Insect Science (2006) 13, 409–420

Impact of insect-resistant transgenic rice on target insect pests and non-target arthropods in China

MAO CHEN[1], JIAN-ZHOU ZHAO[1], GONG-YIN YE[2], QIANG FU[3], and ANTHONY M. SHELTON[1]

[1]Department of Entomology, Cornell University-NYSAES, Geneva, New York, USA, [2]State Key Laboratory of Rice Biology, Institute of Applied Entomology, Zhejiang University and [3]State Key Laboratory of Rice Biology, China National Rice Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, Zhejiang, China

Abstract Progress on the research and development of insect-resistant transgenic rice, especially expressing insecticidal proteins from *Bacillus thuringiensis* (Bt), in China has been rapid in recent years. A number of insect-resistant transgenic rice lines/varieties have passed restricted and enlarged field testing, and several have been approved for productive testing since 2002 in China, although none was approved for commercial use until 2006. Extensive laboratory and field trials have been conducted for evaluation of the efficiency of transgenic rice on target lepidoteran pests and potential ecological risks on non-target arthropods. The efficacy of a number of transgenic rice lines currently tested in China was excellent for control of the major target insect pests, the rice stem borers (*Chilo suppressalis*, *Scirpophaga incertulas*, *Sesamia inferens*) and leaffolder (*Cnaphalocrocis medinalis*), and was better than most insecticides extensively used by millions of farmers at present in China. No significantly negative or unintended effects of transgenic rice on non-target arthropods were found compared with non-transgenic rice. In contrast, most of the current insecticides used for the control of rice stem borers and leaffolders proved harmful to natural enemies, and some insecticides may directly induce resurgence of rice planthoppers. Studies for developing a proactive insect resistance management of transgenic rice in the future are discussed to ensure the sustainable use of transgenic rice.

Key words Bt toxin, insect resistance, non-target effect, target insects, transgenic rice
DOI 10.1111/j.1744-7917.2006.00110.x

Introduction

As one of the centers of the origin of rice, China has more than a 7000-year history of planting rice, which is a staple food not only for China, but also for over half of those living in Asia and for nearly two billion people worldwide (Khush, 1997). Since the 1980s, rice yields have increased due to adoption of high-yielding rice varieties and hybrid rice. However, the growth rate of the rice-consuming population is now higher than that of rice yields. Therefore, severe food shortages will probably occur in the future if the trend is not reversed (Khush, 1995). Simultaneously, rice yield loss caused by rice insect pests, such as the striped stem borer *Chilo suppressalis* (Walker), yellow stem borer *Scirpophaga incertulas* (Walker), pink stem borer *Sesamia inferens* (Walker) and leaffolder *Cnaphalocrocis medinalis* (Guenée), is a threat to rice production. Savary *et al*. (2000) reported that 24%–41% of rice yield was lost every year because of rice stem borers and other insect pests, diseases and weeds. Rice stem borers could annually cause 5%–10% yield loss (Pathak & Khan, 1994). In China, although different chemical insecticides were applied frequently in order to control rice stem borers, 3.1% of gross rice production, being equal to 6.45

Correspondence: Jian-Zhou Zhao, Department of Entomology, Cornell University-NYSAES, Geneva, NY 14456, USA. Tel: +1 315 787 2453; fax: +1 315 787 2326; email: jz49@cornell.edu, zhaojz@yahoo.com

billion yuan, was lost annually because of the damage of stem borers (Sheng *et al*., 2003). The cryptic habits of stem borers protect them from insecticidal sprays, making careful timing of systemic insecticide applications at high rates necessary for effective control. Resistance development to the major classes of insecticides in rice stem borers further decreased their efficacy in rice fields (Jiang *et al*., 2001, 2005b; Li *et al*., 2001; Chi *et al*., 2005; Peng *et al*., 2001).

Transgenic plants expressing insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) were grown in 2005 on 26.3 million ha worldwide (James, 2005). These crops have provided economic benefits to growers and reduced the use of insecticides (Shelton *et al*., 2002; James, 2005). Advances in insect-resistant transgenic rice in China and other countries in recent years also offer a promising alternative to chemical insecticides for control of lepidopteran pests in rice (Zhu, 2001; High *et al*., 2004). Bt rice has the potential to eliminate yield losses caused by lepidopteran pests of up to 2%–10% of Asia's annual rice yield of 523 million tons (High *et al*., 2004). In the past decade, numerous transgenic rice lines conferring high resistance against rice stem borers and leaffolders have been developed (Tu *et al*., 2000; High *et al*., 2004; Chen *et al*., 2005b). Academic institutions and scientists in China, the leading country in rice production and consumption, have been involved in Bt rice research in recent years, supported primarily by the government (Huang *et al*., 2002, 2005). Although not yet commercialized in China, large-scale productive testing of different Bt rice lines were approved in 2002 both in experimental station fields and in farmer fields in at least 13 sites. Restricted (up to 0.26 ha in total in one or two provinces and up to three locations in each province) and enlarged (0.27–2.0 ha in total in one or two provinces and up to seven locations in each province) field testing has been conducted for more transgenic rice lines since 1998 before applying the productive testing (2.1–66.7 ha in total in one or two provinces and up to five locations in each province for each application). These large-scale trials verified yield increases of approximately 6%–9% with an 80% reduction in pesticide usage (Huang *et al*., 2005) and an overall increase in net income per hectare of US$80–100 (James, 2005). All these efforts have made China one of the most advanced countries for research and development of transgenic insecticidal rice, although Iran was the first country to commercialize Bt rice in 2005 (James, 2005) and several other Asian countries are interested in commercial use of Bt rice in the future. This paper reviews briefly the transgenic insecticidal rice developed or field-tested in China, focusing on evaluating the impact of transgenic rice on target and non-target arthropods.

Insect-resistant transgenic rice lines in China

Several insect-resistant transgenic rice events and over a dozen transgenic rice lines have been developed or field-tested in China (Table 1). Some of these contain a single Bt insecticidal gene such as *cry1Ab* in Kemingdo (KMD) (Shu *et al*., 2000), *cry1Ab*/*1Ac* fusion gene in hybrid line Bt Shanyou 63 (Tu *et al*., 2000) or *cry2A* (Chen *et al*., 2005a). Others are pyramided with dual insecticidal genes of modified *CpTI* (cowpea trypsin inhibitor) and *cry1Ac* in MSA/MSB (Zhao *et al*., 2004) that have different binding sites and insecticidal mechanisms with increased potential to delay insect resistance development (Zhao *et al*., 2003). Some transgenic Bt rice lines were stacked with other types of genes, including *bar* for herbicide tolerance (Zhu *et al*., 1999; Yao *et al*., 2002), and *xa21* for disease resistance (Wang *et al*., 2002a). Such stacked traits reflect the needs of rice growers who have to simultaneously address the multiple yield constraints associated with various biotic and abiotic stresses, and will likely become widely adapted (James, 2005). The different insecticidal genes with different promoters were transferred into several rice varieties, and the expression levels of the insecticidal proteins varied in different events or lines, as shown in Table 1.

