Research Article

Received: 20 September 2012 Revised: 11 October 2013 Accepted article published: 17 October 2013 Published online in Wiley Online Library: 11 November 2013

(wileyonlinelibrary.com) DOI 10.1002/ps.3667

Single and fused transgenic *Bacillus thuringiensis* rice alter the species-specific responses of non-target planthoppers to elevated carbon dioxide and temperature

Guijun Wan,[a] Zhihao Dang,[a] Gang Wu,[b] Megha N Parajulee,[c] Feng Ge[d] and Fajun Chen[a*]

Abstract

BACKGROUND: The approval of transgenic *Bacillus thuringiensis* (*Bt*) rice by China was momentous for biotech crops, although it has yet to be approved for commercial production. Non-target pest problems in rice paddies, such as the three ecologically similar species of planthoppers *Nilaparvata lugens*, *Laodelphax striatellus* and *Sogatella furcifera*, could become increasingly serious under global climate change. Fused (*Cry1Ab*/*Cry1Ac*) and single (*Cry1Ab*) transgenic *Bt* rice were evaluated for effects on species-specific responses of planthoppers to elevated carbon dioxide (CO_2) and temperature.

RESULTS: Transgenic *Bt* rice lines significantly modified species-specific responses of the planthoppers to elevated CO_2 and temperature. High temperature appears to favour outbreaks of *S. furcifera* relative to *N. lugens* and *L. striatellus* when feeding upon fused transgenic *Bt* rice, especially at elevated CO_2. Elevated CO_2 at high temperature appears to be a factor reducing *S. furcifera* occurrence when feeding upon single transgenic *Bt* rice.

CONCLUSION: Different types of transgenic *Bt* rice alter the species-specific responses of non-target planthoppers to elevated CO_2 and temperature. Compared with their non-transgenic parental lines, the single transgenic *Bt* rice shows better performance in controlling the non-target planthopper *S. furcifera* by comparison with the fused transgenic *Bt* rice under elevated CO_2 and temperature. It is suggested that multitypes of transgenic *Bt* rice be used in the field simultaneously in order to take advantage of high transgenic diversity for optimal performance against all pests in paddy fields.

Keywords: transgenic *Bt* rice; climate change; fused/single transgene; transgenic diversity; population pattern/dynamics; non-target resistance

1 INTRODUCTION

Carbon dioxide (CO_2) and temperature levels have increased by 31% and 0.7 °C, respectively, since the mid-1800 s, and air temperature is predicted to increase by 2–4 °C, with doubling of the current CO_2 concentration by the 2100 s.[1,2] This projected atmospheric warming, combined with projected atmospheric CO_2 level increases, may have marked ecological effects on the vegetative growth responses of forest and agricultural plants, and there is an emerging consensus that elevated CO_2 and temperature levels are stimulatory (positive) and inhibitory (negative) respectively.[3] As elevated CO_2 and temperature also alter plant quality (nutritional and defensive characteristics) and quantity, these effects may, in turn, cascade through food chains and affect higher trophic levels, such as insect herbivores.[4,5]

Transgenic *Bacillus thuringiensis* (*Bt*) crops have been commercially adopted worldwide and have provided excellent performance against target lepidopteran pests (mainly chewing insects) in diverse cropping systems, and they simultaneously play a great potential role in integrated pest management (IPM).[6–8] On 27 November 2009, China's Ministry of Agriculture (MOA) granted biosafety certificates issued for a rice restorer line (cv. *Bt* Huahui-1) and a hybrid rice line (cv. *Bt* Shanyou-63), both of which expressed fused *Cry1Ab*/*Cry1Ac* genes.[9] It is estimated that 75% of all rice in China is commonly infested with rice stem borer (*Chilo suppressalis*), a target pest for which *Bt* rice provides control.[24,25]

Bt rice offers the potential to generate economic benefits of around $US 4 billion annually, associated with an average yield increase of up to 8% and an 80% decrease in insecticide use in China.[9,10] Following the early 2000s, the sucking

* Correspondence to: Fajun Chen, Department of Entomology, Nanjing Agricultural University, Nanjing 210095, China. E-mail: fajunchen@njau.edu.cn

a Department of Entomology, Nanjing Agricultural University, Nanjing, China

b Department of Plant Protection, Huazhong Agricultural University, Wuhan, China

c Texas A&M AgriLife Research, Lubbock, TX, USA

d Institute of Zoology, Chinese Academy of Sciences, Beijing, China

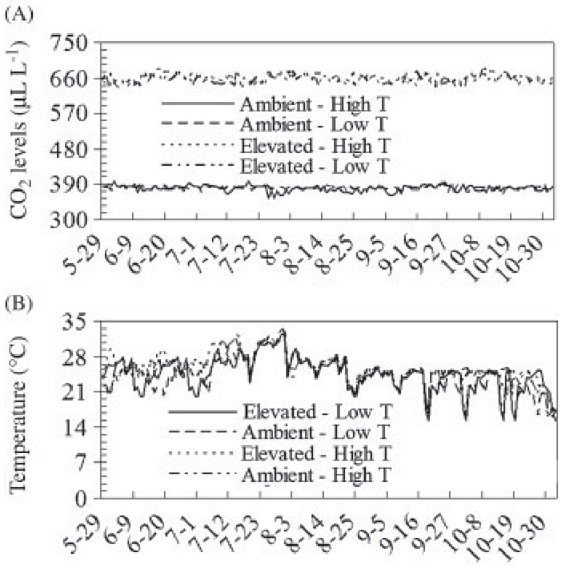

Figure 1. The dynamics of CO_2 levels (A) and temperature levels (B) of the four CO_2 × temperature level treatments from 29 May to 2 November 2010 (Ambient–High T = treatment with ambient CO_2 and high temperature; Ambient–Low T = treatment with ambient CO_2 and low temperature; Elevated–High T = treatment with elevated CO_2 and high temperature; Elevated–Low T = treatment with elevated CO_2 and low temperature).

insects – brown planthopper *Nilaparvata lugens*, small brown planthopper *Laodelphax striatellus* and white-backed planthopper *Sogatella furcifera*, none of which is controlled by *Bt* rice – caused serious annual yield losses and became secondary pests in high-yielding agricultural systems. Planthopper outbreaks may have been partially triggered by climate change, especially global warming.[11,12]

In fact, warmer temperatures are likely to accompany future ambient CO_2 concentration increases. Some potentially significant consequences of higher future CO_2 and temperature levels have been elucidated in both target and non-target *Bt* cotton insect pests.[13–20] Temperature–CO_2 interactions have been studied in plants,[21,22] but few studies have investigated such interactions in herbivorous insects, even though such investigations are critically needed.[23]

In the present experiment, the species-specific population abundance responses of *N. lugens*, *L. striatellus* and *S. furcifera* to elevated CO_2 and temperature levels were studied in open-top chambers in order to ascertain non-target resistance against planthoppers and their population patterns in *Bt* rice. In simultaneous experiments, the effectiveness of single transgenic *Bt* rice (cv. *Bt* Kemingdao-2 expressing the *Cry1Ab* gene)[24] and of double-stacked fused transgenic *Bt* rice (cv. *Bt* Huahui-1 expressing *Cry1Ab/Cry1Ac*)[25] was compared with that of their respective parental lines to ascertain biotech crop resistance features against serious non-target insect pests under realistic environmental conditions as predicted by global climate change scenarios.

