are resistant against yellow stem borer (*Scirpophaga incertulas*). Proc Natl Acad Sci USA 94:2111–2116

Obrycki JJ, Kring TJ (1998) Predaceous Coccinellidae in biological control. Annu Rev Entomol 43:295–321

Pilcher CD, Obrycki JJ, Rice ME, Lewis LC (1997) Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Env Entomol 26:446–454

Riddick EW, Barbosa P (1998) Impact of Cry3A-intoxicated *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) and pollen on consumption, development, and fecundity of *Coleomegilla maculata* (Coleoptera: Coccinellidae). Ann Entomol Soc Am 91:303–307

Schoenly KG, Cohen MB, Barrion AT, Zhang WJ, Gaolach B, Viajante VD (2003) Effects of *Bacillus thuringiensis* on non-target herbivore and natural enemy assemblages in tropical irrigated rice. Env Biosafety Res 2:181–206

Shu QY, Ye GY, Cui HR, Xiang YB (1998) Development of transgenic *Bacillus thuringiensis* rice resistant to rice stem borers and leaf folders. J Zhejiang Agric Univ 24:579–580

Shu QY, Ye QY, Cui HR, Cheng XY, Xiang YB, Wu DX, Gao MW, Xia YW, Hu C, Sardana R, Altosaar I (2000) Transgenic rice plants with a synthetic *cry1Ab* gene from *Bacillus thuringiensis* were highly resistant to eight lepidopteran rice pest species. Mol Breeding 6:433–439

Sims SR (1995) *Bacillus thuringiensis* var. *kurstaki* (CryIA (C)) protein expressed in transgenic cotton: effects on beneficial and other non-target insects. SW Entomol 20:493–500

Smith SF, Krischik VA (1999) Effects of systemic imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae). Env Entomol 28:1189–1195

Song HY, Wu LY, Chen GF, Wang ZC, Song QM (1988) Biological characters of lady-beetle, *Propylaea japonica* (Thunberg). Nat Enemies Insects 10(1):22–33

SPSS (2002) SPSS 11.5 for Windows. SPSS Inc.

Wei JH, Ran RB (1983) A study on *Propylaea japonica* (Thunb.) Nat Enemies Insects 5(2):89–93

Wu C, Fan Y, Zhang C, Oliva N, Datta SK (1997) Transgenic fertile japonica rice plants expressing a modified *cryIA(b)* gene resistant to yellow stem borer. Plant Cell Rep 17:129–132

Wu G, Cui HR, Shu QY, Ye GY, Xie XB, Xia YW, Gao MW, Altosaar I (2001) Expression patterns of *cry1Ab* gene in progenies of "Kemingdao" and the resistance to striped stem borer. Sci Agric Sin 34:496–501

Wünn JA, Kloti A, Burkhardt PK, Biswas GCG, Launis K, Iglesias VA, Potrykus I (1996) Transgenic *indica* rice breeding line IR58 expressing a synthetic *cryIA(b)* gene from *Bacillus thuringiensis* provides effective insect pest control. Biotechnology 14:171–176

Yang JH (1983) Preliminary observations on the habits of *Propylea japonica*. Insect Knowl 20:215–217

Ye GY, Shu QY, Yao HW, Cui HR, Cheng XY, Hu C, Xia YW, Gao MW, Altosaar I (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

Zhou KJ, Xiang JB (1987) Observations on the efficacy of spiders and ladybirds against aphids in the seedling stage of cotton in the cotton fields. Nat Enemies Insects 9(1):17–20

Zou, YD, Wang HF, Tao QZ, Liu DK, Yu W, Holling CS (1986) Studies on predation of *Propylaea japonica* (Thung) larvae on *Aphis gossypii* Glover. Insect Knowl 23:219–222