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ORIGINAL ARTICLE

Influences of elevated CO_2 and pest damage on the allocation of plant defense compounds in Bt-transgenic cotton and enzymatic activity of cotton aphid

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Abstract Plant allocation to defensive compounds by elevated CO_2-grown non-transgenic and transgenic Bt cotton in response to infestation by cotton aphid, *Aphis gossypii* (Glover) in open-top chambers under elevated CO_2 were studied. The results showed that significantly lower foliar nitrogen concentration and Bt toxin protein occurred in transgenic Bt cotton with and without cotton aphid infestation under elevated CO_2. However, significantly higher carbon/nitrogen ratio, condensed tannin and gossypol were observed in transgenic Bt cotton "GK-12" and non-transgenic Bt cotton 'Simian-3' under elevated CO_2. The CO_2 level and cotton variety significantly influenced the foliar nitrogen, condensed tannin and gossypol concentrations in the plant leaves after feeding by *A. gossypii*. The interaction between CO_2 level × infestation time (24 h, 48 h and 72 h) showed a significant increase in cotton condensed tannin concentrations, while the interaction between CO_2 level × cotton variety significantly decreased the true choline esterase (TChE) concentration in the body of *A. gossypi.* This study exemplified the complexities of predicting how transgenic and non-transgenic plants will allocate defensive compounds in response to herbivorous insects under differing climatic conditions. Plant defensive compound allocation patterns and aphid enzyme changes observed in this study appear to be broadly applicable across a range of plant and herbivorous insect interactions as CO_2 atmosphere rises.

Key words *Aphis gossypii*, condensed tannin, elevated CO_2, gossypol, plant allocation, transgenic Bt cotton

Introduction

The atmospheric CO_2 concentration has risen from 280 μL/L to 360 μL/L following the Industrial Revolution, engendering a critical shift in global biogeochemical cycles (IPCC, 2007). This level of CO_2 is anticipated to double by the end of the 21st century (Wigley & Raper, 1992).

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Profound impacts of elevated CO_2 in the terrestrial ecosystem, especially on physiology and growth of plants, are expected (Bazin *et al.*, 2002; Penuelas *et al.*, 2002; Wu *et al.*, 2006): significant increases in photosynthesis, growth, aboveground biomass (Wu *et al.*, 2007a), leaf area, yield (Wu *et al.*, 2007b) and carbon : nitrogen (C : N) ratios of plants, particularly C_3 plants (Oijen *et al.*, 1999) have been reported. As a result of increased photosynthetic rates and faster growth, C : N ratio increases occur through accumulation of non-structural carbohydrate as plants grow in elevated CO_2 (e.g. Wu *et al.*, 2007c; Williams *et al.*, 1998): this, in turn, impacts the production of secondary metabolites, promoting the

manufacture of carbon (C)-based secondary metabolites (e.g. phenolics) over N-based ones (Bryant *et al.*, 1983). Lindroth *et al.* (1995) reported that condensed tannin concentrations in paper birch (*Betula papyrifera*) doubled when trees were grown in elevated CO_2, while Fajer *et al.* (1991) reported that neither iridoid glycoside nor fiber concentrations in *Plantago lanceolata* were affected by CO_2 levels. Condensed tannin and gossypol concentrations have been shown to increase in response to leaf infestation, yet most studies have assayed tannin and gossypol concentrations in elevated CO_2 using only non-damaged leaves (Lindroth *et al.*, 1995). To date, there is a paucity of information on the tannin and gossypol concentrations after insect feeding in elevated CO_2.

