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Effects of elevated CO_2 and plant genotype on interactions among cotton, aphids and parasitoids

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Abstract Effects of CO_2 level (ambient *vs.* elevated) on the interactions among three cotton (*Gossypium hirsutum*) genotypes, the cotton aphid (*Aphis gossypii* Glover), and its hymenoptera parasitoid (*Lysiphlebia japonica* Ashrnead) were quantified. It was hypothesized that aphid-parasitoid interactions in crop systems may be altered by elevated CO_2, and that the degree of change is influenced by plant genotype. The cotton genotypes had high (M9101), medium (HZ401) and low (ZMS13) gossypol contents, and the response to elevated CO_2 was genotype-specific. Elevated CO_2 increased the ratio of total non-structural carbohydrates to nitrogen (TNC : N) in the high-gossypol genotype and the medium-gossypol genotype. For all three genotypes, elevated CO_2 had no effect on concentrations of gossypol and condensed tannins. *A. gossypii* fitness declined when aphids were reared on the high-gossypol genotype versus the low-gossypol genotype under elevated CO_2. Furthermore, elevated CO_2 decreased the developmental time of *L. japonica* associated with the high-gossypol genotype and the low-gossypol genotype, but did not affect parasitism or emergence rates. Our study suggests that the abundance of *A. gossypii* on cotton will not be directly affected by increases in atmospheric CO_2. We speculate that *A. gossypii* may diminish in pest status in elevated CO_2 and high-gossypol genotype environments because of reduced fitness to the high-gossypol genotype and shorter developmental time of *L. japonica*.

Key words *Aphis gossypii*, cotton genotype, elevated CO_2, gossypol, *Lysiphlebia japonica*, tritrophic interaction

Introduction

Global atmospheric CO_2 concentration has increased steadily (from 280 ppm in pre-industrial times to 379 ppm currently) and is predicted to at least double by the end of this century (IPCC, 2007). Elevated CO_2 accelerates the photosynthetic rate, stimulates plant growth, and increases the carbon : nitrogen ratio of most plant

species (Poorter *et al.*, 1997; Curtis & Wang, 1998; Barbehenn *et al.*, 2004). In addition, elevated CO_2 can affect plant quality by inducing changes in allocation to primary and secondary metabolites, which affects tritrophic interactions (Agrell *et al.*, 2000; Hartley *et al.*, 2000; Goverde & Erhardt, 2003). Moreover, fitness of piercing–sucking insects like aphids may increase, decrease or not change when feeding on plants growing under elevated CO_2 (Bezemer & Jones, 1998; Lesley & Fakhri, 2001; Holopainen, 2002; Newman *et al.*, 2003), depending on the insect herbivore species. Although the effects of elevated CO_2 on interactions between herbivores and host plants are becoming better understood, the interactions are complicated by the presence of predator, parasitoids and other natural enemies (Hoover & Newman, 2004). Effects

of elevated CO_2 on tritrophic interactions are frequently "species-specific" and include negative responses (Roth & Lindroth, 1995; Lindroth, 1996), positive responses (Stiling *et al.*, 1999, 2002; Chen *et al.*, 2005a) and no significant effects (Bezemer *et al.*, 1998; Percy *et al.*, 2002; Stacey & Fellowes, 2002; Holton *et al.*, 2003).

Most experiments investigating the effects of elevated CO_2 on natural enemies focused on the bottom-up effects mediated by different species of host plants (Stiling *et al.*, 2002; Holton *et al.*, 2003). These include changes in nutritional and defensive compounds in the plant, which may be passed on to secondary consumers (predators and parasitoids) (Coll & Hughes, 2008). Reduced plant quality may lead to increases in the feeding time of herbivores, thereby exposing them longer to natural enemies (Stiling *et al.*, 2002; Chen *et al.*, 2005a). Moreover, elevated CO_2 also affects the secondary metabolism of plants, leading to increased concentrations of various phenolic compounds, which would act to reduce the pest population and affect natural enemies (Koricheva *et al.*, 1998). However, relatively few studies have examined the effects of CO_2 on tritrophic interactions involving plant genotypes with different levels of chemical defense (Gao *et al.*, 2008). Plant resistance plays an important role in tritrophic interactions and can enhance (Du *et al.*, 2004), reduce (Malcolm, 1992) or have no effect (Giles *et al.*, 2002) on performance of natural enemies under current ambient CO_2. Moreover, the response of plants to elevated CO_2 varies among genotypes with different levels of chemical defense (Lindroth *et al.*, 2001), which may change the interactions between herbivores and their natural enemies. For example, Peltonen *et al.* (2006) found that two genotypes of silver birch (clones 4 and 80 of *Betula pendula* Roth) responded differently to elevated CO_2 and that elevated CO_2 consequently increased the number of aphid eggs laid on clone 4 but not in clone 80. Thus, it is tempting to hypothesize that aphid–parasitoid interactions in crop systems may be altered by elevated CO_2, and that the degree of change is influenced by plant genotype.

The cotton aphid *Aphis gossypii* is an important pest of cotton and is managed in part by use of resistant cultivars. Resistance to *A. gossypii* in cotton is based on the content of gossypol, a phenolic sesquiterpenoid aldehyde. We previously studied the effects of three cotton genotypes (M9101, HZ401 and ZMS13) differing in gossypol content on *A. gossypii* and its predator, the ladybird beetle *Propylaea japonica*. The genotype with the high gossypol content (M9101) suppressed *A. gossypii* but enhanced the performance of *P. japonica* (Du *et al.*, 2004). Importantly, the three cotton genotypes responded differently to elevated CO_2 levels, and the effects of elevated CO_2 on the cotton aphid and *P. japonica* differed among the

genotypes (Gao *et al.*, 2008). In addition to the predator *P. japonica*, the parasitoid *Lysiphlebia japonica* also attacks the cotton aphid (Deng & Tsai, 1998). However, the effects of elevated CO_2 on interactions among this parasitoid, the cotton aphid, and cotton genotypes differing in gossypol contents are unknown. Such information complements our study on tritrophic interactions in crop systems and will help guide the use of crop plant resistance for integrated pest management (IPM) under future CO_2-enriched environments.

