

The New Transgenic *cry1Ab/vip3H* Rice Poses No Unexpected Ecological Risks to Arthropod Communities in Rice Agroecosystems

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Received 12 August 2015; Accepted 2 December 2015

Abstract

The ecological risks to nontarget organisms should be rigorously assessed before Bt crops are released. Here, the impacts of a new Cry1Ab/Vip3H rice line on arthropod communities in rice agroecosystems were evaluated across 3 yr. Arthropods collected via vacuum were sorted into five guilds. The abundance and proportion of each guild as well as community-level parameters were determined in Cry1Ab/Vip3H and control rice fields. Changes in arthropod species assemblage over sampling dates were investigated by principal response curves (PRCs). Cry1Ab/Vip3H rice did not exert significant impacts on the seasonal density and proportion of each guild, except parasitoids. Detritivore seasonal density, but not its relative abundance, was significantly affected by Cry1Ab/Vip3H rice. Four community indices (species richness *S*, Shannon–Wiener index *H'*, Simpson index *D'*, and evenness index *J'*) were similar between rice types. PRCs revealed a slight community difference between rice types in the past two tested years, with rice types accounting for 1.0–3.5% of the variance among arthropod communities. However, sampling dates explain 32.1–67.6% for these community differences. Of the 46 taxa with higher species weights, 26.1% of the taxa were significantly different, including seven taxa with higher abundance and five with lower density in Cry1Ab/Vip3H rice fields. These differences may be attributed to change in abundance of prey or hosts but not to direct effects of Bt proteins. We infer that this new Cry1Ab/Vip3H rice line poses no unintended ecological risks to the arthropod community.

Key words: Transgenic rice, *cry1Ab*, *vip3H*, *epsps*, arthropod

Many invertebrates inhabit Chinese rice agroecosystems (Cheng and He 1996), including more than 1,375 species of natural enemies (He et al. 1991, Lou et al. 2013). These natural enemies effectively suppress insect pest populations, such as rice planthoppers, leaf folders, and stem borers (Cheng and He 1996). However, natural enemies operate in the context of chronic overuse of chemical insecticides, which have caused serious environmental problems. Among the environmental problems, chemical insecticides reduce population sizes and performance of natural enemies, and they induce outbreaks of insect pest populations (Heinrichs 1994, Heong 2009, Bottrell and Schoenly 2012, Lou et al. 2013).

Genetically modified rice producing δ -endotoxins from *Bacillus thuringiensis* Berliner (Bt) have been developed and deployed to reduce the adverse impacts of chemical insecticides. Several transgenic

Bt rice lines effectively control target rice pests (Tu et al. 2000; Ye et al. 2000, 2001a, b, 2003; High et al. 2004; Cohen et al. 2008; Chen et al. 2011a, b). Results of farm-level trials show that production of Bt rice surpassed control cultivars by 6–9% and decreased use of chemical pesticides by 80% (Huang et al. 2005). These crops are also economically beneficial to growers and enhance biological control services provided by natural enemies (Shelton et al. 2002; Chen et al. 2011a, b; Lu et al. 2012). These desirable outcomes of Bt rice drive research designed to rigorously assess the actual and potential large-scale risks associated with transgenic rice production.

Some insect pests have evolved resistance to current transgenic crops, possibly threatening their sustainable deployment, because different Cry1A proteins recognize or share the same midgut binding sites of rice pest insects (Lee et al. 1997, Karim and Dean 2000).

Stacking several Bt genes in a single rice line has been proposed as a strategy to delay the evolution of insect resistance (Zhao et al. 2003, Bravo and Soberon 2008). Vegetative insecticidal proteins (Vips) are produced during vegetative growth stage of *B. thuringiensis*. These toxins, with modes of action different from Bt toxins, are active against several lepidopteran larvae (Estruch et al. 1996; Lee et al. 2003). Transgenic cotton and maize, expressing a single Vip protein or a Vip stacked with one or two Cry proteins, have been developed. Some are in commercial production (Dively 2005; Llewellyn et al. 2007; Whitehouse et al. 2007, 2014; Bommireddy and Leonard 2008; Raybould and Vlachos 2011; Gulzar et al. 2012; Mahon et al. 2012). So far, cross-resistance between these two different insecticidal proteins has not been recorded (Lee et al. 2006, Jackson et al. 2007).

Assessments of whether these crops pose potential ecological risks to nontarget organisms have been conducted. Raybould and Vlachos (2011) showed that Vip3A protein did not impact the selected parameters of 11 of 12 species tested. The lone exception, the water flea, *Daphnia magna* Straus, had significantly shorter length and lighter body weight than unexposed controls. Dively (2005) reported that transgenic *vip3A/cry1Ab* maize had no unintended effects on nontarget arthropod communities. Whitehouse et al. (2007) found a slight difference in arthropod communities between Vip and conventional cotton, and that abundances of some species, specifically, predatory beetles, *Creotia dilutus* (Stål), and whiteflies (Aleyrodidae), were significantly higher, and the densities of tangle web spiders (Theridiidae/Linyphiidae) and green jassids (Cicadellidae) were significantly reduced in Vip cotton fields.

A new transgenic Bt rice line expressing a fused Cry1Ab/Vip3H protein has also been developed in China (Fang 2008, Chen et al. 2010), although little information on the ecological safety of this crop has been documented (Chen et al. 2011a, b; Lu et al. 2013, 2014a). Like all new transgenic crops, this Cry1Ab/Vip3H rice line should be fully evaluated before commercial production. Here, a 3-yr field trial was carried out to examine the impacts of this new rice line (G6H1) on arthropod communities. The goal of this study was to confirm whether the arthropod community structure differs between G6H1 and control rice plots.

Materials and Methods

Plants

The new rice line, G6H1, was constructed by the *Agrobacterium*-mediated method. It contains *cry1Ab* and *vip3H* genes under the control of maize *ubiquitin* promoter and links with a *epsps* gene encoding 5-enolpyruvylshikimate-3-phosphate synthase (Fang 2008). These transgenic plants showed high activity against rice stem borers (Chen et al. 2010).

Field Experiments

The experiments were conducted at the Experiment Station of Zhejiang University in Changxing, Zhejiang province during the growing seasons of 2010–2012. The field was divided into six experimental plots in a 2 (treatments, G6H1 and XS110) $\times$ 3 (replications) with completely randomized block design. Each plot was 30 by 25 m and separated from each other by an unplanted 50-cm-wide walkway. There was a buffer of five border rows of control plants (XS110) surrounding all the study plots. Rice seeds were sown on 12 June in 2010 and 1 June in 2011 and 2012, and after 1 mo, the seedlings were hand-transplanted at one seedling per hill spaced 16.5 by 16.5 cm apart. No pesticides were applied across the experiments, and

fertilization and irrigation were consistent with the practices of local farmers.