The reduction in food protein and nitrogen content often leads to poorer insect performance as measured by behavioral or physiological adaptation (Scriber & Slansky, 1981; Wu *et al.*, 2008). Carbohydrates as well as nitrogen and nitrogenous compounds are important for insect herbivores. Buse *et al.* (1998) found that *Operophtera brumata* produced more eggs when reared on oak leaves of *Quercus robur* grown under elevated CO_2. The cotton aphid, *Aphis gossypii* (Glover), is an important pest of cotton. Transgenic Bt cotton has recently been widely adopted in China (Men *et al.*, 2003). It was anticipated that the primary CO_2 effect on Bt toxin production would be due to differences in N concentration within the plant (Coviella *et al.*, 2002). Biologically relevant changes in plant (non-transgenic cotton and transgenic Bt cotton) defensive chemistry are expected to have measurable effects on cotton aphid, *A. gossypii*. If conditions of increased carbon (e.g. elevated CO_2) allow plants to allocate significantly more resources to condensed tannins and gossypol, then the enzyme composition in the insect herbivore is expected to also change. Similarly, if Bt toxin production changes due to elevated CO_2, then the insect herbivore's body enzymes should also be changed in this circumstance.

We studied allocation to defensive compounds in transgenic Bt cotton (cv. GK-12) and nontransgenic Bt cotton (cv. Simian-3) in response to infestation by cotton aphid, *Aphis gossypii* (Glover) through open-top chambers under elevated CO_2. We addressed the following objectives: (i) to analyze the impacts of elevated CO_2 and aphid infestation on allocation patterns of defensive compounds in transgenic and non-transgenic Bt cotton plants; (ii) to measure how enzymes were changed in *A. gossypii* when feeding on transgenic and non-transgenic Bt cotton plants growing in ambient or elevated CO_2.

Materials and methods

Open-top chamber

This experiment was carried out in six 4.2-m diameter open-top chambers (OTCs) in Sanhe County, Hebei Province, China (35°57′N, 116°47′E). Two levels of atmospheric CO_2 concentration were continuously applied, that is, current ambient level (370 μL/L) and elevated level (750 μL/L). The double-ambient CO_2 represents the level expected to be present in about 100 years (Chen *et al.*, 2005a). Three open-top chambers were used for each CO_2 level. During the period from seedling emergence to harvesting, CO_2 concentrations were monitored 24 h/day and adjusted with an infrared CO_2 analyzer (Ventostat 8102, Telaire Company, Goleta, California, USA) once every 20 min to maintain the CO_2 concentrations. Details of the automatic-control system for CO_2 and open-top chambers are provided in Chen & Ge (2004) and Chen *et al.* (2005b).

Cotton treatments

Two cotton cultivars were used in the study, including a transgenic Bt cultivar 'GK-12' and a non-transgenic cultivar 'Simian-3' from the same recurrent parent line. Both cultivars were planted in white plastic pots (15 cm diameter, 17 cm height) filled with 8 : 3 : 1 (by volume) loam : cow dung : earthworm frass. The soil mixture was sampled and triturated to analyze its chemical composition (Institute of Soil Science, Chinese Academy of Sciences, 1978). Soil pH was 7.1, organic matter 14.2%, available N 403.7 mg/kg (hydrolic N, 1 N NaOH hydrolysis), available P 270.0 mg/kg (0.5 mol/L $NaHCO_3$ extraction), available K 267.1 mg/kg (1 N CH_3COONH_4 extraction). Forty pots for each cotton cultivar were randomly placed in each chamber and re-randomized once a week to minimize position effects. Water (about 2 000 mL) was added to each pot once every 2 days.

Both cultivars were planted on May 3, 2004 and the induced defense experiment began after the seven-leaf stage (≈35–40 days after planting). Each plant was used only once and discarded. Pure CO_2 mixed with ambient air was supplied to the chamber from seedling emergence to the end of the induced defense experiment. No chemical fertilizers or insecticides were used through the duration of the experiment. Open tops of the chambers were all covered with netting to exclude other insects.