The current study explored how three cotton genotypes that differ in gossypol content responded to elevated CO_2; how these changes affected the fitness of insect herbivores (*A. gossypii*); and how the bottom-up effect of elevated CO_2 influenced the third trophic level (*L. japonica*). We hypothesized that the effect of elevated CO_2 on the cotton aphid and its parasitoid *L. japonica* will depend on the cotton genotype. Thus, we asked the following questions. (i) Does elevated CO_2 influence foliar chemistry in different cotton genotypes? (ii) Do these changes in foliar chemistry affect development, fecundity and population parameters of *A. gossypii*? (iii) Do changes in foliar chemistry in cotton genotypes affect the interactions between *A. gossypii* and its parasitoid *L. japonica*?

Materials and methods

Open-top chambers

All experiments were carried out in six octagonal, open-top chambers (OTCs) (1.6 m wide, 4.2 m diameter and 2.4 m high) at the Observation Station on Global Change Biology of the Institute of Zoology, Chinese Academy of Sciences in Xiaotangshan County, Beijing, China (40°11′N, 116°24′E). The current ambient level of CO_2 (375 ppm) and double the current ambient level (750 ppm, the predicted level in about 100 years) (IPCC, 2007) were applied continuously in the OTCs. Three OTCs were used for each CO_2 treatment. The OTCs were arranged in three blocks, consisting of one ambient CO_2 OTC and one double-ambient CO_2 OTC in each block. During the 3.5 months of the experiment (June 20 to October 3, 2007), double-ambient CO_2 concentrations were monitored and controlled by an infrared CO_2 transmitter (Ventostat 8102, Telaire Company, Goleta, CA, USA) and were maintained throughout the experiment. Details of the automatic control system for CO_2 levels and OTCs were provided in Chen *et al.* (2005a, b). The tops of the OTCs were covered with nylon netting to exclude other insects.

Cotton genotypes

Three cotton genotypes (M9101, HZ401 and ZMS13) were obtained from the Institute of Cotton, Chinese Academy of Agricultural Sciences, China. These cotton genotypes exhibited marked differences in gossypol content (Du *et al.*, 2004; Gao *et al.*, 2008). In this paper, M9101, HZ401 and ZMS13 are referred to as the high-gossypol genotype, the intermediate-gossypol genotype and the low-gossypol genotype, respectively. Seeds of these three cotton genotypes were sown in plastic pots (22 cm diameter and 28 cm high) containing sterilized loamy field soil and placed in OTCs on June 3, 2007. Each OTC contained 66 plants (22 of each cotton genotype × three genotypes). Plants were maintained in the OTCs for 3.5 months. Pot placement was re-randomized within each OTC once every week. No chemical fertilizers and insecticides were used. Water was added to each pot once every 2 days.

Aphid life history parameters

The colony of *A. gossypii* used in this study was initiated with a single collection from cotton fields (Institute of Plant Protection, Chinese Academy of Agricultural Sciences). To obtain a standardized aphid colony for the entire experiment, the colony was maintained on the low-gossypol genotype ZMS13 for two generations in environmental chambers at 25 ± 1°C, 70% ± 5% RH, and a 24-h photoperiod of 14 : 10 (L : D).

Aphids from the standard colony were transferred separately to the three test genotypes (M9101, HZ401 and ZMS13) in each OTC. For each cotton genotype, 12 neonate aphid nymphs (<4 h old) were caged (four aphids per plant) on the second and third cotton leaf of three plants at the 6–7-leaf stage (∼35–45 days old) with mini-cages (2.5 cm long, 2.5 cm wide and 1.5 cm high). Therefore, nine randomly selected plants of each of three cotton genotypes (nine plants per OTC and 54 plants in total) were infected with 36 aphid nymphs (216 nymphs in total). Molting and mortality data for each aphid were recorded in each mini-cage at 12-h intervals. When reproduction began, the number of nymphs produced per aphid was recorded daily and neonate aphid nymphs (<4 h old) were kept and considered as the second generation of aphids. Other nymphs were removed every 24 h until the adult aphid died. The procedures for rearing and recording of life-history parameters of the second generation of aphids were the same as described for the first generation.

Parasitoid life-history parameters

The wasp *L. japonica* was obtained by isolating cotton aphid mummies collected from cotton fields (Institute of Plant Protection, Chinese Academy of Agricultural Sciences) and was reared on cotton aphids associated with ZMS13 for two generations in environmental chambers at 25 ± 1°C, 70% ± 5% RH and a 24-h photoperiod of 14 : 10 (L : D). With these methods, wasp life-stages were synchronous and uniform.

Three randomly selected plants at the 6–7-leaf stage (∼35–45 days old) of each of three cotton genotypes per OTC (= 9 plants per OTC and 54 plants in total) were covered with an air-permeable cellophane bag (25 × 35 cm) and infected with 50 second-instar cotton aphids on the second or third leaf. After aphids had settled for 2 h, a mated parasitoid female was introduced into the cellophane bag for a 24-h oviposition period and then the parasitoid was removed. The exposed aphid nymphs were transferred to 10 intact plants (five aphids per plant) at the 6–7-leaf stage and then caged in separate vials (1.5 cm long, 1.5 cm wide and 1 cm high; one nymph per vial) on the second and third cotton leaves (30 plants per OTC and 180 plants in total). Aphids on each cotton genotype (M9101, HZ401 and ZMS13) and under each CO_2 level (ambient *vs.* elevated CO_2) were checked daily for signs of parasitism (the presence of sedentary and bloated mummies). The parasitism rate was determined, and all mummies were examined daily until all parasitoids emerged to assess the individual developmental time (from oviposition to parasitoid adult emergence) and emergence rate. The sex of adult parasitoids was determined with a stereomicroscope.

Foliar chemical components in the absence of aphids

The second and third growth leaves were collected from four randomly selected uninfected cotton plants from each of three genotypes per OTC (12 plants per OTC and 72 plants total). Leaves were placed in liquid nitrogen for 3 h and then stored at −20°C until chemical analysis, except that a sample of fresh leaves (0.5 g) from each plant was removed and used to analyze total amino acids, as described below. Cotton leaf water content, as a proportion of fresh weight, was calculated after drying at 80°C for 72 h.

