Laboratory and Field Assessments of Prey-Mediated Effects of Transgenic Bt Rice on *Ummeliata insecticeps* (Araneida: Linyphiidae)

J. C. TIAN,[1] Z. C. LIU,[2] M. CHEN,[3] Y. CHEN,[1] X. X. CHEN,[1] Y. F. PENG,[4] C. HU,[1] AND G. Y. YE[1,5]

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ABSTRACT One major concern regarding the release of Bt rice is its potential impact through tritrophic interactions on nontarget arthropods, especially natural enemies. We studied the effects of two Bt transgenic rice varieties, TT9-3 and KMD1, expressing Cry1Ab/Cry1Ac and Cry1Ab, respectively, on a predatory ground spider [*Ummeliata insecticeps* (Bösenberg et Strand)] supplied with Bt rice-fed brown planthopper [*Nilaparvata lugens* (Stål)] nymphs. Although immunoassays confirmed that *U. insecticeps* ingested Bt insecticidal protein when supplied with Bt rice–fed *N. lugens*, no negative effects were found on its survival and development. Furthermore, the fecundity of *U. insecticeps* fed prey reared on Bt rice was not significantly different from that of those fed prey reared on non-Bt rice. A 3-yr field trial indicated that Bt rice did not significantly affect the population density of *U. insecticeps* in comparison with non-Bt rice. In conclusion, the Bt rice lines tested in this study had no adverse effects on the survival, developmental time, or fecundity of *U. insecticeps* in the laboratory or on population dynamics in the field.

KEY WORDS *Bacillus thuringiensis*, Bt rice, nontarget effect, tritrophic interactions, *Ummeliata insecticeps*

The growing area of genetically modified crops expressing Cry proteins derived from the soil bacterium *Bacillus thuringiensis* Berliner (*Bt*) has risen rapidly since the first Bt crops were released commercially in the United States in 1996. Since then, Bt crops have been grown in several countries on a steadily increasing acreage, from 1.1 million ha in 1996 to 42 million ha in 2008 (James 2008). However, the effects of Bt crops on the environment and human health have been long debated. The potential deleterious effects of Bt crops on agro-ecosystems need to be evaluated cautiously and systematically before commercialization (Dale et al. 2002, Conner et al. 2003, Nap et al. 2003, Craig et al. 2008, Romeis et al. 2008).

Rice, *Oryza sativa* L., is one of the most important food staples in the world. More than 50% of the world population (or >3 billion people) depend on rice for their daily lives (FAO 2008). Genetic improvement of rice varieties through modern biotechnology to increase tolerance or resistance to biotic and abiotic stresses is one solution to meet the demands of the growing global populations, especially in developing countries. Since 1993, many transgenic rice lines with insecticidal *Bt* genes (referred to as Bt rice hereafter) have been developed to control lepidopteran caterpillar pests, most notably the striped stem borer, *Chilo suppressalis* (Walker) (Lepidoptera: Crambidae), the yellow stem borer, *Scirpophaga incertulas* (Walker) (Lepidoptera: Pyralidae), and the leaffolder, *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae) (High et al. 2004, Chen et al. 2006a, Wang and Johnston 2007), which cause 3–10% annual losses in yield, despite the intense use of insecticides (Sheng et al. 2003). The first field trials of Bt rice were conducted in China in 1998 (Shu et al. 2000; Tu et al. 2000; Ye et al. 2001a, b, 2003), and larger field trials of several Bt rice lines were continued (Chen et al. 2006a, Wang and Johnston 2007). In a study of farmer's fields in China, the quantity and expenditure of pesticides used for non-genetically modified (GM) rice production was 8 and 10 times higher, respectively, as those used for insect-resistant GM rice, whereas the yields of GM rice varieties were shown to be 6–9% higher than those of non-GM varieties (Huang et al. 2005). To date, most Bt rice lines still have not been approved for commercial release in China, although a Bt rice variety (Huahui1) and its hybrid line (Shanyou63) have been granted the biosafety certificates and approved for limited release in farmer's fields in Hubei Province from 2009 to 2014 (MAPCR 2009). One major reason for the cautious release of GM rice seems to be concerns about the potential impact on nontarget arthropods, especially natural enemies through tritrophic

[1] State Key Laboratory of Rice Biology, Ministry of Agriculture Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Institute of Insect Sciences, Zhejiang University, Hangzhou 310029, China.
[2] School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200240, China.
[3] Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.
[4] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100094, China.
[5] Corresponding author, e-mail: chu@zju.edu.cn.

