Assessing the effects of Cry1C rice and Cry2A rice to *Pseudogonatopus flavifemur*, a parasitoid of rice planthoppers

Jun-Ce Tian¹, Jörg Romeis², Kai Liu³, Fa-Cheng Zhang³, Xu-Song Zheng¹, Hong-Xing Xu³, Gui-Hua Chen³, Xiao-Chan He⁴ & Zhong-Xian Lu¹

Transgenic rice producing insecticidal proteins from *Bacillus thuringiensis* (Bt) could help protect the plants from damage by lepidopteran pests. However, one concern is the potential of Bt rice to harm non-target natural enemies, which play a vital role in pest control. In the present study, the potential effects of Cry1C rice and Cry2A rice on different life-table parameters and population dynamics of *Pseudogonatopus flavifemur*, a parasitoid of rice planthoppers, were evaluated under laboratory and field condition. The exposure of *P. flavifemur* to plant-produced Bt proteins was also analyzed. Results indicated that direct feeding on rice plants was the main exposure pathway of *P. flavifemur* to the Cry1C and Cry2A proteins. No significant difference on the development, survival, longevity, fecundity, and prey consumption of *P. flavifemur* was detected over two generations between the Bt and non-Bt rice treatments. Furthermore, the population dynamics of *P. flavifemur* were not affected by Cry1C rice and Cry2A rice. In conclusion, the tested Cry1C rice and Cry2A rice do not appear to harm the parasitoid *P. flavifemur*.

Rice, *Oryza sativa* L., is one of the principal staple foods in the world. More than 50% of the world populations depend on rice for their daily lives¹. According to various estimates, the global population is expected to reach 9.0 billion by 2050², and 40% more rice must be produced to meet the increasing needs of the projected human population; therefore, improvements in yield are urgently required. However, 13–26% of rice yield are lost due to pests³. Rice stem borers, for example, are responsible for 3–10% annual loss in yield and economic losses of 11.5 billion yuan (US$ 1.85 billion) annually in China alone⁴. Numerous genetically modified (GM) rice lines expressing insecticidal crystal (Cry) proteins from *Bacillus thuringiensis* Berliner (Bt) have been developed to control Lepidoptera pests, i.e., stem borers and leaf folders⁵,⁶. Field studies with a Bt rice line in China revealed an increase in yield by 6–9% and a reduction in pesticides usage by 80%⁷. Due to those potential benefits, the Chinese government has issued the biosafety certificates and approved limited releases of two Cry1Ab/Cry1Ac rice lines in farmers’ fields in Hubei Province from 2009 to 2014⁸ with an extension from 2014–2019⁹. However, before being used widely the impact of Bt rice on the environment should be assessed¹⁰,¹¹. One concern is the potential of Bt rice to adversely affect natural enemies which play a vital role in pest control¹². To date, a series of studies have focused on the impacts of Bt rice on the population dynamics, abundance and diversity of natural enemies¹³–¹⁸. In addition, laboratory studies have been conducted to assess the impact of Bt rice on lethal and sublethal endpoints of important natural enemy species that are common in Chinese rice fields (Li et al. 2017). These include the predators *Cyrtorhinus lividipennis* (Hemiptera: Miridae)¹⁷–¹⁹, *Propylea japonica* (Coleoptera: Coccinellidae)²⁰,²¹, *Paederus fuscipes* (Coleoptera: Staphylinidae)²², *Chrysoperla nipponensis* (Neuroptera: Chrysopidae)²³, *Ummeliata insecticeps* (Araneida: Linyphiidae)²⁴, *Parasita pseudomulata* (Aranea: Lycosidae)²⁵, and the parasitoid *Anagrus nilaparvatae* (Hymenoptera: Mymaridae)²⁶,²⁷.