Total non-structural carbohydrates (TNC), mainly starch and sugar, were assayed by acid hydrolysis following the method of Tissue and Wright (1995). Nitrogen content was assayed using Kjeltec nitrogen analysis (Foss

automated Kjeltec^{TM} instruments, Model 2100: Foss Tecator, Höganäs, Sweden). The gossypol (Stipanovic *et al.*, 1988) and condensed tannin (Zhang & Guo, 2000) content of cotton leaves was assayed with high-pressure liquid chromatography (HPLC).

Fresh cotton leaves (0.5 g from each of 72 plants) were homogenized for 1.5 min at 4°C in 1 : 10 (fresh weight/buffer volume ratio) 100 mmol/L phosphate buffer, pH 7.4, containing 100 mmol/L KCl and 1 mmol/L edetic acid (EDTA) for amino acid analyses. Homogenates were centrifuged at 10 000 *g* for 10 min, and the supernatants were used for protein and total amino acid analysis. Protein concentration was determined by the Bradford (1976) assay. Total amino acids were analyzed with a reagent kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Population parameters of cotton aphid and its parasitoid

Population parameters including net reproductive rate (R_0), mean generation time (T), and intrinsic rate of natural increase (r_m) were analyzed using the age-stage, two-sex life table model (Chi, 1988). The jackknife method (Sokal & Rohlf, 1995) was used to estimate the means and the standard errors of population parameters. A computer program, TWOSEX-MSChart (Chi, 2004), was developed for data analysis and jackknife estimation in Visual BASIC for the Windows operating system. This program is available at: http://140.120.197.173/Ecology/prod02.htm (Chung Hsing University, Taichung, Taiwan) and http://nhsbig.inhs.uiuc.edu/wes/chi.html (Illinois Natural History Survey, University of Illinois at Urbana-Champaign, Urbana, IL, USA).

Statistical analyses

A split-split plot design was used to analyze the effects of CO_2 levels on plant chemical components (i.e., condensed tannins, gossypol, amino acids, TNC : N ratio) and life-history parameters of both cotton aphid and its parasitoid (analysis of variance (ANOVA), SAS Institute, Cary, NC, USA, 2002). CO_2 and block (a pair of ambient and elevated OTCs) were the main effects, and cotton genotype was the subplot effect. Effects were considered significant at $P < 0.05$. The effect of block was not significant ($P > 0.3$), and the effect of block and its interaction with other factors are not presented to facilitate data presentation in tables and in text. Least significant difference (LSD) tests were used to separate the levels within the same variable. Proportional data were transformed using the arcsine square root to satisfy assumptions of normality. Other data were ln($X+10$) transformed if necessary.

Results

Foliar chemical component of three cotton genotypes

CO_2 level significantly affected most of the measured chemical compounds of cotton leaves but not the protein, condensed tannins and gossypol contents (Table 1). Elevated CO_2 significantly increased TNC ($F = 28.2$, df = 1,4, $P = 0.006$) and decreased the nitrogen ($F = 35.8$, df = 1,4, $P = 0.004$) and protein content ($F = 74.8$, df = 1,4, $P = 0.001$) in the intermediate-gossypol genotype but not in the other two genotypes (Fig. 1). Elevated CO_2 significantly increased the foliar TNC : N ratio of the high-gossypol genotype ($F = 10.2$, df = 1,4, $P = 0.033$) and in the intermediate-gossypol genotype ($F = 59.4$, df = 1,4, $P = 0.02$), and decreased the amino

Table 1 *P*-values from analyses of variance for the effect of CO_2 level and cotton genotype on foliar chemical compounds of three cotton genotypes.

Dependent variable	CO_2[†]	Genotype[‡]	$CO_2 \times$ genotype
Nitrogen (mg/g)	>0.001***	>0.001***	0.001**
TNC[§] (mg/g)	0.002**	>0.001***	0.009**
TNC /nitrogen ratio	>0.001***	>0.001***	>0.001***
Soluble protein (mg/mL)	0.140	0.031*	0.538
Free amino acid (mg/g)	>0.001***	>0.001***	>0.001***
Water content (%)	0.002**	0.391	0.389
Condensed tannins (%)	0.076	0.283	0.386
Gossypol (%)	0.534	>0.001***	0.791

[†]Ambient CO_2 *vs* elevated CO_2. [‡]Three genotypes of cotton with different levels of gossypol. [§]TNC, total non-structural carbohydrates. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.
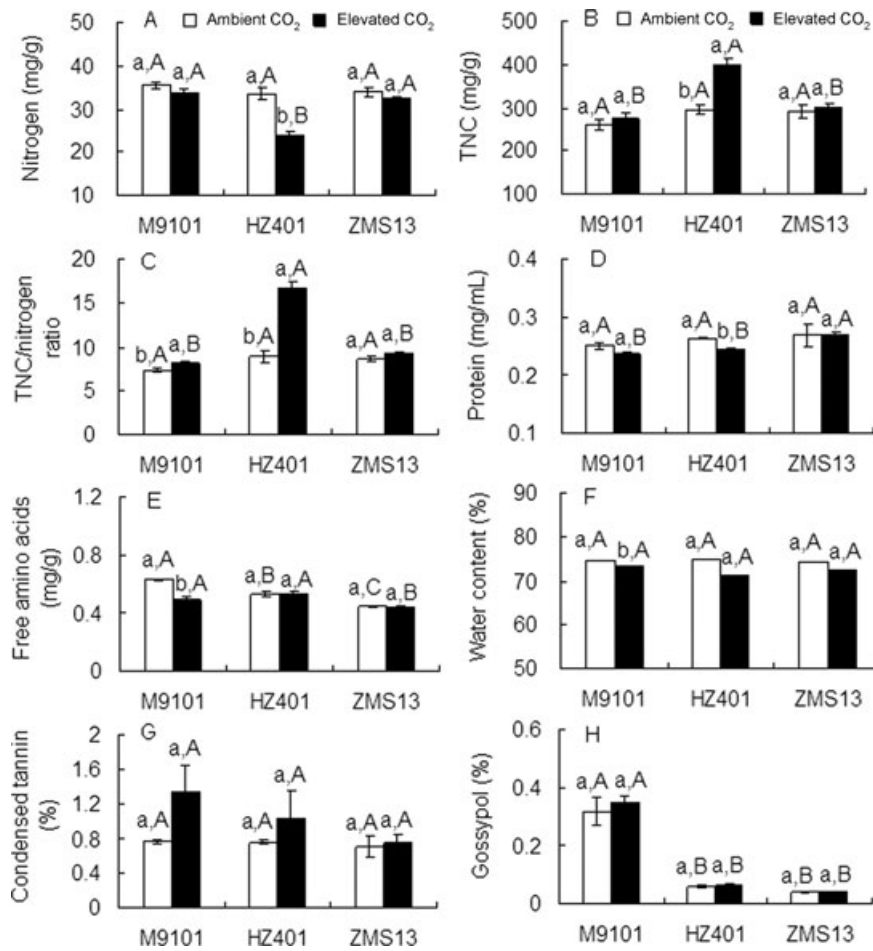
Fig. 1 Foliar chemical components of three cotton genotypes (differing in gossypol content) grown under ambient (370 ppm) and elevated CO$_2$ (750 ppm). Genotypes M9101, HZ401 and ZMS13 have high, intermediate and low gossypol contents, respectively. Values are the means $\pm$ SE of four replicates. Different lowercase letters indicate significant differences between CO$_2$ levels within the same cotton genotype; different uppercase letters indicate significant differences among cotton genotypes within CO$_2$ levels.

