Field Assessment of the Effects of Transgenic Rice Expressing a Fused Gene of *cry1Ab* and *cry1Ac* from *Bacillus thuringiensis* Berliner on Nontarget Planthopper and Leafhopper Populations

M. CHEN,[1,2] G. Y. YE,[1,3] Z. C. LIU,[4] H. W. YAO,[1] X. X. CHEN,[1] Z. C. SHEN,[1] C. HU,[1] and S. K. DATTA[5]

Environ. Entomol. 35(1): 127–134 (2006)

ABSTRACT In 2003 and 2004, field studies were conducted at three sites in Zhejiang Province in China to assess the impacts of *Bacillus thuringiensis* (*Bt*) Berliner rice expressing a fused gene of *cry1Ab* and *cry1Ac* on nontarget planthoppers and leafhoppers. Populations in *Bt* plots were sampled with yellow sticky card traps, Malaise traps, and a vacuum-suction machine, and compared with samples from non-*Bt* control (IR72) plots. The results from yellow sticky card trap samplings indicated no significant differences between *Bt* and non-*Bt* plots in the species composition or densities of each species of planthopper and leafhopper. Three species of planthoppers, *Sogatella furcifera* (Horvath), *Nilaparvata lugens* (Stål), and *Laodelphax striatellus* (Fallén), were collected at all sites, and three species of leafhoppers, *Nephotettix cincticeps* (Uhler), *Thaia subrufa* (Motschulsky), and *Recilia dorsalis* (Motschulsky), were collected at Hangzhou. Another species of leafhopper, *N. virescens* (Distant), was collected at Anji and Jiande instead of *T. subrufa*. The results from the Malaise trap and vacuum-suction samples revealed no significant differences between *Bt* and non-*Bt* plots in species structure of planthoppers and leafhoppers or in population changes of the predominant planthopper species, *S. furcifera*, the predominant leafhopper species, *N. cincticeps*, or *N. virescens* throughout most sampling dates. Densities of planthoppers and leafhoppers were significantly affected by year and site but not by *Bt* rice. In general, our results suggest that the *Bt* rice line tested did not adversely affect nontarget planthopper and leafhopper populations and will not lead to higher populations or damage by planthoppers and leafhoppers.

KEY WORDS transgenic rice, *cry1Ab*/*cry1Ac*, planthopper, leafhopper, population dynamics

The area planted to transgenic crops containing toxin genes from *Bacillus thuringiensis* (*Bt*) Berliner, developed for control of selected lepidopteran or coleopteran pests, has increased ~10-fold since the first *Bt* crops were released to farmers in the United States in 1996 (James 2003). Concerns have been raised regarding the direct or indirect impacts that *Bt* crops may have on various groups of nontarget organisms of ecological and economic value through crop-plant–based food chains (Snow and Palma 1997, Schuler et al.1999, Poppy 2000, Wolfenbarger and Phifer 2000, Obrycki et al. 2001). Such concerns, however, have centered mainly on natural enemies, including predators and parasitoids, of target or nontarget pests of *Bt* crops, and little on nontarget herbivore sucking insects such as planthoppers, leafhoppers, whiteflies, thrips, and aphids, many of which are important crop pests (Shieh et al. 1994, Lozzia et al. 1998, Riddick et al. 1998, Cui and Xia 2000, Ashouri et al. 2001, Reed et al. 2001).

To date, numerous genotypes of *Bt* rice, *Oryza sativa* L. (Poaceae), have been developed, conferring high resistance against stem borers and leaffolders (Lepidoptera: Pyralidae) (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, Tu et al. 1998, Shu et al. 2000). *Bt* rice has not yet been released to farmers, but field trials have been conducted in China since 1998 (Tu et al. 2000, Ye et al. 2001a, b, 2003). However, there has been little published research about the impact of *Bt* rice on nontarget organisms. Bernal et al. (2002) reported that there were no significant negative effects of *Bt* rice on the fitness parameters of the brown planthopper, *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), and its predator, *Cyrtorhinus lividipennis* Reuter (Hemiptera: Miridae), under laboratory conditions. We also found that *Bt* rice did not adversely affect

[1] State Key Laboratory of Rice Biology, Institute of Applied Entomology, Zhejiang University, Hangzhou 310029, China.
[2] Current address: Department of Entomology, Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456.
[3] Corresponding author: Institute of Applied Entomology, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310029, China (e-mail: chu@zju.edu.cn).
[4] College of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 201101, China.
[5] Plant Breeding, Genetics and Biochemistry Division, International Rice Research Institute, DAPO Box 777, Metro Manila, Philippines.

0046-225X/06/0127–0134$04.00/0

predation by the wolf spider, *Pirata subpiraticus* Boesenberg and Strand (Araneida: Lycosidae), on its prey reared on *Bt* rice, including a *Bt* rice target species, *Cnaphalocrocis medinalis* (Guenée) (Lepidoptera: Pyralidae), and a *Bt* rice nontarget species, *N. lugens* (Liu et al. 2003a). The oviposition preference of *N. lugens* was not affected, but significantly less feeding occurred on *Bt* rice under laboratory conditions (Chen et al. 2003). In addition, preliminary studies, conducted with the aid of vacuum-suction sampling, found no marked differences between plots of two *Bt* rice lines and a control line in populations of the main nontarget insect pests and five common spider species (Liu et al. 2002) or in the structure of the arthropod community (Liu et al. 2003b). The effects of *Bt* rice on nontarget sucking insects such as planthoppers (Homoptera: Delphacidae) and leafhoppers (Homoptera: Cicadellidae) in the field remain undefined.