¹Key Laboratory of Information Traceability for Agricultural Products, Ministry of Agriculture of China, Institute of Plant Protection and Microbiology, Zhejiang Academy of Agricultural Sciences, 198 Shiqiao Road, Hangzhou, 310021, China. ²Agroscope, 8046, Zurich, Switzerland. ³Jinhua Plant Protection Station, Jinhua, 321017, China. ⁴Jinhua Research Academy of Agricultural Sciences, Jinhua, 321017, China. Correspondence and requests for materials should be addressed to Z.-X.L. (email: luzxmh@163.com)
However, the risks of Bt rice on parasitoids attacking planthoppers that are not affected by the insecticidal trait have not been assessed so far.

*Pseudonatatorius flavifemur* (Hemiptera: Dryandinidae) is one of the most common parasitoids of *Nilaparvata lugens* (Hemiptera: Delphacidae)\(^34\), which is one of the most serious rice pests in south Asian, and is consistently characterized by a sexual dimorphism\(^35\). The ant-like, wingless female wasps deposit their eggs on nymphs and adults of *N. lugens* in addition to host feeding on the planthoppers\(^36\). The wasps could thus be exposed to Bt proteins when larval or female adults feed on Bt rice-fed *N. lugens*. In the present study, we evaluated the exposure pathways of *P. flavifemur* to Cry1C and Cry2A produced by Bt rice, the tri-trophic effects of Bt rice on different life-table parameters of *P. flavifemur* and the potential effects of Bt rice on *P. flavifemur* populations in the field.

**Results**

**Tri-trophic bioassay with *P. flavifemur***. Studies were conducted to assess the impact of Bt rice feeding by *N. lugens* on the performance of the parasitoid *P. flavifemur*. Eight to eleven days after parasitism, *P. flavifemur* larvae formed cocoons and adults emerged 10.5–14 days later. For both parasitoid generations studied, no significant differences among the two Bt and the non-Bt rice lines were detected on a number of important life-table parameters of *P. flavifemur* (Table 1).

**Bt protein levels in rice plants, *N. lugens* and *P. flavifemur***. Stems of Cry1C rice contained a mean of 3.86 μg/g fresh weight (FW) (Table 2). The average of Cry1C protein detected in *P. flavifemur* that had been exposed to Bt rice plants infested with *N. lugens* or to uninfested Bt rice plants for 48 h was 0.15 μg/g and 0.143 μg/g FW, respectively, which was significantly lower than those in Cry1C rice stem, but significantly higher than those in *N. lugens* (0.053 μg/g FW) \((F = 225.82; df = 3, 19; P < 0.001)\).

Similar results were reported from other tri-trophic studies involving a Bt plant, a herbivore and a parasitoid. When *Cotesia marginiventris* (Hemiptera: Braconidae) developed in Cry1Ac maize-fed *Spodoptera littoralis*\(^32\), Bt protein levels in the predators when compared to those in the prey, i.e., Bt rice-fed *N. lugens*. As expected, no Bt protein was detected in the stem, *N. lugens* and *P. flavifemur* from the respective non-Bt rice treatment.

**P. flavifemur** and **N. lugens** populations in Bt rice and non-Bt rice fields. Field experiments were conducted at two experimental sites. At the Dongyang site, sampling date significantly affected the populations of *N. lugens* \((F = 118.80; df = 3, 35; P < 0.001)\) and *P. flavifemur* \((F = 34.73; df = 3, 35; P < 0.001)\). The factor rice line \((N. lugens: F = 0.97; df = 2, 35; P = 0.39; P. flavifemur: F = 0.17; df = 2, 35; P = 0.85)\) and the interaction between sampling date and rice line were not significant \((N. lugens: F = 1.01; df = 6, 35; P = 0.45; P. flavifemur: F = 0.44; df = 6, 35; P = 0.84)\). The ratio of *N. lugens* to *P. flavifemur* was not significantly affected by Cry1C rice \((F = 0.73; df = 2, 35; P = 0.52)\) or Cry2A rice \((F = 1.14; df = 3, 35; P = 0.41)\) and the interaction between the two factors was not significant \((F = 0.63; df = 6, 35; P = 0.70)\) (Fig. 1A).

