Insect-Resistant Genetically Modified Rice in China: From Research to Commercialization

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Abstract
From the first insect-resistant genetically modified (IRMG) rice transformation in 1989 in China to October 2009 when the Chinese Ministry of Agriculture issued biosafety certificates for commercial production of two cry1Ab/Ac Bacillus thuringiensis (Bt) lines, China made a great leap forward from IRMG rice basic research to potential commercialization of the world’s first IRMG rice. Research has been conducted on developing IRMG rice, assessing its environmental and food safety impacts, and evaluating its socioeconomic consequences. Laboratory and field tests have confirmed that these two Bt rice lines can provide effective and economic control of the lepidopteran complex on rice with less risk to the environment than present practices. Commercializing these Bt plants, while developing other GM plants that address the broader complex of insects and other pests, will need to be done within a comprehensive integrated pest management program to ensure the food security of China and the world.
INTRODUCTION

Rice (Oryza sativa) is the most widely consumed food crop and was grown on over 159 million ha worldwide in 2008, with over 18% grown in China (32). As one of the centers of origin, China has been cultivating rice for over 7,000 years (126). Rice is a staple food for over 1 billion people in China in addition to 2 billion people in other countries (39). Although different synthetic insecticides are applied frequently in order to control insect pests of rice, tremendous economic and environmental losses still occur regularly. For instance, rice stem borers, a major group of lepidopteran pests of rice, cause annual losses of 11.5 billion yuan (US$1.69 billion) (79, 80). In addition, another major group of insect pests, planthoppers, has caused large annual yield losses across the country since the 1970s (24, 25).

Genes from the bacterium Bacillus thuringiensis (Bt) that code for insecticidal Crystal (Cry) proteins were engineered into plants in the mid-1980s to develop the first insect-resistant genetically modified (IRGM) plants (89). Soon after, Chinese scientists began to use genetic engineering techniques to develop new control tactics for insect pests of rice. In 1989, scientists from the Chinese Academy of Agricultural Sciences (CAAS), by means of polyethyl glycol, generated the first IRGM rice plant with a Bt delta-endotoxin gene under the control of the CaMV 35S promoter (108). After 20 years of extensive laboratory and field studies, on October 22, 2009, China’s Ministry of Agriculture issued its first two biosafety certificates for commercial production of two Bt rice lines (cry1Ab/Ac Huahui No. 1 and cry1Ab/Ac Bt Shanyou 63) for Hubei Province (http://www.stee.agri.gov.cn/biosafety/spx/t20091022_819217.htm). By this action, China had not only made great strides from basic research to commercialization of IRGM rice, but likely provided the impetus for the development of other IRGM food crops worldwide, thus moving toward the goal of fighting global poverty and food scarcity (45).

In this review, we examine the history and importance of rice production in China, IRGM rice research, the development of regulations for IRGM crops as they relate to food safety and the environment, and the socioeconomic impact of IRGM rice. Knowledge gaps and future directions for China’s IRGM rice research are also discussed.

RICE PRODUCTION IN CHINA

China is the largest rice producer and consumer in the world and has a long history of rice cultivation in many geographic regions of the country. According to records of pollen, algal, and fungal spores and microcharcoal data from sediments dating back 7,700 years, local communities in the lower Yangtze region of China, a center of rice domestication, cultivated rice in lowland swamps after using fire to clear alder-dominated wetland scrub and dirt banks to control brackish water flooding (126). Today, China’s first priority is to feed its population of 1.3 billion. Among all the agricultural crops (e.g., other grain crops, fruit and vegetable crops, oil crops, fiber crops, sugar, and tobacco) planted in China, the largest share of land is devoted mostly to rice production (ca. 20%), surpassing that devoted to corn (ca. 18%) and cotton (ca. 3–4%) (66).

Traditionally, there are six rice-growing regions in China (Figure 1). Among the six regions, the south China region (region I), central China region (region II), and southwest China region (region III) have the best climatic conditions and are the major rice-growing areas. Both indica and japonica rice subspecies are grown in regions I, II, and III, with approximately two planting seasons a year. However, there is only one season for japonica rice in region IV (north China), V (northeast China), and VI (northwest China) (Figure 1) (34).

It has been estimated that rice yield needs to reach 7.85 × 10^3 kg ha^-1 by 2030 to feed China’s anticipated population of 1.6 billion (26). To do this, China needs 0.2 billion kilograms of rice per year, which is equal to the present total rice production worldwide. Because the yield reduction caused by insect pests is a threat to food security and because the
present heavy reliance on traditional insecticides is recognized as a problem for food and environmental safety, there is increased interest in using other technologies such as genetic engineering (115) and integrated pest management (IPM) (103). The Green Super Rice (GRS) concept integrates traditional, transgenic, and marker-assisted breeding strategies.
to battle rice yield constraints (e.g., insect pests) and improve rice quality (115). From a global standpoint, it should be recognized that if China imports rice to feed its growing population, food scarcity will increase in other parts of the world and may cause a crisis in the price of rice (117). It is important to the world that China is able to meet its own food needs; rice production and the ability to manage rice pests will be key to meeting this goal.

Rice Insect Pest Complex and Damage

Across the rice-growing regions in China, there are more than 200 species of insect pests that damage different life stages and different parts of rice (23, 52). In general, insect pests of rice are divided into two groups: chewing insects (e.g., rice stem borers, leaffolders, rice water weevils) and sucking insects (e.g., planthoppers and leafhoppers). There are five primary insect pests of rice in China, of which three are lepidopterans and two hemipterans (Table 1). Lepidopteran stem borers are chronic pests in rice ecosystems. The earliest documented stem borer infestation on rice in China was during the Song Dynasty (960–1279 AD) (80). Prior to the 1950s, the Asiatic stem borer, *Chilo suppressalis* (Crambidae), was the most dominant stem borer species throughout China. However, during the 1950s–1970s the yellow stem borer, *Scirpophaga incertulas* (Crambidae), became a more important pest (23, 80). Rice stem borers generally caused negligible yield losses to rice production in China in the 1970s. However, since 1993, stem borer infestations have