Among the different transgenic insecticidal rice lines, KMD (containing *cry1Ab*), Bt Shanyou 63 (containing *cry1A*) and GM Youming 86 (containing modified *CpTI*) have been in productive testing in fields since 2002 as the first batch of transgenic rice in China entered the last step before commercialization. KMD was developed at Zhejiang University in collaboration with the University of Ottawa (Cheng *et al*., 1998). From KMD several hybrid lines (such as B1 and B6) (Chen *et al*., 2003c) were developed. The Bt rice lines Shanyou 63 and Huahui 1 (Bt Minghui 63), both expressing *cry1Ab*/*Ac* fusion gene, were developed at Huazhong Agricultural University in collaboration with the International Rice Research Institute (Tu *et al*., 2000), and several hybrid varieties, such as Bt Shanyou 63, were derived from this Bt restorer line (Tu *et al*., 1998, 2000). GM Youming 86, as well as GM Minghui 86 (MSA and MSB restorer lines) and hybrid lines Kefeng 6 (II-32A/MSB) containing *CpTI*+*cry1Ac* genes, were developed at the Institute of Genetics and the Developmental Biology of the Chinese Academy of Sciences collaborated in this with other laboratories (An *et al*., 2001; Zhu, 2001; Zhao *et al*., 2004).

Efficacy of transgenic rice lines on target lepidopteran pests

In order to evaluate the efficacy of transgenic rice on target

Table 1 Transgenic rice with resistance to lepidopteran pests in China.

Gene transformed	Promoter, method of transformation	Parental line or cultivar	Name of transgenic line	Expression level (% soluble protein or ng/mg)	Regulatory stage	Reference
cry1Ab/Ac fusion	Actin1, Biolistic	CMS Minghui 63 (indica rice)	T51-1 (CMS), Bt Shanyou 63 (hybrid)	20.00 ng/mg	Productive testing (2002−current)	Tu *et al*., 2000
cry2A	Ubiquitin, Agrobacterium-mediated	GM Minghui 63	–	9.65−12.11 ng/mg	–	Chen *et al*., 2005a
cry1Ab	Ubiquitin, Agrobacterium-mediated	Xiushui 11 (japonica rice)	KMD (1, 2); PR16, PR18	0.018%−0.136%	Productive testing (2002−current)	Xiang *et al*., 1998; Shu *et al*., 2000
CpTI	Actin1, Biolistic	Minghui 86 (indica rice)	Kefeng (1, 2)	3.30%	Productive testing (2002−current)	An *et al*., 2001; Zhu, 2001
CpTI+cry1Ac	Ubiquitin and Actin1, Biolistic	Minghui 86 (indica rice)	MSA, MSB, Kefeng 6	Up to 1.20 ng/mg; Up to 1.00 ng/mg	Productive testing (2004−current)	Zhao *et al*., 2004; Liu *et al*., 2005a,b
cry1Ab/Ac fusion	Actin1, Biolistic	IR72 (indica rice)	TT9-3, TT9-4	0.01%	–	Ye *et al*., 2001b; Liu *et al*., 2003a
cry1Ab+ pinII+bar	Actin1, Biolistic	Zhonguo 91 (japonica rice)	–	–	–	Zhu *et al*., 1999 Yao *et al*., 2002
cry1Ab+bar+Xa21	Actin1, Biolistic	Zhonguo 91	C48	–	–	Wang *et al*., 2002a

lepidopteran pests, laboratory and field trials have been conducted in China since 1998 and the results indicate that Bt rice has effectively controlled target pests, primarily three species of stem borers (*C. suppressalis*, *S. incertulas*, *S. inferens*) and leaffolder (*C. medinalis*) (Tu *et al.*, 2000; High *et al.*, 2004; Li *et al.*, 2004, 2005; Chen *et al.*, 2005b; Han *et al.*, 2006b) (Table 2). By using leaf-section (Ye *et al.*, 2000) or stem-section (Chen *et al.*, 2005a) bioassays under laboratory conditions, and artificial release or natural infestation of rice borers under field conditions (Ye *et al.*, 2001a, b), the efficacy of different Bt rice lines has been tested against at least eight lepidopteran pests, including *C. suppressalis*, *S. incertulas*, *C. medinalis*, *Herpitogramma licarisalis*, *S. inferens*, *Naranga anescens*, *Mycalesis gotama* and *Parnara guttata* (Shu *et al.*, 2000). Generally, the laboratory results indicated that the mortality of early-stage larvae of different lepidopteran pests fed with transgenic rice was more than 90%, while the mortality was < 5% in the non-transgenic parental lines. However, control of larger stem borer larvae was lower. Hu *et al.* (2005) reported that the corrected mortality of first to sixth instar *C. suppressalis* after feeding on Bt+CpTI rice for 7 days was 89.6%, 87.1%, 72.4%, 50%, 26% and 0%, respectively.

The results of plot trials (plot size up to 60−120 m^2) and large-scale field trials conducted in different sites in China over multiple seasons indicated the percentages of dead-heart or white-head caused by rice stem borers in Bt rice paddies were significantly lower than those in non-Bt rice paddies. Bt rice in the field provided 90%−100% control of rice stem borers (Tu *et al.*, 2000; Wang *et al.*, 2000, 2002c; Ye *et al.*, 2003; Gao *et al.*, 2006b) and 84%−100% control of leaffolders (Tu *et al.*, 2000; Ye *et al.*, 2003; Liu *et al.*, 2005b). Ye *et al.* (2003) found Bt rice line KMD2 gave complete control for rice leaffolder; in contrast, 66.7%−92.2% of plants were damaged by rice leaffolder in non-Bt plots after one spray of a tank mixture of methamidophos and disosultap (87.7%−100% mortality). However, because *S. inferens* was more tolerant to Cry1Ab and Cry1Ac than *C. suppressalis*, the efficacy of MSA, MSB and Kefeng 6 was much lower on *S. inferens* than on *C. suppressalis* in field plot experiments, especially in double-crop late rice fields (Gao *et al.*, 2006b).