Similar results were found at the Jinhua site. Although sampling date significantly affected the populations of *N. lugens* \((F = 13.40; df = 3, 35; P = 0.001)\) and *P. flavifemur* \((F = 6.33; df = 3, 35; P = 0.003)\), the populations did not differ among rice lines \((N. lugens: F = 0.15; df = 2, 35; P = 0.87; P. flavifemur: F = 0.34; df = 2, 35; P = 0.72)\) and the interaction between sampling date and rice line were not significant \((N. lugens: F = 0.03; df = 6, 35; P = 0.99; P. flavifemur: F = 0.21; df = 6, 35; P = 0.97)\). For the ratio of *N. lugens* to *P. flavifemur*, sampling date significantly affect the ratio \((F = 8.21; df = 2, 35; P < 0.001)\), but rice line did not \((F = 0.08; df = 2, 35; P = 0.92)\) and the interaction between sampling date and rice line was also not significant \((F = 0.09; df = 6, 35; P = 0.99)\) (Fig. 1B).

**Discussion**

Natural enemies of crop pests may be at risk from the growing of Bt rice if they are exposed to the insecticidal Cry proteins when attacking their prey or hosts. That the Bt rice produced Cry proteins move through the arthropod food web in rice has recently been confirmed\(^8,9\). In the present study, we confirmed the presence of Cry1C and Cry2A in the tissue of two Bt rice lines and in *N. lugens* that had fed on the plants, albeit at a very low concentration (reduced by a factor of 72 and 135 when compared to the two Bt rice lines, respectively). No Bt protein was detected in *P. flavifemur* larvae, cocoons, and newly emerged adults that had developed in Bt rice-fed *N. lugens*. Similar results were reported from other tri-trophic studies involving a Bt plant, a herbivore and a parasitoid. When *Cotesia marginiventris* (Hemiptera: Braconidae) developed in Cry1Ac maize-fed *Spodoptera littoralis*\(^32\), Bt protein levels in *C. marginiventris* larvae, cocoons, and adults were below the detection limit. In the case of *P. flavifemur*, however, female wasps were found to contain Bt protein when being exposed to Bt rice plants infested with *N. lugens*. The Bt protein levels in female wasps were significantly lower than those in Bt rice (by a factor of 25) but significantly higher (by a factor of 3) that those detected in *N. lugens*. Previous studies conducted with predators of *N. lugens* such as the spiders *U. insecticeps*\(^34\) and *P. pseudoannulata*\(^25\) and the rove beetle *P. fuscipes* had revealed significantly lower Bt levels in the predators when compared to those in the prey, i.e., Bt rice-fed *N. lugens*. Thus, we conducted an additional experiment to test the Bt protein levels in *P. flavifemur* that were exposed to Bt rice alone. The fact that we were able to detect similar high amounts of Bt proteins in the wasps indicates that they have consumed plant material, a fact that has not been reported before. *P. flavifemur*, as many other species of Dryinidae, are known to possess strong mandibles that allow them to bite the host’s integument in order to feed on the leaking haemolymph\(^35\). It is thus possible that they are able to also feed on the plants directly. Consequently, feeding on the rice plants rather than host feeding on *N. lugens* appears to be their main pathway of exposure. A similar finding has been reported for an omnivorous predator, the mirid bug *C. lividipennis*. The Cry2Aa protein levels in...
Their hosts. However, the tested Cry1C rice and Cry2A rice lines did neither affect the development, survival, longevity, and fecundity of *Nephotettix cincticeps* with a number of studies that have not seen any effect of Cry1C rice and Cry2A rice on the population dynamics. Our two-site field experiments showed that Cry1C rice and Cry2A rice did not affect the populations. Our study, however, was conducted at realistic exposure levels. We were exposed to Bt proteins by directly feeding on Bt rice plants rather than through one type of exposure condition. The current study is the first to assess the potential effects of Bt rice on a dryid wasp. Our results are in accordance with those obtained previously for the same Bt rice lines containing Cry1C or Cry2A or purified Cry proteins and different natural enemies. Larvae of the green lacewing *Chrysoperla japonica* (as *C. sinica*) (Neuroptera: Chrysopidae) or the ladybird beetle *P. japonica* where not affected by Cry1C or Cry2A when fed pollen from Bt rice or high doses of purified Bt protein provided within artificial diet.