| Table 1 Primary and secondary insect pests of rice in China |
|-----------------|-----------------|-----------------|
| **Order**       | **Primary insect pests**                                      | **Secondary insect pests** |
| Lepidoptera     | *Scirpophaga incertulas* (Walker)                            | Chrysaespidia festucae (Graeser) |
|                 | *Chilo suppressalis* (Walker)                                 | *Leucania loreyi* (Duponchel) |
|                 | *Cnaphalocrocis medinalis* (Guenée)                           | *Leucania separata* (Walker) |
|                 | *Parnara guttata* (Bremer et Gray)                            | *Parapa guttata* (Bremer et Gray) |
|                 | *Naranga aenescens* (Moore)                                    | *Naranga aenescens* (Moore) |
|                 | *Spodoptera mauritia* (Boisduval)                             | *Pelopidas mathias* (Fabricius) |
| Hemiptera       | *Nilaparvata lugens* (Stål)                                   | *Thuta rubiginosa* (Kuoh) |
|                 | *Sogetella furcifera* (Harváth)                               | *Nephotettix cincticeps* (Uhler) |
|                 | *Sogatella furcifera* (Harváth)                               | *Nephotettix virescens* (Distant) |
|                 | *Recilia dorsalis* (Motschulsky)                              | *Nephotettix virescens* (Distant) |
|                 | *Laodelphax striatellus* (Fallén)                             | *Nephotettix virescens* (Distant) |
|                 | *Macrosiphum avenae* (Fabricius)                              | *Nephotettix virescens* (Distant) |
| Diptera         | –                                                            | *Orseolia oryzae* (Wood-Mason) |
|                 | –                                                            | *Ephydra macellaria* (Egger) |
|                 | –                                                            | *Hydrellia sinica* (Fan et Xia) |
|                 | –                                                            | *Chlorops oryzae* (Matsumura) |
| Coleoptera      | –                                                            | *Oulema oryzae* (Kuwayama) |
|                 | –                                                            | *Donacia prosperi* (Fairmaire) |
|                 | –                                                            | *Echinocnemis squamens* (Billberg) |
| Orthoptera      | –                                                            | *Oxya chinensis* (Thunberg) |
| Thysanoptera    | –                                                            | *Frankliniella intonsa* (Trybom) |
|                 | –                                                            | *Stenchetaothrips bifurmis* (Bagnall) |
|                 | –                                                            | *Haplothrips aculeatus* (Fabricius) |
been severe every year, with a disastrous outbreak in 1996 (80), likely owing to extensive use of synthetic insecticides, insecticide resistance, global warming, changing farming practices (no tillage or reduced tillage), expanded rice production in some areas, and coexistence of early-, mid-, and late-rice varieties (60). Many other foliage-feeding lepidopteran species also occur on rice, the most important of which is the rice leaf folder, *Cnaphalocrocis medinalis* (Pyralidae), which occurs throughout the country.

Yield losses caused by stem borers have been severe in the last decades. Sheng et al. (79, 80) estimated a yearly 3.1% loss nationally (approximately 6.3 billion kilograms of rice), equal to 6.5 billion Chinese yuan (US$956 million), in addition to 3 billion yuan (US$735 million) direct control cost (e.g., insecticides and labor fees). Although yield losses due to leaf folders are generally small because rice plants at the vegetative growth stage have a large capacity to compensate for damage to foliage, leaf folders caused 24–32% yield loss in some rice paddies when no control was applied (22). Further, leaf folder damage is highly visible to farmers and is often the most important stimulus for insecticide applications (63), which adds additional control costs and environmental damage.

Aside from stem borers, the brown planthopper, *Nilaparvata lugens*, and the white-backed planthopper, *Sogatella furcifera* (both Homoptera: Delphacidae), which once were minor insect pests in China prior to 1968, are now primary pests of rice, with 10 disastrous outbreaks in China since 1975. The most recent outbreak occurred in 2005, causing an estimated loss of 2.8 billion kilograms (24). The planthopper outbreaks have been attributed to high adoption of hybrid rice, increased use of insecticides and chemical fertilizers, insecticide resistance, climate change resulting in elevated autumn temperatures, more intense and higher frequency of typhoons that transport hoppers over wider areas, and changes in cropping systems in southern China that may have affected migration patterns (24, 25).

In addition to the primary insect pests listed above, there are 27 common secondary insect pests of rice (Table 1). These insects are distributed in different rice regions in China, with some species sporadically causing high localized losses.

**Present Pest Management Strategies**

Various control strategies for rice pest management have been practiced during the thousands of years of rice cultivation in China, and these include mechanical (e.g., deep plowing for stem borer control and digging ditches for burying locusts), biological, chemical, and cultural control (52). Some of these strategies continue today, although there is more emphasis on chemical control. The development of insect pest management in “New China” (since 1949) has been divided into three stages as the concept of IPM has evolved (67). Most recently, China’s Ministry of Agriculture has promulgated the concept for rice pest management as “public plant protection and green plant protection” (103).

Although different control theories and strategies have been developed for rice pests, in practice farmers in many rice-growing regions still rely heavily on synthetic insecticides because of a lack of education and an incomplete understanding of modern IPM concepts and the quick visible control effects of some effective, cheap and easily accessible synthetic insecticides. The use of all pesticides (including insecticides, fungicides, and herbicides) for agricultural crops including rice in China in 2005 (1.5 billion kg) has doubled since 1990 (65). Consequently, severe insect outbreaks, environmental pollution, insecticide-related food poisoning, and farmer illnesses are frequently reported. Thus, newer and safer pest management strategies are sorely needed for rice production in China.