In Asia, planthoppers and leafhoppers are two important groups of rice insect pests. Some species such as *N. lugens*, the white-backed planthopper, *Sogatella furcifera* (Horvath) (Homoptera: Delphacidae), and the green leafhopper, *Nephotettix cincticeps* (Uhler) (Homoptera: Cicadellidae), often cause more yield loss than either stem borers or leaffolders through the removal of plant sap and by vectoring rice viruses (Dale 1994, Pathak and Khan 1994). It is clear that *Bt* rice lines will be undesirable if they promote significantly higher populations and damage by planthoppers or leafhoppers. Therefore, scientifically sound risk assessments on the impacts of *Bt* rice on the population dynamics of these important nontarget sucking pests in the field are critically needed before releasing *Bt* rice to farmers. We report the results of a 2-yr field study conducted at three sites in Zhejiang Province of China to evaluate the potential impacts of *Bt* rice on planthopper and leafhopper populations using three different sampling methods.

Materials and Methods

Plant Material. Transgenic rice homozygous line, TT9–3 at the 9th and 10th generation after transformation, was chosen for field evaluation in 2003 and 2004, respectively, together with its nontransgenic parental *indica* rice cultivar IR72. The transgenic line was developed through the biolistic method. It contains a fused *Bt* gene that was made from *cry1Ab* and *cry1Ac* after optimization of their codon usage to match the high G + C content in rice and is under the control of the rice *actin1* promoter (Tu et al. 1998). The line is effective against rice stem borers and leaffolders under laboratory (Tu et al. 1998) and field conditions (Ye et al. 2001a).

Field Planting. The experiments were conducted in 2003 and 2004 at three sites in Zhejiang Province of China where planthoppers and leafhoppers occur naturally during rice growing seasons. Each year, transgenic and nontransgenic control plants were sowed and transplanted in three batches. The first batch was sown on 10 April and transplanted on 10 May at the local experimental field in Anji County (119.35° E, 30.88° N). The second batch was sown on 21 April and transplanted on 21 May at the local experimental field outside Jiande City (118.99° E, 29.30° N). The third batch was sown on 27 May and transplanted on 27 June at the Experimental Farm of Zhejiang University at Hangzhou (120.12° E, 30.13° N). Each field was divided into six experimental plots in a 2 (treatments, *Bt* versus non-*Bt*) × 3 (replications) completely randomized design. Each experimental plot was 20 by 35 m^2 in Anji County and Jiande City and 20 by 25 m^2 in Hangzhou City. Each plot 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 surround 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 by Yellow Sticky Card Trap. The yellow sticky card trap developed by Pearsall and Myers (2001) was used to monitor dispersal of planthopper and leafhopper adults between *Bt* and non-*Bt* plots. The dispersal studies were carried out at the Hangzhou site from 24 July to 24 September 2003 and 28 July to 28 September 2004. To measure vertical variation in dispersing adults, yellow cards (25 by 30 cm^2 each) were clamped on a wooden stake at 35 (low), 70 (medium), and 105 cm (high) above the ground. Before placing the traps in the field, each card was uniformly smeared with a glutinous liquid made by boiling a 10:2:1:1 (by weight) mixture of rosin, castor oil, glycerol, and sucrose, respectively. Ten traps were placed in each walkway between plots of *Bt* and non-*Bt* rice at intervals of 2.0 m. Traps alternately faced toward the *Bt* and non-*Bt* plots. Traps were collected and replaced every 2 d, expect on rainy days. Planthoppers and leafhoppers captured by the traps were identified and counted directly on the card with the aid of a dissecting microscope.

Sampling by Malaise Trap. Malaise trapping of planthoppers and leafhoppers was conducted at the Hangzhou site. Insects were collected with a head vial containing 500 ml of 75% ethanol (Walker 1978). The Malaise traps were 2.0 m long by 1.5 m wide by 1.8 m high. One Malaise trap was placed at the center of each tested plot, enclosing ≈96 rice hills (a hill is a group of several tillers grown in one hole). The ethanol head vials containing captured arthropods were collected at 0800 hours each day and replaced by new ones. Planthoppers and leafhoppers in the samples were sorted and counted in the laboratory with the aid of a dissecting microscope. Sampling was initiated 5 wk after transplanting and lasted until the rice plants were fully grown.

Sampling by Vacuum-Suction Machine. A vacuum-suction machine was used for sampling at all test sites to estimate seasonal patterns in population dynamics of planthopper and leafhopper nymphs and adults in *Bt* and non-*Bt* plots. The machine was based on that described by Carino et al. (1979), but was supplemented with a square sampling frame (50 cm by 50 cm by 90 cm high; planar area, 2,500 cm^2) made of mylar sheets to encompass nine rice hills. Samples were taken in all plots on a 15 ± 1 d schedule beginning 1 mo after transplanting and continuing until the rice reached full maturity. On each sampling date, a square sampling frame was placed at random along a diagonal of each plot at each tested location, and five samples were taken per plot. Sample locations in each plot were marked with bamboo stakes left in place for the next sampling dates, during which the marked location was not sampled again. Arthropods inside the frame enclosure that were collected by the vacuum-suction machine were flushed into a labeled glass vial containing 75% ethanol. Samples were taken returned in the laboratory for sorting and counting of planthoppers and leafhoppers.