Arthropod Sampling and Taxonomy

A vacuum-suction machine (model 1612, John W. Hock Company, Gainesville, Florida, USA) was used to collect arthropods in each plot through a three-dimensional sampling frame (50 cm length by 50 width by 90 height; plane area: 0.25 m²; made of Mylar sheets; Carino et al. 1979). This process was usually conducted in the morning from 9:00 to 12:00 on each sampling date. When sampling, the sampling frame was randomly placed along a diagonal of each plot, and five samples (nine rice hills per sample) were taken per plot. Each individual sampling lasted for about 2 min. The collected arthropods were transferred into glass vials containing 100% EtOH and identified to family or species level using appropriate references in the laboratory (He and Pang 1986, Xia et al. 1988, Feng 1990, Song et al. 1999, Zheng and Gui 1999). The arthropods were assorted into five guilds following Liu et al. (2003). The “other” guild mainly included families other than herbivores, predators, parasitoids, and detritivores, with no clear ecological functions. These families were Chironomidae, Culicidae, Tabanidae, Muscidae, and Calliphoridae. The sampling intervals were 7–15 d beginning 1 mo after transplanting until the late growth stage. There were five sampling dates in 2010, and six in 2011 and 2012.

Statistical Analyses

We used four diversity indices, species richness *S*, Shannon–Wiener diversity index *H'*, Simpson diversity index *D*, and evenness index *J'*, for comparing community-level differences between rice types. These diversity indices were calculated using the “vegan” package (Oksanen et al. 2013) based on the statistical software R 3.0.2 (R Core Team 2013). The density for each plot was obtained by averaging the numbers obtained from five samples. To compare the overall impact of rice type on these parameters, the data on the guild density, guild percentage, and community indices over 3 yr (seasonal means for each plot in each year) were analyzed by two-way analysis of variance (ANOVA) based on a Proc Mixed model. Rice type and year were entered as fixed factors, with plot as random effect (Littel et al. 2006). Student's *t*-test was used to compare means between rice types on each sampling date in each year. To achieve normality and homoscedasticity, the arthropod counts and percentage of guilds were transformed by $\ln(x + 1)$ and by arcsine square (x), respectively. Data are presented as untransformed means. The abundance relationships between rice planthoppers (Delphacidae, log-transformation) and all the spiders were tested by linear models for each rice type in each year. The software SAS 9.1 was used to conduct all the statistical analyses (SAS Institute 2001).

The overall responses of all arthropod communities to Cry1Ab/Vip3H rice versus controls were analyzed by principal response curves (PRCs), a community-based multivariate method used to test and present treatment effects that change across time (Van den Brink et al. 1996, Van den Brink and Ter Braak 1999). PRCs are based on the partial redundancy analyses (RDAs), constructed by the program CANOCO 4.5 (Ter Braak and Smilauer 2002). The differences in community composition between Cry1Ab/Vip3H and control rice were tested using the Monte Carlo method on the first canonical axis of the RDA. Taxa with higher species weights that usually determine the shape of treatment curves were further analyzed by ANOVA. The changes in seasonal mean for these taxa over 3 yr were compared by Proc Mixed, and rice type

Table 1. Seasonal density of each guild and total arthropods in Cry1Ab/Vip3H (G6H1) and control rice (XS110) fields

Year	Rice type	Herbivores	Parasitoids	Predators	Detritivores	Others	Total
2010	G6H1	139.98 ± 47.03	2.40 ± 0.42	16.40 ± 3.53	17.82 ± 11.98	13.98 ± 0.89	190.60 ± 62.67
	Control	126.07 ± 31.97	2.29 ± 0.18	19.71 ± 3.81	5.51 ± 2.00	14.31 ± 2.29	167.89 ± 3 6.80
2011	G6H1	30.32 ± 2.35	1.42 ± 0.23*	14.02 ± 1.23*	32.30 ± 11.72*	68.22 ± 23.60	146.27 ± 23.67
	Control	35.02 ± 1.12	3.10 ± 0.29	10.23 ± 0.68	6.52 ± 0.97	62.68 ± 23.35	117.53 ± 23.78
2012	G6H1	46.55 ± 2.92*	1.87 ± 0.21*	24.75 ± 1.03	6.97 ± 2.46	38.30 ± 7.06	118.43 ± 5.77
	Control	26.68 ± 1.33	3.18 ± 0.09	21.25 ± 1.38	7.00 ± 0.85	33.70 ± 5.76	91.82 ± 7.54
Two-way ANOVA		F; df; P					
Rice types		3.13; 1,40; 0.084	17.39; 1,40; <0.001	1.25; 1,40; 0.271	4.99; 1,40; 0.031	0.13; 1,40; 0.717	2.35; 1,40; 0.133
Years		70.99; 2,40; <0.001	1.43; 2,40; 0.251	41.61; 2,40; <0.001	3.43; 2,40; 0.042	8.12; 2,40; 0.001	3.95; 2,40; 0.027
Rice type × Year		10.25; 2,40; <0.001	4.04; 2,40; 0.025	2.57; 2,40; 0.089	4.61; 2,40; 0.016	0.03; 2,40; 0.967	0.16; 2,40; 0.850

The data are expressed as mean ± standard error ($n = 3$, No./0.25 m²). *P* values are calculated based on two-way ANOVA using Proc Mixed, and rice type and year were entered as fixed factors, with plot as random effect. The asterisk indicates there was significant difference for each parameter between rice types (Cry1Ab/Vip3H (G6H1) vs. control) in each year based on Student's *t*-test, *P* level was adjusted by Bonferroni correction, leading to $\alpha = 0.017$.

was entered as fixed factor, with year and plot as random effects. For all tests, significant levels were adjusted by Bonferroni correction.

Results

Arthropod Abundance

We sampled 44,574 individual arthropods from experimental and control rice fields and identified 90 families during the 3-yr experiments. We captured 8,577 (Cry1Ab/Vip3H rice) and 7,555 (control) specimens in 2010; 8,776 (Cry1Ab/Vip3H rice) and 7,052 (control) in 2011; and 7,106 (Cry1Ab/Vip3H rice) and 5,509 (control) in 2012.

Seasonal mean densities of each guild and all arthropods are displayed in Table 1. Analysis by pairwise comparison indicates that densities of some guilds significantly differed between rice types in some, but not all, tested years. For example, predators and detritivores were significantly higher in 2011, and herbivores strikingly increased in 2012 in Cry1Ab/Vip3H rice fields. The situation differed for parasitoids (declined in 2011 and 2012). Overall analyses demonstrate that population densities of herbivores, predators, and other sub-communities, as well as total arthropod abundance, did not significantly differ between rice types. However, the abundance of parasitoids and detritivores was significantly affected by rice types. The densities of each guild, except parasitoids, also showed annual changes. The seasonal means of herbivores, parasitoids, and detritivores were further influenced by interaction between rice types and years.