Aphid stocks

Aphids were collected from nontransgenic cultivar 'Simian-3' fields at the Beiai Science and Technology

Center of Hebei Province and reared in a growth chamber (HPG280H, Orient Electronic Ltd. Co., Haerbin City, China) using fresh leaves of the same cotton cultivar for stock cultures for more than three generations to obtain uniform colonies. Relative humidity was maintained at 60% (day) and 70% (night). Temperature was maintained at 25 ± 1°C (day) / 22 ± 1°C (night) and photoperiod was 14 : 10 L : D at 9 000 μmol/m^2/s of active radiation supplied by twelve 60-W fluorescent lamps in the growth chamber.

Insect feeding treatments

Ten pots per cultivar were randomly selected in each open-top chamber (OTC) and covered with netting (80-mesh nylon gauze) to prevent aphid infestation, which were sampled as the control treatment, that is, 30 control cotton pots for each cultivar × CO_2 treatment. The fourth leaf from the bottom was selected and stored at −20°C to assay foliar chemical composition prior to aphid feeding treatment.

Apterous adult aphids were randomly collected from the stock colony and placed on the undersurface of each leaf above the cotyledon with 20 individuals of each cultivar × CO_2 treatment. Ten pots were randomly selected from the remaining 30 pots per cultivar in each OTC for three treatments of aphid infestation (i.e., feeding period of 24 h, 48 h and 72 h), respectively. At 24 h, 48 h and 72 h after aphid inoculation, the fourth leaf from the bottom was selected to assay foliar chemical composition. Simultaneously, the aphids were also collected for assay of catalase, superoxide dismutase and true choline esterase activity.

Plant chemical compositions assays

Plant leaves from each cultivar in each CO_2 treatment were selected for chemical analysis at the same time that the bioassays were conducted. Leaf materials were kept at –65°C until analysis (Coviella *et al.*, 2002). For the foliar nitrogen concentration, leaves (infested and uninfested) were taken from the plants and dried in an oven at 65°C for 48 h (Coviella *et al.*, 2002). Nitrogen concentration was assayed by using a CNH analyzer (Model ANCA-nt; Europa Elemental Instruments, Okehampton, UK) and total sugar content was tested using the DNS (3,5-dinitrosalicylic acid) method (Suh *et al*., 2002). The Bt toxin protein in the transgenic Bt cotton leaves was analyzed using enzyme-linked immunosorbent assay (ELISA) test (Chen *et al.*, 2005a). For the condensed tannin and gossypol concentration analysis, leaf samples were dried at 38°C for 48 h. Dried leaf samples were ground with liquid nitrogen and quartz sand using a mortar and pestle. Gossypol in the plant leaves was assayed by the method of Wu *et al.* (2007c). The condensed tannin concentration was assayed by the method of Chen *et al.* (2005a).

Insect biochemical assays

Biochemical assays were conducted to test whether there was a biochemically significant change in defensive chemistry in the aphid. Three enzymes, including catalase (CAT), superoxide dismutase (SOD) and true choline esterase (TChE) in the cotton aphid, *A. gossypii* fed on different cotton leaves (GK-12 or Simian-3) grown in ambient CO_2 and double-ambient CO_2 were analyzed according to the reagent label directions (Nanjing Jiancheng Ltd. Co., Nanjing, China).

Data analysis

One-way analyses of variance (ANOVAs) (SAS, 1996) were used to analyze the effect of elevated CO_2 on the foliar chemical compositions of transgenic Bt and non-transgenic Bt cotton. The foliar defensive compounds in cotton leaves damaged by cotton aphid, *A. gossypii*, and the chemical compounds in *A. gossypii* were analyzed using ANOVA with CO_2 and feeding time as sources of variability, where CO_2 level was the main factor and feeding time was a sub-factor deployed in a split-plot design. Difference between means was compared with least significant difference (LSD) test. Test data were transformed prior to LSD test where appropriate, to satisfy assumptions of normally; that is, the absolute value data (i.e., the foliar carbon/nitrogen ratio, gossypol and Bt toxin, and the CAT and SOD) were all log transformed.