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interactions. A growing number of studies on the effects of Bt crops on natural enemies have been carried out under laboratory and field conditions (Romeis et al. 2008, Wolfenbarger et al. 2008, Naranjo 2009). However, few studies have been conducted on natural enemies in Bt rice, especially under field conditions (Chen et al. 2006a).

Spiders, as a group of generalist predators, are the most abundant invertebrate predators in many agroecosystems and play an important role in pest control in many crops including rice (Marc et al. 1999, Symondson et al. 2002). *Ummeliata insecticeps* (Bösenberg et Strand) (Araneida: Linyphiidae) is a small spider (length, 2.5–3.5 mm) and is one of the dominant spider species in rice field in China (Zhang et al. 1995). It can make small webs at the bottom of rice plants and is one of the major natural enemies of the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), which prefer to stay at the bottom of rice plants if undisturbed (Zhang et al. 1999, Zhao et al. 2004). Previous studies have examined the effects of Bt rice on the population dynamics of five common spider species and the predation rate of the wolf spider, *Pirata subpiraticus* Böesenberg and Strand (Araneida: Lycosidae) (Liu et al. 2002, 2003). In this study, we report on prey-mediated effect of Bt rice on the survivorship, development, and fecundity of *U. insecticeps* and a 3-yr field study conducted at two sites of Zhejiang Province of China to evaluate the potential impacts of Bt rice on *U. insecticeps* populations.

Materials and Methods

Plant Materials. Transgenic rice line TT9-3 was developed using a biolistic method. It contains a fused *cry*1Ab/*cry*1Ac gene under the control of the rice *actin*1 promoter, which is expressed everywhere (Tu et al. 1998). The untransformed parental *indica* rice cultivar (IR72) was used as control. The line is effective against rice stem borers and leaffolders under laboratory (Tu et al. 1998) and field conditions (Ye et al. 2001b). Transgenic rice line KMD1 was developed using an *Agrobacterium*-mediated method to transfer a *cry*1Ab gene under the control of the maize *ubiquitin* promoter, which was expressed everywhere. The untransformed parental commercial cultivar (Xiushui 11) was used as control. The line, selected through nine generations, was homozygous for the transgenes (*cry*1Ab, *gus*, *npt*) (Shu et al. 1998).

Insects. A *U. insecticeps* colony was established in April 2005 from the Experimental Farm of Zhejiang University. Forty pairs of spiders were collected from the fields and subsequently maintained in the laboratory. *U. insecticeps* second-instar spiderlings were used in the experiments. A laboratory colony of *N. lugens* was provided by the China National Rice Research Institute. The females and males of *N. lugens* were paired and placed on a rice plant transplanted in a plastic pot and covered with a transparent plastic cylindrical cage (height 40 cm and diameter 11 cm) with a pair of nylon mesh window (diameter 6 cm) in the middle side of the cage and a top nylon mesh window (diameter 11 cm) for ventilation. Nymphs of the second or third instar fed on four genotypes of rice (i.e., KMD1, Xiushui11, TT9-3, and IR72) were fed to the spiders. Both colonies of *U. insecticeps* and *N. lugens* were continuously reared in a controlled temperature room (25 ± 2°C, 14:10-h L:D photoperiod).

Laboratory Experiments. For each of four treatments, 200 individuals of second-instar *U. insecticeps* were separately kept in small glass tubes (length 12 cm and diameter 2 cm). The opening of each tube was covered with a cotton ball. The bottom of each tube was filled with a piece of moist sponge to maintain humidity. The tubes were kept in a controlled temperature room (25 ± 2°C, 14:10-h L:D photoperiod). Spiders were supplied with either KMD1-, Xiushui11-, TT9-3-, or IR72-fed second- or third-instar *N. lugens* nymphs every day and checked daily until maturation or death. The day when spiders molted or died in each group was recorded, and the tubes were cleaned as needed. Spiders (males and females) were paired within each group when they reached the adult stage and kept under the same conditions as described above until the female adults laid their first egg sacs. The number of juveniles hatching from the first egg sac of each female in each group was recorded, as well as the number of unhatched eggs in the sac, because successive egg sacs contain fewer and fewer eggs (Zhao and Liu 1987).