Transgenic insecticidal rice showed much better control of rice stem borers than most insecticides currently and extensively used by farmers in China. Insecticide sprays have been the major method to control rice borers in the past decades in China. However, the efficiency of chemical insecticides declined because of the evolution of resistance. During the past three decades, chemical control of rice borers was difficult because of the rapid development of insect resistance, insecticide residue and effects on the environment. Farmers initially used organochlorine and organophosphate insecticides, then changed to chlordimeform and nereistoxin, and then back to organophosphate because some new insecticides such as triazophos were reintroduced for rice stem borer control in the late 1990s. After the stem borers developed resistance to monosultap and fenutorthion in the late 1990s (Li *et al.*, 2001), a high level of resistance to triazophos was also detected soon after its extensive use (Peng *et al.*, 2001; Qu *et al.*, 2003). In recent years rice stem borers developed resistance to most commonly used insecticides in several provinces in China (Chi *et al.*, 2005; Jiang *et al.*, 2005b). After approval for commercial use, transgenic insecticidal rice will become an important tool both for pest management and insecticide resistance management of the target pests.

Effects of Bt rice lines on non-target arthropods

Controversy about the benefits and ecological risks of transgenic crops has existed since their advent, and became fiercer after the first three Bt crops were commercialized in 1996 (Shelton & Sears, 2001). Potential impact, especially on non-target organisms, was a major concern with the deployment of transgenic crops (Romeis *et al.*, 2006). During the past decade of commercialization of transgenic crops, dozens of research articles or news relating to biosafety of transgenic crops were published monthly, especially after the "Pusztai case" and monarch butterfly debates (Dorcey & Serrano, 2002). Much of the controversy relates to evaluation methods of ecological risks or reasonable explanation of research data/results of risk assessments.

Rice, as a major staple food crop for humans, evokes considerable concern about food and environmental safety. Many trials have evaluated the potential impact of Bt rice on non-target organisms under laboratory and field conditions (Table 3), some over multiple years and sites. In general, compared with non-transgenic rice, transgenic rice did not negatively impact the arthropod community as determined by guild dominance, family composition, diversity index, evenness index and species richness (Chen *et al.*, 2003b, d; Liu *et al.*, 2003a; Liu *et al.*, 2005a), or by the dominant species dynamics of non-target herbivores (Chen *et al.*, 2006). No difference on feeding and oviposition behavior of non-target insects *Nilaparvata lugens* (Chen *et al.*, 2003a, c), insect development (Wang *et al.*, 2002b), or population dynamics (Zhou *et al.*, 2004) was detected. Jiang *et al.* (2004) reported that parasitization rates of *C. suppressalis* larvae feeding on Bt (Cry1Ab) rice by *Apanteles chilonis* and the percentage of cocoon formation were significantly lower than those on non-Bt rice.

Table 2 Efficacy of main transgenic Bt rice events used in China.

Gene transformed	Line	% efficacy to target pests: In laboratory/greenhouse	% efficacy to target pests: In field	References
cry1Ab/Ac	Bt-Shanyou 63	–	100% for *C. medinalis* 98.9% for *S. incertulas*	Tu *et al.*, 2000
	T51-1	100% for *S. incertulas*	84.8% for *C. medinalis* 91.4%–95.7% for *S. incertulas*	
crylAb	KMD1 (PR16)	100% for 8 lepidopteran rice pest species (neonate or 3-instar larvae)	100% for *C. suppressalis*, *S. incertulas* and *C. medinalis*	Shu *et al.*, 2000; Ye *et al.*, 2001a; Ye *et al.*, 2003
	KMD2 (PR18)	100% for *C. suppressalis* and *S. incertulas*	100% for *C. suppressalis* and *C. medinalis*	Wang *et al.*, 2000; Wang *et al.*, 2002c
CpTI+cry1Ac	MSA	92.8%–100% for *C. suppressalis* and 98.1% –100 %for *C. medinalis* on different development stage of *Bt* rice	99.79% for *C. medinalis*	Zhao *et al.*, 2004
	MSB	79.3%–98.9% for *C. suppressalis* and 94.3% –100% for *C. medinalis* on different development stage of Bt rice	> 99.82% for *S. inferens* > 92.7% for *C. suppressalis* > 99.37% for *S. incertulas* > 84% for *C. medinalis*	Zhao *et al.*, 2004; Li *et al.*, 2004; Hu *et al.*, 2005; Li *et al.*, 2005; Liu *et al.*, 2005a,b
	II-32A/MSB	–	> 99% for *C. medinalis*	Liu *et al.*, 2005a,b
cry1Ab/Ac	TT9-3, TT9-4	–	> 90% for *S. inferens, C. suppressalis, S. incertulas, C. medinalis* and *N. anescens*	Ye *et al.*, 2001b
cry1Ab+pinII+bar	–	100% for *C. suppressalis*		Zhu *et al.*, 1999; Yao *et al.*, 2002
cry1Ab+bar+Xa21	C48, SK(1,2,3)	–	> 99% for *C. suppressalis*	Wang *et al.*, 2002a; Ci *et al.*, 2005

Table 3 Impacts of transgenic rice on non-target organisms compared with non-transgenic rice.