### Methods

**Plants.** Two transgenic Bt rice lines, T1C-19 (Cry1C rice) expressing Cry1C protein and T2A-1 (Cry2A rice) expressing the Cry2A protein, and the untransformed parental commercial non-Bt rice MIH63 were used for C. *lividipennis* that had been provided with Cry2A rice plants was higher than those that had consumed Cry2A rice-fed *N. lugens.*

Though female *P. flavifemur* adults were exposed to Cry1C or Cry2A proteins in our tri-trophic bioassays, no significant difference in development, survival, longevity, fecundity, prey consumption, and progeny sex ratio were found between Bt rice and non-Bt rice lines over two generations.

The current study is the first to assess the potential effects of Bt rice on a dryid wasp. Our results are in accordance with those obtained previously for the same Bt rice lines containing Cry1C or Cry2A or purified Cry proteins and different natural enemies. Larvae of the green lacewing *Chrysoperla japonica* (as *C. sinica*) (Neuroptera: Chrysopidae) or the ladybird beetle *P. japonica* where not affected by Cry1C or Cry2A when fed pollen from Bt rice or high doses of purified Bt protein provided within artificial diet.

### Table 1

| Parameters                         | Cry1C rice | Cry2A rice | Non-Bt rice | Statistics |
|-----------------------------------|------------|------------|-------------|------------|
| 1st generation                    |            |            |             |            |
| Development (days)                |            |            |             |            |
| Eggs to cocoons                   | 9.7 ± 0.3  | 9.6 ± 0.4  | 9.4 ± 0.4   | F = 0.14; df = 2.29; P = 0.87 |
| Male eggs to adults               | 22.3 ± 0.4 | 22.2 ± 0.2 | 22.0 ± 0.3  | F = 0.14; df = 2.29; P = 0.87 |
| Female eggs to adults             | 23.0 ± 0.5 | 22.7 ± 0.3 | 22.8 ± 0.5  | F = 0.12; df = 2.29; P = 0.89 |
| Cocoons to adults survival (%)    | 84.7 ± 1.7 | 83.7 ± 2.2 | 81.6 ± 2.5  | F = 0.52; df = 2.29; P = 0.60 |
| Male longevity (days)             | 2.7 ± 0.2  | 3.1 ± 0.3  | 2.9 ± 0.3   | F = 0.53; df = 2.29; P = 0.59 |
| Female longevity (days)           | 11.3 ± 1.5 | 10.1 ± 1.2 | 10.4 ± 1.6  | F = 0.17; df = 2.29; P = 0.84 |
| No. consumed nymphs               | 47.9 ± 5.3 | 45.6 ± 6.5 | 43.9 ± 3.6  | F = 0.14; df = 2.29; P = 0.87 |
| Fecundity                         | 53.0 ± 6.9 | 49.4 ± 6.2 | 51.3 ± 5.7  | F = 0.08; df = 2.29; P = 0.92 |
| Sex ratio (%)                     | 33.3 ± 1.6 | 32.2 ± 1.7 | 31.7 ± 1.5  | F = 0.27; df = 2.29; P = 0.76 |
| 2nd generation                    |            |            |             |            |
| Development (days)                |            |            |             |            |
| Eggs to cocoons                   | 9.8 ± 0.3  | 9.6 ± 0.3  | 9.5 ± 0.3   | F = 0.40; df = 2.29; P = 0.67 |
| Male eggs to adults               | 22.5 ± 0.3 | 22.0 ± 0.2 | 22.4 ± 0.3  | F = 0.20; df = 2.29; P = 0.80 |
| Female eggs to adults             | 23.2 ± 0.4 | 23.1 ± 0.2 | 23.4 ± 0.4  | F = 1.04; df = 2.29; P = 0.37 |