**IRGM RICE DEVELOPMENT IN CHINA**

**The Development of IRGM Rice**

Because of the prominent pest status of stem borers and leaf folders, the limited sources of
Bt rice plants: transgenic plants with insecticidal gene(s) from B. thuringiensis
CpTI: cowpea trypsin inhibitor
GMO: genetically modified organism
Pilot field testing: first stage of field testing for agricultural GMOs in China, also known as restricted field testing; conducted on a small scale (upto 0.26 ha)
Environmental release testing: second stage of field testing for agricultural GMOs in China, also known as enlarged field testing; conducted on a middle scale (0.26–2.0 ha)
Preproduction testing: last stage of field testing for agricultural GMOs in China, also known as productive testing; conducted on a larger scale (2.1–66.7 ha)

Resistance to these pests in rice germplasm (27), and the success of Bt cotton in China and elsewhere (62, 64, 103), the Chinese government, research institutes, and academic researchers have devoted large efforts to finding newer and safer tactics to control rice pests. Agricultural biotechnology has been extensively explored in China for creating such tactics. Since 1985, with the government’s support, many National Key laboratories have been established across the country in the general areas of agricultural biotechnology and crop genetics, which formed an infrastructure for Chinese biotechnology researchers to test their ideas on rice IPM (114). In 1989, scientists from the CAAS generated Bt rice plants (108), which, to our knowledge, was the earliest successful Bt rice transformation in the world. Public research expenditures on GM rice in China increased from 8 million yuan (US$1.18 million) in 1986 to 195 million yuan (US$28.68 million) in 2003 (43). In late 2008, China started a 26 billion yuan (US$3.5 billion) research and development (R&D) initiative on GM plants, which paved the way for commercialization of IRGM rice (19). Since 1989, IRGM rice lines expressing insecticidal genes with lepidopteran activity [e.g., cry1Aa, cry1Ab, cry1Ac, cry1Ab/Ac, cry1G, cry2A, CpTI (cowpea trypsin inhibitor)] or hemipteran activity (e.g., Galanthus nivalis agglutinin, gna, and Pinellia ternata agglutinin, pta) under control of various promoters have been developed and tested at various stages based on the regulatory process for agricultural genetically modified organisms (GMOs) in China (Supplemental Table 1; follow the Supplemental Material link from the Annual Reviews home page at http://www.annualreviews.org). Although IRGM rice research in China has been given great opportunities both in terms of the fast development of research infrastructure and in terms huge input of public research funds, issues of intellectual property rights, government regulations on GM plants, educational outreach programs for farmers about IRGM crops, and effective and well-regulated seed distribution systems have all delayed IRGM rice from reaching full commercialization and reflected some of the weaknesses of the Chinese research and regulatory systems. For instance, there have been reports of illegal planting of unapproved IRGM rice in central China in 2005 (101, 125) and intellectual property rights issues involving foreign-owned patents used in several IRGM rice lines (123).

First Release and Biosafety Certificate Approval for Bt Rice

For the past 20 years in China, numerous IRGM rice lines have been developed (Supplemental Table 1) and the first field tests took place in 1998 (111). Based on the regulation policy for agricultural GMOs in China, GM crops go through three tiers of field testing (pilot field testing, environmental release testing, and preproduction testing) before being submitted to the Office of Agricultural Genetic Engineering Biosafety Administration (OAGEBA) to apply for the “Biosafety Certificate” for commercialization. Each year hundreds of applications are submitted to the OAGEBA for different tiers of field testing. By the first half of 2009, the OAGEBA had approved 357 applications for field testing. These included 228 applications for pilot field testing (or restricted field testing), 95 applications for environmental release testing (or enlarged field testing), and 34 applications for preproduction testing (or productive testing). On October 22, 2009, China’s Ministry of Agriculture issued two biosafety certificates for commercial production of Bt rice lines Huahui No. 1 and Bt Shanyou 63 in Hubei Province. Huahui No. 1 is a CMS (cytoplasmic male sterile) restorer line and Bt Shanyou 63 is a hybrid of Huahui No. 1 and Zhenshan 97A (the CMS line). Both lines express a cry1Ab/Ac fusion gene. China is now poised to become the first nation in the world to commercialize IRGM rice, which will likely result in a positive influence on global acceptance and the speed at which biotech food and feed crops are adopted (45).
Other IRGM Rice in Development

Although two Bt rice lines have been issued biosafety certificates, many other IRGM rice lines have been extensively tested and are now waiting for approval. In addition to the two Bt rice lines, the National Biosafety Committee approved in August 2009 five other IRGM rice lines after preproduction testing, although no biosafety certificate has been issued. These IRGM rice lines are KMD (expressing cry1Ab gene), T1c-9 (expressing cry1C), T2A-1 (expressing cry2A gene), and Kefeng 6 and 8 (both expressing cry1Ac+CpTI genes) (http://www.stec.agri.gov.cn/biosafety/spxx/t20091022_819217.htm).

Although cry1 and cry2 are the primary genes used for IRGM rice, the Bt vip gene (vip3H) (31), plant-derived insect-resistant lectin genes (e.g., gna, pta), protease inhibitor genes [e.g., CpTI, pinII (potato inhibitor II) and SbTI (soybean trypsin inhibitor)], and animal-derived insect-resistant gene (e.g., spider toxin gene, SpI) are also being used for IRGM rice (Supplemental Table 1). New cry genes (e.g., cry4Ca1, cry30Ga1, and cry56Aa1) have been identified as having insecticidal activity on stem borers (53, 121). Therefore, it may be possible to substitute genes for cry1 and cry2 or they could be used for gene pyramiding to broaden activity and delay the evolution of resistance. In addition, DNA shuffling has been used to construct novel insecticidal genes from existing cry genes for rice transformation (92).

In addition to the insecticidal genes, promoters play a vital role in determining where and when the genes are expressed in the plant (6a). Thus, promoters can influence the environmental fate of insecticidal proteins and the evolution of resistance (6a, 35). Constitutive promoters generally allow the genes to be expressed continuously in most parts of the plant. An alternative is to have them expressed only in certain tissues attacked by insects. The tissue-specific promoter rbc (ribulose-1,5-bisphosphate carboxylase/oxygenase) was used for cry1C rice to reduce potential ecological and food risks (112). IRGM rice with stacked traits of herbicide resistance, disease resistance, or both has also been explored (Supplemental Table 1). More recently, suppressing the expression of key genes for insect development or biochemical metabolism through RNA interference (RNAi) using gene fragments from a target pest has been achieved with IRGM corn (8) and cotton (61). Such second-generation IRGM plants hold promise for future work with rice.