Temporal changes in each guild density and total mean abundance are presented in Supp. Fig. 1 [online only]. There were similar trends between rice types in each year, with significant impacts of Cry1Ab/Vip3H rice on densities on one or several sampling dates in 2011 and 2012, but not in 2010.

Dominance Distribution

Herbivores accounted for 24.0–73.5% of total sampled arthropods and were the dominant guild in 2010 (Table 2). In 2011 and 2012, the “others” made up 31.1–43.9% of total insects and spiders sampled. Predators ranked second or third among the five guilds, depending on the year, and comprised 9.3–23.9% of total arthropods. Parasitoids were rare in all 3 yr. In general, dominance distribution of each guild, except parasitoids, was not significantly impacted by rice types across 3 yr, although percent of parasitoids was significantly lower in Cry1Ab/Vip3H rice plots. The relative abundance of guilds varied annually, and the proportions of herbivores and

parasitoids were also significantly affected by interaction between rice types and years.

Diversity Indices

Diversity indices are presented in Table 3. Multiyear analysis showed that these four diversity indices were not significantly impacted by rice types, or by the interaction between rice types and years. However, all these indices varied annually.

There were consistent trends between experimental and control plots in each year, with five significantly decreased in Cry1Ab/Vip3H rice plots in 2011 and 2012 (Supp. Fig. 2 [online only]).

Multivariate Analysis

PRC diagrams showed that entire arthropod communities in Cry1Ab/Vip3H and control rice fields differed significantly in 2011 and 2012, but not in 2010 (Fig. 1). Of the total variance in arthropod abundance in all 3 yrs, 1.0, 1.3, and 3.5% are explained by rice types and 67.6% (2011), 32.1% (2011), and 40.9% (2012) are explained by sampling times. Thus, a large percentage of the arthropod community difference can be attributed to sampling times.

Taxa with higher species weights (higher than +0.5 or lower than −0.5) strongly influenced the shape of treatment response curves. There were 46 species groups with high species weights, which included 19 taxa with negative, 15 taxa with positive, and 12 taxa with annual variations in experimental plots relative to control (Fig. 1). Multiyear analysis showed that Cry1Ab/Vip3H rice significantly impacted the abundance of Delphacidae, Chloropidae, and Anthomyiidae from herbivores; Braconidae, Ichneumonidae, Pteromalidae, and Eurytomidae from parasitoids; Tetragnathidae and Salticidae from predators; and Isotomidae, Psychodidae, and Scatopsidae from detritivores (Supp. Table 1 [online only]).

Relationships Between Planthopper Density and Spider Abundance

Linear regression analyses showed that abundances of all the spiders were positively correlated with the density of rice planthoppers for each rice type in each year (Table 4).

Discussion

Our findings demonstrate that abundance of parasitoids (Braconidae, Ichneumonidae, Pteromalidae, and Eurytomidae) was significantly lower in Cry1Ab/Vip3H rice fields, compared with controls (Table 1 and Supp. Table 1 [online only]). Parasitoids may be

Table 2. Dominance distribution (%) of each guild in Cry1Ab/Vip3H (G6H1) and control rice (XS110) fields

Year	Rice type	Herbivores	Parasitoids	Predators	Detritivores	Others
2010	G6H1	72.87 ± 2.14	1.38 ± 0.19	9.27 ± 1.04	7.39 ± 3.00	9.09 ± 3.01
	Control	73.48 ± 4.96	1.47 ± 0.25	12.31 ± 1.38	3.15 ± 0.54	9.77 ± 3.38
2011	G6H1	23.96 ± 2.45*	1.12 ± 0.24*	11.42 ± 1.65	21.38 ± 5.25*	42.12 ± 6.65
	Control	35.80 ± 3.79	3.23 ± 0.49	10.27 ± 1.12	6.79 ± 1.26	43.91 ± 5.85
2012	G6H1	40.21 ± 3.30	1.61 ± 0.19*	21.07 ± 0.82	6.02 ± 2.12	31.08 ± 4.46
	Control	29.79 ± 1.30	3.34 ± 0.26	23.93 ± 1.73	7.67 ± 0.75	34.97 ± 3.01
Two-way ANOVA		F; df; P				
Rice types		0.08; 1,40; 0.774	18.90; 1,40; <0.001	1.08; 1,40; 0.306	3.29; 1,40; 0.078	0.28; 1,40; 0.597
Years		42.71; 2,40; <0.001	4.59; 2,40; 0.016	39.15; 2,40; <0.001	4.32; 2,40; 0.020	15.52; 2,40; <0.001
Rice type × Year		6.86; 2,40; 0.003	2.85; 2,40; 0.070	1.00; 2,40; <0.3767	5.09; 2,40; 0.012	0.05; 2,40; 0.950

The data are expressed as mean ± standard error ($n = 3$, No./0.25 m²). *P* values are calculated based on two-way ANOVA using Proc Mixed, and rice type and year were entered as fixed factors, with plot as random effect. The asterisk indicates there was significant difference for each parameter between rice types (Cry1Ab/Vip3H (G6H1) vs. control) in each year based on Student's *t*-test, *P* level was adjusted by Bonferroni correction, leading to $\alpha = 0.017$.

Table 3. Seasonal diversity indices of arthropod communities in Cry1Ab/Vip3H (G6H1) and control rice (XS110) fields

Year	Rice type	<i>S</i>	<i>H'</i>	<i>J'</i>	<i>D</i>
2010	G6H1	48.33 ± 1.45	2.37 ± 0.20	0.42 ± 0.04	0.58 ± 0.04
	Control	47.67 ± 0.88	2.29 ± 0.23	0.41 ± 0.04	0.56 ± 0.06
2011	G6H1	34.60 ± 1.38	2.80 ± 0.24	0.55 ± 0.05	0.72 ± 0.06
	Control	38.20 ± 0.68	2.93 ± 0.23	0.56 ± 0.04	0.73 ± 0.05
2012	G6H1	36.60 ± 0.82	3.22 ± 0.08	0.62 ± 0.02	0.80 ± 0.02
	Control	38.00 ± 1.20	3.58 ± 0.10	0.68 ± 0.02	0.83 ± 0.02
Two-way ANOVA		F; df; P			
Rice types		1.68; 1,40; 0.203	0.49; 1,40; 0.488	0.24; 1,40; 0.629	0.03; 1,40; 0.856
Years		31.29; 2,40; <0.001	10.41; 2,40; <0.001	12.50; 2,40; <0.001	9.03; 2,40; <0.001
Rice type × Year		1.20; 2,40; 0.312	0.44; 2,40; 0.647	0.46; 2,40; 0.637	0.13; 2,40; 0.880

The data are expressed as Mean ± standard error ($n = 3$, No./0.25 m²). *P* values are calculated based on two-way ANOVA using Proc Mixed, and rice type and year were entered as fixed factors, with plot as random effect. The asterisk indicated there was significant difference for each parameter between rice types (Cry1Ab/Vip3H (G6H1) vs control) in each year based on Student's *t* test, *P* level was adjusted by Bonferroni correction, leading to $\alpha = 0.017$.