acids ($F = 70.4$, df $= 1,4$, $P = 0.001$) and water content ($F = 8.00$, df $= 1,4$, $P = 0.047$) in the high-gossypol genotype (Fig. 1C, E, and F). Furthermore, with the exception of water content (Fig. 1F) and condensed tannins (Fig. 1G), cotton genotype significantly affected all the measured chemical compounds of cotton leaves (Table 1). Among the three cotton genotypes, the high-gossypol genotype had the highest gossypol content regardless of treatment (Fig. 1H).

Developmental time, adult life span, fecundity and the population parameters of A. gossypii

Effect of elevated CO$_2$ CO$_2$ levels significantly affected the developmental time of the second instar

and the duration of total nymphal stage of *A. gossypii* (Table 2 and 3). Elevated CO$_2$ reduced the developmental time of the second ($F = 9.54$, df $= 1,58$, $P = 0.003$) and the third ($F = 11.4$, df $= 1,58$, $P = 0.001$) instar, as well as the duration of total nymphal stage ($F = 15.7$, df $= 1,58$, $P < 0.001$) in the first generation of *A. gossypii* reared on the high-gossypol genotype. Elevated CO$_2$ also reduced the developmental time of the second instar in the first generation of *A. gossypii* reared on the intermediate-gossypol genotype ($F = 7.39$, df $= 1,58$, $P = 0.009$). In contrast, elevated CO$_2$ did not affect the life history parameters in the first generation of *A. gossypii* reared on the low-gossypol genotype (Table 3).

In the second generation of *A. gossypii*, elevated CO$_2$ significantly increased the adult life span ($F = 5.77$, df $= 1,56$, $P = 0.02$) of *A. gossypii* reared on the

Table 2 P-values from analyses of variance for the effect of CO_2 level, cotton genotype and generation on developmental time, adult life span, fecundity and the population parameters of cotton aphid.

Measured indices	CO_2[†]	Genotype[‡]	Generation[§]	$CO_2 \times$ genotype	$CO_2 \times$ generation	Genotype $\times$ generation	$CO_2 \times$ genotype $\times$ generation
1st instar (h)	0.676	0.220	>0.001***	0.363	0.915	0.069	0.989
2nd instar (h)	>0.001***	0.758	0.009**	0.826	0.083	0.150	0.520
3rd instar (h)	0.043*	0.805	0.688	0.440	0.127	0.545	0.020*
4th instar (h)	0.543	0.914	0.266	0.101	0.629	0.646	0.260
Nymphal stage (h)	>0.001***	0.308	0.361	0.898	0.680	0.342	0.010*
Adult life span (d)	0.169	0.580	>0.001***	0.021*	0.117	0.064	0.851
Fecundity[¶]	0.817	0.635	0.001**	0.298	0.285	0.387	0.801
R_0	0.769	0.737	0.001**	0.430	0.738	0.366	0.837
T	0.771	0.002**	0.039*	0.462	0.144	0.014*	0.653
r_m	0.699	0.010*	0.315	0.864	0.188	0.068	0.622

[†]Ambient CO_2 *vs.* elevated CO_2.
[‡]Three genotypes of cotton with different levels of gossypol.
[§]Aphid first generation *vs.* second generation.
[¶]Fecundity represents the numbers of nymphs laid by per aphid.
R_0, net reproductive rate; T, generation time; r_m, intrinsic rate of natural increase. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

high-gossypol genotype, and decreased the developmental time ($F = 4.15$, df $= 1,56$, $P = 0.046$) of the second instar of *A. gossypii* reared on the intermediate-gossypol genotype. Moreover, elevated CO_2 reduced the developmental time of the second instar ($F = 6.02$, df $= 1,60$, $P = 0.017$) and the duration of total nymphal stage ($F = 5.88$, df $= 1,60$, $P = 0.018$) when the second generation of *A. gossypii* was reared on the low-gossypol genotype (Table 3).

Effect of genotype Genotype significantly affected the generation time (T) and intrinsic rate of natural increase (r_m) of *A. gossypii* (Table 2). For the first generation of *A. gossypii* under ambient CO_2, duration of the total nymphal stage ($F = 4.15$, df $= 1,56$, $P = 0.046$) was greater and adult life span ($F = 8.29$, df $= 1,59$, $P = 0.006$) was shorter with the high-gossypol genotype than with the other two genotypes. Furthermore, for the first generation of *A. gossypii* under elevated CO_2, the developmental time of the second instar was longer and the developmental time of the fourth instar and the generation time (T) were shorter with the low-gossypol genotype than with the intermediate-gossypol genotype (Table 3).

In the second generation of *A. gossypii* under ambient CO_2, the developmental time of the first instar was shorter with the low-gossypol genotype than with the intermediate-gossypol genotype ($F = 4.65$, df $= 1,61$, $P = 0.035$). Furthermore, the high-gossypol genotype increased the duration of the total nymphal stage and gen-

eration time (T) and reduced the intrinsic rate of natural increase (r_m) of *A. gossypii* in their second generation under elevated CO_2 versus ambient CO_2 (Table 3). In addition, with the exception of adult lifespan, the statistical interaction between CO_2 and genotype was not significant (Table 2).