BIOSAFETY REGULATION AND POLICIES ON GENETICALLY MODIFIED RICE

The first biosafety regulation for GMOs in China was issued in 1993 by the Chinese Ministry of Science and Technology. Since then, the regulations have been updated and revised (44), with the latest version issued by the State Council in 2001, followed by three additional regulations focusing on agricultural GMOs by the Ministry of Agriculture and one food hygiene regulation by the Ministry of Public Health in 2002 (http://www.agri.gov.cn/xzsp/xgzl/).

The National Biosafety Committee was formed under OAGEBA to evaluate all biosafety assessment applications relating to agricultural GMOs and to provide positive or negative recommendations to OAGEBA based on the results of biosafety assessments. However, OAGEBA is responsible for the final decision. Safety assessment for agricultural GMOs including GM rice in China is conducted on a case-by-case scientific examination using safety regulations appropriate to the testing stage. Safety assessment of GM plants is divided into five stages: (a) laboratory research, (b) pilot field testing, (c) environmental release field testing, (d) preproduction testing, and (e) application for biosafety certificates. In addition, after being issued biosafety certificates, GM rice lines along with other GM plants need to pass seed variety testing standards regulated by The Seed Law prior to entering production and marketing (44, 93).
Although the current regulations on agricultural GMOs in China are comprehensive and elaborate, criticisms and challenges exist. For instance, the biosafety decision making process for agricultural GMOs in China relies primarily on the National Biosafety Committee, which has 75 members, of which the majority are biotechnologists (44). Thus, different but well-informed voices may be needed to achieve a balanced decision on the biosafety of agricultural GMOs. In addition, appropriate biosafety assessment practices and approaches, such as tier-based risk assessment (71), proper test species selection, and statistical analysis (69), are needed to reduce some repeated and unnecessary studies; to ensure sound experimental design for risk assessments, good quality data, and interpretation; and to harmonize biosafety assessment processes in China with those in other countries.

LABORATORY AND FIELD TESTING OF IRGM RICE

Summary of Peer-Reviewed Publications

Since the first Bt rice plant was developed in China in 1989 (108), numerous laboratory and field studies have been conducted on its environmental and food safety. Although many peer-reviewed papers on IRGM rice in China were published in English language journals, most were published in Chinese journals that are unknown or inaccessible to many scientists in the western world. Two searching methods were used to summarize the publications: papers published in Chinese on IRGM rice were found using China Academic Journals Full-Text Database under the China National Knowledge Infrastructure (http://www.cnki.net/), which is the largest searchable full-text Chinese database in the world; papers published in English on China’s IRGM rice were found using the Web of Science® (including Science Citation Index Expanded, Social Sciences Citation Index, and Arts & Humanities Citation Index databases). From January 1995 to December 2009, there were 378 and 108 peer-reviewed papers published in Chinese (including MS and PhD dissertations) and in English, respectively (Supplemental Figure 1). Those papers included laboratory and field studies on GM rice development; on the efficacy on target insects; and the effects on nontarget insects including soil biota, gene flow, transgene detection, food safety, and agronomic traits. Publications on field studies of IRGM rice accounted for 24% of all papers published in Chinese sources and 36% of papers published in English sources. From 1995 to 2009, an average of 34.7 peer-reviewed papers were published annually on IRGM rice, with a maximum of 70 papers in 2005. This exceeds the publication record of both IRGM cotton and corn, and this large source of references serves as the source for the various topics on Bt rice discussed in this manuscript.

Environmental Risk Assessment

Interactions of IRGM rice with biological control agents. Rice insecticides accounted for nearly 15% of the global crop insecticide market value (98). Farmers tend to overreact to slight infestations caused by leaf-feeding pests, such as rice leaffolders, and make routine preventive applications, which usually removes most natural enemies from the system and leaves the field open for pest buildup (63). The evolution of resistance to the major classes of insecticides in rice stem borers decreased their efficacy and often led farmers to compensate by increasing the amounts used (46, 68). These factors are detrimental to biological control and pest management in the rice ecosystem.

Although field surveys indicated that there were approximately 200 species of insect pests injurious to rice in China, in rice-growing region I alone more than 228 species of natural enemies of insect pests were found, including 111 parasitoid species and 117 predator species (23, 52). Because of the critical role biological control has in rice IPM and the previous deleterious experiences with indiscriminate use of
another insect control technology (i.e., broad-spectrum insecticides), the need to carefully evaluate the ecological effects of IRGM rice before its release has been generally recognized in China. Many trials have assessed the potential impacts of IRGM rice on parasitoids and predators under laboratory and field conditions (summarized in Reference 18). These trials include studies of direct toxicity of purified insecticidal proteins or IRGM rice conducted in the laboratory and ecological studies conducted in the field. The field studies have examined populations of target and nontarget herbivores and their natural enemies using various insect sampling methods (e.g., vacuum sampling, sweep nets, and sticky traps). In general, negative effects of IRGM rice on natural enemies have not been observed, as measured by indicators of fitness, population density and dynamics, and biodiversity indices (18). For instance, the fitness of *Propylea japonica* (Coleoptera: Coccinellidae) and *Chrysoptera sinica* (Neuroptera: Chrysopidae) was not negatively affected by *Bt* rice through direct or indirect feeding (2, 3, 5).

Several multiple-year per site field studies indicated that the population dynamics of *Cyrtothrips lividipennis* (Hemiptera: Miridae) and of five common spider species was similar in *Bt* and non-*Bt* rice fields (15, 16, 57). As expected, some negative effects on parasitoids have been observed when *Bt*-susceptible herbivores are used as hosts (47, 48, 87), but this is most likely attributed to poor host quality than to toxic effects of the parasitoid (72). However, the dispersal dynamics of the parasitoids (18) and the overall temporal dynamics of species richness, diversity, and evenness of the parasitoid communities (50, 56) were similar between *Bt* and non-*Bt* rice fields. More conclusively, 14 parasitic arthropod families and 26 predatory arthropod families belonging to Hemiptera, Neuroptera, Coleoptera, Diptera, Hymenoptera, and Araneae were collected from both *Bt* and non-*Bt* rice fields, with no consistent differences in population structure in the two rice ecosystems (58). Although such studies were usually conducted on fields less than 1 ha in size, the results provide initial evidence that changes in natural enemy populations on a landscape level would be minimal, if at all. From our review of the Chinese and English literature, we believe that the results from studies with IRGM rice on natural enemies are consistent with those from other *Bt* crops, as reviewed in individual studies (72) or meta-analyses (64).