Results

Changes in foliar nitrogen and condensed tannin with and without aphid infestation under elevated CO_2

From Table 1, CO_2 level, cotton cultivar and aphid infestation time all significantly affected foliar nitrogen and condensed tannin. However, only the interaction between CO_2 level × infestation time significantly affected foliar condensed tannin concentration.

As shown in Table 2, foliar nitrogen was significantly lower in the leaves of non-transgenic cotton and transgenic Bt cotton after feeding for 0 h, 24 h, 48 h and 72 h by *A. gossypii* under the elevated CO_2 treatment compared with

Table 1 Effects of CO_2, cotton variety, infestation time, CO_2 × cotton variety, infestation time × CO_2, cotton variety × infestation time and CO_2 × cotton variety × infestation time interactions on the foliar chemical compounds of non-transgenic cotton and transgenic Bt cotton after feeding by *A. gossypii* (split-plot ANOVA).

Source of variation	CO_2[†]	Variety[‡]	Time[§]	CO_2 × variety	CO_2 × time	Variety × time	Variety × CO_2 × time
Foliar nitrogen	0.0001***	0.0001***	0.0001***	0.56	0.75	0.79	0.97
Carbon/nitrogen ratio	0.0001***	0.94	0.09	0.39	0.87	0.56	0.88
Condensed tannin	0.0001***	0.0001***	0.0001***	0.91	0.02*	0.77	0.50
Gossypol	0.0017**	0.0001***	0.0001***	0.95	0.31	0.78	1.00
Bt toxin protein	0.0011**		0.0001***		0.88		

[†]CO_2 levels (ambient and double-ambient CO_2).
[‡]Cotton variety (Simian-3 and GK-12).
[§]Infestation time (24 h, 48 h and 72 h).
*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

the ambient CO_2 treatment. Significantly lower ($P < 0.05$) foliar nitrogen was observed in the leaves of transgenic Bt cotton compared with non-transgenic cotton after feeding by *A. gossypii* for 24 h, 48 h and 72 h under two CO_2 levels.

Significantly higher condensed tannin was found in the two cotton leaves after feeding by *A. gossypii* for 24 h, 48 h and 72 h under elevated CO_2 treatment compared with ambient CO_2 treatment. Condensed tannin was significantly decreased in the leaves of transgenic Bt cotton

Table 2 Changes in foliar chemical compounds (mean ± SE) of non-transgenic cotton (cv. Simian-3) and transgenic Bt cotton (cv. GK-12) separately infested by *A. gossypii* in ambient CO_2 and double-ambient CO_2.