Cry1Ab Insecticidal Protein Analyses. Another 40 second-instar *U. insecticeps* for each treatment were reared as describe in laboratory experiments. After all spiders reached the adults stage, rice stems, *N. lugens* nymphs, and *U. inseciceps* adults were collected individually, transferred into 1.5-ml Eppendorf tubes, and frozen at −70°C. The Cry1Ab level in the samples was assayed using a double sandwich ELISA kit for Bt-Cry1Ab/1Ac protein (Agdia, Elkhart, IN). For each treatment, samples (five for rice stems and *N. lugens* nymphs [100 mg as one replicate], three for *U. insecticeps* adults [two spiders as one replicate]) were weighed, homogenized, and diluted (×500 mg/μl for rice stems; ×5 mg/μl for *N. lugens* and *U. insecticeps*) in phosphate-buffered saline solution in Tween20. The solution was mixed for 1 min on a vortex mixer, centrifuged for 5 min at 12,000*g*, and loaded at 100 μl per test well. After dispensing 100 μl enzyme conjugate per well and incubating 2 h in a humid box at room temperature, 100 μl of the 3,3′,5,5′-tetramethylbenzidine (TMB) substrate solution was added for color development. At the end of the 15-min incubation with TMB substrate, 50 μl of 3 M sulfuric acid was added to each well. Spectrophotometric measurements were taken using a multidetection microplate reader (Synergy HT; Bio-Tek, Winooski, VT) at 450 nm. Purified Cry1Ab (EnviroLogix, Portland, ME) toxin at concentrations of 0, 0.3125, 0.625, 1.25, 2.5, 5, 10, and 20 ng/ml was used as calibrators.

Field Planting. The experiments were conducted during 2005–2007 at the Experimental Farm of Zhejiang University at Hangzhou (120.12° E, 30.13° N) and the local experimental field in Anji County (119.35° E, 30.88° N). Each year, rice seeds of KMD1 and Xiushui 11 were sown on 20 June, and the seedlings were transplanted on 20 July; rice seeds of TT9-3 and IR72 were sown on 30 March, and the seedlings weretransplanted on 1 May. Four months after transplantation, the rice reached full maturity. At both test locations, two separate fields were set up for the experiments with two pairs of Bt rice versus its corresponding non-Bt control rice. Each field was divided into six experimental plots in a 2 (treatments, Bt versus non-Bt) × 3 (replications) completely randomized design. Each experimental plot measured 20 by 25 m and was bordered on all sides by a 50-cm-wide unplanted walkway. Seedlings were hand transplanted at one seedling per hill spaced 16.5 by 16.5 cm apart, and the entire experimental field was surrounded by five border rows of nontransgenic control plants. Normal cultural practices, such as fertilization and irrigation, for growing rice were followed during the course of the experiment, except that no insecticide was applied after sowing and transplanting.

Sampling Method. A vacuum-suction machine was used to evaluate the seasonal patterns of *U. insecticeps* populations in Bt and non-Bt plots as described in our previous studies (Chen et al. 2006b, 2009). Samples (0.25 m^2/sample) were taken in all plots on a 15 ± 1-d schedule beginning 36 d after transplanting and continuing until the rice reached full maturity. There were five sampling dates at both the Anji site and Hangzhou site each year. On each sampling date, five samples were taken per plot at random along the diagonal of each plot at both test locations. Arthropods in each sample collected by the vacuum-suction machine were flushed into a labeled glass vial containing 75% ethanol and returned to the laboratory for sorting and counting.

Data Analyses. ELISA data were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple-range test. Survival analyses of *U. insecticeps* fed on Bt and non-Bt rice–reared *N. lugens* were conducted using the Wilcoxon test for homogeneity. Data on the developmental time and reproduction of *U. insecticeps* fed on Bt and non-Bt rice–reared *N. lugens* were analyzed using Student's *t*-test. Densities (seasonal means) of *U. insecticeps* in Bt and non-Bt plots in different years at two sites were analyzed using three-way ANOVA and Tukey's multiple-range test. The means on each sampling date compared were conducted using Student's *t*-test. All statistical calculations were performed with SAS version 9.1 package (SAS Institute 2001). For all tests, $\alpha = 0.05$.