**Nontarget Herbivores**

Because the deployment of lepidopteran-resistant GM rice in China may potentially release competition pressure for planthoppers in the rice ecosystem and further worsen their already severe pest status (24, 25), planthoppers and leafhoppers have been identified as a key group of nontarget herbivores for IRGM rice research in China. In tests of direct toxicity, Bai et al. (5) reported that *N. lugens* ingested Cry proteins from *Bt* rice lines, but that they had no detectable negative effects on its fitness. Similarly, *Bt* rice had no significant effect on the feeding and oviposition behavior of planthoppers and leafhoppers (18, 85). Multiple-year per site studies indicated that populations of planthoppers and leafhoppers in *Bt* and non-*Bt* rice fields were similar (15, 17). A recent two-year field trial indicated that *cry1Ab/Ac* *Bt* Shanyou 63 rice harbored higher planthopper populations than did non-*Bt* rice at the late growth stage of rice, although not at early and middle growth stages (94). However, this result may have been caused by migration from nearby non-*Bt* rice fields where non-*Bt* rice leaf tissues were severely damaged by rice stem borers and leaffolders. A field study with six *Bt* rice lines indicated that *Bt* rice posed no risk of causing higher *Stenchaetothrips biformis* (Thysanoptera: Thripidae) populations in the field compared with non-*Bt* rice (1).

The nontarget effects on storage pests have also been studied. IRGM rice grains did not cause negative effects on four nonlepidopteran storage pests but did result in less damage by *Sitotroga cerealella* (Lepidoptera: Gelechiidae) compared with non-GM rice grains (10, 11, 51).
Effect of IRGM Rice Pollen on the Silkworm

The culture of silkworms, *Bombyx mori* (Lepidoptera: Bombycidae), or sericulture, has a long history in China. Silkworm larvae feed exclusively on fresh mulberry leaves. In southeast China, mulberry trees are generally planted near or around the edges of paddy fields, so-called mulberry-rice mixed cropping (30). Thus, mulberry leaves could be contaminated with IRGM rice pollen. Because silkworms and stem borers belong to the same order, this could be problematic. Fan et al. (30) reported that rice pollen escaped to nearby mulberry trees and contaminated mulberry leaves with an average concentration of 93 pollen grains per cm$^2$, which was slightly lower than a threshold concentration of $10^9$ Bt rice pollen grains per cm$^2$, under which development of silkworm larvae could be negatively affected.

Different effects of IRGM rice lines expressing different Cry proteins on silkworm larvae under laboratory conditions have been reported; these effects might be due to a different insecticidal spectrum of the Cry protein or to different expression levels in rice pollen (95, 96, 113). Yao et al. (110) reported that Bt rice line TT9-3 expressing a cry1Ab/Ac gene had no significant adverse effects on young silkworm larvae, even after the neonates had been exposed to Bt pollen at the highest density of 3395.0 grains per cm$^2$ for 48 h. Such pollen density is more than twofold greater than the highest pollen density on mulberry leaves, 1635.9 grains per cm$^2$, naturally occurring in the field. However, in a worst-case-scenario laboratory feeding bioassay, pollen from cry1Ab rice lines (KMD1 and B1) was toxic to silkworm larvae and caused pathological midgut changes (109).

The data suggest that some IRGM rice pollen may be toxic and therefore a hazard to silkworm larvae. However, risk is a function of hazard $\times$ exposure (77), and the deposition of rice pollen on mulberry leaves is very limited under field conditions and appears to pose minimal risk to silkworms (110). Furthermore, because the silkworm has been completely domesticated, routine colony maintenance practices (including leaf cleaning prior to feeding) dramatically decrease the amount of IRGM rice pollen on mulberry leaves. This, in conjunction with some environmental factors in southeast China (such as more rainfall) and the physical requirement of temporal and spatial overlap between silkworm larval occurrence and plant anthesis, makes IRGM rice pollen negligible on silkworm (109).

Soil Biota

Soil-dwelling detritivores, such as collembolans, play an important role in rice ecosystems (36, 37). Bai et al. (4) found that Cry1Ab could be detected in *Entomobrya griseoolivata* (Collembola: Entomobryidae) feeding on Bt rice tissue in the laboratory. However, field studies indicated the populations of common collembolan families (e.g., Scatopsidae, Sminthuridae, and Tomoceridae) and detritivorous dipteran families (e.g., Ceratopogonidae, Mycetophilidae, Phoridae, and Psychodidae) were similar in Bt and non-Bt rice fields (6, 58).

Wang et al. (91) found that degradation of Cry1Ab from Bt rice occurred in soils under aerobic conditions with half-lives ranging from 19.6 to 41.3 days. However, under water-flooded conditions, the half-life of Cry1Ab was prolonged to 45.9–141 days, indicating that soil microbial organisms may be exposed to Cry proteins for longer periods in flooded Bt rice fields than in a dry Bt cotton or Bt corn field. Under laboratory conditions, Bt rice straw could significantly increase the number of hydrolytic-fermentative and anaerobic nitrogen-fixing bacteria in flooded paddy soil (106). However, the numbers of anaerobic fermentative bacteria, denitrifying bacteria, hydrogen-producing acetogenic bacteria, methanogenic bacteria, and colony-forming units of culturable bacteria and actinomycetes were similar between soil amended with Bt rice straw and non-Bt rice straw (70, 104). Yang et al. (107) identified 303 bacteria strains belonging to 20 genera from two cry1Ab rice fields and
one non-Bt isoinline rice field, with no statistical differences in Shannon-Wiener, Simpson, and Pielou indexes for the total bacterial community among the three rice fields. Based on published laboratory and field studies of 1–2 years, the results to date indicate that IRGM rice has not caused significant changes to soil biota in China.