| Cocoons to adults survival (%)    | 79.5 ± 2.1 | 76.0 ± 1.3 | 78.5 ± 2.6  | F = 0.55; df = 2.29; P = 0.59 |
| Male longevity (days)             | 3.1 ± 0.3  | 3.1 ± 0.3  | 3.0 ± 0.3   | F = 0.08; df = 2.29; P = 0.92 |
| Female longevity (days)           | 9.3 ± 1.1  | 9.0 ± 0.8  | 9.9 ± 1.4   | F = 0.15; df = 2.29; P = 0.86 |
| No. consumed nymphs               | 48.0 ± 7.2 | 46.8 ± 5.1 | 50.2 ± 7.9  | F = 0.05; df = 2.29; P = 0.96 |
| Fecundity                         | 45.6 ± 5.4 | 48.4 ± 2.9 | 43.7 ± 3.9  | F = 0.31; df = 2.29; P = 0.74 |
| Sex ratio (%)                     | 30.7 ± 2.2 | 29.3 ± 2.1 | 31.8 ± 2.0  | F = 0.25; df = 2.29; P = 0.79 |
laboratory and field evaluation. The gene cry1C and cry2A gene were synthesised on the basis of the amino acid sequence of the corresponding wild-type cry1Ca5 gene and cry2Aa gene of B. thuringiensis and both driven by maize ubiquitin promoter. Both transgenic Bt rice lines have high resistance to stem borers and leaffolders under laboratory and field conditions. MH63 is an elite indica restorer strain for cytoplasmic male-sterility in China and served as the control. All the above rice lines were supplied by the National Key Laboratory of Crop Genetic Improvement and National Centre of Plant Gene Research (Wuhan), Huazhong Agricultural University, China. Taichung Native 1 (TN1), a pest-susceptible rice variety obtained from the International Rice Research Institute (Los Baños, Laguna, Philippines), was used to maintain the N. lugens colony.

The rice plants were cultured in a plastic tank (200 cm length × 50 cm width × 15 cm height) in Yoshida culture solution in the greenhouse. 45-day-old rice seedlings were used in the laboratory experiments. All the plants were maintained at 26 ± 2°C and the relative humidity was 75 ± 5%.

| Sample                                | Amount (μg/g FW)          |
|---------------------------------------|---------------------------|
|                                       | Cry1C rice  | Cry2A rice  | non-Bt rice |
| Rice stem                             | 3.86 ± 0.50 a  | 9.07 ± 0.44 a  | n.d. |
| N. lugens                             | 0.053 ± 0.008 c | 0.067 ± 0.007 c | n.d. |
| P. flavifemur larvae                   | n.d.         | n.d.         | n.d. |
| P. flavifemur cocoons                  | n.d.         | n.d.         | n.d. |
| Newly emerged male P. flavifemur       | n.d.         | n.d.         | n.d. |
| Newly emerged female P. flavifemur     | n.d.         | n.d.         | n.d. |
| P. flavifemur exposed to Bt rice infested with N. lugens | 0.150 ± 0.010 b | 0.275 ± 0.022 b | n.d. |
| P. flavifemur exposed to uninfested Bt rice | 0.143 ± 0.007 b | 0.236 ± 0.028 b | n.d. |

Table 2. Bt protein levels in Bt rice plants, Nilaparvata lugens and Pseudogonatopus flavifemur. Means (±SE) within a column followed by different letters are significantly different (One-way ANOVA, P < 0.05); N = 5. n.d. – not detectable. The detection limit for the two Cry proteins was 1 ng/g.