Results

Cry1Ab Detection in *N. lugens* and *U. insecticeps*. *Cry1Ab* was detected from the tested Bt rice lines TT9-3 and KMD1 (Bt+) (Fig. 1). The average Cry1Ab concentration in TT9-3 rice–reared *N. lugens* nymphs (10.47 ± 1.58 ng/g flesh weight [FW]) was significantly higher than those in *U. insecticeps* adults fed on TT9-3 rice–reared *N. lugens* nymphs (2.04 ± 0.29 ng/g FW). However, the concentrations of the insecticidal protein detected in arthropods were significantly lower than those in TT9-3 rice stems (0.578 ± 0.05 μg/g of FW; $F = 639.76$; df = 2,10; $P < 0.001$). Likewise, in KMD1 rice stems, high concentration of Cry1Ab was detected (2.02 ± 0.17 μg/g FW); however, Cry1Ab concentrations in KMD1 rice–reared *N. lugens* nymphs (11.67 ± 1.45 ng/g FW) and *U. insecticeps* adults fed on KMD1 rice–reared *N. lugens* nymphs (2.29 ± 0.29 ng/g FW) were significantly lower. Cry1Ab concentration in prey was significantly higher than those in predators ($F = 1188.87$; df = 2,10; $P < 0.001$). As expected, no Cry1Ab was detected in non-Bt controls (IR72 and Xiushui 11).

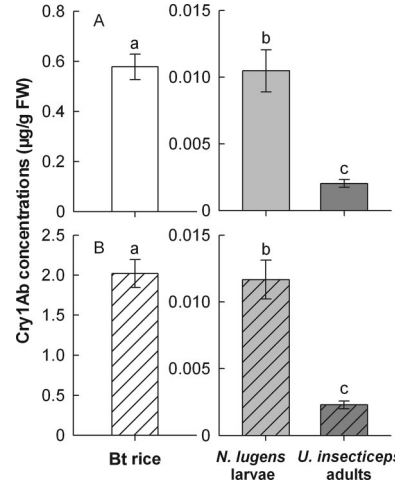

Fig. 1. Levels of Cry1Ab protein (mean ± SE) detected from Bt rice and Bt rice–reared *N. lugens* nymphs, and *U. insecticeps* adults fed on Bt rice–fed *N. lugens* larvae using ELISA. (A) TT9-3, (B) KMD1. $n = 5$ for Bt rice and *N. lugens* nymphs; $n = 3$ for *U. insecticeps* adults. Means denoted with different lowercase letters were significantly different (one-way ANOVA, $P < 0.05$).

Survival of *U. insecticeps*. The survival probability of *U. insecticeps* was not significantly affected when the spiders were supplied with Bt rice–reared (TT9-3 and KMD1) *N. lugens* compared with non-Bt rice–reared (IR72 and Xiushui11) *N. lugens* over a period of 100 d (TT9-3 and IR72: $\chi^2 = 0.0021$; df = 1; $P = 0.99$; KMD1 and Xiushui11: $\chi^2 = 0.1272$; df = 1; $P = 0.72$; Fig. 2).

Developmental Time of *U. insecticeps*. After preying on Bt rice-fed or non-Bt rice–fed *N. lugens*, *U. insecticeps* second-instar spiderlings had four molts before they reached the adult stage. The larval development time (from second instars to adult stage) of *U. insecticeps* fed on TT9-3 (Bt+) rice-reared *N. lugens* was not significantly different from that fed on IR72 (Bt−) rice-reared *N. lugens* ($t = 0.2504$; df = 73; $P = 0.8107$; Table 1). However, the duration of the third

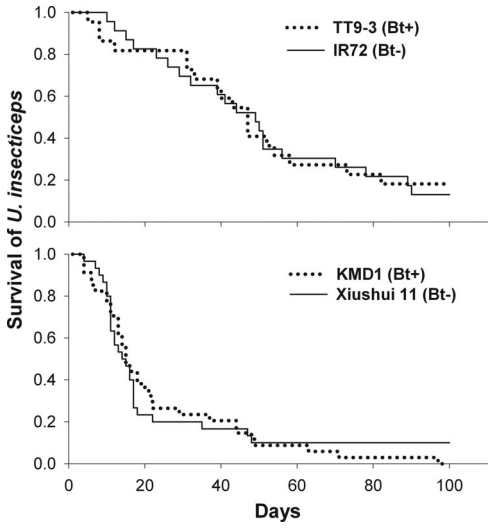

Fig. 2. Survival of *U. insecticeps* over a 100-d period when fed either Bt rice–fed or non-Bt rice–fed *N. lugens* nymphs. There was no significant difference between Bt rice and control treatment, based on Wilcoxon test. $n = 200$ for TT9-3 and 200 for IR72; $n = 200$ for KMD1 and 200 for Xiushui 11.