Non-target organisms	Insecticidal protein	Parameters measured	Result	Reference
Herbivorous	Cry1Ab/Ac	Feeding and oviposition behavior of *N. lugens*; species composition, dispersal and dominant species dynamics of Delphacidae and Cicadellidae; guild dominance in the arthropod community, family composition, dynamics of arthropod individual density, dominant index, diversity index, evenness index and species richness, Bray-Curtis distance index	No negative impacts	Liu *et al.*, 2003a; Liu *et al.*, 2004; Chen *et al.*, 2006; Chen *et al.*, 2003a
	CpTI+Cry1Ac	Species richness, number of individuals, diversity, evenness and dominant concentration indices; nymphal duration, fresh weight of new adult, emergence rate, adult fecundity, rate of eggs fertilized, number of offsprings per female and their development, host preference behavior of *N. lugens* and *Sogatella fucifera*	No negative impacts except for the impact of one Bt rice line MSB on fresh weight of new adult and brachypterous rate of *S. fucidera*, fresh weight of new adult of *N. lugens*	Chen *et al.*, 2003c; Fu *et al.*, 2003; Liu *et al.*, 2005a
	Cry1Ab	Development and midgut sub-microstructure of *Bombyx mori* L.; feeding and oviposition behavior of *Nilaparvata lugens*; innate capacity of increase of *Nephotettix cincticeps*	No negative impacts except for weight of larvae and cocoons, cocoons layers and the number of grown *B. mori*; longevity and fecundity of *N. cincticeps* as well	Wang *et al.*, 2002b; Chen *et al.*, 2003a; Zhou *et al.*, 2004
Parasitoids	Cry1Ab/Ac	Dispersal dynamics of *Anagrus* spp. and *Lymaenon longicrus*; guild dominance in the arthropod community, family composition and dominance, dynamics of arthropod individual density, dominant index, diversity index, evenness index and species richness, Bray-Curtis distance index	No negative impacts	Chen *et al.*, 2003b; Liu *et al.*, 2003a
	CpTI+Cry1Ac	Pupa formation, duration of egg and larva, cocoon number of simple cocoon mass, wasp emergence rate, sex ratio, longevity and forewing length of *Apanteles chilonis* on *C. suppressalis*	No negative impacts except for pupal duration, length of cocoon of *A. chilonis*	Jiang *et al.*, 2005a
	Cry1Ab	Parasitized rates, percentages of cocoon formation, developmental period of the parasitoid pupa, longevity of male and length of cocoon of *A. chilonis* on *C. suppressalis*	Negative impacts	Jiang *et al.*, 2004a
Predators	Cry1Ab/Ac	Guild dominance in the arthropod community, family composition, dynamics of arthropod individual density, dominant index, diversity index, evenness index and species richness, Bray-Curtis distance index	No negative impacts	Liu *et al.*, 2003a
	Cry1Ab	Predation and functional response of *Pirata subpiraticus* and *Microvelia horvathi*	No negative impacts	Liu *et al.*, 2003b; Bai *et al.*, 2005; Chen *et al.*, 2005c;
Detritivores	Cry1Ab/Ac	Guild dominance in the arthropod community, family composition, dynamics of arthropod individual density, dominant index, diversity index, evenness index and species richness, Bray-Curtis distance index	No negative impacts	Liu *et al.*, 2003a
	Cry1Ab	Population densities of *Entomobrya griseoolivata* and *Bourletiella christianseni*	No negative impacts	Bai *et al.*, 2005
Others	Cry1Ab/Ac	Guild dominance in the arthropod community, family composition, dynamics of arthropod individual density, dominant index, diversity index, evenness index and species richness, Bray-Curtis distance index; the number and species of aquatic organisms	No negative impacts	Chen *et al.*, 2003d; Liu *et al.*, 2003a

However, this finding is likely caused by the poor quality of the insect host rather than direct toxicity of Bt protein (Romeis *et al.*, 2006) because *C. suppressalis* was not able to complete development on Bt rice.

Bt rice did not adversely impact predation and functional response of predatory natural enemies compared with non-Bt rice (Bai *et al.*, 2005; Chen *et al.*, 2005c; Liu *et al.*, 2003b). Liu *et al.* (2004) did not find any significant differences in non-target organisms between Bt and non-Bt rice; however, the guild dominance of herbivores and parasitoids and family composition of predators and detritivores in non-Bt rice paddies were significantly impacted by chemical insecticides compared with non-insecticide treatments in Bt or non-Bt rice fields. In fact, most insecticides currently used for the control of rice stem borers and leaffolder in China are broad-spectrum chemicals that are known to be harmful to natural enemies in rice fields (Tanaka *et al.*, 2000). Insecticide-induced resurgence has been a serious problem in the management of rice planthoppers in recent years (Wang *et al.*, 1994; Wu *et al.*, 2001), especially after the outbreak of *N. lugens* in 2005 in several provinces in China (Gao *et al.*, 2006a).

Proactive insect resistance management consideration

While transgenic plants offer many unique opportunities for the management of pest populations, one of the major concerns for long-term use of Bt plants is the potential for the evolution of insect resistance (Gould, 1998; Shelton *et al.*, 2002). Insect resistance management (IRM) has generated more data, meetings, and public comments than any other issue related to the re-registration of Bt crops in the US by the USEPA (2001). After 8–10 years of extensive use of Bt crops in the field, there have been no reports of product failure caused by increased insect resistance (Tabashnik *et al.*, 2003, 2005). However, because of the evolution of resistance to Bt proteins in commercial settings by two insect species, the diamondback moth (*Plutella xylostella*) in the fields (Tabashnik *et al.*, 1990; Shelton *et al.*, 1993) and the cabbage looper (*Trichoplusia ni*) in commercial greenhouses (Janmaat & Myers, 2003; Kain *et al.*, 2004), there is concern that some insects may also evolve resistance to Bt crops in the field. Proactive IRM strategies will be critical for a sustainable use of Bt rice in the future (High *et al.*, 2004). An IRM strategy, especially the structured refuge, is a mandatory requirement for registration and commercial use of Bt crops in some countries such as the US and Australia, but not in China.

Among the various options that have been considered for IRM for Bt crops, "high dose/refuge" and gene pyramiding strategies are most promising over the foreseeable future (Gould, 1998; Bates *et al.*, 2005). As the major IRM strategy currently used in the US, the "high dose/refuge" calls for high expression of Bt protein in Bt plants, that is, 25 times the protein concentration needed to kill susceptible target insects (SS genotype), which is expected to cause very high mortality (> 95%) of resistant heterozygotes (RS genotype) (USEPA, 1998). It is known that the Bt protein expression level in Bt rice is much lower than that in Bt corn (Tu *et al.*, 2000), and there were differences in the susceptibility of each target pest of Bt rice to the same or different Bt proteins (Fu *et al.*, unpublished data, 2006). A 95% or 100% kill of a target pest by Bt rice plants in the greenhouse or fields, or a confirmed Bt protein expression as high as 20 ng/mg might be a good indication of excellent control for certain insect species. However, an excellent control efficacy by itself cannot meet the definition of "high dose" for the IRM strategy.

Before a resistant strain (RR genotype) of target insects is available to produce the RS genotype for a direct assessment of high dose, a Bt plant could be considered to provide a "high dose" if verified by at least two of the five approaches proposed by USEPA (1998, 2001). Grower acceptance and logistical feasibility is also a key factor in designing a "high dose/refuge" strategy and program (USEPA, 1998, 2001). Similar to the Bt cotton situation in China (Zhao & Rui, 2004), a structured refuge is not feasible for millions of small farmers who grow Bt cotton or Bt rice. The uncertainty of a "high dose" of a single Bt protein in Bt rice plus the non-feasibility of planting a structured refuge by most farmers is a great challenge for the development and sustainable use of Bt rice in China and other Asian countries. Cohen *et al.* (2000) suggested that single-gene Bt rice not be released because of IRM considerations.