2 MATERIALS AND METHODS

2.1 Open-top chambers

This experiment was conducted in 12 open-top chambers (OTCs), each 2.5 m in height × 3.2 m in diameter, in Ningjin County, Shandong Province, China (37° 38′ 30.7″ N, 116° 51′ 11.0″ E). Two CO_2 levels, ambient (375 μL L^{-1}) and elevated (650 μL L^{-1}), and two temperature levels, low (ambient) and high (ambient + 0.6 °C, to simulate predicted future atmospheric warming based on data for the last 100 years), were applied continuously from 23 May to 2 November in 2010. Three OTCs were used for each CO_2 × temperature treatment, and the CO_2 concentrations in each OTC were monitored continuously and adjusted using an infrared CO_2 analyser (Ventostat 8102; Telaire Company, Goleta, CA). Temperature levels in each OTC were maintained via an air-exchange-system (eight fans for low temperature and four fans for high temperature) frequency adjustment, and were monitored continuously using an automatic temperature analysis system (U23-001, HOBO Pro V2 Temp/RH Data Logger; MicroDAQ.com,

Table 1. Statistical analysis of the differences in CO_2 level dynamics among four CO_2 × temperature level treatments by comparison using the paired *t*-test (*t*/*P* values, df = 157)[a]

		Ambient CO_2 (μL L^{-1})		
		High temperature	Low temperature	Group-paired *t*-test
Elevated CO_2 (μL L^{-1})	High temperature	263.45/<0.0001***	296.11/<0.0001***	0.40/0.6903
	Low temperature	233.03/<0.0001***	258.47/<0.0001***	
Group-paired *t*-test		0.44/0.6628		

[a] *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. Statistical analysis of the differences in temperature level dynamics among four CO_2 × temperature level treatments by comparison using the paired *t*-test (*t*/*P* values, df = 157)[a]

		Low temperature (°C)		
		Elevated CO_2	Ambient CO_2	Group-paired *t*-test
High temperature (°C)	Elevated CO_2	3.68/0.0003***	2.34/0.021*	0.31/0.7632
	Ambient CO_2	3.32/0.0011**	3.67/0.0003***	
Group-paired *t*-test		0.40/0.6910		

[a] *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

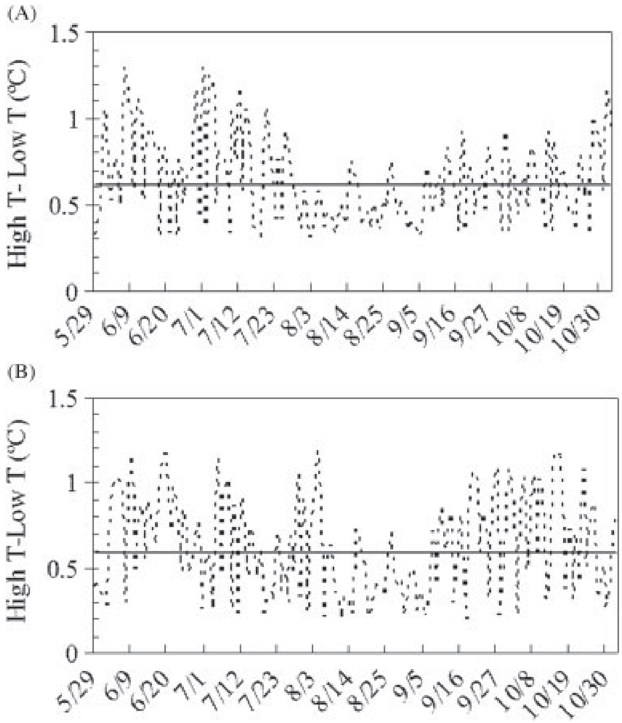

Figure 2. The dynamics (dotted line) and mean values (solid line) of the temperature level difference between high temperature and low temperature under elevated CO_2 (A) and ambient CO_2 (B) from 29 May to 2 November 2010 (paired *t*-test: $t = 0.99$, $P = 0.33 > 0.05$).

Ltd, Contoocook, NH). The accuracy of temperature was defined as ±0.02 °C from 0 to 50 °C.

Each OTC was divided into four similar units using plastic netting (mesh size 0.15 mm × 0.15 mm), and the open tops of these OTCs were also covered with the same mesh size of plastic netting to prevent insects escaping and mixing. The OTCs were specifically designed and improved on the basis of previously installed OTCs.[26] For the entire OTC field experiments, the dynamics of climate data, including daily mean CO_2 (μL L^{-1}) and temperature (°C) from 29 May to 2 November, is shown in Fig 1. The daily mean CO_2 and temperature were 353–399 μL L^{-1}/16.43–33.20 °C (mean = 381 μL L^{-1}/26.19 °C) in ambient CO_2 and at high temperature, 365–396 μL L^{-1}/15.33–32.56 °C (mean = 383 μL L^{-1}/25.60 °C) in ambient CO_2 and at low temperature, 637–674 μL L^{-1}/16.06–32.96 °C (mean = 656 μL L^{-1}/26.10 °C) in elevated CO_2 and at high temperature, and 654–683 μL L^{-1}/15.95–32.29 °C (mean = 658 μL L^{-1}/25.55 °C) in elevated CO_2 and at low temperature. The differences in daily mean temperature level were 0.50–0.64 °C between the treatments of high temperature and low temperature at ambient CO_2 or elevated CO_2, which is enough to simulate the atmospheric warming (+0.7 °C) based on data for the last 100 years.[1,2] The paired *t*-test (SAS PROC TTEST, 2002; SAS Institute Inc., Cary, NC) indicated no significant differences in the CO_2 level dynamics between the treatments of high temperature and low temperature at ambient or elevated CO_2 ($P > 0.05$) (Table 1), and no significant differences in the temperature level dynamics between the treatments of elevated CO_2 and ambient CO_2 at high or low temperature ($P > 0.05$) (Table 2), while significant differences were shown between elevated CO_2 and ambient CO_2 ($P < 0.0001$) (Table 1) and between high temperature and low temperature ($P < 0.05$, 0.01 and 0.001) (Table 2). The dynamics of temperature level difference over time between high temperature and low temperature at elevated and ambient CO_2 is shown in Fig. 2. The temperature level difference range between high temperature and low temperature was 0.31–1.30 °C with a mean of 0.62 °C at elevated CO_2 (Fig. 2A) and 0.20–1.19 °C with a mean of 0.59 °C at ambient CO_2 (Fig. 2B) respectively, and no significant difference of high temperature and low temperature was found between the treatments of elevated and ambient CO_2 (paired *t*-test: $t = 0.99$, $P = 0.33 > 0.05$). Analysis of the dynamics demonstrates that differences between high and low temperature treatments were consistently maintained over time and were similar in both CO_2 treatment groups in spite of daily variation.