Developmental time, parasitism rate, emergence rate and female proportion of L. japonica

Effect of elevated CO_2 CO_2 level significantly affected the developmental time of *L. japonica* (Table 4), and elevated CO_2 markedly decreased the developmental time of *L. japonica* associated with the high-gossypol genotype ($F = 43.7$, df $= 1,31$, $P < 0.001$; Table 5) and the low-gossypol genotype ($F = 14.6$, df $= 1,42$, $P < 0.001$). Neither parasitism rate nor emergence rate were influenced by CO_2 level. Elevated CO_2 significantly decreased the proportion of *L. japonica* females associated with the low-gossypol genotype ($F = 20.0$, df $= 1,16$, $P < 0.001$).

Effect of genotype Genotype did not affect developmental time, parasitism rate, emergence rate or female proportion of *L. japonica* (Table 4). Furthermore, for the ambient CO_2 level, the parasitism rate was significantly higher with the low-gossypol genotype than with the intermediate-gossypol genotype ($F = 5.03$, df $= 1,16$,

Table 3 Developmental time, adult life span, fecundity and the population parameters of two successive generations of *A. gossypii* reared on different cotton genotypes (M9101, HZ401, and ZMS13) under ambient (370 ppm) and elevated CO_2 (750 ppm).

Generation[†]	Measured indices	Ambient CO_2			Elevated CO_2		
		M9101	HZ401	ZMS13	M9101	HZ401	ZMS13
F1	1st instar (h)	33.5 ± 1.5 a,A	31.9 ± 1.5 a,A	31.5 ± 1.4 a,A	33.7 ± 1.5 a,A	30.4 ± 2.0 a,A	33.8 ± 1.4 a,A
	2nd instar (h)	26.9 ± 1.0 a,A	26.3 ± 0.8 a,A	27.0 ± 0.9 a,A	24.0 ± 0.0 b,B	23.6 ± 0.4 b,B	27.0 ± 1.7 a,A
	3rd instar (h)	29.0 ± 1.4 a,A	27.8 ± 1.3 a,A	27.4 ± 1.1 a,A	24.0 ± 0.6 b,A	25.7 ± 1.2 a,A	25.1 ± 0.8 a,A
	4th instar (h)	29.8 ± 1.9 a,A	27.0 ± 2.0 a,A	29.3 ± 1.2 a,A	26.7 ± 2.0 a,B	30.0 ± 1.6 a,A	26.3 ± 2.2 a,AB
	Nymphal stage (h)	119.2 ± 1.9 a,A	112.9 ± 2.0 a,B	115.1 ± 1.2 a,AB	108.4 ± 2.0 b,A	109.7 ± 1.6 a,A	112.1 ± 2.2 a,A
	Adult life span (d)	17.3 ± 1.5 a,B	23.6 ± 1.5 a,A	21.5 ± 1.5 a,AB	20.5 ± 1.3 a,A	21.3 ± 1.0 a,A	20.1 ± 1.5 a,A
	Fecundity[‡]	39.1 ± 2.9 a,B	47.2 ± 2.6 a,A	43.2 ± 2.0 a,AB	45.9 ± 3.1 a,A	46.9 ± 2.1 a,A	41.6 ± 2.8 a,A
	R_0	39.1 ± 2.9 a,B	47.2 ± 2.6 a,A	43.2 ± 2.0 a,AB	44.5 ± 3.3 a,A	43.7 ± 2.9 a,A	41.6 ± 2.80 a,A
	T	16.0 ± 0.3 a,A	16.7 ± 0.46 a,A	16.0 ± 0.26 a,A	15.8 ± 0.30 a,AB	16.4 ± 0.25 a,A	15.7 ± 0.22 a,B
	r_m	0.230 ± 0.005 a,A	0.231 ± 0.005 a,A	0.236 ± 0.004 a,A	0.240 ± 0.005 a,A	0.230 ± 0.006 a,A	0.238 ± 0.006 a,A
F2	1st instar (h)	28.6 ± 1.8 a,AB	30.0 ± 2.4 a,A	24.0 ± 1.6 a,B	28.6 ± 2.4 a,A	29.1 ± 2.2 a,A	26.5 ± 1.6 a,A
	2nd instar (h)	30.6 ± 2.0 a,A	30.0 ± 1.6 a,A	29.5 ± 1.5 a,A	26.5 ± 1.5 a,A	25.7 ± 1.3 b,A	24.4 ± 1.4 b,A
	3rd instar (h)	24.8 ± 1.9 a,A	27.6 ± 1.9 a,A	28.7 ± 2.0 a,A	29.4 ± 1.8 a,A	24.0 ± 2.0 a,B	26.5 ± 1.1 a,AB
	4th instar (h)	28.6 ± 2.6 a,A	28.0 ± 1.8 a,A	31.3 ± 1.2 a,A	30.6 ± 1.7 a,A	28.7 ± 2.4 a,A	28.1 ± 2.0 a,A
	Nymphal stage (h)	112.6 ± 3.4 a,A	115.6 ± 2.7 a,A	113.5 ± 2.2 a,A	115.0 ± 3.2 a,A	107.6 ± 3.4 a,AB	105.5 ± 2.4 b,B
	Adult life span (d)	13.2 ± 1.7 b,A	15.0 ± 1.4 a,A	13.3 ± 1.4 b,A	19.0 ± 1.7 a,A	14.4 ± 1.8 a,A	15.9 ± 1.7 a,A
	Fecundity	36.4 ± 5.0 a,A	39.9 ± 3.3 a,A	39.6 ± 3.8 a,A	37.2 ± 3.5 a,A	33.8 ± 4.2 a,A	37.4 ± 3.7 a,A
	R_0	35.3 ± 5.0 a,A	36.2 ± 3.6 a,A	39.6 ± 3.8 a,A	36.0 ± 3.5 a,A	33.7 ± 4.2 a,A	37.8 ± 3.72 a,A
	T	15.9 ± 0.4 a,A	15.6 ± 0.3 a,A	15.0 ± 0.3 a,A	16.8 ± 0.4 a,A	15.9 ± 0.4 a,AB	14.9 ± 0.42 a,B
	r_m	0.225 ± 0.010 a,A	0.230 ± 0.009 a,A	0.245 ± 0.008 a,A	0.214 ± 0.008 a,B	0.222 ± 0.009 a,AB	0.244 ± 0.008 a,A

[†]Generation: F1 *vs.* F2 represents the first generation *vs* the second generation of cotton aphid.