In both years, there were five sampling dates at the Anji site and seven sampling dates at the Jiande and Hangzhou sites.

Data Analysis. Densities of planthopper and leafhopper species collected by vacuum-suction throughout the sampling period between *Bt* and non-*Bt* plots at all of three sites were analyzed using repeated measures analysis of variance (ANOVA). Differences in numbers of the predominant species [*S. furcifera* and *N. cincticeps* or *N. virescens* (Distant)] between *Bt* and non-*Bt* plots at each site collected by vacuum-suction were analyzed by Student's *t*-test. Comparisons of numbers of the predominant species (*S. furcifera* and *N. cincticeps*), collected by yellow sticky card and Malaise trap throughout the entire sampling period between *Bt* and non-*Bt* plots, were carried out using the Mann-Whitney *U* test, because capture variances for the two methods were not homogeneous ($P < 0.05$). All statistical calculations were performed using the STATISTICA software package (StatSoft 1994). For all tests, $\alpha = 0.05$.

Results

Population Composition and Density of Planthoppers and Leafhoppers. In both 2003 and 2004, the species composition of planthopper and leafhopper groups in *Bt* and non-*Bt* rice plots at Hangzhou was very similar, although the mean proportion of each species throughout the entire sampling period varied numerically among plots, years, and sampling methods. All three sampling methods collected two species of planthoppers, namely the white-backed planthopper, *S. furcifera,* and *N. lugens,* and three species of leafhoppers, namely, the green leafhopper, *N. cincticeps,* the white-winged leafhopper, *Thaia subrufa* (Motschulsky), and the zigzag leafhopper, *Recilia dorsalis* (Motschulsky). In addition, the small brown planthopper, *Laodelphax striatellus* (Fallén), was captured by vacuum-suction sampling. In both *Bt* and non-*Bt* plots, *S. furcifera* was predominant, accounting for >93% of all planthopper individuals collected by all three sampling methods combined. Likewise, *N. cincticeps,* constituted >51% of all leafhoppers captured. No significant differences were found between *Bt* and non-*Bt* plots in the average proportions of each species during the whole sampling period at the Hangzhou site.

Another species of leafhopper, *N. virescens,* was found in *Bt* and non-*Bt* plots using vacuum-suction at Anji and Jiande instead of *T. subrufa* in both years. *N. virescens* was the predominant species of leafhoppers at Jiande instead of *N. cincticeps.* The vacuum-suction method was more effective for sampling planthoppers and leafhoppers in *Bt* and non-*Bt* rice plots than the other two sampling methods. Densities of different planthopper and leafhopper species collected by vacuum-suction are shown in Table 1. The densities of *S. furcifera* were significantly affected by test site ($F = 235.29$, df = 2, $P = 0.046$). However, rice treatment (*Bt*/non-*Bt;* $F = 152.32$, df = 1, $P = 0.082$) and year ($F = 148.00$, df = 1, $P = 0.07$) did not significantly affect the densities of *S. furcifera.* Densities of *N. lugens* were significantly affected by year ($F = 20.01$, df = 1, $P = 0.042$) and test site ($F = 382.66$, df = 2, $P = 0.036$), but not by rice type ($F = 55.21$, df = 1, $P = 0.074$). Densities of *L. striatellus* were significantly impacted by year ($F = 6012.84$, df = 1, $P < 0.001$) and test site ($F = 3559.49$, df = 2, $P = 0.004$), but not by rice type ($F = 0.031$, df = 1, $P = 0.876$). The same trends were found for leafhoppers. Densities of *N. virescens, T. subrufa,* and *R. dorsalis* were statistically affected by year ($F = 7643.79$, df = 1, $P < 0.001$ for *N. virescence;* $F = 223.81$, df = 1, $P = 0.04$ for *T subrufa;* $F = 417.54$, df = 1, $P = 0.035$ for *R dorsalis*) and test site ($F = 2547.43$, df = 2, $P < 0.001$ for *N. virescence;* $F = 1809.24$, df = 2, $P = 0.017$ for *T subrufa;* $F = 1809.24$, df = 2, $P = 0.017$ for *R dorsalis*), but not by rice treatment ($F = 16.70$, df = 1, $P = 0.055$ for *N. virescence;* $F = 0.12$, df = 1, $P = 0.762$ for *T. subrufa;* $F = 5.16$, df = 1, $P = 0.151$ for *R dorsalis*), except that densities of *N. cincticeps* were significantly affected by year ($F = 19.51$, df = 1, $P = 0.048$), site ($F = 21833.72$, df = 2, $P = 0.005$), and rice treatment ($F = 401.77$, df = 1, $P = 0.02$).