S, Species richness; *H'*, Shannon–Wiener diversity index; *J'*, Evenness index; *D*, Simpson diversity index.

exposed to Bt toxins after their larvae consume hosts that had fed on transgenic plants (Dively 2005). Such host-mediated effects depend on whether the hosts were lethally or sub-lethally affected by Bt insecticidal proteins (Romeis et al. 2006). Braconidae, Ichneumonidae, Pteromalidae, and Eurytomidae are important parasitoid wasps in rice agroecosystems, particularly in larvae of rice stem borers and leaf rollers, targets of Bt rice (He and Pang 1986). The decline in parasitoid density is expected because the density of rice pest larvae is reduced in Bt rice fields (Chen et al. 2010). Similar results were reported elsewhere (Liu et al. 2003, Dively 2005, Tian et al. 2008, Lu et al. 2014b). The density of parasitoids in Cry1A, Cry1C, and Cry2A rice fields was significantly lower compared with the control fields, especially Braconidae, Ichneumonidae and/or Pteromalidae. Dively (2005) showed that populations of Braconids and Ichneumonids in Vip3A/Cry1Ab maize plots were 28% lower than in control maize fields. Wolfenbarger et al. (2008) reported lower populations of parasitoids in Bt maize, which may be owing to lower numbers of *Macrocentrus grandii* Brischke, a parasitoid of European corn borers, a Bt maize-target.

Spider populations, families Tetragnathidae and Salticidae, were significantly higher in Cry1Ab/Vip3H rice plots (Supp. Table 1 [online only]). Recalling that spider and planthopper populations are tightly correlated, the higher abundance of these two spider families may be linked to larger populations of rice planthoppers (Delphacidae) in experimental fields. Other studies also document that spider populations are correlated with the populations of their prey (Heong et al. 1991, Lu et al. 2014b). Spiders are the most

abundant arthropod predators and they effectively control pests in agroecosystems (Cheng and He 1996, Li et al. 2011). They can ingest Bt insecticidal proteins when foraging on prey that had consumed Bt proteins or may feed directly on transgenic plant material, such as pollen (Meissle 2013). Bt insecticidal proteins can be detected in spider tissue after they feed on Bt crop pollen or prey feeding on Bt plants. However, parameters (survival rate, development duration, number of offspring, and predation capacity) of studies species were not affected by Bt insecticidal proteins (Ludy and Lang 2006, Meissle and Romeis 2009; Tian et al. 2010, 2012; Han et al. 2015). Field studies showed that spider population dynamics were similar between rice types (Liu et al. 2002, Chen et al. 2009, Tian et al. 2010, Han et al. 2011). We infer that Bt insecticidal proteins pose no risks for spider populations.

Detritivore population numbers were significantly higher in Cry1Ab/Vip3H rice fields, especially Isotomidae, Psychodidae, and Scatopsidae. Detritivores contribute to degrading organic matter and enhance the nutrient cycling in the soil (Bitzer et al. 2005). Most detritivores captured in this study were Collembola. Bai et al. (2005) reported higher population densities of two Collembolan species, *Entomobrya griseoolivata* (Packard) and *Bourletiella christianseni* Snider, in experimental Cry1Ab rice plots. This finding differs from results of other Collembolan studies that used pitfall traps instead of vacuum collection (Bai et al. 2010, 2012). Laboratory tests confirmed that Cry1A protein and Vip3A protein had no direct impacts on the ecological parameters of a standard test soil organism, the collembolan *Folsomia candida* Willem (Bai et al. 2011, Raybould