Sampling time	Measured indices	Simian-3		GK-12	
		370 μL/L	750 μL/L	370 μL/L	750 μL/L
No infestation	Foliar nitrogen (mg/g)	4.26 ± 0.03 aC	4.03 ± 0.04 bC	4.12 ± 0.04 bC	3.88 ± 0.04 cD
	Carbon/nitrogen ratio	11.7 ± 0.1 bA	13.7 ± 0.1 aA	11.7 ± 0.1 bA	13.6 ± 0.2 aA
	Condensed tannin (%)	0.79 ± 0.01 bcC	0.90 ± 0.02 aC	0.72 ± 0.03 cC	0.82 ± 0.02 bD
	Gossypol (mg/kg)	79.9 ± 1.6 bD	85.6 ± 2.2 abC	84.1 ± 2.2 abD	89.4 ± 1.4 aD
	Bt toxin (ng/g)			445 ± 6 aC	416 ± 4 bC
24 h after infestation	Foliar nitrogen (mg/g)	4.36 ± 0.04 aBC	4.13 ± 0.03 bC	4.20 ± 0.04 bC	3.92 ± 0.02 cC
	Carbon/nitrogen ratio	11.7 ± 0.1 bA	13.7 ± 0.1 aA	11.8 ± 0.1 bA	13.7 ± 0.0 aA
	Condensed tannin (%)	0.83 ± 0.01 cC	1.01 ± 0.01 aB	0.76 ± 0.01 dC	0.91 ± 0.02 bC
	Gossypol (mg/kg)	87.6 ± 1.6 bC	94.9 ± 0.7 aC	90.8 ± 1.3 bC	97.5 ± 0.7 aC
	Bt toxin (ng/g)			448 ± 4 aBC	419 ± 3 bBC
48 h after infestation	Foliar nitrogen (mg/g)	4.48 ± 0.04 aB	4.27 ± 0.03 bB	4.29 ± 0.05 bB	4.08 ± 0.04 cB
	Carbon/nitrogen ratio	11.7 ± 0.1 bA	13.7 ± 0.0 aA	11.7 ± 0.0 bA	13.7 ± 0.0 aA
	Condensed tannin (%)	0.96 ± 0.01 bB	1.04 ± 0.01 aB	0.86 ± 0.02 cB	0.98 ± 0.01 bB
	Gossypol (mg/kg)	94.9 ± 1.3 bB	103 ± 1 aB	96.6 ± 1.4 bB	105 ± 2 aB
	Bt toxin (ng/g)			456 ± 2 aAB	426 ± 2 bAB
72 h after infestation	Foliar nitrogen (mg/g)	4.62 ± 0.02 aA	4.43 ± 0.03 bA	4.45 ± 0.03 bA	4.23 ± 0.03 cA
	Carbon/nitrogen ratio	11.8 ± 0.1 bA	13.8 ± 0.1 aA	11.8 ± 0.0 bA	13.8 ± 0.0 aA
	Condensed tannin (%)	1.02 ± 0.01 cA	1.14 ± 0.01 aA	0.95 ± 0.01 dA	1.08 ± 0.02 bA
	Gossypol (mg/kg)	99.9 ± 1.6 bA	109 ± 2 aA	102 ± 2 bA	112 ± 1 aA
	Bt toxin (ng/g)			461 ± 5 aA	436 ± 2 bA

Means within a row indicated by different lowercase letters are significantly different; means of each measured parameter across three sampling times within a column indicated by different uppercase letters are significantly different (LSD test, $P < 0.05$).

compared with non-transgenic cotton after feeding by *A. gossypii* for 24 h, 48 h and 72 h under the ambient CO_2 treatment and also for 0 h, 24 h, 48 h and 72 h under elevated CO_2 treatment. Significant increase occurred in the condensed tannin of two cotton leaves after feeding for 72 h compared to that observed after 0 h, 24 h and 48 h of infestation by *A. gossypii* under two CO_2 levels (Table 2).

Changes in foliar gossypol and Bt toxin with and without aphid infestation under elevated CO_2

CO_2 level and aphid infestation time all significantly affected foliar gossypol and Bt toxin ($P < 0.01$). Cotton variety also significantly affected foliar gossypol (Table 1).

Significantly higher gossypol was also found in the leaves of non-transgenic and transgenic Bt cotton after feeding by *A. gossypii* for 24 h, 48 h and 72 h under elevated CO_2 treatment compared with ambient CO_2 treatment (Table 2). Significantly increased gossypol was also observed in the cotton leaves after feeding for 72 h, more so than those for 0 h, 24 h and 48 h by *A. gossypii* in the two cotton leaves under two CO_2 levels. Significantly lower Bt toxin was found in the leaves of transgenic Bt cotton after feeding by *A. gossypii* for 0 h, 24 h, 48 h and 72 h under elevated CO_2 treatment compared with ambient CO_2 treatment (Table 2). Bt toxin increased significantly in the leaves of transgenic Bt cotton after feeding for 72 h than those for 0 h, 24 h and 48 h by *A. gossypii* under elevated CO_2 treatment (Table 2).

Enzyme changes in A. gossypii *fed on non-transgenic cotton and transgenic Bt cotton grown in elevated CO_2*

The CO_2 level, cotton variety and feeding time significantly affected the concentration of CAT, SOD and TChE in the body of *A. gossypii* ($P < 0.001$). The interaction between CO_2 level × cotton variety significantly affected the TChE concentration in *A. gossypii* (Table 3).