Dispersal of Planthoppers and Leafhoppers. In both 2003 and 2004, yellow sticky card traps revealed that dispersal of both planthopper and leafhopper adults between *Bt* and non-*Bt* plots was most frequent at the height of 35 cm. Differences in direction of dispersal of the predominant species, *S. furcifera* and *N. cincticeps,* moving between *Bt* and non-*Bt* plots was not significantly different at any tested heights of the trap over the entire sampling period (Figs. 1 and 2). Densities of the dispersing minor species, *N. lugens, T. subrufa,* and *R. dorsalis,* moving from *Bt* to non-*Bt* plots in 2003 (0.26 ± 0.09, 5.79 ± 1.03, and 6.37 ± 0.87; mean ± SE; $n = 3$) were not significantly different than corresponding values reflecting movement from non-*Bt* to *Bt* plots (0.11 ± 0.06, 4.68 ± 0.93, and 4.16 ± 0.70, respectively). The same was true in 2004. The values were 0.57 ± 0.10, 5.16 ± 0.77, and 4.53 ± 0.82, respectively, in the direction of *Bt* to non-*Bt,* and 0.26 ± 0.09, 4.68 ± 1.11, and 4.16 ± 0.94, respectively, in the direction of non-*Bt* to *Bt.*

Population Dynamics of Planthoppers and Leafhoppers. Data from the Malaise trap indicate that the patterns of population changes of the predominant

Table 1. Densities (mean ± SE) of each species of planthopper or leafhopper collected by the vacuum-suction from *Bt* rice (*Bt*) and the control (non-*Bt*) plots across the entire sampling period at three sites in China

Year	Species	Anji		Hangzhou		Jiande	
		Bt	non-*Bt*	*Bt*	non-*Bt*	*Bt*	non-*Bt*
2003				Delphacidae			
	S. furcifera	4.00 ± 0.45	4.24 ± 0.69	20.89 ± 2.03[a]	10.51 ± 1.18	12.03 ± 1.06	16.69 ± 1.05
	N. lugens	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.05	0.14 ± 0.01	0.34 ± 0.01	0.09 ± 0.01
	L. striatellus	0.36 ± 0.01	0.32 ± 0.02	0.60 ± 0.06	0.63 ± 0.05	1.29 ± 0.02	1.57 ± 0.03
				Cicadellidae			
	N. cincticeps	2.88 ± 0.36	2.52 ± 0.47	9.20 ± 1.04[a]	22.91 ± 1.85	0.09 ± 0.00	0.11 ± 0.01
	N. virescens	0.16 ± 0.01	0.16 ± 0.01	0	0	0.91 ± 0.01	1.29 ± 0.05
	T. subrufa	0	0	0.43 ± 0.09	0.86 ± 0.10	0	0
	R. dorsaslis	0.12 ± 0.01	0.20 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.26 ± 0.01	0.14 ± 0.02
2004				Delphacidae			
	S. furcifera	4.84 ± 0.55	4.32 ± 0.96	19.83 ± 1.60	16.06 ± 1.05	16.31 ± 1.60	14.34 ± 1.21
	N. lugens	0.12 ± 0.01	0.08 ± 0.00	0.20 ± 0.01	0.20 ± 0.00	0.14 ± 0.00	0.14 ± 0.00
	L. striatellus	0.40 ± 0.01	0.48 ± 0.01	0.66 ± 0.10	0.37 ± 0.01	0.89 ± 0.02	0.86 ± 0.01
				Cicadellidae			
	N.cincticeps	2.72 ± 0.46	2.52 ± 0.48	18.97 ± 1.46	11.69 ± 0.96	0.29 ± 0.01	0.23 ± 0.01
	N. virescens	0.24 ± 0.01	0.20 ± 0.02	0	0	0.60 ± 0.01	0.49 ± 0.01
	T. subrufa	0	0	0.71 ± 0.05	0.54 ± 0.02	0	0
	R. dorsaslis	0.12 ± 0.01	0.24 ± 0.01	0.09 ± 0.01	0.17 ± 0.01	0.17 ± 0.01	0.09 ± 0.00

$n = 3$ at each site in 2003 and 2004.

[a] Significantly different from the control non-*Bt* ($P < 0.05$); otherwise, there were no significant differences between *Bt* and non-*Bt* plots, based on repeated measures ANOVA.

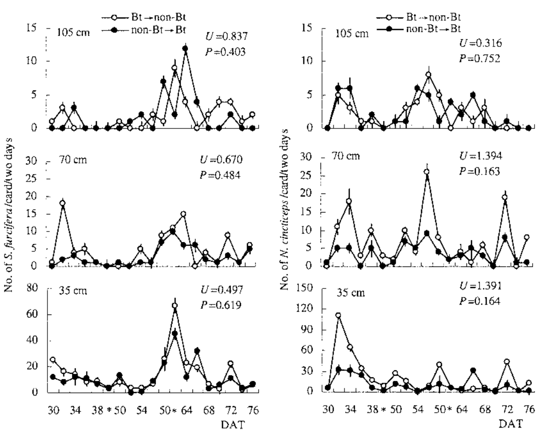

Fig. 1. Dispersing adults of *S. furcifera* and *N. cincticeps* captured between *Bt* and non-*Bt* plots on yellow sticky card traps at different vertical heights and facing different plots in 2003 at Hangzhou, China. The values are mean ± SE ($n = 3$). DAT, days after transplanting; asterisk, rainy day.

species, *S. furcifera* and *N. cincticeps,* were very similar in the *Bt* and non-*Bt* plots at Hangzhou (Fig. 3). Likewise, the results of the vacuum-suction samples revealed no apparent differences in population levels of the predominant species of planthoppers and leafhoppers at any sampling date in *Bt* and non-*Bt* plots at Hangzhou (Fig. 4), Anji (Fig. 5), and Jiande (Fig. 6) in either year. Exceptions were significantly more *S. furcifera* and less *N. cincticeps* individuals collected in the *Bt* plots at Hangzhou in 2003.