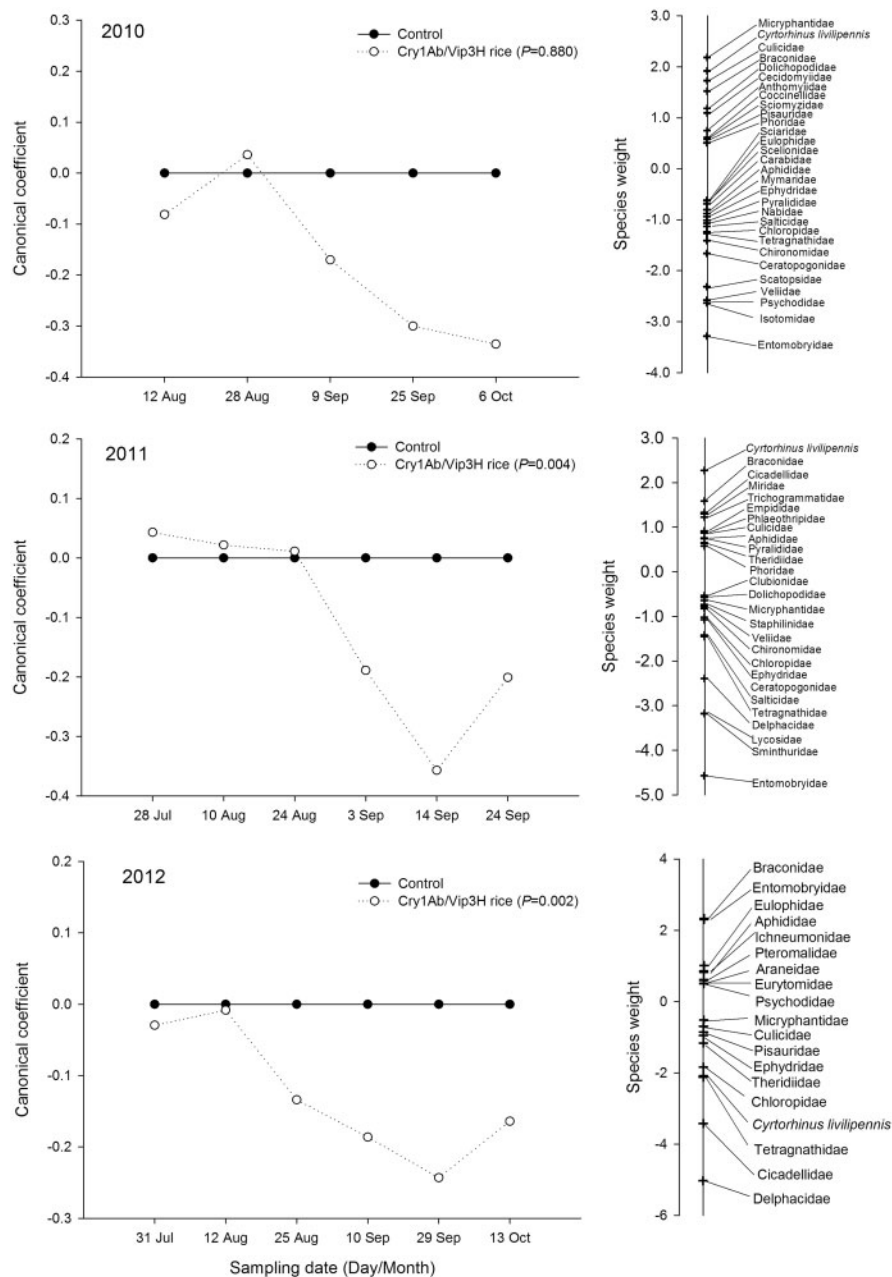


Fig. 1. PRC plots and species weights of arthropod community in Cry1Ab/Vip3H (G6H1) and control rice (XS110) plots at Changxing over three seasons. Taxa with species weights between 0.5 and -0.5 are not shown, because these taxa have little influence on the shape of treatment curves. Species weights greater than +0.5 are most likely to follow the shape of the curves, whereas those less than -0.5 indicated an opposite pattern.

Table 4. The relationship between abundance of spiders and rice planthoppers in Cry1Ab/Vip3H (G6H1) and control rice (XS110) fields in each year

Year	Rice type	Linear regression	R^2	F	df	P
2010	G6H1	$y = 1.88x + 1.30$	0.43	9.74	1, 13	0.008
	Control	$Y = 2.53x - 0.83$	0.51	13.41	1, 13	0.003
2011	G6H1	$y = 3.39x + 1.90$	0.20	14.28	1, 58	<0.001
	Control	$y = 1.49x + 2.42$	0.16	10.81	1, 58	0.002
2012	G6H1	$y = 3.31x + 5.68$	0.07	4.63	1, 58	0.004
	Control	$y = 3.31x + 5.21$	0.07	4.53	1, 58	0.038

y and x are the spiders abundance and planthoppers log-transformed ($\ln(n+1)$) abundance (No./0.25 m^2), respectively.

and Vlachos 2011, Yuan et al. 2013). A meta-analysis of 146 observations also reported similar abundance of detritivores between crop types (Wolfenbarger et al. 2008). Thus, Cry1Ab/Vip3H rice likely had no direct effect on the populations of detritivores, given that significant density differences were recorded on no more than one or two sampling dates.

Cry1Ab/Vip3H rice fields also contained higher abundances of herbivores in the families Delphacidae and Chloropidae. Delphacidae is a nontarget herbivore of Bt rice, serving as the main prey and host for beneficial arthropods (Cheng and He 1996). Several studies document increased populations of some sap-sucking species in Bt crop fields (Cui and Xia 1998, Zhou et al. 2005, Lu et al. 2010, Wang et al. 2010). Zhou et al. (2005) found that

population densities of *Nephotettix cincticeps* (Uhler) in Cry1Ab rice plots were 1–2 times higher than controls. Wang et al. (2010) showed that populations of *Nilaparvata lugens* (Stål) significantly increased in Bt rice fields. However, because Cry1Ab or Vip3 toxins do not directly influence Delphacidae and Chloropidae, their higher densities in Cry1Ab/Vip3H rice may be due to other factors, such as differences in chemical constituents or nutrition between rice types (Zhou et al. 2005, Faria et al. 2007).

Results of this 3-yr study indicate the Cry1Ab/Vip3H rice poses very slight influence on arthropod communities, which can be attributed to population fluctuations in a few taxa. The density changes in parasitoids, detritivores, and a few other taxa are primarily indirect effects of prey or host abundances. In congruence with other investigations of Bt cotton (Whitehouse et al. 2005, 2007, 2014), community-level biodiversity and seasonal densities of some guilds (herbivores, predators, and others) as well as total arthropod abundance were not significantly influenced by the Cry1Ab/Vip3H rice line. Also in agreement with our findings, Dively (2005) reported no unintended tri-trophic effects of Vip3Aa/Cry1Ab corn on nontarget arthropods. Naranjo (2005) indicated that the effects of Cry1Ac cotton on 22 taxa of arthropod natural enemies are minor. Single-event rice lines, Cry1A, Cry1C, or Cry2A rice, exerted no significant impacts on the arthropod communities (Liu et al. 2003, Li et al. 2007, Lu et al. 2014b). Taken together with contemporary literature, our findings support the idea that Cry1Ab/Vip3H rice does not pose undue risk to arthropod communities in rice agroecosystems.

Supplementary Data

Supplementary data are available at Environmental Entomology online.

Acknowledgments

We thank Prof. Q. Y. Tang (Zhejiang University, Zhejiang, China) for helping with our data analyses and Dr M. Chen (Monsanto Company, 700 Chesterfield Parkway W., GG3I Chesterfield, MO 63017, USA) for critically polishing our manuscript. Financial support was provided by the Special Research Projects for Developing Transgenic Plants (2014ZX08011-001) and China National Science Fund for Innovative Research Group of Biological Control (Grant no. 31321063). Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. All programs and services of the U.S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap.