[‡]Fecundity represents the numbers of nymphs laid per aphid.

R_0, Net reproductive rate; T, generation time; r_m, intrinsic rate of natural increase. Each value represents the mean (± SE) of four replicates. Different lowercase letters indicate significant differences between CO_2 treatments within cotton genotype; different uppercase letters indicate significant differences among cotton genotypes within CO_2 level (LSD test, $P < 0.05$).

Table 4 *P*-values from analyses of variance for the effect of CO_2 level and cotton genotype on developmental time and the parasitism parameters of *L. japonica*.

Measured indices	CO_2[†]	Genotype[‡]	$CO_2 \times$ genotype
Developmental time (day)	>0.001***	0.648	0.008**
Parasitism rate (%)	0.938	0.333	0.215
Emergence rate (%)	0.492	0.607	0.940
Female (%)	0.025*	0.941	0.078

[†]Ambient CO_2 *vs* elevated CO_2.

[‡]Three cotton genotypes that differ in gossypol content.

*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

$P = 0.039$; Table 5). For the elevated CO_2 level, *L. japonica* developmental time was significantly longer with the high-gossypol genotype than with the intermediate-gossypol genotype ($F = 14.4$, df $= 1,54$, $P < 0.001$).

Discussion

In the present study, three cotton genotypes differing in gossypol content exhibit different or "genotype-specific" responses to elevated CO_2. Elevated CO_2 affected the following characteristics in the high-gossypol genotype and intermediate-gossypol genotype: TNC : N ratio and contents of amino acids, proteins, nitrogen and water. However, elevated CO_2 did not affect any of these characteristics in the low-gossypol genotype, suggesting that the low-gossypol genotype is insensitive to elevated CO_2 levels in this system. In contrast, Gao *et al.* (2008) found that TNC : N ratio of the low-gossypol genotype increased with elevated CO_2 in the cotton–aphid–predator system, which is possibly owing to the difference of nitrogen content in plant soil. Elevated CO_2 accumulating excess carbon in plant tissues is probably allocated to more carbon-based secondary metabolites, such as phenolics, condensed tannins and terpenoids (Bazin *et al.*, 2002). For example, Hartley *et al.* (2000) found that changes in phenolic biosynthesis in response to elevated CO_2 was species-specific (four plant species were studied) and that the responses changed markedly between generations. However, Holton *et al.* (2003) showed that elevated CO_2 had little effect on both total phenolic glycosides and condensed tannins of aspen. Our study showed that, although the TNC : N ratio increased in the high-gossypol genotype and the intermediate-gossypol genotype under elevated CO_2, gossypol and condensed tannin content did not change. Moreover, the interactive effect of CO_2 and genotype did not affect the gossypol and condensed tannin contents. This suggests that, although nutrient content (proteins and amino acids) was decreased with elevated

CO_2, the resistance of the high-gossypol genotype was unaffected by elevated CO_2.

Cotton gossypol content has been considered the most important resistance mechanism against cotton aphids. Bottger *et al.* (1964) observed that aphid infestations increased in cotton cultivars lacking the gossypol gland. Our results also showed that the high-gossypol genotype adversely affected development, survivorship, longevity and reproduction of the first generation of *A. gossypii* when reared under ambient CO_2, which is in accordance with our earlier findings (Du *et al.*, 2004; Gao *et al.*, 2008). However, for the second generation of *A. gossypii*, adult longevity and fecundity did not change in response to cotton genotypes when reared on different genotypes under ambient CO_2. This suggests that, although the first generation of *A. gossypii* was sensitive to the gossypol content of the host plant, the offspring may somehow adapt so as to tolerate high gossypol content under ambient CO_2. Furthermore, with the exception of genotype, no factor or interaction affected the intrinsic rate of natural increase (r_m) (Table 2). Our data showed that, for the second generation under elevated CO_2, *A. gossypii* had higher fitness (r_m) on the low-gossypol genotype than on high-gossypol genotype.

Some studies have reported that elevated CO_2 had little or no effect on natural enemies of herbivores (Salt *et al.*, 1995; Stacey & Fellowes, 2002; Holton *et al.*, 2003), while others suggested the elevated CO_2 would enhance the performance of natural enemies (Stiling *et al.*, 1999, 2002; Chen *et al.*, 2005a). In this study, elevated CO_2 reduced the developmental time of the parasitoid *L. japonica* attacking aphids on the high-gossypol genotype and the low-gossypol genotype and decreased female : male proportion of *L. japonica* associated with the low-gossypol genotype. It was suggested that elevated CO_2 may alter population growth of parasitoids on different host plants. Furthermore, phenolics and condensed tannins were negatively correlated with the performance of insect herbivores, and those compounds that limit the parasitoid

Table 5 The developmental time, parasitism rate and biological parameters of the wasp *L. japonica* parasitizing cotton aphids reared on different cotton genotypes (M9101, HZ401, and ZMS13) under ambient (370 ppm) and elevated CO$_2$ (750 ppm).