Discussion

Our 2-yr field monitoring results at three sites indicated that transgenic (*Bt*) *cry1Ab*/*cry1Ac* rice did not significantly impact the species composition or the densities of planthoppers and leafhoppers throughout the whole sampling period (Table 1) and did not affect the population dynamics of the predominant species (Figs. 2–6). No noticeable differences were found in the total dispersal densities of each species of planthoppers and leafhoppers between *Bt* and non-*Bt* plots. These results are supported by our previous studies conducted in 2000 using the vacuum-suction sampling method, which revealed no significant differences in adult and nymph densities of *S. furcifera* and *N. cincticeps* between the plots of two *Bt* rice lines, i.e., TT9–3 and TT9–4, and their control (non-*Bt*) throughout the rice growing season (Liu et al. 2002). In a 1992≈1993 study of *Bt* potatoes, Reed et al. (2001) found no marked impacts on the abundance of nontarget sucking insect pests on potato plants, except that in 1992, leafhoppers were observed in beat-cloth samples over the entire season from *Bt* than conventional potato plants. No significant differences were evident in population densities of the corn leaf aphid, *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae), on *Bt* and non-*Bt* corn plants (Wold et al. 2001). In contrast, leafhoppers, primarily *Empoasca fabae* (Harris) (Homoptera: Cicadellidae), were significantly more abundant in a mixed planting of transgenic and nontransgenic *Bt* potatos than in 100% transgenic potato fields (Riddick et al. 1998). Significantly more sucking cotton pests were found on *Bt* than non-*Bt* cotton plants (Cui and Xia 2000). The differences between our findings and those of previous studies may be related to numerous factors, including differences in crop, the insertion site, and expression pattern of the *Bt* gene after being inserting into the crop genome; in tritrophic interactions involving *Bt* crop plants, their target or nontarget herbivores and their natural enemies (parasitoids and predators); and in the competition between target and nontarget herbivores of the *Bt* crop. In other words, it is necessary to specifically evaluate the impacts of different *Bt* crops, even different lines of the same *Bt* crop, on nontarget herbivores, so that suitable management tactics can be applied to nontarget herbivore pests.

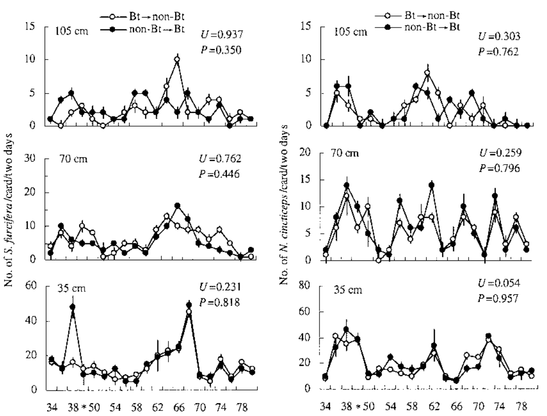

Fig. 2. Dispersing adults of *S. furcifera* and *N. cincticeps* captured between *Bt* and non-*Bt* plots on yellow sticky card traps at different vertical heights and facing different plots in 2004 at Hangzhou, China. The values are mean ± SE ($n = 3$). DAT, days after transplanting; asterisk, rainy day.

Our findings that the patterns of population dynamics of planthoppers and leafhoppers on *Bt* rice were not significantly different from those on non-*Bt* rice imply that the fitness parameters (e.g., survival to the adult stage, male and female weight, male and female developmental time, fecundity) of planthoppers or leafhoppers might not be significantly affected by *Bt* rice either. This inference is supported by Bernal et al. (2002) for *N. lugens.* We may also infer from these results that the abundance of natural enemies of planthoppers and leafhoppers may not be impacted by *Bt* rice, because planthoppers and leafhoppers are as available in a *Bt* rice field as in a non-*Bt* rice field. To a certain extent, this supposition is supported by our previous results that no significant differences between the plots of two *Bt* rice lines and their control line were found in the abundance of five common spider species regarded as important predators of planthoppers and leafhoppers (Liu et al. 2002).

Overall, given these results and that *Bt* rice did not adversely affect the fitness parameters of *N. lugens* and

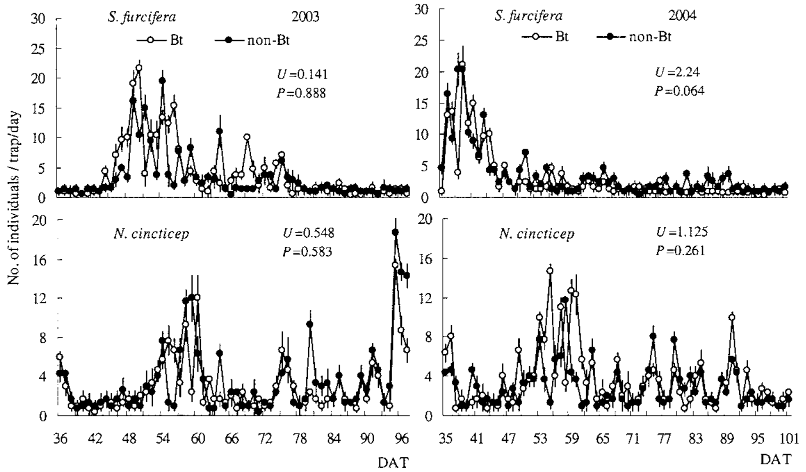

Fig. 3. Mean ± SE number of adult *S. furcifera* and *N. cincticeps* captured by Malaise trap in *Bt* and non-*Bt* plots in 2003 (left) and 2004 (right) at Hangzhou, China. DAT, days after transplanting.