References Cited

- Bai, Y. Y., M. X. Jiang, and J. A. Cheng. 2005. Impacts of transgenic *cry1Ab* rice on the two collembolan species and predation of *Microvelia horvathi* (Hemiptera: Veliidae). *Acta Entomol. Sin.* 48: 42–47.
- Bai, Y. Y., R. H. Yan, G. Y. Ye, F. N. Huang, and J. A. Cheng. 2010. Effects of transgenic rice expressing *Bacillus thuringiensis* Cry1Ab protein on ground-dwelling collembolan community in postharvest seasons. *Environ. Entomol.* 39: 243–251.
- Bai, Y. Y., R. H. Yan, X. Ke, G. Y. Ye, F. N. Huang, Y. M. Luo, and J. A. Cheng. 2011. Effects of transgenic Bt rice on growth, reproduction, and superoxide dismutase activity of *Folsomia candida* (Collembola: Isotomidae) in laboratory studies. *J. Econ. Entomol.* 104: 1892–1899.
- Bai, Y. Y., R. H. Yan, G. Y. Ye, F. N. Huang, D. S. Wangila, J. J. Wang, and J. A. Cheng. 2012. Field response of above ground non-target arthropod community to transgenic Bt-Cry1Ab rice plant residues in postharvest seasons. *Transgenic Res.* 21: 1023–1032.
- Bitzer, R. J., M. E. Rice, C. D. Pilcher, C. L. Pilcher, and W.K.F. Lam. 2005. Biodiversity and community structure of epedaphic and euedaphic springtails (Collembola) in transgenic rootworm Bt corn. *Environ. Entomol.* 34: 1346–1376.
- Bommireddy, P. L., and B. R. Leonard. 2008. Survivorship of *Helicoverpa zea* and *Heliothis virescens* on cotton plant structures expressing a *Bacillus thuringiensis* vegetative insecticidal protein. *J. Econ. Entomol.* 101: 1244–1252.
- Bottrell, D. G., and K. G. Schoenly. 2012. Resurrecting the ghost of green revolutions past: The brown planthopper as a recurring threat to high-yielding rice production in tropical Asia. *J. Asia Pac. Entomol.* 15: 122–140.
- Bravo, A., and M. Soberon. 2008. How to cope with insect resistance to Bt toxins? *Trends Biotechnol.* 26: 573–579.
- Carino, F. A., P. E. Kenmore, and V. A. Dyck. 1979. The FARMCOP suction sampler for hoppers and predators in flooded rice fields. *Int. Rice Res. News* 4: 21–22.
- Chen, M., G. Y. Ye, Z. C. Liu, Q. Fang, C. Hu, Y. F. Peng, and A. M. Shelton. 2009. Analysis of Cry1Ab toxin bioaccumulation in a food chain of Bt rice, an herbivore and a predator. *Ecotoxicology* 18: 230–238.
- Chen, M., A. Shelton, and G. Y. Ye. 2011a. Insect-resistant genetically modified rice in China: From research to commercialization. *Annu. Rev. Entomol.* 56: 81–101.
- Chen, Y., J. C. Tian, Z. C. Shen, Y. F. Peng, C. Hu, Y. Y. Guo, and G. Y. Ye. 2010. Transgenic rice plants expressing a fused protein of Cry1Ab/Vip3H has resistance to rice stem borers under laboratory and field conditions. *J. Econ. Entomol.* 103: 1444–1453.
- Chen, Y., J. C. Tian, Y. F. Peng, Y. Y. Guo, and G. Y. Ye. 2011b. Multi-generation effects of transgenic Cry1Ab/Vip3H rice G6H1 on development and reproduction of the non-target pest, *Nilaparvata lugens* (Stål). *Chin. J. Biol. Control* 27: 490–497.
- Cheng, J. A., and J. H. He. 1996. Rice insect pests. China Agriculture Press, Beijing, China.
- Cohen, M. B., M. Chen, J. S. Bentur, K. L. Heong, and G. Y. Ye. 2008. Bt rice in Asia: Potential benefits, impact, and sustainability, pp. 223–248. *In* J. Romeis, A. M. Shelton and E. J. Kennedy (eds.), *Integration of insect-resistant genetically modified crops within iPM programs*. Springer, Dordrecht, The Netherlands.
- Cui, J. J., and J. Y. Xia. 1998. Effects of Bt transgenic cotton (with early maturity) on population dynamic of main pests and their natural enemies. *Acta Gossypii Sin.* 10: 255–262.
- Dively, G. P. 2005. Impact of transgenic VIP3A x Cry1Ab lepidopteran-resistant field corn on the nontarget arthropod community. *Environ. Entomol.* 34: 1267–1291.
- Estruch, J. J., G. W. Warren, M. A. Mullins, G. J. Nye, J. A. Craig, and M. G. Koziel. 1996. Vip3A, a novel *Bacillus thuringiensis* vegetative insecticidal protein with a wide spectrum of activities against lepidopteran insects. *Proc. Natl. Acad. Sci. USA* 93: 5389–5394.
- Fang, J. 2008. The vegetative insecticidal protein genes of *Bacillus thuringiensis* and expression in transgenic rice. Ph.D., Zhejiang University Hangzhou, Zhejiang Province, China.
- Faria, C. A., F. L. Wäckers, J. Pritchard, D. A. Barrett, and T.C.J. Turlings. 2007. High susceptibility of Bt maize to aphids enhances the performance of parasitoids of lepidopteran pests. *PLoS ONE* 2: e600.
- Feng, Z. Q. 1990. Spiders of China in colour. Hunan Science and Technology. Changsha, China.
- Gulzar, A., B. Pickett, A. H. Sayyed, and D. J. Wright. 2012. Effect of temperature on the fitness of a Vip3A resistant population of *Heliothis virescens* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 105: 964–970.
- Han, Y., X. L. Xu, W. H. Ma, B. Q. Yuan, H. Wang, F. Z. Liu, M. Q. Wang, G. Wu, and H. X. Hua. 2011. The influence of transgenic *cry1Ab/cry1Ac*, *cry1C* and *cry2A* rice on non-target planthoppers and their main predators under field conditions. *Agric. Sci. China* 10: 1739–1747.
- Han, Y., J. Chen, H. Wang, J. Zhao, Y. P. He, and H. X. Hua. 2015. Prey-mediated effects of transgenic *cry2Aa* rice on the spider *Hylyphantes graminicola*, a generalist predator of *Nilaparvata lugens*. *Biocontrol* 60: 251–261.
- He, J. H., and X. F. Pang. 1986. Atlas of natural enemies on rice pests, pp. 208–236. Shanghai Scientific and Technical Publishers, Shanghai, China.