Table 3. *P*-values derived from four-way repeated-measures ANOVA on species-specific responses of brown planthopper, *Nilaparvata lugens*, small brown planthopper, *Laodelphax striatellus*, and white-backed planthopper, *Sogatella furcifera*, to elevated CO_2 and temperature levels, as fed on *Bt* rice relative to non-transgenic rice[a]

Variables	Single transgene[b] (cv. KMD versus cv. XSD)	Fused transgenes[c] (cv. HH1 versus cv. MH63)
CO_2[d]	0.061*	0.50
Temperature[e]	0.60	0.048**
Transgenic[f]	0.12	0.000***
Species[g]	0.000***	0.000***
CO_2 × temperature	0.70	0.84
CO_2 × transgenic	0.60	0.95
CO_2 × species	0.21	0.58
Temperature × transgenic	0.85	0.37
Temperature × species	0.99	0.71
Transgenic × species	0.70	0.93
CO_2 × temperature × transgenic	0.53	0.43
CO_2 × temperature × species	0.31	0.80
CO_2 × transgenic × species	1.00	0.93
Temperature × transgenic × species	0.99	0.94
CO_2 × temperature × transgenic × species	0.58	0.84

[a] * $P < 0.10$; ** $P < 0.05$; *** $P < 0.01$.
[b] Single *Bt* transgene *Cry1Ab*.
[c] Fused *Bt* transgenes *Cry1Ab* + *Cry1Ac*.
[d] CO_2 level (382 μL L^{-1} versus 657 μL L^{-1}).
[e] Temperature level (low temperature at 25.6 °C versus high temperature at 26.1 °C).
[f] Transgenic treatment (*Bt* rice versus non-transgenic rice).
[g] Planthopper species (*N. lugens*, *L. striatellus* and *S. furcifera*).

2.2 Transgenic *Bt* rice cultivars

Two types of transgenic *Bt* rice expressing transgenes from *Bacillus thuringiensis kurstaki* (*Bt*) Berliner, one expressing the single *Bt* transgene *Cry1Ab* (cv. KMD and its parental line cv. XSD), provided by the Institute of Atomic Energy Research of Zhejiang University, and another expressing fused *Bt* transgenes *Cry1Ab*/*Cry1Ac* (cv. HH1 and its parental line cv. MH63), provided by the College of Plant Science and Technology of Huazhong Agricultural University, were used in this study. Seeds were sowed in white plastic pots (45 cm in height × 35 cm in diameter) filled with an 8:2 (by volume) loam:manure mixture on 23 May 2010. The soil was then sampled and triturated for analysis of its chemical composition (Institute of Soil Science and Chinese Academy of Sciences, 1978). Soil pH was 7.3, organic matter 12.3%, available N 215.2 mg kg^{-1}

Table 4. *P*-values of three-way repeated-measures ANOVA on species-specific responses of *N. lugens*, *L. striatellus* and *S. furcifera* to elevated CO_2 and temperature levels, as fed on the same cultivar of *Bt* rice and non-transgenic rice[a]

	Single transgene (*Cry1Ab*)		Fused transgenes (*Cry1Ab* + *Cry1Ac*)	
Variables	*Bt* rice (cv. KMD)	Non-*Bt* rice (cv. XSD)	*Bt* rice (cv. HH1)	Non-*Bt* rice (cv. MH63)
CO_2	0.084*	0.35	0.67	0.61
Temperature	0.81	0.63	0.048**	0.44
Species	0.001***	0.001***	0.000***	0.000***
CO_2 × temperature	0.86	0.49	0.49	0.68
CO_2 × species	0.44	0.48	0.69	0.79
Temperature × species	0.98	1.00	0.78	0.85
CO_2 × temperature × species	0.35	0.51	0.74	0.92

[a] *$P < 0.10$; **$P < 0.05$; ***$P < 0.01$.

Table 5. *P*-values of three-way repeated-measures ANOVA on species-specific responses of *N. lugens*, *L. striatellus* and *S. furcifera* to global warming and elevated CO_2, as fed on transgenic *Bt* rice relative to non-transgenic rice[a]

	Single transgene (cv. KMD versus XSD)			Fused transgenes (cv. HH1 versus MH63)		
Variables	*N. lugens*	*L. striatellus*	*S. furcifera*	*N. lugens*	*L. striatellus*	*S. furcifera*
CO_2	0.79	0.093*	0.012**	0.92	0.98	0.14
Temperature	0.74	0.85	0.65	0.18	0.67	0.065*
Transgenic	0.28	0.28	0.71	0.011**	0.031**	0.001***
CO_2 × temperature	0.91	0.15	0.36	0.70	0.84	0.53
CO_2 × transgenic	0.81	0.72	0.70	0.76	0.87	0.97
Temperature × transgenic	0.99	0.89	0.77	0.44	0.73	0.67
CO_2 × temperature × transgenic	0.75	0.67	0.13	0.75	0.92	0.26

[a] *$P < 0.10$; **$P < 0.05$; ***$P < 0.01$.

(hydrolic N, 1 M NaOH hydrolysis), available P 145.8 mg kg^{-1} (0.5 M $NaHCO_3$ extraction), and available K 105.9 mg kg^{-1} (1 M CH_3COONH_4 extraction). The two partitions in each OTC were used for KMD/HH1 and its parental line, and five pots per rice cultivar were placed randomly and rerandomised in the same part of the OTC every other day to minimise positional effects. Fifty tillers were maintained in each pot after 30 days of planting. Throughout the growing season, single CO_2 mixed with ambient air was continuously supplied to the OTCs to maintain the desired CO_2 level. Pots were watered regularly to ensure sufficient moisture, and no pesticide was used throughout the experiment.

2.3 Insect stocks

Three species of rice planthopper, brown planthopper (*Nilaparvata lugens*), small brown planthopper (*Laodelphax striatellus*) and white-backed planthopper (*Sogatella furciferai*), were collected from the Jiangpu paddy field in Nanjing, Jiangsu Province, in September 2009, and were continuously reared on the susceptible rice cultivar TN1 (provided by the International Rice Research Institute, Philippines) in greenhouses until they were used for inoculation treatments on 24 July 2010.