**Outcrossing of Insect Resistance Transgenesc**

In general, cultivated rice is primarily self-pollinating with very little cross-pollination between GM and non-GM rice cultivars (crop-to-crop) under field conditions (59). Field studies with \( Bt + CpTI \) rice lines indicated that risk of pollen-mediated crop-to-crop gene flow from IRGM rice to non-GM-cultivated rice in China is at a manageable level (73, 75). However, transgenic outcrossing from IRGM rice varieties to weedy rice and wild species (e.g., *Oryza rufipogon* and *O. nivara*) (crop-to-wild species) could occur at a higher level because these plants are present in and around cultivated rice fields where pollen from cultivated rice is at a high level (59). Pollen from cultivated rice can fertilize weedy rice (14) and *O. rufipogon* (14, 83) and produce fertile progeny. The rate of outcrossing declines rapidly with distance, but weedy and wild rice could occur within and around rice fields (59). Song et al. (81, 83) found that the maximum frequency of gene flow from cultivated rice to adjacent wild rice was less than 3%. Some fitness costs (producing fewer seeds) of the F1 hybrid of cultivated rice and wild rice could reduce the rate of transgene introgression into wild populations (82).

A recent field study (12) in China compared the field performances of three weedy rice strains and their six F1 hybrids with two IRGM rice lines (\( CpTI \) and \( Bt + CpTI \)), and the results indicated an enhanced relative performance of the crop-weed hybrids (e.g., taller plants and more tillers and panicles). Such results call for careful evaluation of the potential consequence of crop-to-wild gene outcrossing. Because seed trading is common in households, counties, and provinces. This suggests that seed-mediated gene flow should also be closely evaluated.

To better control gene flow from IRGM rice, Lin et al. (55) developed a built-in strategy for containing transgenes in GM rice. In addition, Rong et al. (74) recently constructed a model that takes into account the outcrossing rates of recipients and cross-compatibility between rice and its wild relatives to better predict pollen-mediated crop-to-wild gene flow. Based on such studies, strategies such as developing future IRGM rice lines with limited or no gene flow or releasing lines in areas where wild rice is absent can be incorporated into management programs that reduce the risk of outcrossing.

**Food Safety Assessment for IRGM Rice**

Food safety of IRGM rice in China has been studied primarily on the basis of the principle of substantial equivalence, the method used in the United States. Various biochemical methods have been used to compare the nutritional components of IRGM and non-GM parental rice grains. In addition, feeding experiments have been conducted on small animals. No significant differences in major nutritional components (e.g., crude protein, crude lipid, free amino acids, and mineral elements) and physicochemical properties (e.g., amylose content, alkali spreading value, and starch viscosity) were found between \( cry1Ab \) or \( CpTI+cry1Ac \) rice lines and their non-GM counterparts (54, 99). However, a compositional difference (three amino acids, two fatty acids, and two vitamins) between disease-resistant and insect-resistant GM rice grains and non-GM controls was recently reported (49). A 90-day laboratory feeding test on rats indicated that \( cry1Ab \) rice flour had no effects on the development of the rats. Necropsy indicated that neither pathological lesions nor histopathological abnormalities were present in liver, kidneys, and intestines of rats in either the IRGM rice group or the non-GM rice group (97). Similar results were reported for mice (21),
rats (20, 124), and pigs (38) fed CptTI rice grains. Different proactive measures, such as cooking (99) or gamma irradiation (100), have also been tested to further reduce insecticidal proteins in IRGM rice grains.

A recent laboratory study indicated that cry1Ab rice could accumulate more heavy metals in grain and straw than non-Bt rice could (90). This study calls for more attention to IRGM rice food safety in areas with heavy-metal pollution, which is not uncommon in China (90).

**SOCIOECONOMIC IMPACTS OF IRGM RICE**

**Yield and Gross Income**

The rice-growing area in China has been decreasing in the last decade due to a lack of agricultural labor (migration into cities), water shortages, and poor profitability of rice production (105), which is in striking contrast to the increasing trend of growing cotton (Supplemental Figure 2). From 2002 to 2004, two IRGM rice lines (cry1Ab/Ac Bt Shanyou 63 and CptTI GM II-youming 86), as a part of pre-production trials, were tested at 17 villages located in eight different counties in Hubei and Fujian Provinces. A three-year survey was conducted with rice farmers to address whether IRGM rice could increase rice yields, reduce insecticide use, and increase farmers’ incomes by adopting the new technology.

In a small-scale field trial conducted in Hubei Province in 1999, no insecticides were applied to both Bt and non-Bt rice fields, and the former rice field yielded 29% more rice (88). In the preproduction field trials in Hubei and Fujian Provinces, based on a survey of 330 households, Bt rice increased yield by up to 9% compared with non-Bt rice (42, 43). IRGM rice farmers spent only 31 yuan per hectare per season on insecticides (US$4.56), whereas non-GM rice farmers spent 243 yuan per hectare per season (US$35.74). Moreover, 3–11% of non-GM rice farmers reported insecticide poisonings, whereas there were no such reports from IRGM rice farmers (41, 43). Recently, a two-year field trial with cry1Ab/Ac Bt Shanyou 63 in Wuhan indicated that Bt rice could increase rice yield by 60–65% compared with non-Bt rice without insecticide applications (94). Clearly, IRGM rice will help rice farmers save on labor and insect control costs and increase their profit.

**Impact of IRGM Rice on Farmers’ Pest Management Practices**

Preproduction trials on IRGM rice indicated that IRGM rice could substantially reduce insecticide use while increasing rice yield (42, 43, 94), which may lead to a change in farmers’ attitudes regarding insecticide use, reduce unnecessary insecticide use, and result in increased profits. However, the impact of IRGM rice on farmers’ pest management practices may be more complicated. Current IRGM rice lines developed in China are primarily first-generation biotech crops with one insecticidal gene. Even if two genes (e.g., CptTI+Cry1Ac rice) are used, both genes are often targeting the same group of lepidopteran pests (stem borers and leaffolders) (Supplemental Table 1). Although other insecticidal genes with different...
pest spectrums, such as gna, were also used for IRGM rice, unsatisfactory control of the target planthopper species made them less desirable for commercial release. Considering the complex of rice pest species in China, enhanced varieties are needed to address the other insects and pest organisms, including planthoppers. This is similar to the situation with Bt cotton in China, which effectively suppressed the target pest, cotton bollworm, but required increasing amounts of insecticide applications for sucking insect species (116). Taking into account the additional cost that an IRGM rice farmer needs to pay for higher-priced seed and control of sucking insect pests, the impact of current IRGM rice lines on farmers’ pest management practices in China needs careful evaluation. Developing future IRGM rice lines with stacked traits targeting multiple groups of insect pests will likely have a more profound effect on farmers’ pest management practices. In addition, proper training and education in agricultural biotechnology and IPM will be crucial to achieve a positive impact on Chinese rice farmers’ pest management practices.