- He, J. H., Y. Ma, H. Q. Wang, Y. S. Shi, N. Z. Qiu, S. Z. Ren, X. X. Chen, Z. F. Chen, L. Y. Zhen, T. S. Pu, et al. 1991. Directory of natural enemies of rice insect pests in China, Science Press, Beijing, China.
- Heinrichs, E. A. 1994. Impact of insecticides on the resistance and resurgence of rice planthoppers, pp. 571–598. *In* R. F. Denno and T. J. Perfect (eds.), *Planthoppers: Their ecology and management*. Chapman & Hall, New York, NY.
- Heong, K. L., G. B. Aquino, and A. T. Barrion. 1991. Arthropod community structures of rice ecosystems in the Philippines. *Bull. Entomol. Res.* 81: 407–416.
- Heong, K. L. 2009. Are planthopper problems caused by a breakdown in ecosystem services, pp. 221–231. *In* K. L. Heong and B. Hardy (eds.), *Planthoppers: New threats to the sustainability of intensive rice production systems in Asia*. IRRI, Los Baños, Philippines.
- High, S. M., M. B. Cohen, Q. Y. Shu, and I. Altosaar. 2004. Achieving successful deployment of Bt rice. *Trends Plant Sci.* 9: 286–292.
- Huang, J. K., R. F. Hu, S. Rozelle, and C. Pray. 2005. Insect-resistant GM rice in farmers' fields: Assessing productivity and health effects in China. *Science* 308: 688–690.
- Jackson, R. E., M. A. Marcus, F. Gould, J. R. Bradley, and J. W. Van Duyn. 2007. Cross-resistance responses of Cry1Ac-selected *Heliothis virescens* (Lepidoptera: Noctuidae) to the *Bacillus thuringiensis* protein Vip3A. *J. Econ. Entomol.* 100: 180–186.
- Karim, S., and D. H. Dean. 2000. Toxicity and receptor binding properties of *Bacillus thuringiensis* delta-endotoxins to the midgut brush border membrane vesicles of the rice leaf folder, *Cnaphalocrocis medinalis* and *Marasmia patnalis*. *Curr. Microbiol.* 41: 276–283.
- Lee, M. K., R. M. Aguda, M. B. Cohen, F. L. Gould, and D. H. Dean. 1997. Determination of binding of *Bacillus thuringiensis* δ -endotoxin receptors to rice stem borer midguts. *Appl. Environ. Microbiol.* 63: 1453–1459.
- Lee, M. K., F. S. Walters, H. Hart, N. Palekar, and J. S. Chen. 2003. The mode of action of the *Bacillus thuringiensis* vegetative insecticidal protein Vip3A differs from that of Cry1Ab δ -endotoxin. *Appl. Environ. Microbiol.* 69: 4648–4657.
- Lee, M. K., P. Miles, and J. S. Chen. 2006. Brush border membrane binding properties of *Bacillus thuringiensis* Vip3A toxin to *Heliothis virescens* and *Helicoverpa zea* midguts. *Biochem. Biophys. Res. Commun.* 339: 1043–1047.
- Li, F. F., G. Y. Ye, Q. Wu, Y. F. Peng, and X. X. Chen. 2007. Arthropod abundance and diversity in Bt and non-Bt rice fields. *Environ. Entomol.* 36: 646–654.
- Li, K., J. Tian, Q. Wang, Q. Chen, M. Chen, H. Wang, Y. Zhou, Y. Peng, J. Xiao, and G. Ye. 2011. Application of a novel method PCR-ligase detection reaction for tracking predator-prey trophic links in insect-resistant GM rice ecosystem. *Ecotoxicology* 20: 2090–2100.
- Littel, R., G. A. Milliken, W. W. Stroup, R. D. Wolfinger, and O. Schabenberger. 2006. SAS[®] for mixed models. SAS Institute Inc., Cary, NC.
- 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. Sin.* 29: 138–144.
- Liu, Z. C., G. Y. Ye, C. Hu, and S. K. Datta. 2003. Impact of transgenic *indica* rice with a fused gene of *cry1Ab/cry1Ac* on the rice paddy arthropod community. *Acta Entomol. Sin.* 46: 454–465.
- Llewellyn, D. J., C. L. Mares, and G. P. Fitt. 2007. Field performance and seasonal changes in the efficacy against *Helicoverpa armigera* (Hübner) of transgenic cotton expressing the insecticidal protein Vip3A. *Agric. For. Entomol.* 9: 93–101.
- Lou, Y. G., G. R. Zhang, W. Q. Zhang, Y. Hu, and J. Zhang. 2013. Biological control of rice insect pests in China. *Biol. Control* 67: 8–20.
- Lu, Y. H., K. M. Wu, Y. Y. Jiang, B. Xia, P. Li, H. Q. Feng, K. A. G. Wyckhuys, and Y. Y. Guo. 2010. Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of Bt cotton in China. *Science* 328: 1151–1154.
- Lu, Y. H., K. M. Wu, Y. Y. Jiang, Y. Y. Guo, and N. Desneux. 2012. Widespread adoption of Bt cotton and insecticide decrease promotes bio-control services. *Nature* 487: 362–365.
- Lu, Z. B., N. S. Han, G. Xu, Y. E. Liu, C. Hu, Y. F. Peng, Y. Y. Guo, and G. Y. Ye. 2013. Impacts of transgenic *cry1Ab/vip3H+epsps japonica* rice on the population dynamics of its non-target herbivores, rice leafhoppers, under field conditions. *Acta Entomol. Sin.* 56: 1275–1285.
- Lu, Z. B., N. S. Han, J. C. Tian, Y. F. Peng, C. Hu, Y. Y. Guo, Z. C. Shen, and G. Y. Ye. 2014a. Transgenic *cry1Ab/vip3H+epsps* rice with insect and herbicide resistance acted no adverse impacts on the population growth of a non-target herbivore, the white-backed planthopper, under laboratory and field conditions. *J. Integr. Agric.* 13: 2678–2689.
- Lu, Z. B., J. C. Tian, N. S. Han, C. Hu, Y. F. Peng, D. Stanley, and G. Y. Ye. 2014b. No direct effects of two transgenic Bt rice lines, T1C-19 and T2A-1, on the arthropod communities. *Environ. Entomol.* 43: 1453–1463.
- Ludy, C., and A. Lang. 2006. Bt maize pollen exposure and impact on the garden spider, *Araneus diadematus*. *Entomol. Exp. Appl.* 118: 145–156.
- Mahon, R. J., S. J. Downes, and B. James. 2012. Vip3A resistance alleles exist at high levels in Australian targets before release of cotton expressing this toxin. *PLoS ONE* 7: e39192.
- Meissle, M., and J. Romeis. 2009. The web-building spider *Theridion impresum* (Araneae: Theridiidae) is not adversely affected by Bt maize resistant to corn rootworms. *Plant Biotechnol. J.* 7: 645–656.
- Meissle, M. 2013. Side effects of *Bacillus thuringiensis* toxins on spiders, pp. 429–439. *In* W. Nentwig (ed.), *Spider ecophysiology*. Springer-Verlag, Berlin.
- Naranjo, S. E. 2005. Long-term assessment of the effects of transgenic Bt cotton on the abundance of nontarget arthropod natural enemies. *Environ. Entomol.* 34: 1193–1210.
- Oksanen, J., F. G. Blanchet, R. Kindt, P. Legendre, P. R. Minchin, R. B. O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, and H. Wagner. 2013. vegan: Community ecology package R package version 2.0-9. (<http://CRAN.R-project.org/package=vegan>).
- R Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. (<http://www.R-project.org/>).
- Raybould, A., and D. Vlachos. 2011. Non-target organism effects tests on Vip3A and their application to the ecological risk assessment for cultivation of MIR162 maize. *Transgenic Res.* 20: 599–611.
- Romeis, J., M. Meissle, and F. Bigler. 2006. Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nat. Biotechnol.* 24: 63–71.
- SAS Institute. 2001. SAS V9.1 Help. SAS Institute Inc, Cary, NC.
- Shelton, A. M., J. Z. Zhao, and R. T. Roush. 2002. Economic, ecological, food safety, and social consequences of the deployment of Bt transgenic plants. *Annu. Rev. Entomol.* 47: 845–881.
- Song, D. X., M. S. Zhu, and J. Chen. 1999. The spiders of China. Hebei Science and Technology Publishing House, Shijiazhuang, China.
- Ter Braak, C. J. F., and P. Smilauer. 2002. Canoco 4.5: Reference manual and canodraw for windows user's guide: Software for Canonical community ordination (version 4.5), Microcomputer Power, Ithaca, NY.
- Tian, J. C., Z. C. Liu, H. W. Yao, G. Y. Ye, and Y. F. Peng. 2008. Impact of transgenic rice with a *cry1Ab* gene on parasitoid subcommunity structure and the dominant population dynamics of parasitoid wasps in rice paddy. *J. Environ. Entomol.* 30: 1–7.
- Tian, J. C., Z. C. Liu, M. Chen, Y. Chen, X. X. Chen, Y. F. Peng, C. Hu, and G. Y. Ye. 2010. Laboratory and field assessments of prey-mediated effects of transgenic Bt rice on *Ummeliata insecticeps* (Araneida: Linyphiidae). *Environ. Entomol.* 39: 1369–1377.
- Tian, J. C., Y. Chen, Z. L. Li, K. Li, M. Chen, Y. F. Peng, C. Hu, A. M. Shelton, and G. Y. Ye. 2012. Transgenic *Cry1Ab* rice does not impact ecological fitness and predation of a generalist spider. *PLoS ONE* 7: e35164.
- Tu, J. M., G. A. Zhang, K. Datta, C. G. Xu, Y. Q. He, Q. F. Zhang, G. S. Khush, and S. K. Datta. 2000. Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* delta-endotoxin. *Nat. Biotechnol.* 18: 1101–1104.
- Van den Brink, P. J., R. P. A. Van Wijngaarden, W. G. H. Lucassen, T. C. M. Brock, and P. Leeuwangh. 1996. Effects of the insecticide Dursban[®] 4E