Figure 1. Population dynamics of Nilaparvata lugens and Pseudogonatopus flavifemur in 2013. Data are represented as mean ± SE. (A) Dongyan field site; (B) Jinha field site. There was no significant difference between the Cry1C, Cry2A and non-Bt rice fields (repeated-measured ANOVA and Tukey’s multiple comparison tests, P < 0.05).
Insects. A colony of *N. lugens* was collected from paddy fields (30.31° N, 120.19° E) in the suburb of Hangzhou, Zhejiang Province, China, and maintained on TN1 at 26 ± 2°C, 75 ± 5% RH, under a light and dark regime of 14:10 h. Prior to the tri-trophic bioassays, independent colonies of *N. lugens* were established on Cry1C rice, Cry2A rice and non-Bt rice and maintained for more than 10 generations before being used in the experiments.

*P. flavifemur* adults were collected from the same paddy fields where *N. lugens* was collected and maintained on TN1 with *N. lugens* for 3 generations before being used in the bioassay.

Tri-trophic bioassay with *P. flavifemur*. Newly emerged female and male *P. flavifemur* adults from TN1 rice were paired in a glass tube (Diameter 2 cm, Height 25 cm) that contained a 45-day-old Cry1C, Cry2A or non-Bt rice seedling in 10 mL Yoshida culture solution. Cotton wool was warped around the rice plants and sealed the glass tube to prevent insects escaping. After allowing 24 h for mating, twenty 3rd instar *N. lugens* nymphs from the corresponding rice line were introduced to the wasps. After a 24 h exposure period, alive *N. lugens* nymphs were transferred into a new glass tube containing a corresponding rice seedling. Dead *N. lugens* nymphs were removed and checked under the microscope for signs of host feeding. The number of *N. lugens* killed by host feeding was recorded. Subsequently, a second batch of twenty *N. lugens* nymphs from the corresponding rice line was exposed to the same pair of *P. flavifemur* for another 24 h. Alive nymphs were transferred into a new glass tube and dead nymphs were counted in the same manner. New *N. lugens* nymphs were provided to *P. flavifemur* daily until the female wasp had died. Parasitized *N. lugens* nymphs were checked twice per day (9 am and 9 pm) and the time when parasitoids cocoons formed and adults emerged was recorded. Ten pairs of *P. flavifemur* were utilized for the Cry1C rice, Cry2A rice and non-Bt rice treatments. The offspring of *P. flavifemur* underwent another generation as described above. The developmental time, adult longevity and fecundity of *P. flavifemur* were estimated.

Transfer of Cry1C and Cry2A through tri-trophic levels. An additional 10 pairs of *P. flavifemur* were set-up for each of the three rice lines parallel to the second generation study, as described above. For each treatment, five samples (replications) of the following materials were collected and analysed by ELISA: rice stem (100 mg per sample), *N. lugens* nymphs (3 insects pooled per sample), *P. flavifemur* larvae (10 larvae pooled per sample), *P. flavifemur* cocoons (10 cocoons pooled per sample), newly emerged male *P. flavifemur* (5 males pooled per sample), and newly emerged female *P. flavifemur* (5 females pooled per sample). In addition, groups of 5 female *P. flavifemur* were contained in a glass tube containing a rice seedling and *N. lugens* nymphs, and groups of 5 female *P. flavifemur* were contained in a glass tube containing a rice seedling and *P. flavifemur* nymphs. The developmental time, adult longevity and fecundity of *P. flavifemur* were estimated.