Theoretical models and greenhouse experiments suggested that Bt plants pyramiding two dissimilar insecticidal protein genes in the same plant have the potential to delay resistance much more effectively than single toxin plants used sequentially or in mosaics or seed mixtures, even with relatively smaller and more economically acceptable refuge sizes (Roush, 1998; Zhao *et al.*, 2003, 2005). The Bt rice lines pyramided with dual insecticidal genes of modified *CpTI* and *cry1Ac* have the potential to delay insect adaptation to a single Bt protein because there are different modes of actions and insecticidal mechanisms between CpTI and Cry1Ac. The recent development of Bt rice expressing Cry2A protein (Chen *et al.*, 2005a) is an important step for pyramiding both *cry1A* and *cry2A* genes in the same Bt rice plant, as reported by Bashir *et al.* (2004) and is similar to Bollgard II in Bt cotton. Further studies to explore new insecticidal genes, to test different insecticidal protein combinations, to develop new Bt rice with pyramided Bt genes with higher Bt protein expression, and to detect the rare allele frequency of Bt resistance in field populations of target insect pests (Meng *et al*., 2003; Han *et al*., 2006b) will be important for proactive IRM strategies in China in the future.

Final remarks

Extensive greenhouse and field studies documented that most of the Bt rice lines could effectively control the target lepidopteran pests and reduce chemical insecticide application both in China and some other countries, with important benefits to environmental quality and the health of rice growers and consumers. Ecological risk assessments of insect-resistant transgenic rice confirmed that transgenic Bt rice has no markedly negative or unintended impact on non-target organisms, compared with non-transgenic rice, and is safer than chemical insecticides.

The rapid adoption of Bt crops in the past decade has already reflected the substantial multiple benefits. Deployment of Bt cotton or Bt corn has resulted in significant decreases in insecticide use in developed and developing countries, and in some cases, increases in yield and profitability (Shelton *et al*., 2002; Huang *et al*., 2002; Qaim & Zilberman, 2003). More than 50 field studies varying in size, duration and sampling method have been carried out both in experimental and commercial fields for evaluating the impact of Bt plants on natural enemies, and no consistent negative effect has been found compared with non-Bt plants (Romeis *et al*., 2006). In December 2005, the journal *Environmental Entomology* published 13 papers about 11 field studies, which were carried out in the US and Australia and focused on the long-term assessment of potential impact of Bt cotton and corn, including five different insecticidal proteins on non-target organisms (Naranjo *et al*., 2005). All the studies conducted over multiple sites/years on moderately sized research plots or in commercial fields with different sampling methods did not show any significantly negative effects of Bt cotton and corn on non-target organisms. Reduced parasitization rates and cocoon formation of *A. chilonis* from *C. suppressalis* larvae feeding on Bt rice (Jiang *et al*., 2004a) were predictable responses caused by poor quality of the target host insect instead of direct toxicity from Bt protein (Romeis *et al*., 2006). A tiered testing strategy (Rose, 2006) will be useful for ecological risks assessment of Bt rice on non-target organisms in China in the future. The gene flow from transgenic rice to wild rice or weed rice and resistant evolution of target pest to Bt rice may be the main ecological risks for commercialization of Bt rice (Han *et al*., 2006a). However, there were concerns on the conflicts of intellectual property rights for commercial use of the transgenic rice lines as most of the lines with Bt genes were developed in China in collaboration with international institutions (Wang, 2000). Proactive IRM considerations before and after commercial release of Bt rice lines in China will be critical for long-term sustainable use in the future.

In China, Bt cotton has been commercialized since 1997 (Hebei) and 1998 (other provinces) and has provided us with valuable experience with transgenic crops. The experience with Bt cotton, plus increased government investment in crop biotechnology, including rice (Huang *et al*., 2005), will have a positive impact on commercial planting of transgenic rice in China in the future. Obviously, commercialization of insect-resistant transgenic rice in China will not only have crucial implications for China but for the rest of the world.

Acknowledgments

We are grateful to Dr Michael Cohen and Dr Kong-ming Wu for the helpful comments and suggestions on the manuscript.

References

An, H.B., Zhu, Z., Li, H.F., Wu, Q., Zhou, Z.L., Xu, H.L., Xu, J. W., An, L.J. and Li, X.H. (2001) Obtaining fertile transgenic plants of resistant-insect rice (*Oryza sativa* L.) via particle bombardment. *High Technology Letters*, 11, 12–16.

Bai, Y.Y., Jiang, M.X. and Cheng, J.A. (2005) Impacts of transgenic *cry1Ab* rice on two collembolan species and predation of *Microvelia horvathi* (Hemiptera: Veliidae). *Acta Entomologica Sinica*, 48, 42–47. (in Chinese)

Bashir, K., Husnain, T., Fatima, T., Latif, Z., Mehdi, S.A. and Riazuddin, S. (2004) Field evaluation and risk assessment of transgenic indica basmati rice. *Molecular Breeding*, 13, 301–312.

Bates, S.L., Zhao, J.-Z., Roush, R.T. and Shelton, A.M. (2005) Insect resistance management in GM crops: Past, present and future. *Nature Biotechnology*, 23, 57–62.

Chen, H., Tang, W., Xu, C.G., Li, X.H., Lin, Y.J. and Zhang, Q. F. (2005a) Transgenic indica rice plants harboring a synthetic *cry2A* gene of *Bacillus thuringiensis* exhibit enhanced resistance against lepidopteran rice pests. *Theoretical and Applied Genetics*, 111, 1330–1337.

Chen, M. (2005b) Ecological safety assessments of transgenic rice with insect or virus resistance on their non-target organisms. PhD Dissertation, Zhejiang University, Hangzhou, China. (in Chinese)

Chen, M., Ye, G.Y., Liu, Z.C., Yao, H.W., Chen, X.X., Sheng, Z. C., Hu, C. and Datta, S.K. (2006) Field assessment of the effects of transgenic rice expressing a fused gene of *cry1Ab* and *cry1Ac* from *Bacillus thuringiensis* Berliner on non-target planthoppers and leafhoppers. *Environmental Entomology*, 35, 127–134.

Chen, M., Ye, G.Y., Hu, C. and Datta, S.K. (2003a) Effects of transgenic *Bt* indica rice on the feeding and oviposition behavior of the brown planthopper, *Nilaparvata lugens*. *Acta Phytophylacica Sinica*, 30, 366–370. (in Chinese)

Chen, M., Ye, G.Y., Hu, C. and Datta, S.K. (2003b) Effect of transgenic *Bt* rice on the dispersal of planthoppers and leafhoppers as well as their egg parasitic wasps. *Journal of Zhejiang University (Agriculture and Life Science)*, 29, 29–33. (in Chinese)

Chen, M., Ye, G.Y., Lu, X.M., Hu, C., Peng, Y.F., Shu, Q.Y. and Altosaar, I. (2005c) Biotransfer and bioaccumulation of Cry1Ab insecticidal protein in rice plant-brown planthopper-wolf spider food chain. *Acta Entomologica Sinica*, 48, 208–213. (in Chinese)

Chen, M., Ye, G.Y., Yao, H.W., Hu, C. and Shu, Q.Y. (2003c) 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). *Agricultural Sciences in China*, 2, 1000–1006.