its most important predator, *C. lividipennis* (Bernal et al. 2002), the abundance of predominant spider species (Liu et al. 2002), the oviposition preference of *N. lugens* (Chen et al. 2003), or predation by the wolf spider on *N. lugens* feeding on *Bt* rice (Liu et al. 2003a), we concluded that the *Bt* rice line tested has no marked negative effects in the short term on nontarget planthoppers and leafhoppers and their natural enemies, especially their predators. Thus, the potential risks of more severe damage by planthoppers and leafhoppers in *Bt* rice than in its nontransgenic control are relatively minor, and we can manage their populations on *Bt* rice using the same tactics being used in non-*Bt* rice fields. However, field experiments in this study were designed to detect large differences among planthopper and leafhopper populations in *Bt* and non-*Bt* rice and were only conducted over 2 yr with a relatively small plot area and number of replications. Also, because the densities of natural enemies were relatively low, the data are not presented in this paper.

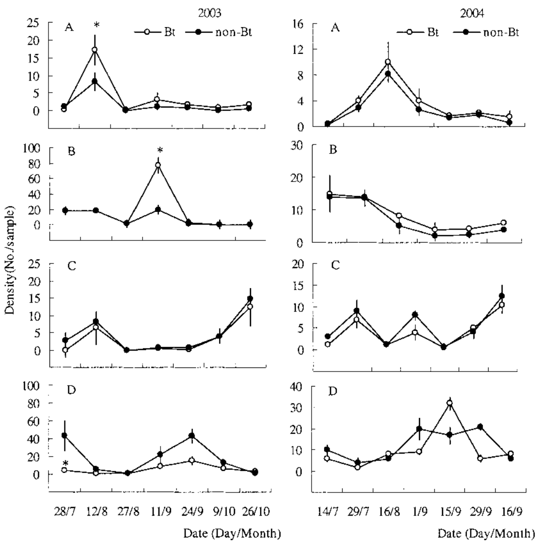

Fig. 4. Mean ± SE number of *S. furcifera* and *N. cincticeps* captured by the vacuum-suction machine in *Bt* and non-*Bt* plots in 2003 (left) and 2004 (right) at Hangzhou, China. (A) *S. furcifera* nymphs. (B) *S. furcifera* adults. (C) *N. cincticeps* nymphs. (D) *N. cincticeps* adults. $n = 3$. *Bt* point marked * is significantly different from non-*Bt* ($P < 0.05$); otherwise, there are no significant differences between *Bt* and non-*Bt*, based on Student's *t*-test.

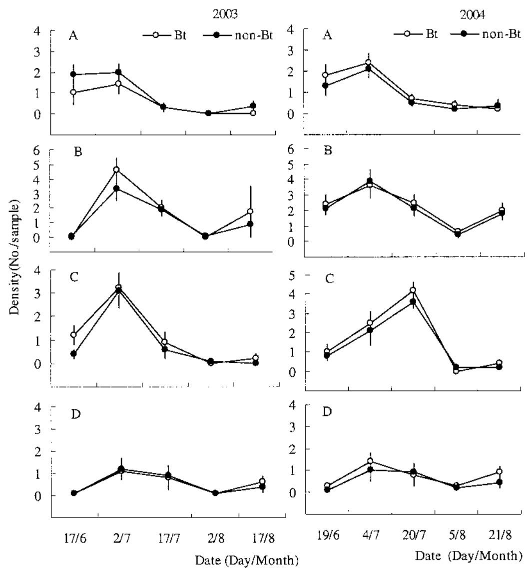

Fig. 5. Mean ± SE number of *S. furcifera* and *N. cincticeps* captured by the vacuum-suction machine in *Bt* and non-*Bt* plots in 2003 (left) and 2004 (right) at Anji, China. (A) *S. furcifera* nymphs. (B) *S. furcifera* adults. (C) *N. cincticeps* nymphs. (D) *N. cincticeps* adults. $n = 3$. No significant differences between *Bt* and non-*Bt* at any date, based on Student's *t*-test.

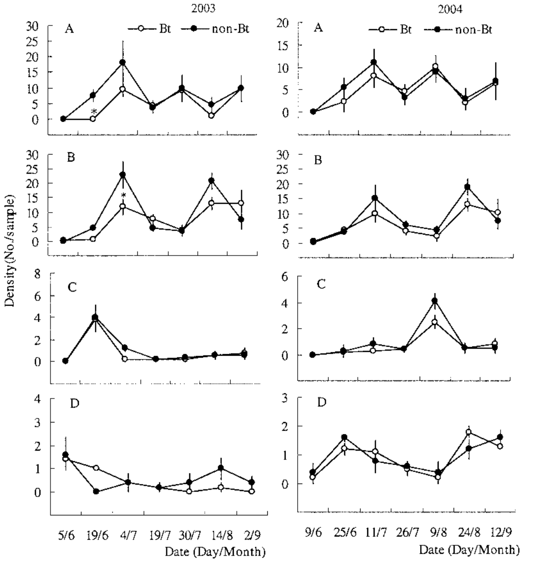

Fig. 6. Mean ± SE number of *S. furcifera* and *N. virescens* captured by the vacuum-suction machine in *Bt* and non-*Bt* plots in 2003 (left) and 2004 (right) at Jiande, China. (A) *S. furcifera* nymphs. (B) *S. furcifera* adults. (C) *N. virescens* nymphs. (D) *N. virescens* adults. $n = 3$. *Bt* point marked * is significantly different from the control non-*Bt* ($P < 0.05$); otherwise, there are no significant differences between *Bt* and non-*Bt*, based on Student's *t*-test.