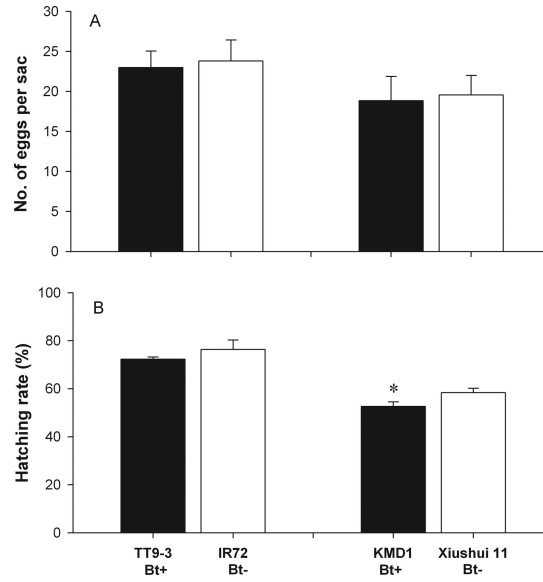

Fig. 3. Effect of Bt rice on egg production (A) and egg hatching rate (B) of *U. insecticeps* when fed either Bt rice–fed or non-Bt rice–fed *N. lugens* nymphs. $n = 36$ for TT9-3 and IR72; $n = 20$ for KMD1 and Xiushui 11. *Significantly different from the control non-Bt rice treatment ($P < 0.05$; Student t-test).

instar was significantly shorter in KMD1 (Bt+) than in Xiushui11 (Bt−) ($t = 3.4029$; df = 87; $P = 0.0059$), at 10.1 and 14.2 d, respectively. The development time of other instars was not significantly different. Overall, there was no difference in the developmental time for the entire juvenile stage ($t = 0.8310$; df = 38; $P = 0.4669$; Table 1).

Fecundity of *U. insecticeps*. After adult *U. insecticeps* were assigned to breeding pairs and fed either Bt rice–fed or non-Bt rice–fed *N. lugens*, the number of eggs in the first egg sac of *U. insecticeps* was not significantly different between Bt rice and non-Bt rice, whereas the difference in egg hatching rate between Bt and non-Bt rice was dependent on tested Bt rice lines (Fig. 3). *U. insecticeps* females supplied with TT9-3 (Bt+)-fed *N. lugens* laid 23.0 ± 2.0 eggs per sac and 72.3 ± 0.9% eggs hatched, whereas females from the IR72 treatment (Bt−) laid 23.8 ± 2.6 eggs per sac and 76.4 ± 3.9% eggs hatched (number of eggs: $t = 0.2309$; df = 36; $P = 0.8203$; egg hatching rate: $t = 1.0119$; df = 36; $P = 0.3355$). Likewise, *U. insecticeps* females supplied with KMD1 (Bt+)-fed *N. lugens* laid 18.9 ± 3.0 eggs, which was not significantly from that (19.6 ± 2.4 eggs per sac) laid by females in the Xiushui11 (Bt−) treatment ($t = 0.1852$; df = 20; $P = 0.8561$). However, the egg hatching rate in the KMD1 (Bt+) treatment was 52.7 ± 1.9%, which was significantly lower than that (58.4 ± 1.8%) in the Xiushui11 (Bt−) treatment ($t = 2.1758$; df = 20; $P = 0.0503$).

***Ummeliata insecticeps* Population Dynamics in Bt and Non-Bt Rice Fields.** In 2005, 2006, and 2007, *U. insecticeps* was found in Bt and non-Bt plots using vacuum-suction at Anji and Hangzhou. *U. insecticeps* densities (seasonal means) in TT9-3 (Bt+) and IR72 (Bt−) plots were significantly affected by test site ($F = 12.50$; df = 1,35; $P = 0.0009$). However, rice type ($F = 0.01$; df = 1,35; $P = 0.9067$), year ($F = 1.51$; df = 2,35; $P = 0.2313$), the interaction between rice type and test site ($F = 0.00$; df = 1,35; $P = 1.0000$), the interaction between rice type and year ($F = 0.01$; df = 2,35; $P = 0.9896$), the interaction between test site and

Table 1. Developmental time (mean ± SE) of *U. insecticeps* from second instars to adult emergence when fed either Bt rice-fed (TT9-3, KMD1, Bt+) or non-Bt rice-fed (IR72, Xiushui11, Bt−) *N. lugens*