Chen, X.J., Hu, S.L., Cheng, K.L. and Ren, G.J. (2003d) Effect of *Bt*-transgenic rice on organism community in paddy-field. *Journal of Sichuan Agricultural University*, 21, 185–186. (in Chinese)

Cheng, X.Y., Sardana, R., Kaplan H. and Altosaar, I. (1998) *Agrobacterium*-transformed rice plants expressing synthetic *cry1Ab* and *cry1Ac* genes are highly toxic to striped stem borer and yellow stem borer. *Proceedings of the National Academy of Sciences of United States of America*, 95, 2767–2772.

Chi, S.Y., Peng, Y., Wang, Y.C., Han, Z.J. and Chen, C.K. (2005) Advance in the research of insecticide resistance of *Chilo suppressalis*. *Plant Protection*, 31(6), 3–6. (in Chinese)

Ci, X.Y., Yao, F.Y., Zhu, C.X., Song, Y.Z., Wen, F.J. and Jiang M.S. (2005) Breeding resistant rice varieties from transgenic parental lines with *cry1Ab* and *Xa21* genes and their field performance. *Scientia Agricultura Sinica*, 38, 313–319. (in Chinese)

Cohen, M.B., Gould, F. and Bentur, J.S. (2000) *Bt* rice: practical steps to sustainable use. *International Rice Research Notes*, 25, 4–10.

Dorcey, E. and Serrano, A. (2002) GM foods in Spanish newspaper. *Trends in Biotechnology*, 20, 285–286.

Fu, Q., Wang, F., Li, D.H., Yao, Q., Lai, F.X. and Zhang, Z.T. (2003) Effects of insect-resistant transgenic rice lines MSA and MSB on non-target pests *Nilaparvata lugens* and *Sogatella fucifera*. *Acta Entomologica Sinica*, 46, 697–704. (in Chinese)

Gao, X.W., Peng, L.N. and Liang, D.Y. (2006a) Factors causing the outbreak of brown planthopper, *Nilaparvata lugens* Stal in China in 2005. *Plant Protection*, 32(2), 23–24. (in Chinese)

Gao, Y.L., Fu, Q., Wang, F., Lai, F.X., Luo, J., Peng, Y.F. and Zhang, Z.T. (2006b) Effects of transgenic *cry1Ac+CpTI* rice on survival and field composition of *Chilo suppressalis* and *Sesamia inferens*. *Chinese Journal of Rice Science*, 20, 543–548. (in Chinese).

Gould, F. (1998) Sustainability of transgenic insecticidal cultivars–integrating pest genetics and ecology. *Annual Review of Entomology*, 43, 701–726.

Han, L.Z., Wu, K.M., Peng, Y.F. and Guo, Y.Y. (2006a) Advances in ecological safety of insect-resistant rice. *Chinese Journal of Applied and Environmental Biology*, 12(3), 431–436. (in Chinese)

Han, L.Z., Wu, K.M., Peng, Y.F., Wang, F. and Guo, Y.Y. (2006b) Evaluation of transgenic rice expressing Cry1Ac and CpTI against *Chilo suppressalis* and its intrapopulation variation in susceptibility to Cry1Ac. *Environmental Entomology*, 35, 1453–1459.

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

Hu, Y.Q., Li, Y.R., Zheng, Y., Hu, X.B., Zhang, M.J. and Li, B. J. (2005) Insecticidal activity of transgenic rice expressing *CpTI* or *CpTI+Bt* to *Chilo suppressalis* Walker. *Journal of Fujian Agriculture and Forestry University (Nature Science Edition)*, 34, 185–188. (in Chinese)

Huang, J.K., Hu, R.F., Rozelle, S. and Pray, C. (2005) Insect-resistant GM rice in farmers' fields: Assessing productivity and health effects in China. *Science*, 308, 688–690.

Huang, J.K., Rozelle, S., Pray, C. and Wang, Q.F. (2002) Plant biotechnology in China. *Science*, 295, 674–677.

James, C. (2005) Executive summary: Global Status of Commercialized Biotech/GM Crops: 2005. *ISAAA Briefs*, No. 34, ISAAA: Ithaca, NY.

Janmaat, A.F. and Myers, J. (2003) Rapid evolution and the cost of resistance to *Bacillus thuringiensis* in greenhouse populations of cabbage looper, *Trichoplusia ni*. *Proceedings of the Royal Society B: Biological Sciences*, 270, 2263–2270.

Jiang, X.H., Zhang, Q.H., Hu, S.M., Xie, S.J. and Xu, S.G. (2001) The status of pesticide resistance of rice stalk borer in Zhejiang Province and their management tactics. *Plant Protection Technology and Extension*, 21(3), 27–29. (in Chinese)

Jiang, Y.H., Fu, Q., Cheng, J.A., Ye, G.Y., Bai, Y.Y. and Zhang, Z.T. (2004) Effects of transgenic *Bt* rice on the biological characteristics of *Apanteles chilonis* (Munakata) (Hymenoptera: Braconidae). *Acta Entomologica Sinica*, 47, 124–129. (in Chinese)

Jiang, Y.H., Fu, Q., Cheng, J.A., Zhu, Z.R., Jiang, M.X. and Zhang, Z.T. (2005a) Effect of transgenic *sck+cry1Ac* rice on the survival and growth of *Chilo suppressalis* (Walker) and its parasitoid *Apanteles chilonis* (Munakata)(Hymenoptera:

Braconidae). *Acta Entomologica Sinica*, 48, 554–560. (in Chinese)

Jiang, W.H., Han, Z.J. and Hao, M.L. (2005b) Primary study on resistance of rice stem borer (*Chilo suppressalis*) to fipronil. *Chinese Journal of Rice Science*, 19, 577–579. (in Chinese)

Kain, W.C., Zhao, J.-Z., Janmaat, A.F., Myers, J., Shelton, A.M. and Wang, P. (2004) Inheritance of resistance to *Bacillus thuringiensis* toxin Cry1Ac in cabbage looper (Lepidoptera: Noctuidae). *Journal of Economic Entomology*, 97, 2073–2078

Khush, G.S. (1995) Breaking the yield frontier of rice. *GeoJournal*, 35, 329–332.