To elucidate the specific effects of *Bt* rice on nontarget insect populations over the long term, studies with larger sample sizes over large spatial scales or periods of time will be required in the future.

It is important to choose a suitable sampling method for accurately assessing the impacts of *Bt* crops on nontarget arthropods under field conditions, as influenced by the study aims and biological characteristics of the subject arthropods. In some cases, more than one suitable sampling method must be used to avoid drawing inappropriate conclusions. The yellow sticky card trap only provided information about dispersal of adult arthropods with the ability to fly between *Bt* and non-*Bt* fields, and thus can suitably assess only whether *Bt* crops promote dispersal of nontarget insect pests into adjacent non-*Bt* fields. It cannot directly measure population densities of arthropods, especially immatures or adults without the capacity of flight. Similarly, the Malaise trap only can offer information on seasonal abundance of adult arthropods capable of flight in the *Bt* or non-*Bt* fields. In contrast, the vacuum-suction method is more effective. It can supply information on population densities of immature and adult arthropods with and without the ability to fly and is appropriate to assess the impacts of *Bt* crops on any species of arthropods residing on the surface of crop plants. Given the biological characteristics of the arthropods investigated in this study, neither the yellow sticky card trap nor the Malaise trap are suitable for evaluating the potential risk of *Bt* rice on planthopper populations when *N. lugens* is the predominant species instead of *S. furcifera*. *N. lugens* is dimorphic, with full-winged "macropterous" and truncated-winged "brachypterous" forms. The flightless form is predominant in the rice field after colonization, in contrast to *S. furcifera*, which often produces long-winged form with the ability to fly (Dale 1994). Thus, sampling by vacuum-suction should be the preferred method for detecting the effects of *Bt* rice on planthopper populations when the predominant species is *N. lugens*. Consequently, documentation of biological and ecological information (e.g., the relative abundance of dimorphic forms) on subject arthropods at different trial sites should be referenced before the choice of sampling methods is made.

Acknowledgments

We thank C. Yang and X. Y. Yu for assistance in the field and laboratory and three anonymous reviewers, M. Cohen, G. F. Barry, and O. Adimo for comments on a draft of the manuscript and for reviewing it. This study was supported by the National Program on Key Basic Research Projects (973 Program, 001CB109004), the Ministry of Science and Technology of China, the National Natural Science Foundation of China (39970507), and the Special Foundation for the Winner of National Excellent Doctoral Dissertation, the Ministry of Education of China (199944).

References Cited

Ashouri, A., D. Michaud, and C. Cloutier. 2001. Unexpected effects of different potato resistance factors to the Colorado potato beetle (Coleoptera: Chrysomelidae) on the potato aphid (Homoptera: Aphididae). Environ. Entomol. 30: 524–532.

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.

Carino, F. O., P. E. Kenmore, and V. A. Dyck. 1979. The Farmcop suction sampler for hoppers and predators in flooded rice fields. Rice Res. Newsl. 4: 21–22.

Chen, M., G. Y. Ye, H. W. Yao, C. Hu, and Q. Y. Shu. 2003. Impact evaluation of insect-resistant transgenic rice on the feeding and oviposition behavior of its non-target insect, the brown planthopper, *Nilaparvata lugens* (Homptera: Delphacidae). Agric. Sci. China. 2: 1000–1006.

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

Cui, J. J., and J. Y. Xia. 2000. Effects of transgenic *Bt* cotton R93–6 on the insect community. Acta Entomol. Sinica. 43: 43–51.

Dale, D. 1994. Insect pests of the rice plant—their biology and ecology, pp. 363–485. *In* E. A. Heinrichs (ed.), Biology and management of rice insects. Wiley Eastern Limited and New Age International Limited, New Delhi, India.

Datta, K., A. Vasquez, J. Tu, L. Torrizo, M. F. Alam, N. Oliva, E. Abrigo, G. S. Khush, and S. K. Datta. 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.

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

Ghareyazie, B., F. G. Alinia, C. A. Menguito, L. G. Rubia, J. M. dePalma, E. A. Liwangag, M. B. Cohen, G. S. Khush, and J. Bennett. 1997. Enhanced resistance to two stem borers in an aromatic rice containing a synthetic *cry1A(b)* gene. Mol. Breed. 3: 401–414.

James, C. 2003. Preview: global status of commercialized transgenic crops: 2003. ISAAA, Ithaca, NY.

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

Liu, Z. C., G. Y. Ye, Q. Fu, Z. T. Zhang, and C. Hu. 2003a. Indirect impact assessment of transgenic rice with *cry1Ab* gene in predations by the wolf spider, *Pirata subpiraticus.* Chinese J. Rice Sci. 17: 175–178.