Rice varieties	Developmental time (d)				
	Second instar	Third instar	Fourth instar	Fifth instar	Juveniles–adult
Group 1					
TT9-3, Bt+	7.7 ± 2.0 (167)	10.8 ± 1.1 (133)	12.0 ± 2.0 (75)	18.5 ± 2.6 (33)	56.3 ± 3.7 (33)
IR72, Bt−	8.8 ± 1.5 (183)	8.8 ± 1.6 (108)	8.6 ± 1.5 (58)	19.2 ± 2.7 (42)	53.5 ± 10.3 (42)
Group 2					
KMD1, Bt+	8.8 ± 1.1 (100)	10.1 ± 0.8 (46)[a]	12.2 ± 2.9 (39)	25.3 ± 4.6 (21)	53.5 ± 4.5 (21)
Xiushui 11, Bt−	5.8 ± 1.1 (77)	14.2 ± 0.9 (43)	19.0 ± 0.6 (35)	21.3 ± 1.9 (19)	58.7 ± 4.1 (19)

[a] Significantly different from the control non-Bt rice treatment ($P < 0.05$; Student t-test).
n, no. of individuals at each development stage.

Table 2. Seasonal densities (mean ± SE) of *U. insecticeps* in TT9-3 (Bt+), IR72 (Bt−), KMD1 (Bt+), and Xiushui11 (Bt−) plots detected using vacuum-suction sampling at two sites in China in 2005–2007

Rice varieties	Anji			Hangzhou		
	2005	2006	2007	2005	2006	2007
Group 1						
TT9-3, Bt+	2.28 ± 0.51	2.84 ± 0.56	3.00 ± 0.60	1.84 ± 2.09	0.68 ± 0.39	2.00 ± 1.70
IR72, Bt−	2.16 ± 0.37	2.68 ± 0.69	3.16 ± 0.77	1.76 ± 1.13	0.88 ± 0.30	1.76 ± 1.11
Group 2						
KMD1, Bt+	1.08 ± 0.31	1.36 ± 0.29	1.24 ± 0.33	0.60 ± 0.57	0.56 ± 0.50	0.68 ± 0.46
Xiushui11, Bt−	1.20 ± 0.47	1.32 ± 0.26	1.32 ± 0.29	1.28 ± 1.08	0.88 ± 0.23	1.04 ± 0.26

$n = 3$ at both sites in 2005, 2006, and 2007. There were no significant differences between Bt rice and control plots, based on three-way ANOVA. Seasonal density, an average density of *U. insecticeps* over all sampling dates.

year ($F = 1.76$; df = 2,35; $P = 0.1829$), and the interaction between rice type, test site, and year ($F = 0.11$; df = 2,35; $P = 0.9002$) did not significantly affect *U. insecticeps* densities (Table 2). In KMD1 (Bt+) and Xiushui11 (Bt−) fields, test site ($F = 5.75$; df = 1,35; $P = 0.0204$) significantly influenced the density of *U. insecticeps* but not rice treatment ($F = 2.16$; df = 1,35; $P = 0.1480$), year ($F = 0.02$; df = 2,35; $P = 0.9807$), the interaction between rice type and test site ($F = 1.35$; df = 1,35; $P = 0.2515$), the interaction between rice type and year ($F = 0.20$; df = 2,35; $P = 0.8201$), the interaction between test site and year ($F = 0.50$; df = 2,35; $P = 0.6123$), and the interaction between rice type, test site, and year ($F = 0.06$; df = 2,35; $P = 0.9434$) (Table 2).

Population dynamics (means of each sampling date) of *U. insecticeps* at Hangzhou and at Anji are presented in Figs. 4 and 5. Only at one sampling date was a significant difference found in population densities of *U. insecticeps* between Bt and non-Bt plots.

Discussion

Because Bt rice is on the verge of being commercially released in China (Cohen et al. 2008), data on its potential effects through tritrophic interactions on nontarget arthropods, especially insect natural enemies, are timely. In this study, our data indicated that Bt insecticidal protein can be transferred from lower trophic levels to higher trophic levels in the rice sys-