Khush, G.S. (1997) Origin, dispersal, cultivation and variation of rice. *Plant Molecular Biology*, 35, 25–34.

Li, D.H., Fu, Q., Wang, F., Yao, Q., Lai, F.X., Wu, J.C. and Zhang, Z.T. (2004) Resistance of transgenic rice containing both *sck* and *cry1Ac* genes against *Chilo suppressalis* and *Cnaphalocrocis medinalis*. *Chinese Journal of Rice Science*, 18, 43–47. (in Chinese)

Li, X.F., Han, Z.J., Chen, C.K., Li, G.Q. and Wang, Y.C. (2001) Monitoring for resistance of rice stem borer (*Chilo suppressalis* Walker) to 4 conventional insecticides. *Journal of Nanjing Agricultural University*, 24, 43–46. (in Chinese)

Li, Y.R., Hu, Y.Q., Zheng, Y., Hu, X.B., Zhang, M.J. and Li, B. J. (2005) Field evaluation of the resistance of transgenic rice expressing CpTI or CpTI+*Bt* to lepidopterous pests. *Journal of Fujian Agriculture and Forestry University (Nature Science Editon)*, 34, 182–185. (in Chinese)

Liu, Y.F., Su, J., You, M.S., Wang, Q., Hu, S.Q., Liu, W.H., Zhao, S.X. and Wang, F. (2005a) Effect of transgenic pest-resistant rice on pest insect communities in paddy fields. *Acta Entomologica Sinica*, 48, 544–553. (in Chinese)

Liu, Y.F., Wang, F., You, M.S., Wang, Q., Hu, S.Q., Liu, W.H. and Zhao, S.X. (2005b) Resistance of *cry1Ac+SCK* transgenic rice and its filial generation to the rice leaf roller *Cnaphalocrocis medinalis*. *Scientia Agricultura Sinica*, 38, 725–729. (in Chinese)

Liu, Z.C., Ye, G.Y. and Hu, C. (2004) Effects of *Bacillus thuringiensis* transgenic rice and chemical insecticides on arthropod communities in paddy-fields. *Chinese Journal of Applied Ecology*, 15, 2309–2314. (in Chinese)

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

Liu, Z.C., Ye, G.Y., Fu, Q., Zhang, Z.T. and Hu, C. (2003b) Indirect impact assessment of transgenic rice with *cry1Ab* gene on predations by the wolf spider, *Pirata subpiraticus*. *Chinese Journal of Rice Science*, 17, 175–178.

Meng, F.X., Wu, K.M., Gao, X.W., Peng, Y.F. and Guo, Y.Y. (2003) Geographic variation in susceptibility of *Chilo suppressalis* (Lepidoptera: Pyralidae) to *Bacillus thuringiensis* toxins in China. *Journal of Economic Entomology*, 96, 1383–1842.

Naranjo, S.E., Head, G. and Dively, G.P. (2005) Field studies assessing arthropod non-target effects in *Bt* transgenic crops: Introduction. *Environmental Entomology*, 34, 1178–1180.

Pathak, M.D. and Khan, Z.R. (1994) *Insect Pests of Rice*. International Rice Research Institute, Manila, The Philippines.

Peng, Y., Chen, C.K., Han, Z.J. and Wang, Y.C. (2001) Resistance measurement of *Chilo suppressalis* from Jiangsu province and its resistance mechanism to methamidophos. *Acta Phytophylacica Sinica*, 28, 173–177. (in Chinese)

Qaim, M. and Zilberman, D. (2003) Yield effects of genetically modified crops in developing countries. *Science*, 299, 900–902.

Qu, M.J., Han, Z.J., Xu, X.J. and Yue, L.N. (2003) Triazophos resistance mechanisms in the rice stem borer (*Chilo suppressalis* Walker). *Pesticide Biochemistry and Physiology*, 77, 99–105.

Romeis, J., Meissle, M. and Bigler, F. (2006) Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. *Nature Biotechnology*, 24, 63–71.

Rose, R.I. (2006) Tier-based testing for effects of proteinaceous insecticidal plant-incorporated protectants on non-target arthropods in the context of regulatory risk assessments. *IOBC/WPRS Bulletin*, 29, 145–252.

Roush, R.T. (1998) Two-toxin strategies for management of insect resistant transgenic crops: Can pyramiding succeed where pesticide mixtures have not? *Philosophical Transactions of the Royal Society B: Biological Sciences*, 353, 1777–1786.

Savary, S., Willocquet, L., Elazegui, F.S., Castilla, N.P. and Teng, P.S. (2000) Rice pest constraints in tropical Asia: quantification of yield losses due to rice pests in a range of production situations. *Plant Disease*, 84, 357–369.

Shelton, A.M., Robertson, J.L., Tang, J.D., Perez, C., Eigenbrode, S.D., Preisler, H.K., Wilsey, W.T. and Cooley, R.J. (1993) Resistance of diamondback moth to *Bacillus thuringiensis* subspecies in the field. *Journal of Economic Entomology*, 86, 697–705.

Shelton, A.M. and Sears, M.K. (2001) The monarch butterfly controversy: Scientific interpretations of a phenomenon. *The Plant Journal*, 27, 483–488.

Shelton, A.M., Zhao, J.-Z. and Roush R.T. (2002) Economic, ecological, food safety, and social consequences of the deployment of *Bt* transgenic plants. *Annual Review of Entomology*, 47, 845–881.

Sheng, C.F., Wang, H.T., Gao, L.D. and Xuan, W.J. (2003) The occurrence status, damage cost estimate and control strategies of stem borers in China. *Plant Protection*, 29, 37–39. (in Chinese)

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

Tabashnik, B.E., Cushing, N.L., Finson, N. and Johnson, M.W. (1990) Field development of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae). *Journal of Economic Entomology*, 83, 1671−1676.

Tabashnik, B.E., Carrière, Y., Dennehy, T.J., Morin, S., Sisterson, M.S., Roush, R.T., Shelton, A.M. and Zhao, J.-Z. (2003) Insect resistance to transgenic Bt crops: lessons from the laboratory and field. *Journal of Economic Entomology*, 96, 1031−1038.

Tabashnik, B.E., Dennehy, T.J. and Carrière, Y. (2005) Delayed resistance to transgenic cotton in pink bollworm. *Proceedings of the National Academy of Sciences of United States of America*, 102, 15389−15393.

Tanaka, K., Endo, S. and Kazano, H. (2000) Toxicity of insecticides to predators of rice planthoppers: Spiders, the mirid bug and the dryinid wasp. *Applied Entomology and Zoology*, 35, 177−187.