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

Lozzia, G. C., C. Furlanis, B. Manachini, and I. E. Rigamonti. 1998. Effects of *Bt*-corn on *Rhopalosiphum padi* L. (Rhynchota: Aphididae) and on its predator *Chrysoperla carnea* Stephen (Neuroptera: Chrysopidae). Boll. Zool. Agraria Bachiocolt. Ser. II. 30: 153–164.

Nayak, P., D. Basu, S. Das, A. Basu, D. Ghosh, N. A. Ramakrishna, M. Ghosh, and S. K. Sen. 1997. Transgenic elite *indica* rice plants expressing CryIA(c) δ-endotoxin of *Bacillus thuringiensis* are resistant against yellow stem borer (*Scirpophaga incertulas*). Proc. Natl. Acad. Sci. U.S.A. 94: 2111–2116.

Obrycki, J. J., J. E. Losey, O. R. Taylor, and L. C. Jesse. 2001. Transgenic insecticidal corn: beyond insecticidal toxicity to ecological complexity. Bioscience. 51: 353–361.

Pathak, M. D., and Z. R. Khan. 1994. Insect pests of rice. International Rice Research Institute, Manila, Phillipines.

Pearsall, I. A., and J. H. Myers. 2001. Spatial and temporal patterns of dispersal of Western flower thrips (Thysanoptera: Thripidae) in nectarine orchards in British Columbia. J. Econ. Entomol. 94: 831–843.

Poppy, G. M. 2000. GM crops: environmental risks and non-target effects. Trends Plant Sci. 5: 4–6.

Reed, G. L., A. S. Jensen, J. Riebe, G. Head, and J. J. Duan. 2001. Transgenic *Bt* potato and conventional insecticides for Colorado potato beetle management: comparative efficacy and non-target impacts. Entomol. Exp. Appl. 100: 89–100.

Riddick, E. W., G. Dively, and P. Barbosa. 1998. Effect of a seed-mix deployment of Cry3A-transgenic and nontransgenic potato on the abundance of *Lebia grandis* (Coleoptera: Carabidae) and *Coleomegilla maculata* (Coleoptera: Coccinellidae). Ann. Entomol. Soc. Am. 91: 647–653.

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

Shieh, J. N., R. E. Berry, G. L. Reed, and P. A. Rossignol. 1994. Feeding activity of green peach aphid (Homoptera: Aphididae) on transgenic potato expressing a *Bacillus thuringiensis spp. tenbrionis* δ-endotoxin gene. J. Econ. Entomol. 87: 618–622.

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.

Snow, A. A., and P. M. Palma. 1997. Commercialization of transgenic pants: potential ecological risks. BioScience. 47: 86–96.

StatSoft. 1994. Statistica for Windows. StatSoft Inc.

Tu, J., K. Datta, M. F. Alam, Y. Fan, G. S. Khush, and S. K. Datta. 1998. Expression and function of a hybrid *Bt* toxin gene in transgenic rice conferring resistance to insect pest. Plant Biotechnol. 15: 195–203.

Tu, J., G. Zhang, K. Datta, C. Xu, Y. He, Q. Zhang, G. S. Khush, and S. K. Datta. 2000. Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* δ-endotoxin. Nat. Biotechnol. 18: 1101–1104.

Walker, T. J. 1978. Migration and re-migration of butterflies through north peninsular Florida: quantification with Malaise traps. J. Lepid. Soc. 32: 178–190.

Wold, S. J., E. C. Burkness, W. D. Hutchison, and R. C. Venette. 2001. In-field monitoring of beneficial insect populations in transgenic corn expressing a *Bacillus thuringiensis* toxin. J. Entomol. Sci. 36: 177–187.

Wolfenbarger, L. L., and P. R. Phifer. 2000. The ecological risks and benefits of genetically engineered plants. Science. 290: 2088–2093.

Wu, C., Y. Fan, C. Zhang, N. Oliva, and S. K. Datta. 1997. Transgenic fertile *japonica* rice plants expressing a modified *cry1A(b)* gene resistant to yellow stem borer. Plant Cell Rep. 17: 129–132.

Wünn, J., A. Klöti, P. K. Burkhardt, G. C. Ghosh Biswas, K. Launis, V. A. Iglesia, and I. Potrykus. 1996. Transgenic *indica* rice breeding line IR58 expressing a synthetic *cry1A(b)* gene from *Bacillus thuringiensis* provides effective insect pest control. Biotechnology. 14: 171–176.

Ye, G. Y., J. Tu, H. Cui, K. Datta, and S. K. Datta. 2001a. Transgenic IR72 with fused *Bt* gene *cry1Ab/cry1Ac* from *Bacillus thuringiensis* is resistant against four lepidopteran species under field conditions. Plant Biotechnol. 18: 125–133.

Ye, G. Y., Q. R. Shu, H. W. Yao, H. R. Cui, X. Cheng, C. Hu, Y. W. Xia, M. W. Gao, and I. Altosaar. 2001b. Field evaluation of resistance of transgenic rice containing a synthetic *cry1Ab* gene from *Bacillus thuringiensis* Berliner to two stem borers. J. Econ. Entomol. 94: 270–276.

Ye, G. Y., H. W. Yao, Q. R. 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 synthetic *cry1Ab* gene from *Bacillus thuringiensis* Berliner to rice leaffolder, *Cnaphalocrocis medinalis* (Guenée) under field conditions. Crop Prot. 22: 171–178.

Received for publication 18 May 2005; accepted 26 October 2005.