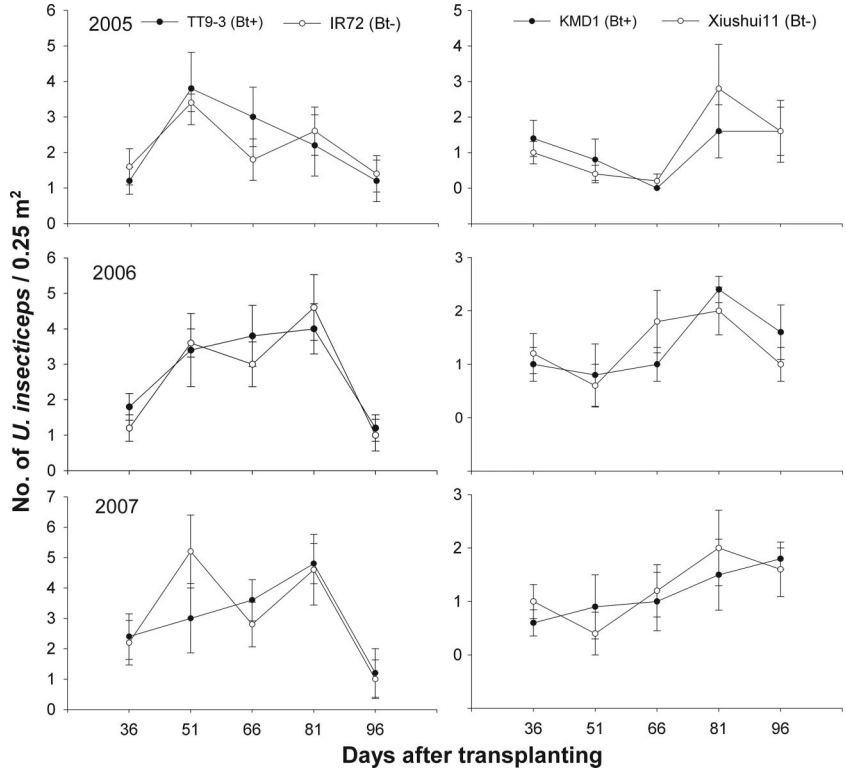

Fig. 4. Mean (±SE) number of *U. insecticeps* in TT9-3 and IR72 (left) and KMD1 and Xiushui11 (right) detected using vacuum-suction at Anji, China, in 2005–2007. $n = 3$ in 2005, 2006, and 2007. There was no significant difference between Bt rice and control plots, based on Student's *t*-test.

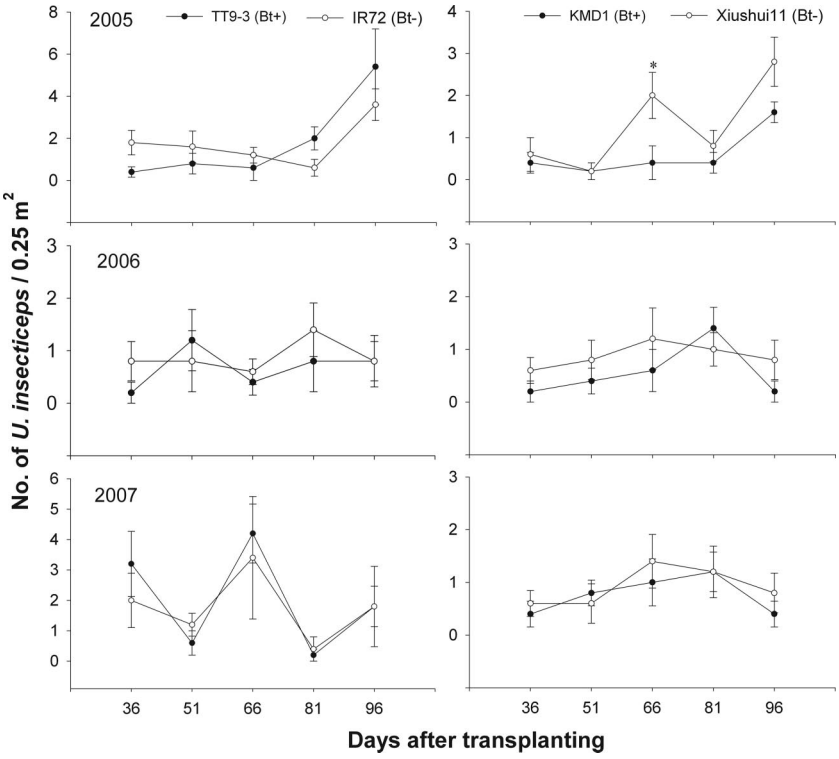

Fig. 5. Mean (±SE) number of *U. insecticeps* in TT9-3 and IR72 (left) and KMD1 and Xiushui11 (right) detected using vacuum-suction at Hangzhou, China, in 2005–2007. $n = 3$ in 2005, 2006, and 2007. Control plot marked * was significantly different from Bt rice plot ($P < 0.05$); otherwise, there were no significantly differences between Bt rice and control plots, base on Student's *t*-test.