Field experiments. Cry1C rice (T1C-19), Cry2A rice (T2A-1) and non-Bt rice (MH63) were planted at the Jinhua Plant Protection Experimental Station (Jinhua) and the Zhejiang Middle Experimental Station (Dongyang) in 2013 at the restricted field testing site. The experiments were managed following the Implementation Regulations on Safety Assessment of Agricultural Genetically Modified Organisms issued by the Ministry of Agriculture of the People's Republic of China. At Jinhua, rice seeds were sown on 25 June, and seedlings were transplanted on 25 July. At Dongyang, rice seeds were sown on 1 July, and seedlings were transplanted on 25 July. At both sites, the field was divided into nine experimental plots in a 3 (treatments: Cry1C rice, Cry2A and non-Bt rice) × 3 (replications) completely randomized design. Each experimental plot was 15 × 15 m. Each plot was bordered on all sides by a 50-cm-wide unplanted walkway. Seedlings were hand transplanted at one seedling per hill spaced 16.5 × 16.5 cm apart, and the entire experimental field was surrounded by five border rows of non-Bt plants (MH63). Normal cultural practices for growing rice, such as fertilization and irrigation, were followed during the course of the experiment, except that no pesticides were applied. A white porcelain plate (46 cm length × 36 cm width × 3.5 cm height), as described by [45], was used to monitor the density of *N. lugens* and *P. flavifemur*. This plate is made of metal, and its surface is painted white. On each sampling date, 30 randomly selected hills were sampled in each plot. When sampling, the plate was held at a 45° angle to the ground, and a single hill was carefully grasped at the lower stem and then quickly bent into the plate. The sampled hill was beaten vigorously against the side of the plate for 4–5 s periods (about 13–15 beats). Subsequently, *N. lugens* and female *P. flavifemur* on the plate were counted immediately. Samples were taken in all plots on a 7–15 day schedule, beginning 1 month after transplanting until the rice reached full maturity. There were four sampling dates at both sites.

Statistical analyses. Data on life table parameters of *P. flavifemur*, Bt protein residues in plants and insects, and were all analyzed using one-way ANOVA and Tukey’s multiple comparison tests. Field data were analyzed by repeated-measured ANOVA and Tukey’s multiple comparison tests. Before analysis, all percentage data were arcsine transformed, but untransformed means are presented. All statistical calculations were performed with SAS version 9.1 package.
References