Measured indices	Ambient CO$_2$			Elevated CO$_2$		
	M9101	HZ401	ZMS13	M9101	HZ401	ZMS13
Developmental time (day)	13.2 ± 0.970 a,A	11.7 ± 0.619 a,A	12.0 ± 0.315 a,A	10.0 ± 0.126 b,B	11.0 ± 0.221 a,A	10.5 ± 0.239 b,AB
Parasitism rate (%)	10.2 ± 0.042 a,AB	4.67 ± 0.019 a,B	17.1 ± 0.052 a,A	17.6 ± 0.085 a,A	7.78 ± 0.058 a,A	6.67 ± 0.031 a,A
Emergence rate (%)	58.7 ± 0.160 a,A	51.4 ± 0.167 a,A	73.7 ± 0.141 a,A	54.0 ± 0.171 a,A	42.2 ± 0.168 a,A	57.4 ± 0.155 a,A
Female (%)	41.4 ± 0.166 a,A	51.9 ± 0.168 a,A	73.5 ± 0.142 a,A	44.0 ± 0.156 a,A	33.3 ± 0.167 a,A	38.9 ± 0.261 b,A

Each value represents the mean (± SE). Different lowercase letters indicate significant differences between CO$_2$ treatments within cotton genotype; different uppercase letters indicate significant differences among cotton genotypes within CO$_2$ level (LSD test, $P < 0.05$).

may be different from those that limit its insect host (Holton *et al.*, 2003). Our previous research found that the high-gossypol genotype enhanced the growth and development of the aphid predator *P. japonica* (Gao *et al.*, 2008). However, in this study cotton genotypes differing in gossypol content had no effect on the performance of the aphid parasitoid *L. japonica* (Table 4). Moreover, no factor or interaction affected the parasitism rate of *L. japonica*. Thus, we suggest that depending on cotton genotype, elevated CO$_2$ may shorten the developmental time of *L. japonica*, while having no effect on its parasitism rate.

Our results advance the understanding of the effects of elevated CO$_2$ on aphid populations in crop systems by assessing both bottom-up and top-down effects. In cotton–aphid–predator systems, considering increased survivorship of the aphid and longer development time of the predator, cotton aphid may become a more serious pest under elevated CO$_2$ environments (Gao *et al.*, 2008). However, in this study, elevated CO$_2$ shortened the developmental time of *A. gossypii* but did not affect the intrinsic rate of natural increase (r_m) of *A. gossypii*, which suggests that the abundance of *A. gossypii* on cotton will not be directly affected by increases in atmospheric CO$_2$. Additionally, the performances of predator and parasitoid in the cotton–aphid system, respond differently to elevated CO$_2$, which differs in this study versus our previous work (Gao *et al.*, 2008).

In summary, the plant-mediated effect caused by elevated CO$_2$ is likely to accelerate the development of *A. gossypii* and its parasitoid *L. japonica* but have minimal effect on the parasitism rate of *L. japonica*. Although CO$_2$ level and the interaction between CO$_2$ level and genotype had no effect on the fitness (as measured by r_m) of *A. gossypii* reared on different genotypes, fitness declined when aphids were reared on the high-gossypol genotype versus the low-gossypol genotype under elevated CO$_2$. Thus, fitness of *A. gossypii* on cotton will not be directly affected by increases in atmospheric CO$_2$. Considering increased susceptibility to high-gossypol genotype and shorter developmental time of *L. japonica*, we speculate that *A. gossypii* may not be successful in elevated CO$_2$ environments.

Acknowledgments

References

Agrell, J., McDonald, E.P. and Lindroth, R.L. (2000) Effects of CO_2 and light on tree-insect interactions. *Oikos*, 88, 259–272.

Barbehenn, R.V., Chen, Z., Karowe, D.N. and Spickard, A. (2004) C_3 grasses have higher nutritional quality than C_4 grasses under ambient and elevated atmospheric CO_2. *Global Change Biology*, 10, 1565–1575.

Bazin, A., Goverde, M., Erhardt, A. and Shykoff, J. (2002) Influence of atmospheric carbon dioxide enrichment on induced response and growth compensation after herbivore injury in *Lotus corniculatus*. *Ecological Entomology*, 27, 271–278.

Bezemer, T.M., Jones, T.H. and Knight, K.J. (1998) Long-term effects of elevated CO_2 and temperature on populations of the peach potato aphid *Myzus persicae* and its parasitoid *Aphidius matricariae*. *Oecologia*, 116, 128–135.

Bezemer, T.M. and Jones, T.H. (1998) Plant-insect herbivore interactions in elevated atmospheric CO_2: quantitative analysis and guild effects. *Oikos*, 82, 212–222.

Bottger, G.T., Sheehan, E.T. and Lukefahr, M.J. (1964) Relation of gossypol content of cotton plants to insect resistance. *Journal of Economic Entomology*, 57, 283–285.

Bradford, M.M. (1976) A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye-binding. *Analytical Biochemistry*, 72, 248–254.

Chen, F.J., Ge, F. and Parajulee, M.N. (2005a) Impact of elevated CO_2 on tri-trophic interaction of *Gossypium hirsutum*, *Aphis gossypii*, and *Leis axyridis*. *Environmental Entomology*, 34, 37–46.

Chen, F.J., Wu, G., Ge, F., Parajulee, M.N. and Shrestha, R.B. (2005b) Effects of elevated CO_2 and transgenic Bt cotton on plant chemistry, performance, and feeding of an insect herbivore, the cotton bollworm. *Entomologia Experimentalis et Applicata*, 115, 341–350.

Chi, H. (1988) Life-table analysis incorporating both sexes and variable development rate among individuals. *Environmental Entomology*, 17, 26–34.

Chi, H. (2004) TWOSEX-MSChart: a computer program for the age-stage, two-sex life table analysis. http://140.120.197.173/Ecology/Download/Twosex-MSChart.zip.

Coll, M. and Hughes, L. (2008) Effects of elevated CO_2 on an insect omnivore: A test for nutritional effects mediated by host plants and prey. *Agriculture, Ecosystems and Environment*, 123, 271–279.

Curtis, P.S. and Wang, X. (1998) A meta-analysis of elevated CO_2 effects on woody plant mass, form and physiology. *Oecologia*, 113, 299–313.

Deng, Y.X. and Tsai, J.H. (1998) Biology of *Lysiphlebia japonica*. *Florida Entomologist*, 81, 415–423.

Du, L., Ge, F. and Zhu, S.R. (2004) Effect of cotton cultivar on development and reproduction of *Aphis gossypi* (Homoptera: Aphiddae) and its predator *Propylaea japonica* (Coleoptera: Coccinellidae). *Journal of Economic Entomology*, 97, 1278–1283.

Gao, F., Zhu, S.R., Sun, Y.C., Du, L., Parajulee, M., Kang, L. and Ge, F. (2008) Interactive effects of elevated CO_2 and cotton cultivar on tri-trophic interaction of *Gossypium hirsutum*, *Aphis gossyppii*, and *Propylaea japonica*. *Environmental Entomology*, 37, 29–37.