tem through tritrophic interactions (Fig. 1). However, the level of Cry1Ab protein detected in the predator *U. insecticeps* supplied with Bt rice–fed prey *N. lugens* was ≈5 times lower than that in the prey, which implies that no bioaccumulation of Cry1Ab protein occurred in this food chain. Our results are consistent with previous studies. For example, Torres and Ruberson (2008) showed that, although the Bt insecticidal protein detected in herbivores [the twospotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae)] was highly concentrated, the amount conveyed to their predators [the big-eye bug, *Geocoris punctipes* (Say) (Heteroptera: Lygaeldae), and the damsel bug, *Nabis roseipennis* Reuter (Hemiptera: Nabidae)] was only 4 and 14% in the Bt cotton system. Alvarez-Alfageme et al. (2008) also reported that a significantly lower concentration of Bt insecticidal protein was detected in the predator *Stethorus punctillum* Weise (Coleoptera: Coccinellidae) compared with the prey *T. urticae* in Bt maize system under field conditions.

Although *U. insecticeps* ingested measurable amounts of Cry1Ab protein when it was supplied with Bt rice–fed *N. lugens*, Bt rice lines (TT9-3 and KMD1) did not have negative effects on the developmental time and fecundity of *U. insecticeps.* Liu et al. (2006) reported that Bt cotton expressing Cry1Ac protein had no adverse prey-mediated effects on the larvae and adults of two spiders, *Hylyphantes graminicola* (Sundevall) (Araneae: Linyphiidae) and *Coleosoma octomaculatum* (Böesenberg et Strand) (Araneae: Theridiidae), which are commonly found in cotton fields. Similarly, Cry1Ab protein expressed in Bt maize pollen had no negative effects on the weight increase, survival, molt frequency, reaction time, and various web variables of the garden spider, *Araneus diadematus* Korsedderkop (Araneae: Araneidae), in maize fields (Ludy and Lang 2006a). Meissle and Romeis (2009) also showed that no difference in mortality, weight development, or offspring production of the web-building spider, *Theridion impressum* L. Koch (Araneae: Theridiidae) was observed between spiders provided with food containing or not containing Cry3Bb1.

Although *U. insecticeps* had a relatively high mortality in our laboratory experiments, there was no difference in survival between Bt and control prey-fed spiders in the 100-d feeding experiment (Fig. 2). Chen et al. (2009) reported that Cry1Ab protein did not have binding receptors on the brush border membrane vesicles (BBMVs) of the wolf spider *P. subpiraticus* (one of the most important predators of rice insect pests), whereas several proteins on BBMVs in the midgut of the rice leaf folder *C. medinalis* (one of the target insect of Bt rice) were found to bind Cry1Ab using Western blot analysis.

The high mortality of *U. insecticeps* in this study might be because of the monotonous diet of only one prey species, which might have been suboptimal for spiders (Miyashita 1968, Uetz et al. 1992, Li 2002). We also noticed that the mortality in the laboratory experiments was different in the two groups of rice varieties tested, which might result from the different nutrition of prey fed on the two different groups of rice varieties. Many studies reported that the type and quality of the diet could affect the survival of the target organism (Riddick 2009). Burgess et al. (2009) also showd that an exclusive diet of tobacco-reared *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) had a negative impact on the survival of the male predator, *Ctenognathus novaezelandiae* (Fairmaire) (Coleoptera: Carabidae). In a number of studies, pollen, silk, and leaf tissue from plants was supplemented with prey to improve the predator survival (Romeis et al. 2006).

Our 3-yr field trials at Anji and Hangzhou indicated that the *U. insecticeps* population density did not differ between Bt and non-Bt rice plots (Table 2), which corroborated our laboratory results. Previous studies that investigated the impact of Bt rice on the abundance of spider communities showed similar results (Liu et al. 2002, Li et al. 2007). Furthermore, Bt maize expressing Cry1Ab protein did not impact spider communities negatively in the Czech Republic (Řezáč et al. 2006), Germany (Volkmar and Freier 2003, Meissle and Lang 2005, Ludy and Lang 2006b, Toschki et al. 2007), Spain (Farinos et al. 2008), and the United States (Rose and Dively 2007). When comparing the spider populations in Bt cotton, expressing Cry1Ac protein, and non-Bt cotton fields, no significant differences were detected by Head et al. (2005) or Torres and Ruberson (2005) in the United States, whereas a slight drop in some spider taxa were observed in Australia (Whitehouse et al. 2005) and the United States (Naranjo 2005).

In conclusion, the Bt rice lines tested in this study, TT9-3 and KMD1, had no adverse effects on the survival, developmental time, and fecundity of *U. insecticeps* in the laboratory or on population dynamics in the field. These results, together with the published literature, suggest that spiders are not likely to be harmed by the cultivation of Bt rice.

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