INSECTICIDE RESISTANCE MANAGEMENT

Challenges to IRM for IRGM Rice

Although transgenic plants offer many unique opportunities for the management of pest populations, one major concern regarding long-term use of IRGM plants is the potential for insect resistance (7, 35). Among the various options that have been considered for insecticide resistance management (IRM) for IRGM crops, especially Bt crops, the high dose/refuge and gene pyramiding strategies have strong theoretical support (33, 35, 118) and have been broadly implemented in the United States, Canada, and Australia. After more than a decade of widespread IRGM crops, there have only been two clear-cut cases of resistance involving cry1F and cry1Ab corn (62, 84). However, the few reported resistance incidences in IRGM crops does not mean the failure of the high dose/refuge strategy; instead, insecticidal proteins not expressed at a high dose level in the IRGM crops, plus an insufficient refuge area, are probably the key reasons (84).

The high dose/refuge strategy calls for high expression of insecticidal protein in IRGM plants. The Bt protein expression level in Bt rice is much lower than that in Bt corn (88), and most of the current IRGM rice lines developed in China could not achieve 100% kill of late instars of target pests (18). Hu et al. (40) reported that the 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. Having <99% mortality is not ideal and can lead to a more rapid evolution of resistance, as was seen with Spodoptera frugiperda (Lepidoptera: Noctuidae) (84).

On-farm refuges are not required for Bt cotton in China because its principal target pest Helicoverpa armigera is highly polyphagous and natural refuges can function as unstructured refuges for this pest (102, 120). It is not clear whether there will be a refuge requirement for IRGM rice, but there are no significant alternative wild or cultivated host plants to serve as natural refuges for rice stem borers in most rice-growing regions (28). Thus, it will be difficult to have a highly effective IRM program for IRGM rice in China based on natural refuges alone. In addition, a mixture of single-gene and dual-gene IRGM rice lines is currently under various testing stages in China, and this may result in sequential or concurrent planting of single-gene and dual-gene IRGM rice lines in the fields, a practice that may further challenge IRM for IRGM rice in China (118).

Lastly, in rice fields most stem borer larvae move from plant to plant (29). Such movement can decrease the dose to which pests are exposed and decrease the effective size of refuges in a seed mix IRM strategy (33, 78, 86). Due to routine farming practices such as planting and trading self-kept rice seeds by rice farmers, deliberate or inadvertent mixing of IRGM and non-GM rice seeds could occur and
challenge the sustainable use of IRGM rice in China.

**Options to Increase Durability**

Rice stem borers, the key target pests of the current IRGM rice lines in China, have multiple generations per year [e.g., up to seven generations for *Sesamia inferens* (Noctuidae) and *Scirpophaga incertulas* in rice region I]. Moreover, the number of generations of stem borers per year on rice in China has increased owing to a warming climate (60). Insecticidal proteins could become ineffective within a few years of deployment in IRGM rice unless a proper IRM program is deployed (27). For instance, under laboratory conditions, after 17 generations of selection on *CpTI* rice plants, the percentage striped stem borers that survived on *CpTI* rice plants increased from 10.7% to 42.7% (122).

To help rice farmers fully benefit from IRGM rice, the following practical actions need to be considered.

First, for the longer term it is vital to develop and release IRGM rice lines with pyramided insecticidal genes in which each gene has a different mode of action. IRGM crops with pyramided genes require smaller refuges than do one-toxin lines (7, 76) and are more durable (119). Aside from *CpTI* and *gna*-*SbTI* rice, more IRGM rice lines with pyramided genes in China are being actively investigated, including *cry1Ab+cry1C*, *cry1Ab+cry2A*, *cry1Ac+cry1C*, *cry1Ac+cry2A*, *cry2A+cry1C*, and *cry1Ab+vip3H+G6-epsps* (13, 31). These pyramided lines will certainly benefit IRM programs in the future.

Second, given the difficulty of implementing structured refuges in China, regulatory authorities may concurrently release GM rice lines with different traits (e.g., insect resistant, disease resistant, herbicide resistant, and drought tolerant). Thus, disease-resistant or herbicide-resistant GM rice can serve as refuges for IRGM rice while saving farmers additional costs for disease or weed control. In addition, ensuring adequate seed supplies of popular non-GM varieties may help maintain a certain amount of non-GM rice in the field (27).

Finally, developing education programs on agricultural biotechnology and basic understanding of IPM and IRM for rice farmers will certainly help achieve a sustainable use of IRGM rice. However, because a large number of Chinese farmers are illiterate, on-site or audio-visual interactions will be essential.

**FUTURE DIRECTIONS AND RESEARCH NEEDS**

In the past 20 years, a tremendous amount of research has been conducted on IRGM rice; however, to meet the demand for food from the increasing population in China and to fully benefit from the technology, knowledge gaps on IRGM rice need to be better understood and it should be recognized that additional challenges are yet to come.

With the majority of first-generation IRGM rice lines targeting stem borers, urgent attention should be given to identifying new insecticidal genes with different modes of action targeting different groups of insect pests. Furthermore, emphasis should be placed on developing rice lines with pyramided genes for IRM and GM lines with stacked traits to battle the various rice yield constraints in the field. The new 26 billion yuan (US$3.5 billion) R&D initiative on GM plants in China is helping Chinese scientists work on these aspects (19). Environmental and food safety assessments have been conducted primarily on *Bt* rice in comparison with other IRGM rice (e.g., *gna* rice and *gna*-*SbTI* rice), but research is needed on other non-*Bt* insecticidal genes that are or will be used in IRGM rice lines because some of them have broader insecticidal spectrums than *cry* genes. In addition, some studies have been conducted with unfocused research objectives and unclear hypotheses that have little use in risk assessments. An appropriate evaluation approach, such as tier-based risk assessment on nontarget organisms (71) and proper species selection (69), is needed to
optimize risk assessments for IRGM rice. Strengthening regulations on IRGM rice seed distribution to prevent seed-mediated gene flow and studying its potential ecological and social impacts are urgently needed.