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

Tu, J.M., Zhang, G.A., Datta, K., Xu, C.G., He, Y.Q., Zhang, Q. F., Khush, G.S. and Datta, S.K. (2000) Field performance of transgenic elite commercial hybrid rice expressing *Bacillus thuringiensis* δ-endotoxin. *Nature Biotechnology*, 18, 1101−1104.

US EPA (1998) *Final Report of the Subpanel on Bacillus thuringiensis (Bt) Plant-Pesticides and Resistance Management*, Meeting held on February 9−10, 1998. Available at: http://www.epa.gov/scipoly/sap/meetings/1998/february/finalfeb.pdf.

US EPA (2001) *Biopesticides Registration Action Document−Bacillus thuringiensis Plant-Incorporated Protectants*. Available at: http://www.epa.gov/pesticides/biopesticides/pips/bt_brad.htm.

Wang, A.J., Yao, F.Y., Wen, F.J., Zhu, C.X., Li, G.X., Yang, L., Zhu, Q.S. and Zhang H.R. (2002a) Obtaining of transgenic rice plants resistant to both stem borer and Bacterial blight disease from *Bt* and *Xa21* genes transforming. *Acta Agronomica Sinica*, 28, 857−860. (in Chinese)

Wang, F. (2000) Progress and prospects in the development of transgenic rice breeding. *Fujian Journal of Agricultual Sciences,* 15 (Suppl.), 141−144. (in Chinese)

Wang, Y.C., Fan, J.Q. and Tian, X.Z. (1994) Studies on the resurgent question of plant-hoppers induced by deltamethrin and methamidophos. *Entomology Knowledge*, 31, 257−262. (in Chinese)

Wang, Z.H., Shu, Q.Y., Cui, H.R., Xu, M.K., Xie, X.B. and Xia, Y.W. (2002b) The effect of *Bt* transgenic rice flour on the development of silkworm larvae and the sub-microstructure of its midgut. *Scientica Agricultura Sinica*, 35, 714−718. (in Chinese)

Wang, Z.H., Shu, Q.Y., Ye, G.Y., Cui, H.R., Wu, D.X., Altosaar, I. and Xia, Y.W. (2002c) Genetic analysis of resistance of *Bt* rice to stripe stem borer (*Chilo supperssalis*). *Euphytica*, 123, 379−386.

Wang, Z.H., Shu, Q.Y., Cui, H.R., Ye, G.Y., Wu, D.X., Gao, M. W., Xia, Y.W., Cheng, X.Y. and Altosaar, I. (2000) Resistance of progenies of crosses between *Bt* transgenic rice and conventional rice varieties to stripe stem borer *Chlio suppressalis*. *Acta Agronomica Sinica*, 26, 310−314. (in Chinese)

Wu, J.C., Xu, J.X., Yuan, S.Z., Liu, J.L., Jiang, Y.H. and Xu, J. F. (2001) Pesticide-induced susceptibility of rice to brown planthopper *Nilaparvata lugens*. *Entomologia Experimentalis et Applicata*, 100, 119−126.

Yao, F.Y., Zhu, C.X., Li, G.X. and Wen, F.J. (2002) Identification of *Bt* rice resistance to stripe stem borer and genetic analysis of transgenes. *Scientia Agricultura Sinica*, 35, 142−145. (in Chinese)

Ye, G.Y., Shu, Q.Y., Cui, H.R., Gao, M.W., Xia, Y.W., Cheng, X.Y. and Altosaar, I. (2000) A leaf-section bioassay for evaluating rice stem borer resistance in transgenic rice containing a synthetic *cry1Ab* gene from *Bacillus thuringiensis* Berliner. *Bulletin of Entomological Research*, 90, 179−182.

Ye, G.Y., Shu, Q.Y., Yao, H.W., Cui, H.R., Cheng, X.Y., Hu, C., Xia, Y.W., Gao, M.W. and Altosaar, I. (2001a) Field evaluation of resistance of transgenic rice containing a synthetic *cry1Ab* gene from *Bacillus thuringiensis* Berliner to two stem borers. *Journal of Economic Entomology*, 94, 270−276.

Ye, G.Y., Tu, J.M., Hu, C., Datta, K. and Datta, S.K. (2001b) Transgenic non-*Bt* with fused *Bt* gene *cry1Ab*/*cry1Ac* from *Bacillus thuringiensis* is resistant against four lepidopteran species under field conditions. *Plant Biotechnology*, 18, 125−133.

Ye, G.Y., Yao, H.W., Shu, Q.Y., Cheng, X.Y., Hu, C., Xia, Y.W., Gao, M.W. and Altosaar, I. (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 Protection*, 22, 171−178.

Zhao, H.Y., Zhang, Y.J., Wu, K.M., Zhao, K.J., Peng, Y.F. and Guo, Y.Y. (2004) Expression of Cry1Ac/CpTI transgenic rice and its resistance in different stages to *Chilo suppressalis*. *Journal of Agricultural Biotechnology*, 12, 76−79.

Zhao, J.-Z., Cao, J., Li, Y.X., Collins, H.L., Roush, R.T., Earle, E.D. and Shelton, A.M. (2003) Transgenic plants expressing two *Bacillus thuringiensis* toxins delay insect resistance evolution. *Nature Biotechnology*, 21, 1493−1497.

Zhao, J.-Z., Cao, J., Collins H.L., Bates, S.L., Roush, R.T., Earle, E.D. and Shelton, A.M. (2005) Concurrent use of transgenic plants expressing a single and two *Bacillus thuringiensis* genes speeds insect adaptation to pyramided plants. *Proceedings of the National Academy of Sciences of United States of America*, 102, 8426−8430.

Zhao, J.-Z. and Rui, C.H. (2004) Insect resistance management for transgenic Bt cotton. *Transgenic Cotton* (English Edition), (ed. S.R. Jia), pp. 184–204. Science Press, Beijing.

Zhou, X., Cheng, J.A., Hu, Y. and Lou, Y.G. (2004) Effects of transgenic *Bt* rice on the population development of *Nephotettix cincticeps*. *Chinese Journal of Rice Science*, 19, 74–78. (in Chinese)

Zhu, C.X., Hu, A.Q., Wen, F.J., Zheng, C.C. and Zhang, J. (1999) Production of insect-resistant rice plants transformed with *cry1Ab* and *pinII* genes. *Journal of Agricultural Biotechnology*, 7, 259–266. (in Chinese)

Zhu, Z. (2001) Research and development of highly insect-resistant transgenic rice. *Bulletin of the Chinese Academy of Sciences*, 5, 353–358. (in Chinese)

Accepted August 15, 2006