2.4 Insect inoculation and abundance sampling

On 24 July 2010, five pairs of brachypterous females and males within 24 h of adult emergence were randomly collected from available insect stocks for inoculation in each pot of transgenic *Bt* rice (cv. KMD and cv. HH1) and non-transgenic rice (cv. XSD and cv. MH63) in the OTCs of four CO_2 × temperature treatments respectively. After 1 month of continuous rearing, two pots of each rice cultivar were randomly selected from each chamber, and all planthopper nymphs and adults were counted every 7 days from 25 August to 5 October. Population abundances were converted to the number of planthoppers per 100 plants.

2.5 Data analysis

All data were analysed with SPSS 16.0 (2008; SPSS Institute, Chicago, IL). In order to highlight the subtle but marked effects of CO_2 and temperature levels on planthopper population abundances, significance levels of $P < 0.10$, 0.05 and 0.01 were used in subsequent repeated-measures analysis of variance (ANOVA) to indicate significant effects of CO_2 and temperature levels and their interactions (Tables 3 to 6); significance levels of $P < 0.05$, 0.01 and 0.001 were used in the paired *t*-test for comparison of differences in CO_2 levels and temperature levels (Tables 1 and 2, Fig. 2). Four-way repeated-measures ANOVA was used to examine the species-specific responses of *N. lugens*, *L. striatellus* and *S. furcifera* to elevated CO_2 and temperature levels, as fed on *Bt* rice with single or fused transgenes (versus their parental lines respectively), with CO_2 and temperature levels and transgenic treatments as the main factors, planthopper species as a subfactor and sampling dates as repeated measures (Table 3). Because no significant interactions were found among CO_2 and temperature levels, transgenic treatments or planthopper species ($P > 0.10$) (Table 3), three-way repeated ANOVA was used to analyse the effects of CO_2 and temperature levels on planthopper species as fed on *Bt* rice or non-transgenic rice (Table 4). Simultaneously, three-way repeated ANOVA was used to analyse further the

Table 6. *P*-values of two-way repeated-measures ANOVA on the effects of CO_2 and temperature levels on the population dynamics of *S. furcifera* fed on single and fused transgenic *Bt* rice, and of *L. striatellus* fed on single transgenic *Bt* rice[a]

	Single transgene (*Cry1Ab*)		Fused transgenes (*Cry1Ab* + *Cry1Ac*)
Variables	*S. furcifera*	*L. striatellus*	*S. furcifera*
CO_2	0.042**	0.033**	0.33
Temperature	0.92	0.95	0.046**
CO_2 × temperature	0.11	0.24	0.27

[a] $^{*}P < 0.10$; $^{**}P < 0.05$; $^{***}P < 0.01$.

impacts of CO_2 levels, temperature levels and transgenic fused or single treatments (versus their parental lines respectively) on the population dynamics of each tested rice planthopper species (Table 5). Because the effect ($P = 0.065 < 0.10$) of temperature level on *S. furcifera* population abundance in fused transgenic *Bt* rice (versus its parental line) and the effects ($P = 0.093 < 0.10$ and $P = 0.012 < 0.05$) of CO_2 level on *L. striatellus* and *S. furcifera* population abundances in single transgenic *Bt* rice (versus its parental line) were significant (Table 5), two-way repeated ANOVA was also used to analyse them further (Table 6). Moreover, the pairwise differences between treatments were separated by LSD tests at a significance level of $P < 0.05$ (Figs 6 to 8). Abundance data were log transformed to normalise them prior to analysis.

3 RESULTS

3.1 Effects of different transgenes on the responses of rice planthoppers to CO_2 and temperature levels

When *N. lugens* (Fig. 3), *L. striatellus* (Fig. 4) and *S. furcifera* (Fig. 5) were allowed to feed on transgenic *Bt* rice with different transgenes, the population abundances of the three ecologically similar planthopper species responded differently to various CO_2 levels (Table 3). CO_2 level effects were significant in the single-transgene treatment ($P = 0.061 < 0.10$), while temperature level ($P = 0.048 < 0.05$) and transgene ($P = 0.000$) were significant in the fused-transgene treatment (Table 3). CO_2 and temperature levels affected planthopper responses significantly when fed on *Bt* rice with single *Cry1Ab* (CO_2 levels: $P = 0.084 < 0.10$) (Table 4) and fused *Cry1Ab/Cry1Ac* (temperature levels: $P = 0.048 < 0.05$) (Table 4). In addition, species-specific responses were indicated for both *Bt* transgene treatments owing to the significant effects of planthopper species ($P = 0.000$ or 0.001) (Tables 3 and 4).

3.2 Species-specific planthopper responses to temperature and CO_2 levels, as fed on fused and single transgenic *Bt* rice

The species-specific responses of *N. lugens*, *L. striatellus* and *S. furcifera* to elevated CO_2 and temperature levels, when allowed to feed upon transgenic *Bt* rice versus non-transgenic rice, were analysed by three-way repeated-measures ANOVA (Table 5). The results indicated that fused transgenic *Bt* rice significantly reduced the population abundances of these three planthopper species ($P = 0.031 < 0.05$) compared with feeding on the non-transgenic parental line, and high temperature only significantly enhanced *S. furcifera* abundance ($P = 0.065 < 0.10$) (Fig. 5 and Table 5). CO_2 level significantly reduced *S. furcifera* population abundance ($P = 0.012 < 0.05$) (Fig. 5 and Table 5) and markedly reduced *L. striatellus* population abundance ($P = 0.093 < 0.10$) (Fig. 5 and Table 5).

3.3 Effects of elevated CO_2 and temperature levels on *S. furcifera* and *L. striatellus* population dynamics when fed on transgenic *Bt* rice

Temperature levels significantly affected *S. furcifera* population dynamics when feeding on transgenic *Bt* rice (fused *Cry1Ab/Cry1Ac* cv. HH1) ($P = 0.046 < 0.05$) (Table 6). Compared with low temperature, high temperature only significantly increased the population abundances of *S. furcifera* at elevated CO_2 ($P < 0.05$) (Fig. 6). On the other hand, CO_2 level significantly influenced the population dynamics of *S. furcifera* ($P = 0.042 < 0.05$) and *L. striatellus* ($P = 0.033 < 0.05$) when feeding on transgenic *Bt* rice with single *Cry1Ab* cv. KMD (Table 6). Compared with ambient CO_2, elevated CO_2 only significantly reduced the population abundances of *S. furcifera* at high temperature ($P < 0.05$) (Fig. 7) and *L. striatellus* at low temperature ($P < 0.05$) (Fig. 8).