Rice is of great cultural importance throughout Asia and is the predominant staple food for over 3 billion people worldwide. Near and long-term effects of commercial release of IRGM rice in China on the rice trade among different countries will be profound. Although it is clear that Chinese farmers and the Chinese public will benefit from IRGM rice that decreases environmental and food safety risks, the release of IRGM rice may affect rice exports from China to some trading partners (27). China is in a difficult position of balancing its own production needs with the evolving regulations of international trade of GM crops. However, as two biosafety certificates for commercial production of *cry1Ab/Ac* Bt Shanyou 63 and Huahui No. 1 have been issued, this suggests that China sees IRGM rice as an important part of the future.

### SUMMARY POINTS

1. China is the largest producer and consumer of rice in the world, with a >7,000-year history of rice cultivation and six rice-growing regions.

2. There are over 200 insect pests of rice in China. Stem borers and planthoppers are the two major groups of insects that cause losses in rice production totaling billions of yuan annually.

3. In 1989, scientists from the CAAS generated the first Bt rice line, and the first field tests of insect-resistant genetically modified rice took place in 1998 in China.

4. On October 22, 2009, China’s Ministry of Agriculture issued its first two biosafety certificates for commercial production of Bt rice (*cry1Ab/Ac* Huahui No. 1 and *cry1Ab/Ac* Bt Shanyou 63) for Hubei Province.

5. A new generation of GM rice with pyramided genes and stacked traits is needed in China to battle the complex of insect pests of rice and other yield constraints in rice.

6. Effective IRM strategies for IRGM rice are needed to sustain their effectiveness and continued benefits.

7. Education programs on agricultural biotechnology and basic understanding of IPM and IRM will help achieve a positive impact on rice farmers’ pest management practices and sustainable use of IRGM rice.

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Contents

*Bemisia tabaci*: A Statement of Species Status
Paul J. De Barro, Shu-Sheng Liu, Laura M. Boykin, and Adam B. Dinsdale ............ 1

Insect Seminal Fluid Proteins: Identification and Function
Frank W. Avila, Laura K. Sirot, Brooke A. LaFlamme, C. Dustin Rubinstein,
and Mariana F. Wolfner .................................................................................. 21

Using Geographic Information Systems and Decision Support Systems
for the Prediction, Prevention, and Control of Vector-Borne Diseases
Lars Eisen and Rebecca J. Eisen ....................................................................... 41

Salivary Gland Hypertrophy Viruses: A Novel Group of Insect
Pathogenic Viruses
Verena-Ulrike Lietze, Adly M.M. Abd-Alla, Marc J.B. Vreysen,
Christopher J. Geden, and Drion G. Boucias .............................................. 63

Insect-Resistant Genetically Modified Rice in China: From Research
to Commercialization
Mao Chen, Anthony Shelton, and Gong-yin Ye ............................................. 81

Energetics of Insect Diapause
Daniel A. Hahn and David L. Denlinger ...................................................... 103

Arthropods of Medicoveterinary Importance in Zoos
Peter H. Adler, Holly C. Tuten, and Mark P. Nelder ..................................... 123

Climate Change and Evolutionary Adaptations at Species’
Range Margins
Jane K. Hill, Hannah M. Griffiths, and Chris D. Thomas ............................... 143

Ecological Role of Volatiles Produced by Plants in Response
to Damage by Herbivorous Insects
J. Daniel Hare ................................................................................................... 161

Native and Exotic Pests of Eucalyptus: A Worldwide Perspective
Timothy D. Paine, Martin J. Steinbauer, and Simon A. Lawson .................... 181
Urticating Hairs in Arthropods: Their Nature and Medical Significance  
Andrea Battisti, Göran Holm, Bengt Fagrell, and Stig Larsson .............................. 203
The Alfalfa Leafcutting Bee, *Megachile rotundata*: The World’s Most Intensively Managed Solitary Bee  
Theresa L. Pitts-Singer and James H. Cane .............................................................. 221
Vision and Visual Navigation in Nocturnal Insects  
Eric Warrant and Marie Dacke .................................................................................. 239
The Role of Phytopathogenicity in Bark Beetle–Fungal Symbioses: A Challenge to the Classic Paradigm  
Diana L. Six and Michael J. Wingfield ...................................................................... 255
Micky D. Eubanks, Michael J. Raupp, and Deborah L. Finke .................................... 273
The Role of Resources and Risks in Regulating Wild Bee Populations  
Tai H. Roulston and Karen Goodell ......................................................................... 293
Venom Proteins from Endoparasitoid Wasps and Their Role in Host-Parasite Interactions  
Sassan Asgari and David B. Rivers ........................................................................... 313
Recent Insights from Radar Studies of Insect Flight  
Jason W. Chapman, V. Alistair Drake, and Don R. Reynolds .................................. 337
Arthropod-Borne Diseases Associated with Political and Social Disorder  
Philippe Brouqui ......................................................................................................... 357
Ecology and Management of the Soybean Aphid in North America  
David W. Ragsdale, Douglas A. Landis, Jacques Brodeur, George E. Heimpel, and Nicolas Desneux ................................................................. 375
A Roadmap for Bridging Basic and Applied Research in Forensic Entomology  
Visual Cognition in Social Insects  
Aurore Avarguès-Weber, Nina Deisig, and Martin Giurfa ......................................... 423
Evolution of Sexual Dimorphism in the Lepidoptera  
Cerisse E. Allen, Bas J. Zwaan, and Paul M. Brakefield ........................................... 445
Forest Habitat Conservation in Africa Using Commercially Important Insects  
Suresh Kumar Raina, Esther Kioko, Ole Zethner, and Susie Wren .............................. 465
Systematics and Evolution of Heteroptera: 25 Years of Progress  
Christiane Weirauch and Randall T. Schub ................................................................ 487