Significantly higher CAT was found in *A. gossypii* after feeding on the non-transgenic and transgenic Bt cotton leaves for 48 h under elevated CO_2 treatment compared with ambient CO_2 treatment. Significantly higher CAT was found in the body of *A. gossypii* after feeding on the transgenic Bt cotton leaves for 48 h than in those for 24 h and 72 h under the ambient and elevated CO_2 treatments (Table 4).

Significantly higher SOD was found in the body of *A. gossypii* after feeding on the transgenic Bt cotton leaves for 24 h, 48 h and 72 h under elevated CO_2 treatment compared with ambient CO_2 treatment. Significantly higher SOD was also observed in the body of *A. gossypii* after feeding on the transgenic Bt cotton leaves for 48 h than in those for 24 h and 72 h under two CO_2 levels (Table 4).

TChE significantly decreased in the body of *A. gossypii* after feeding on the non-transgenic cotton leaves for 24 h, 48 h and 72 h and also in transgenic Bt cotton leaves for 72 h under elevated CO_2 treatment compared with ambient CO_2 treatment. Significantly higher TChE was found in the body of *A. gossypii* after feeding on transgenic Bt cotton leaves for 48 h than in those for 24 h and 72 h under two CO_2 levels (Table 4).

Discussion

In the present study, there was significant increase in carbon-based secondary metabolites (condensed tannins in this study) while a significant decrease in nitrogen-based compounds (here, Bt toxin protein and foliar nitrogen) in the leaves of transgenic Bt cotton without aphid infestation under elevated CO_2 treatment compared with ambient CO_2 treatment. These studies suggest an overflow mechanism for carbon allocation when nitrogen is limited and carbon-based secondary metabolites will be fixed in excess of growth demands when plants are grown under elevated CO_2. The cotton plant can show a shift in allocation from nitrogen-based compounds to carbon-based defensive compounds, especially with regard to atmospheric CO_2 concentration. We suggest that transgenic Bt cotton is able to shift allocation between N-based and C-based defensive compounds owing to the changes in relative availability of carbon and nitrogen inputs in elevated CO_2.

Bacillus thuringiensis has been available for insect control since the mid 1930s (Tabashnik, 1997). Several studies have been conducted to determine the influence of transgenic Bt cotton on target and non-target arthropods (Fitt, 1994; Chen *et al.*, 2005c). The cotton aphid, *A. gossypii*, is an important pest of cotton. Although transgenic Bt cotton plants have been grown over a wide geographical range in north China to manage herbivorous insect pests, little is known of their effects on non-target herbivorous insects (e.g., *A. gossypii*). Toxins from *B. thuringiensis* (Bt) are a critical component in many current pest management programs (Koppenhofer & Kaya, 1997; Coviella & Trumble, 1999). In our study, *B. thuringiensis* concentrations in the transgenic Bt cotton leaves significantly increased in cotton grown under elevated CO_2 compared with ambient CO_2 or in cotton damaged by *A. gossypii*.

From the present experiment, the interaction between CO_2 level × infestation time resulted in a significant

Table 3 Effects of CO_2, cotton variety, infestation time, CO_2 × cotton variety, infestation time × CO_2, cotton variety × infestation time and CO_2 × cotton variety × infestation time interactions on the enzymes of *A. gossypii* after feeding on non-transgenic and transgenic Bt cotton.

Source of variation	CO_2[†]	Variety[‡]	Time[§]	CO_2 × variety	CO_2 × time	Variety × time	Variety × CO_2 × time
CAT	0.0001***	0.0001***	0.0001***	0.47	0.88	0.21	0.96
SOD	0.0001***	0.0001***	0.0001***	0.41	0.91	0.52	0.97
TChE	0.0001***	0.0001***	0.0001***	0.0002***	0.39	0.80	0.23

[†]CO_2 levels (ambient and double-ambient CO_2).