4 DISCUSSION

In the modern sustainable agroecosystem, transgenic plants are being used to develop environmentally benign, yet profitable, practices to aid in minimising pest resistance and reducing insecticide use.[6,9] Since 1996, transgenic *Bt* crops have

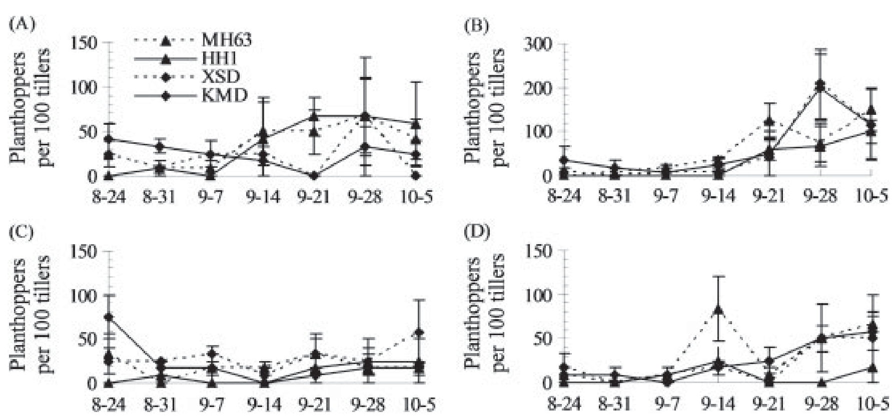

Figure 3. Population dynamics of the brown planthopper, *Nilaparvata lugens*, fed on transgenic *Bt* rice (cv. KMD expressing single *Cry1Ab*, and cv. HH1 expressing fused *Cry1Ab/Cry1Ac*) and non-transgenic rice (parental line cv. XSD and cv. MH63) grown in open-top chambers under elevated CO_2 at high (A) and low (B) temperature and under ambient CO_2 at high (C) and low (D) temperature from 24 August to 5 October 2010.

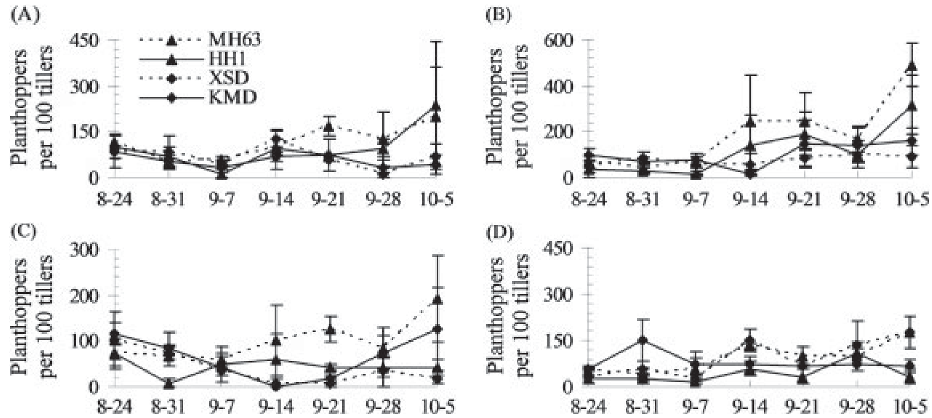

Figure 4. Population dynamics of the small brown planthopper, *Laodelphax striatellus*, fed on transgenic *Bt* rice (cv. KMD expressing single *Cry1Ab*, and cv. HH1 expressing fused *Cry1Ab/Cry1Ac*) and non-transgenic rice (parental line cv. XSD and cv. MH63) grown in open-top chambers under elevated CO_2 at high (A) and low (B) temperature and under ambient CO_2 at high (C) and low (D) temperature from 24 August to 5 October 2010.

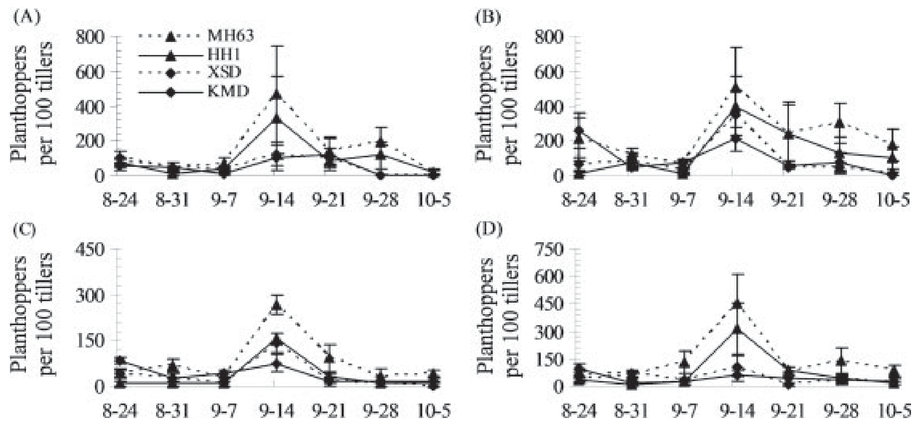

Figure 5. Population dynamics of the white-backed planthopper, *Sogatella furcifera*, fed on transgenic *Bt* rice (cv. KMD expressing single *Cry1Ab*, and cv. HH1 expressing fused *Cry1Ab/Cry1Ac*) and non-transgenic rice (parental line cv. XSD and cv. MH63) grown in open-top chambers under elevated CO_2 at high (A) and low (B) temperature and under ambient CO_2 at high (C) and low (D) temperature from 24 August to 5 October 2010.

been used to provide excellent control performance against target lepidopteran pests in diverse cropping systems.[6,9] Soon, transgenic *Bt* rice may become commercially available in China,[27] as *Bt* rice Huahui-1 and *Bt* Shanyou-63 are under preparation for commercialisation.[9,28]

While the potential adverse consequences of global climate change for *Bt* crops have received increasing attention in recent years, general decreases in exogenous-toxin (*Bt*) and foliar nitrogen and marked increases in foliar carbon and C:N ratio have been found in *Bt* cotton under elevated CO_2[13,15–18] and high temperature,[14,20] and the same findings have been obtained for *Bt* rice under elevated CO_2.[18] It has also been shown that exposure to high temperature during cotton boll development results in a reduction in glutamic-pyruvic transaminase (GPT) activity, a significant decrease in soluble protein content and significant increases in protease activity.[14] Owing to the general decrease in exogenous-toxin content in *Bt* crops at elevated CO_2 and temperature levels, it is hypothesised that *Bt* crops will face a new ecological risk of reduced effectiveness against target-insect pests under climate change.[14,18] At the same time, changes in plant tissue nutritional composition and defensive characteristics may cascade through food chains and ultimately affect both target and non-target insect herbivores.[29,30] However, many insects, including the plant-sucking hemipterans such as aphids, vary in their responses to plants grown at elevated CO_2 and temperature levels.[4,31]