Giles, K.L., Berberet, R.C., Zarrabi, A.A. and Dillwith, J.W. (2002) Influence of alfalfa cultivar on suitability of *Acyrthosiphon kondoi* (Homoptera: Aphididae) for survival and development of *Hippodamia convergens* and *Coccinella semptempunctata* (Coleoptera: Coccinellidae). *Journal of Economic Entomology*, 95, 552–557.

Goverde, M. and Erhardt, A. (2003) Effects of elevated CO_2 on development and larval food-plant preferences in the butterfly, *Coenonympha pamphilus* (Lepidoptera: Satyridae). *Global Change Biology*, 9, 74–83.

Hartley, S.E., Jones, C.G., Couper, G.C. and Jones, T. (2000) Biosynthesis of plant phenolic compounds in elevated atmospheric CO_2. *Global Change Biology*, 6, 497–506.

Holopainen, J.K. (2002) Aphid response to elevated ozone and CO_2. *Entomologia Experimentalis et Applicata*, 104, 137–142.

Holton, M.K., Lindroth, R.L. and Nordheim, E.V. (2003) Foliar quality influences tree-herbivore-parasitoid interactions: effects of elevated CO_2, O_3, and plant genotype. *Oecologia*, 137, 233–244.

Hoover, J.K. and Newman, J.A. (2004) Tritrophic interactions in the context of climate change: a model of grasses, cereal aphids and their parasitoids. *Global Change Biology*, 10, 1197–1208.

Intergovernmental Panel on Climate Change (2007) Climate Change 2007; the physical science basis. Summary for policy makers. Report of Working Group I of the Intergovernmental Panel on Climate Change. http://www.ipcc.ch/pub/spm 18-02.pdf.

Koricheva, J., Larsson, S., Haukioja, E. and Keinanen, M. (1998) Regulation of woody plant secondary metabolism by resource availability: hypothesis testing by means of meta-analysis. *Oikos*, 83, 212–226.

Lesley, H. and Fakhri, A.B. (2001) Effects of elevated CO_2 on five plant-aphid interactions. *Entomologia Experimentalis et Applicata*, 99, 87–96.

Lindroth, R.L., Roth, S. and Nordheim, E.V. (2001) Genotypic variation in response of quaking aspen (*Populus tremuloides*) to atmospheric CO_2 enrichment. *Oecologia*, 126, 371–379.

Lindroth, R.L. (1996) CO_2-mediated changes in tree chemistry and tree-Lepidoptera interactions. *Carbon Dioxide and Terrestrial Ecosystems* (eds. G.W. Koch & J. Roy), pp. 105–120. Academic Press, San Diego, CA, USA.

Malcolm, S.B. (1992) Prey defence and predator foraging. *The Population Biology of Predators, Parasites and Diseases* (ed. M.J. Crawley), pp. 458–475. Blackwell, Oxford, UK.

Newman, J.A., Gibson, D.J., Parsons, A.J. and Thornley, J.H.M. (2003) How predictable are aphid population responses to elevated CO_2? *Journal of Animal Ecology*, 72, 556–566.

Peltonen, P.A., Julkunen-tiitto, R., Vapaavuori, E. and Holopainen, J.K. (2006) Effects of elevated carbon dioxide and ozone on aphid oviposition preference and birch bud exudate phenolics. *Global Change Biology*, 12, 1670–1679.

Percy, K.E., Awmack, C.S., Lindroth, R.L., Kubiske, M.E., Kopper, B.J., Isebrands, J.G., Pregitzer, K.S., Hendrey, G.R., Dickson, R.E., Zak, D.R., Oksanen, E., Sober, J., Harrington, R. and Karnosky, D.F. (2002) Altered performance of forest pests under atmospheres enriched by CO_2 and O_3. *Nature*, 420, 403–407.

Poorter, H., van Berkel, Y., Baxter, R., den Hertog, J., Dijkstra, P., Gifford, R., Griffin, K.L., Roumet, C., Roy, J. and Wong, S.C. (1997) The effect of elevated CO_2 on the chemical composition and construction costs of leaves of 27 C_3 species. *Plant Cell and Environment*, 20, 472–482.

Roth, S.K. and Lindroth, R.L. (1995) Elevated atmospheric CO_2 effects on phytochemistry, insect performance and insect-parasitoid interactions. *Global Change Biology*, 1, 173–182.

Salt, D.T., Brook, G.L. and Whittaker, J.B. (1995) Elevated carbon dioxide affects leaf-miner performance and plant growth in docks (*Rumex* spp.). *Global Change Biology*, 1, 153–156.

Sokal, R.R. and Rohlf, F.J. (1995) *Biometry: the Principles and Practice of Statistics in Biological Research*. 3rd ed. W. H. Freeman and Co., New York, 887 pp.

Stacey, D.A. and Fellowes, M.D.E. (2002) Influence of elevated CO_2 on interspecific interactions at higher trophic levels. *Global Change Biology*, 8, 668–678.

Stiling, P., Cattell, M., Moon, D.C., Rossi, A., Hungate, B.A., Hymus, G. and Drake, B. (2002) Elevated atmospheric CO_2 lowers herbivore abundance, but increases leaf abscission rates. *Global Change Biology*, 8, 658–667.

Stiling, P., Rossi, A.M., Hungate, B., Dijkstra, P., Hinkle, C.R., Knott, W.M. and Drake, B. (1999) Decreased leaf-miner abundance in elevated CO_2: reduced leaf quality and increased parasitoid attack. *Ecological Applications*, 9, 240–244.

Stipanovic, R.D., Altman, D.W., Begin, D.L., Greenblatt, G.A. and Benedict, J.H. (1988) Terpenoid aldehydes in upland cottons: Analysis by aniline and HPLC methods. *Journal of Agricultural and Food Chemistry*, 36, 509–515.

Tissue, D.T. and Wright, S.J. (1995) Effects of seasonal water availability on phenology and the annual shoot carbohydrate cycle of tropical forest shrubs. *Functional Ecology*, 9, 518–527.

Zhang, Y.J. and Guo, Y.Y. (2000) Interactions between condensed tannin and Bt crystal protein in cotton. *Cotton Science*, 12, 294–297. (in Chinese)