1. FAO. A regional rice strategy for sustainable food security in Asia and the Pacific. http://www.fao.org/3/aa162896-2f7b-51d2-8b63-4644fa320d061/13643e.pdf (2014).
2. Godfray, H. C. et al. Food security: the challenge of feeding 9 billion people. Science 327, 812–818 (2010).
3. Oerke, E.-C. Crop losses to pests. J. Agr. Sci. 144, 31–43 (2006).
4. Dang, C., Bernal, C. C., Aguda, R. M. & Cohen, M. B. Effect of rice lines transformed with Bacillus thuringiensis on performance and predation of a generalist spider. Transgenic Res. 23, 257–264 (2014).
5. Jervis, M. A. & Kidd, N. A. C. Host-feeding strategies in hemipteran parasitoids. Biol. Rev. 61, 395–434 (1986).
6. Tian, J.-C. et al. Laboratory and field assessments of prey-mediated effects of transgenic Bt rice on the parasitoid Anagrus nilaparvatae. Transgenic Res. 20, 1–22 (2011).
7. Yu, Z. et al. Impacts of Bt rice expressing Cry1C protein on the performance of nontarget leafflower, Nephotettix cincticeps (Hemiptera: Cicadellidae). Environ. Entomol. 39, 1330–1337 (2010).
8. Liu, Z. B. et al. Transgenic indica rice plants harboring a synthetic cry2Aa gene of Bacillus thuringiensis exhibit enhanced resistance against lepidopteran pest, NP. Theor. Appl. Genet. 111, 1330–1337 (2005).
9. Tian, J.-C. et al. Transgenic Cry1Ab rice does not impact ecological fitness and predation of a generalist spider. Transgenic Res. 20, 46–51 (2011).
10. Tian, J.-C. et al. Laboratory and field assessments of prey-mediated effects of transgenic Bt rice on the parasitoid Anagrus nilaparvatae. Transgenic Res. 23, 257–264 (2014).
11. Jervis, M. A. & Kidd, N. A. C. Host-feeding strategies in hemipteran parasitoids. Biol. Rev. 61, 395–434 (1986).
12. Li, Y. et al. Bt rice expressing Cry2Aa does not cause direct detrimental effects on larvae of Chrysoperla sinica. Environ. Toxicol. Chem. 33, 1391–1397 (2014).
13. Han, Y. et al. Bt rice expressing Cry2Aa does not harm Cry1Ab, Cry1Ac, Cry1C and cry2Aa rice on non-target plant hoppers and their main predators under field conditions. J. Integr. Agr. 10, 1739–1747 (2011).
14. Han, Y. et al. Bt rice in China - focusing the non-target risk assessment. Plant Biotechnol. J. 18, 7679 (2015).
15. Ministry of Agriculture of the People’s Republic of China (MAPRC). The second list of approval agricultural genetically modified organisms' safety certificates in 2009. Available: http://www.stee.agri.gov.cn/biosafety/pxpy/P020091127591594359668.pdf [In Chinese]. Accessed 2017 March 27 (2009).
16. Ministry of Agriculture of the People’s Republic of China (MAPRC). The third list of approval agricultural genetically modified organisms' safety certificates in 2014. Available: http://www.moa.gov.cn/zjzl/syzwqy/sxpx/201502/P020160524354739618071.pdf [In Chinese]. Accessed 2017 March 27 (2014).
17. Han, Y. et al. Assessment of risk of insect-resistant transgenic crops to non-target arthropods. Nat. Biotechnol. 26, 203–208 (2008).
18. Bernal, C. C., Aguda, R. M. & Cohen, M. B. Effect of rice lines transformed with Bacillus thuringiensis on performance and predation of a generalist spider. Transgenic Res. 23, 257–264 (2014).
19. Tian, J.-C. et al. Laboratory and field assessments of prey-mediated effects of transgenic Bt rice on the parasitoid Anagrus nilaparvatae. Transgenic Res. 20, 1–22 (2011).
20. Yu, Z. B. et al. Transgenic cry1C or cry2Aa rice has no adverse impacts on the life-table parameters and population dynamics of the brown planthopper, Nilaparvata lugens (Hemiptera: Delphacidae). Pest Manag. Sci. 71, 937–945 (2015).
21. Tian, J.-C. et al. Eliminating host-mediated effects demonstrates Bt maize producing Cry1F has no adverse effects on the parasitoid Cotesia marginiventris. Transgenic Res. 23, 257–264 (2014).
22. Tian, J.-C. et al. Laboratory and field assessments of prey-mediated effects of transgenic Bt rice on the parasitoid Anagrus nilaparvatae. Transgenic Res. 20, 1–22 (2011).
23. Liu, Z. et al. Impacts of Bt rice expressing Cry1C or Cry2Aa protein on the performance of nontarget leafflower, Nephotettix cincticeps (Hemiptera: Cicadellidae). Environ. Entomol. 39, 1330–1337 (2010).
24. Yu, Z. B. et al. Transgenic cry1C or cry2Aa rice has no adverse impacts on the life-table parameters and population dynamics of the brown planthopper, Nilaparvata lugens (Hemiptera: Delphacidae). Pest Manag. Sci. 71, 937–945 (2015).
Acknowledgements
This project was supported by China Agriculture Research System (CARS-01-17) and the National Special Key Project for Transgenic Breeding (2016ZX08001001).

Author Contributions
J.T. and Z.L. designed the experiments, J.T., K.L., F.Z., X.Z., H.X. and X.H. conducted the experiments, J.T. and Z.L. conducted the statistical analyses and J.T., J.R., G.C. and Z.L. wrote the manuscript. All authors reviewed the manuscript.

Additional Information
Competing Interests: The authors declare that they have no competing interests.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2017