In the present study, rice planthoppers, which are hemipteran sucking insects, showed species-specific responses in population abundance to elevated CO_2 and temperature levels relative to their non-transgenic parental lines when feeding on single and fused transgenic *Bt* rice. Elevated CO_2 and transgenic treatments both had adverse effects, while high temperature only affected rice planthopper abundance. Moreover, high temperature (positive) and fused *Bt* transgenes (negative) significantly affected the population dynamics of the three rice planthopper species relative to the non-transgenic parental lines, while no significant effects were found for CO_2 level, temperature level or single *Bt* transgene, nor for their interactions. Fused *Cry1Ab/Cry1Ac* transgenic *Bt* rice significantly reduced the population abundances of the three rice planthopper species relative to the non-transgenic parental line.[12] High temperature only significantly increased white-backed planthopper abundances when fed on transgenic *Bt* rice grown under elevated CO_2. With single *Cry1Ab Bt* rice, elevated CO_2 only significantly reduced white-backed planthopper and small brown planthopper abundances at high temperature relative to the non-transgenic parental line.

The present results suggest that, under the predicted global climate changes of elevated CO_2 and temperature levels, transgenic *Bt* rice can significantly modify the population patterns of rice planthoppers. Predicted global change conditions, especially elevated CO_2, appear to be a potential factor

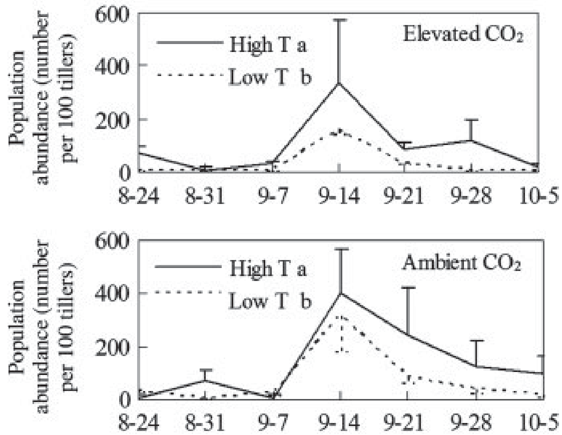

Figure 6. Effects of temperature levels on the population dynamics of the white-backed planthopper, *S. furcifera*, fed on transgenic *Bt* rice (cv. HH1 expressing fused *Cry1Ab/Cry1Ac*) grown under elevated and ambient CO_2 from 24 August to 5 October 2010 (different lower-case letters indicate a significant difference between the treatments of high temperature and low temperature by the LSD test, $P < 0.05$; one-way repeated ANOVA with df = 20).

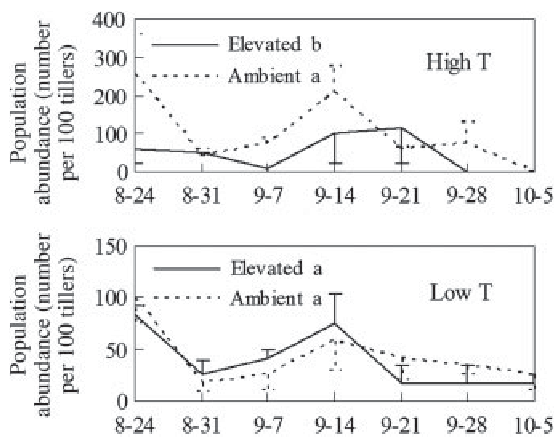

Figure 7. Effects of CO_2 levels on the population dynamics of the white-backed planthopper, *S. furcifera*, fed on transgenic *Bt* rice (cv. KMD expressing single *Cry1Ab*) grown at high temperature and low temperature from 24 August to 5 October 2010 (different lower-case letters indicate a significant difference between the treatments of elevated CO_2 and ambient CO_2 by the LSD test, at $P < 0.05$; one-way repeated ANOVA with df = 20).

that could accelerate outbreaks of white-backed planthopper, *S. furcifera*, relative to brown planthopper, *N. lugens*, and small brown planthopper, *L. striatellus*, when fed on fused transgenic *Bt* rice with double-stacked traits relative to the non-transgenic parental line. In addition, elevated CO_2 appears to be a factor that could reduce the abundance of white-backed planthopper, *S. furcifera*, and small brown planthopper, *L. striatellus*, relative to brown planthopper, *N. lugens*, when fed on single transgenic *Bt* rice with single traits relative to the non-transgenic parental line. The different responses of the different species of rice planthopper imply that the effects of transgene insertion, which can consist of changes in composition and content of nutrients and antinutrients (e.g. secondary metabolites, inhibitors, metabolic enzymes and endosymbiotes),[32–37] may affect the planthoppers differently[37–40] because of different biology and physiology of the three species of planthoppers, leading to different population abundances of the three species of planthopper. Furthermore,

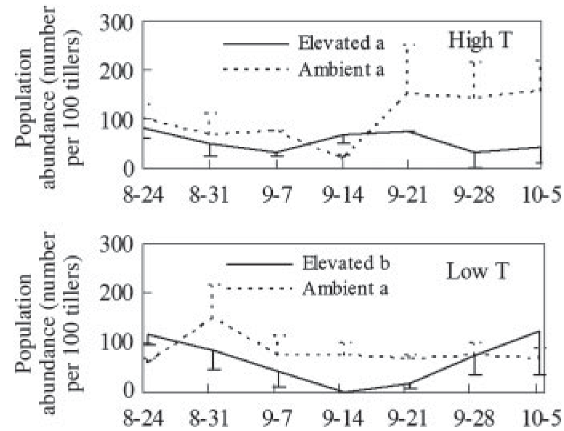

Figure 8. Effects of CO_2 levels on the population dynamics of the small brown planthopper, *L. striatellus*, fed on transgenic *Bt* rice (cv. KMD expressing single *Cry1Ab*) grown at high temperature and low temperature from 24 August to 5 October 2010 (different lower-case letters indicate a significant difference between the treatments of elevated CO_2 and ambient CO_2 by the LSD test, $P < 0.05$; one-way repeated ANOVA with df = 20).

it has also been reported that the CO_2 concentration and temperature level could also affect the composition and content of nutrients and secondary chemicals,[16,41–43] as well as the different *Bt* expression patterns,[14,17] in transgenic plants, leading to different insect responses. More work is necessary to determine the precise mechanisms underlying the species-specific responses observed here.