- (active ingredient chlorpyrifos) in outdoor experimental ditches: II. Invertebrate community responses and recovery. *Environ. Toxicol. Chem.* 15: 1143–1153.
- Van den Brink, P. J., and C.J.F. Ter Braak. 1999. Principal response curves: Analysis of time-dependent multivariate responses of biological community to stress. *Environ. Toxicol. Chem.* 18: 138–148.
- Wang, Y. M., G. A. Zhang, J. P. Du, B. Liu, and M. Q. Wang. 2010. Influence of transgenic hybrid rice expressing a fused gene derived from *cry1Ab* and *cry1Ac* on primary insect pests and rice yield. *Crop Prot.* 29: 128–133.
- Whitehouse, M.E.A., L. J. Wilson, and G. P. Fitt. 2005. A comparison of arthropod communities in transgenic Bt and conventional cotton in Australia. *Environ. Entomol.* 34: 1224–1241.
- Whitehouse, M.E.A., L. J. Wilson, and G. A. Constable. 2007. Target and non-target effects on the invertebrate community of Vip cotton, a new insecticidal transgenic. *Aust. J. Agric. Res.* 58: 273–285.
- Whitehouse, M.E.A., L. J. Wilson, A. P. Davies, D. Cross, P. Goldsmith, A. L. Thompson, S. Harden, and G. Baker. 2014. Target and nontarget effects of novel “triple-stacked” Bt-transgenic cotton 1: Canopy arthropod communities. *Environ. Entomol.* 43: 218–241.
- Wolfenbarger, L.L.R., S. E. Naranjo, J. G. Lundgren, R. J. Bitzer, and L. S. Watrud. 2008. Bt crop effects on functional guilds of non-target arthropods: A meta-analysis. *PLoS ONE* 3: e2118.
- Xia, S. Y., H. F. Wu, and Z. P. Wang. 1988. Primary colored iconograph of entomophagous insects in paddy fields. Hunan Press of Science and Technology, Changsha, China.
- Ye, G. Y., Q. Y. Shu, H. R. Cui, C. Hu, M. W. Gao, Y. W. Xia, X. Cheng, and I. Altosaar. 2000. A leaf-section bioassay for evaluating rice stem borer resistance in transgenic rice containing a synthetic *cry1Ab* gene from *Bacillus thuringiensis* Berliner. *Bull. Entomol. Res.* 90: 179–182.
- Ye, G. Y., Q. Y. Shu, H. W. Yao, H. R. Cui, X. Y. Cheng, C. Hu, Y. W. Xia, M. W. Gao, and I. Altosaar. 2001a. Field evaluation of resistance of transgenic rice containing a synthetic *cry1Ab* gene from *Bacillus thuringiensis* Berliner to two stem borers. *J. Econ. Entomol.* 94: 271–276.
- Ye, G. Y., J. M. Tu, C. Hu, and S. K. Datta. 2001b. 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., H. W. Yao, Q. Y. Shu, X. Cheng, C. Hu, Y. W. Xia, M. W. Gao, and I. Altosaar. 2003. High levels of stable resistance in transgenic rice with a *cry1Ab* gene from *Bacillus thuringiensis* Berliner to rice leaffolder, *Cnaphalocrocis medinalis* (Guenée) under field conditions. *Crop Prot.* 22: 171–178.
- Yuan, Y. Y., N. W. Xiao, P. H. Krogh, F. J. Chen, and F. Ge. 2013. Laboratory assessment of the impacts of transgenic Bt rice on the ecological fitness of the soil non-target arthropod, *Folsomia candida* (Collembola: Isotomidae). *Transgenic Res.* 22: 791–803.
- Zhao, J. Z., J. Cao, Y. X. Li, H. L. Collins, R. T. Roush, E. D. Earle, and A. M. Shelton. 2003. Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. *Nat. Biotechnol.* 21: 1493–1497.
- Zheng, L. Y., and H. Gui. 1999. Insect classification (Volume 1 and 2). Nanjing Normal University Press, Nanjing, China.
- Zhou, X., J. A. Cheng, Y. Hu, and Y. G. Lou. 2005. Effects of transgenic Bt rice on the population development of *Nephotettix cincticeps*. *Chin. J. Rice Sci.* 19: 74–78.