[‡]cotton variety (Simian-3 and GK-12).

[§]Time (24 h, 48 h and 72 h). CAT, catalase; SOD, superoxide dismutase; TChE, true choline esterase.

***$P < 0.001$.

increase in the plants' condensed tannin concentrations. Apparently, the mechanisms whereby non-transgenic and transgenic Bt cotton leaves produce condensed tannin contents in response to aphid infestation are similar in the ambient CO_2 and elevated CO_2 treatments.

Most herbivores are affected by the physiological and nutritional states of their host plants (Coviella & Trumble, 1999). Thus, changes in chemical components of host plants can be expected to affect the changes in activity of some enzymes of these herbivores. In our study, significantly higher activity of SOD and significantly lower activity of TChE contents were observed in *A. gossypii* after feeding on the non-transgenic cotton under elevated CO_2 treatment compared with ambient CO_2 treatment. These results show that *A. gossypii* appears to be negatively affected by elevated CO_2 because of the reduction in TChE caused by elevated CO_2. Furthermore, the CO_2 level, cotton variety and infestation time can significantly affect the active enzymes (CAT, SOD and TChE) contents in *A. gossypii*. However, only the interaction between CO_2 level × cotton variety had a significant effect on the TChE content in *A. gossypii*, which means the influence of the transgenic gene in plant leaves far outweighed the influence of infestation time by *A. gossypii* to affect the TChE level in *A. gossypii* under elevated CO_2 conditions.

This study exemplifies the complexities of predicting plant allocation defensive compounds and herbivorous insects in response to future climatic conditions. Similarly, the effects of CO_2 treatment and transgenic Bt cotton also affected the enzyme composition in *A. gossypii.* Atmospheric conditions do affect the allocation of plant defensive compounds and this modifies the enzyme activities in *A. gossypii* in response to transgenic Bt cotton. If the plant defensive compound allocation patterns observed in this study prove broadly applicable across a range of plant and

Table 4 Changes (mean ± SE) in catalase (CAT), superoxide dismutase (SOD) and true choline esterase (TChE) of *A. gossypii* after feeding on host plants (Simian-3 or GK-12) for different times in ambient CO_2 and double-ambient CO_2.

Sampling time	Measured indices	Simian-3		GK-12	
		370 μL/L	750 μL/L	370 μL/L	750 μL/L
24 h after infestation	CAT U/mg protein	124 ± 3 bAB	134 ± 5 abAB	131 ± 2 abB	139 ± 3 aB
	SOD U/mg protein	96.1 ± 4.7 cA	105 ± 1 bcAB	114 ± 3 bAB	126 ± 3 aAB
	TChE U/mg protein	0.38 ± 0.01 aA	0.31 ± 0.01 bA	0.30 ± 0.01 bcA	0.28 ± 0.00 cB
48 h after infestation	CAT U/mg protein	131 ± 3 cA	143 ± 2 bA	144 ± 1 bA	152 ± 3 aA
	SOD U/mg protein	101 ± 2 dA	111 ± 2 cA	118 ± 2 bA	132 ± 2 aA
	TChE U/mg protein	0.40 ± 0.00 aA	0.34 ± 0.01 bA	0.32 ± 0.01 bcA	0.30 ± 0.00 cA
72 h after infestation	CAT U/mg protein	121 ± 3 cB	132 ± 3 bB	134 ± 5 bB	144 ± 5 aAB
	SOD U/mg protein	92 ± 2 cA	103 ± 3 bB	107 ± 2 bB	120 ± 1 aB
	TChE U/mg protein	0.35 ± 0.01 aB	0.31 ± 0.01 bA	0.27 ± 0.01 cB	0.25 ± 0.00 dC

Means within a row indicated by different lowercase letters are significantly different; means of each measured parameter across three sampling times within a column indicated by different uppercase letters are significantly different (LSD test, $P < 0.05$).

herbivorous insects, changes in plant–insect interactions due to elevated CO_2 are likely to be substantial.

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