Regarding the different responses of planthopper species to single-gene versus fused transgenic *Bt* rice, the objective of the present experiment was not directly to compare insect responses to *Cry1Ab* versus *Cry1Ab/Cry1Ac* transgenes. The observed effects of transgenic treatments were based on comparisons of the transgenic lines with their respective non-*Bt* parental lines. Any differences in response to single gene (*Cry1Ab*) and fused gene (*Cry1Ab/Cry1Ac*) could be due to differences other than the specific transgenes, including parental genetic background or different pleiotropic effects of the transgenic events on the genome.[25] Given these potential sources of variation, the present results suggest that the single *Cry1Ab* transgenic *Bt* line (i.e. cv. KMD versus the parental line cv. XSD) may be a superior option for minimising non-target planthopper abundances to the effects of fused *Cry1Ab/Cry1Ac* transgenic *Bt* lines (i.e. cv. HH1 versus the parental line cv. MH63) under elevated CO_2 and temperature.

5 CONCLUSIONS

Transgenic *Bt* rice offers the potential to generate economic benefits through yield increase and decrease in insecticide use in China. Since the early 2000s, the sucking planthoppers *N. lugens*, *L. striatellus* and *S. furcifera*, none of which is controlled by *Bt* rice, have become significant secondary pests in high-yielding agricultural systems. Planthopper outbreaks may have been partially triggered by climate change, especially global warming.[11,43,44] As such, it is necessary to test the effectiveness of different types of transgenic *Bt* line (single *Cry1Ab* and fused *Cry1Ab/Cry1Ac*) for biotech crop resistance against serious non-target planthoppers. The present study has shown that different types of transgenic *Bt* rice can alter the species-specific responses of non-target planthoppers to elevated CO_2 and temperature. Compared with non-transgenic parental lines, the present results indicate that the single transgenic *Bt* line is better at reducing the abundance of *S. furcifera* than the fused *Bt* variety under elevated CO_2 and temperature. It is suggested that multiple types of transgenic *Bt* rice might be used simultaneously in paddy fields to take advantage of the high transgenic diversity for optimal performance against not only target lepidopterans[24,25] but also non-target planthoppers.

ACKNOWLEDGEMENTS

The authors offer their sincere thanks to Prof. Illimar Altosaar, University of Ottawa, Canada, and to Prof. Shu Qing-Yao, Prof. Zhu Zeng-Rong and Prof. Li Dian-Xing, Zhejiang University, China, who provided seeds of transgenic *Bt* rice with the single *Bt* transgene *Cry1Ab* (cv. KMD). They are also very grateful to Prof. Hongxia Hua, College of Plant Sciences and Technology, Huanzhong Agricultural University, China, who provided seeds of transgenic *Bt* rice with fused *Bt* transgenes *Cry1Ab/Cry1Ac* (cv. HH1). Owen McSpadden, Texas A&M AgriLife Research, provided a review of the penultimate draft of this paper. Many thanks also to Prof. Dr Gregory A Sword, Texas A&M University, College Station, TX, for his help in revising the manuscript. This research was supported by the National Basic Research Programme of China (973) (2010CB126200), the State Public Industry (Agriculture) Research Project (200903051), the National Nature Science Foundation of China (31272051, 31101491), the Fok Ying Tung Education Foundation from the Ministry of Education of the People's Republic of China (122033) and the Major Projects of Cultivated New Varieties of Genetically Modified Organisms (2012ZX08011002, 2013ZX08012-005, 2014ZX08012-005).

REFERENCES

1 Houghton JT, Ding YH, Griggs DJ, Noquer M, Linden PJ and Xiaosu D, *Climate Change 2001: the Scientific Basis*. Cambridge University Press, Cambridge, UK, 944 pp. (2001).

2 *Climate Change 2007: the Physical Science Basis. Summary for Policy Makers. Report of Working Group I of the Intergovernmental Panel on Climate Change*. [Online]. IPCC (2007). Available: http://www.ipcc.ch/publications_and_data/ar4/wg1/en/spm.html.

3 Peters RL and Darling JD, The greenhouse effect and nature reserves. *Bioscience* **35**:707–715 (1985).

4 Newman JA, Climate change and cereal aphids: the relative effects of increasing CO_2 and temperature on aphid population dynamics. *Global Change Biol* **10**:5–15 (2004).

5 Stiling P, Rossi AM, Hungate B, Dijkstra P, Hinkle CR, Knott WM *et al.*, Decreased leaf-miner abundance in elevated CO_2: reduced leaf quality and increased parasitoid attack. *Ecol Appl* **9**:240–244 (1999).

6 Huang F, Detection and monitoring of insect resistance to transgenic *Bt* crops. *Insect Sci* **13**:73–84 (2006).

7 Lu YH, Wu KM, Jiang YY, Xia B, Li P, Feng HQ *et al.*, Mirid bug outbreaks in multiple crops correlated with wide-scale adoption of *Bt* cotton in China. *Science* **328**:1151–1154 (2010).

8 Wu KM, Lu YH, Feng HQ, Jiang YY and Zhao JZ, Suppression of cotton bollworm in multiple crops in China in areas with *Bt* toxin-containing cotton. *Science* **321**:1676–1678 (2008).

9 James C, Global status of commercialized biotech/GM crops: 2009. ISAAA Brief No. 41, 36 pp. (2009).

10 Huang JK, Hu RF, Rozelle S and Pray C, Insect-resistant GM rice in farmers' fields: assessing productivity and health effects in China. *Science* **308**:688–690 (2005).

11 Cheng J, Rice planthopper problems and relevant causes in China, in *Planthoppers: New Threats to the Sustainability of Intensive Rice Productive Systems in Asia*, ed. by Heong KL and Hardy B. International Rice Research Institute, Los Baños, Philippines, pp. 157–178 (2009).

12 Wang BJ, Xu HX, Zheng XS, Fu Q and Lu ZX, High temperature modifies resistance performances of rice varieties to brown planthopper, *Nilaparvata lugens* (Stål). *Rice Sci* **17**:334–338 (2010).

13 Coviella CE, Morgan DJW and Trumble JT, Interactions of elevated CO_2 and nitrogen fertilization: effects on production of *Bacillus thuringiensis* toxins in transgenic plants. *Environ Entomol* **29**:781–787 (2000).

14 Chen DH, Ye GY, Yang CQ, Chen Y and Wu YK, The effect of high temperature on the insecticidal properties of *Bt* cotton. *Environ Exp Bot* **53**:333–342 (2005).

15 Chen FJ, Wu G, Ge F, Parajulee MN and Shrestha RB, Effects of elevated CO_2 and transgenic *Bt* cotton on plant chemistry, performance, and feeding of an insect herbivore, the cotton bollworm *Helicoverpa armigera* (Hübner). *Entomol Exp Appl* **115**:341–350 (2005).

16 Chen FJ, Wu G, Parajulee MN and Ge F, Impacts of elevated CO_2 and transgenic *Bt* cotton on performance and feeding of three generations of cotton bollworm in a long-term experiment. *Entomol Exp Appl* **124**:27–35 (2007).

17 Chen FJ, Wu G, Ge F and Parajulee MN, Relationships between exogenous-toxin quantity and increased biomass of transgenic *Bt* crops under elevated carbon dioxide. *Ecotoxicol Environ Saf* **74**(4):1074–1080 (2011).

18 Chen FJ, Ge F and Parajulee MN, Impact of elevated CO_2 on Tri–Trophic interaction of *Gossypium hirsutum*, *Aphis gossypii*, and *Leis axyridis*. *Environ Entomol* **34**:37–46 (2005).

19 Li ZY, Liu TJ, Xiao NW, Li JS and Chen FJ, Effects of elevated CO_2 on the interspecific competition between two sympatric species of *Aphis gossypii* and *Bemisia tabaci* fed on transgenic *Bt* cotton. *Insect Sci* **18**(4):426–434 (2011).

20 Zhou GS, Zhou FC, Xie YM, Feng CN and Yang YZ, Effects of temperature stress on *Bt* insecticidal protein expression in *Bt* transgenic cotton leaves and death rate of cotton bollworm. *Cotton Sci* **21**:302–306 (2009).

21 Luomala EM, Laitinen K, Sutinen S, Kellomäki S and Vapaavuori E, Stomatal density, anatomy and nutrient concentrations of Scots pine needles are affected by elevated CO_2 and temperature. *Plant Cell Environ* **28**:733–749 (2005).

22 Morison JIL and Lawlor DW, Interactions between increasing CO_2 concentration and temperature on plant growth. *Plant Cell Environ* **22**:659–682 (1999).

23 Johns CV and Hughes A, Interactive effects of elevated CO_2 and temperature on the leaf-miner *Dialectica scalariella* Zeller (Lepidoptera: Gracillariidae) in Paterson's Curse, *Echium plantagineum* (Boraginaceae). *Global Change Biol* **8**:142–152 (2002).

24 Wang ZS, Shui QY, Cui HR, Ye GY, Wu DX, Gao MW *et al.*, Resistance of progenies from crosses between *Bt* transgenic rice and conventional rice varieties to stripe stem borer *Chilo suppressalis*. *Acta Agron Sin* **26**:310–314 (2000).

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

26 Chen FJ, Ge F and Su JW, An improved top-open chamber for research on the effects of elevated CO_2 on agricultural pests in field. *Chin J Ecol* **24**(5):585–590 (2005).

27 Han LZ, Wu KM, Peng YF and Guo YY, Research advances in ecological safety of insect-resistant transgenic rice. *Chin J Appl Environ Biol* **12**:431–436 (2006).

28 *The Approved List of Security Certificates for the Second Batch of Agricultural Genetically Modified Organisms in 2009*. [Online]. GMO Safety Management Office, Ministry of Agriculture of the People's Republic of China (2009). Available: http://www.stee.agri.gov.cn/biosafety/spxx/P020090324584455137271.pdf.

29 Fritshi FB, Bote KJ and Sollenberger LE, Carbon dioxide and temperature effects on forage establishment: photosynthesis and biomass production. *Global Change Biol* **5**:441–453 (1999).

30 Johns CV, Beaumont LJ and Hughes L, Effects of elevated CO_2 and temperature on development and consumption rate of *Octotoma championi* and *O. scabripennis* feeding on *Lantana camara*. *Entomol Exp Appl* **108**:169–178 (2003).

31 Dury SJ, Good JEG, Perrins CM, Buse A and Kaye T, The effects of increasing CO_2 and temperature on oak leaf palatability and the implications for herbivorous insects. *Global Change Biol* **4**:55–61 (1998).

32 Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P *et al.*, Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* **287**(5451):303–305 (2000).

33 Jiao Z, Si XX, Li GK, Zhang ZM and Xu XP, Unintended compositional changes in transgenic rice seeds (*Oryza sativa* L.) studied by

spectral and chromatographic analysis coupled with chemometrics methods. *J Agric Food Chem* **58**(3):1746–1754 (2010).

34 Keymanesh K, Darvishi MH and Sardari S, Metabolome comparison of transgenic and non-transgenic rice by statistical analysis of FTIR and NMR spectra. *Rice Sci* **16**(2):119–123 (2009).

35 Saxena D and Stotzky G, *Bt* corn has a higher lignin content than non-*Bt* corn. *Am J Bot* **88**(9):1704–1706 (2001).

36 Wang Y, Xu W, Zhao W, Hao J, Luo Y, Tang X *et al.*, Comparative analysis of the proteomic and nutritional composition of transgenic rice seeds with *Cry1ab/ac* genes and their non-transgenic counterparts. *J Cereal Sci* **55**(2):226–233 (2012).

37 He Y, Zhang J, Chen J, Wu Q, Chen L, Chen L *et al.*, Influence of pymetrozine on feeding behaviors of three rice planthoppers and a rice leafhopper using electrical penetration graphs. *J Econ Entomol* **104**(6):1877–1884 (2011).

38 Liu YK, Wang WX, Fu Q, Lai FX and Luo J, Effects of host plants on activities of detoxification and protective enzymes in three rice planthoppers. *Chin J Rice Sci* **25**(6):659–666 (2011).

39 Chen YH, Bernal CC, Tan J, Horgan FG and Fitzgerald MA, Planthopper 'adaptation' to resistant rice varieties: changes in amino acid composition over time. *J Insect Physiol* **57**(10):1375–1384 (2011).

40 Noda H, Koizumi Y, Zhang Q and Deng K, Infection density of *Wolbachia* and incompatibility level in two planthopper species, *Laodelphax striatellus* and *Sogatella furcifera*. *Insect Biochem Mol* **31**(6/7):727–737 (2001).

41 Himanen SJ, Nissinen A, Dong WX, Nerg AM, Stewart CN, Poppy GM *et al.*, Interactions of elevated carbon dioxide and temperature with aphid feeding on transgenic oilseed rape: are *Bacillus thuringiensis* (*Bt*) plants more susceptible to nontarget herbivores in future climate? *Global Change Biol* **14**(6):1437–1454 (2008).

42 Ge F and Chen FJ, Impacts of elevated CO_2 on insects. *Acta Ecol Sin* **26**(3):935–944 (2006).

43 Yamamura K and Kiritani K, A simple method to estimate the potential increase in the number of generations under global warming in temperate zones. *Appl Entomol Zool* **33**:289–298 (1998).

44 Hu G, Xie MC, Lin ZX, Xin DY, Huang CY, Chen W *et al.*, Are outbreaks of *Nilaparvata lugens* (Stål) associated with global warming? *Environ Entomol* **39**(6):1705–1714 (2010).

