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Impacts of elevated CO_2 on expression of plant defensive compounds in Bt-transgenic cotton in response to infestation by cotton bollworm

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Abstract

1 The allocation of defensive compounds of transgenic Bt (cv. GK-12) and nontransgenic cotton (cv. Simian-3) grown in elevated CO_2 in response to infestation by cotton bollworm *Helicoverpa armigera* (Hübner) was studied in closed-dynamics CO_2 chambers.

2 A significant reduction in foliar nitrogen content and Bt toxin protein occurred when transgenic Bt cotton grew under elevated CO_2. A significantly higher carbon/nitrogen ratio as well as condensed tannin and gossypol contents was observed for transgenic Bt (cv. GK-12) and nontransgenic cotton in elevated CO_2, in partial support of the carbon nutrient balance hypothesis as a result of limiting nitrogen and excess carbon in cotton plants in response to elevated CO_2.

3 The CO_2 level and infestation time significantly affected the foliar nitrogen, condensed tannin, gossypol and Bt toxin protein contents of cotton plants after feeding by *H. armigera.* The interaction between CO_2 levels × cotton variety had a significant effect on foliar nitrogen content after injury by *H. armigera.*

Keywords Closed-dynamics CO_2 chamber, condensed tannin, cotton bollworm, defensive compounds, elevated CO_2, *Helicoverpa armigera* (Hübner), transgenic Bt cotton.

Introduction

Climate change is defined as 'any change in climate over time, whether as a result of natural variability or as a result of human activity' (Intergovernmental Panel on Climate Change, IPCC, 2007). The global atmospheric carbon dioxide (CO_2) concentration is expected to increase from approximately 350–650 μL/L by the year 2080 (Houghton *et al*., 2001), and is anticipated to reach 700 μL/L within the 21st Century (Goverde *et al*., 1999; Johns & Hughes, 2002).

In general, elevated CO_2 can lead to a significant increase in plant photosynthesis, growth, aboveground biomass, leaf area, yield and carbon : nitrogen (C : N) ratios, particularly in C_3 plants (Pritchard *et al*., 1999; Chen *et al*., 2005a; Schadler *et al*., 2007; Wu *et al*., 2007). All these changes in chemical components of plants arising from elevated CO_2, which, in turn, affect the production of secondary metabolites (Bryant *et al*., 1983; Stiling & Cornelissen, 2007; Kretzschmar *et al*., 2009). The carbon nutrient balance (CNB) hypothesis (Bryant *et al*., 1983) predicts that the pattern of plant allocation to defensive compounds depends on the relative availability of carbon and nutrients as well as their relationship with the plant growth rate (Lindroth *et al*., 1993; Coviella *et al*., 2002). Coviella *et al*. (2002) and Chen *et al*. (2005b) reported that elevated CO_2 can result in a significant increase in carbon-based secondary compounds (e.g. starch, condensed tannins, gossypol) and a reduction in nitrogen-based compounds (i.e Bt toxin protein) in transgenic Bt cotton plants, as predicted by the CNB hypothesis. The effects of elevated CO_2 and nutrient availability on plant defensive chemistry generally support the CNB hypothesis (Koricheva *et al*., 1998; Bazin *et al*., 2002); however, the detection of such environmental effects on defensive compounds is complicated because their concentrations also change with leaf ontogeny (Gleadow *et al*., 1998; Williams *et al*., 1998) and differ among genotypes

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(Briggs, 1990). Ruohomäki *et al*. (1996) reported that the factor with the greatest influence on total phenolics and condensed tannins in *Betula pubescens* ssp. *tortuosa* is environment (fertilization and shade), followed distantly by genotype and injury. In *Betula pendula*, however, the most important factor appears to be genotype. Reichardt *et al*. (1991) even concluded that the CNB hypothesis did not apply to 'dynamic metabolites' (i.e. those metabolites that rapidly degrade, are mobile, or are metabolically labile).

Cotton, a C_3 plant, appears to be particularly responsive to CO_2. Chen *et al*. (2005b) found that elevated CO_2 resulted in a reduction of Bt toxin, which may reduce the ability of transgenic Bt cotton to resist *Helicoverpa armigera* (Hübner). An increase in condensed tannin, gossypol and other compounds may, however, occur in response to an increasing CO_2 atmosphere, especially in combination with injury caused by herbivorous insects (Drury *et al*., 1998; Roth & Lindroth, 1994), and these may compensate for the Bt toxin loss in the transgenic Bt cotton. Thus, the effects of transgenic Bt cotton on cotton bollworm infestations may become more complex under elevated CO_2 conditions as a result of changes that may occur in the allocation pattern of defensive chemicals in transgenic Bt cotton.

Plant defense responses to herbivory are dependent on the plants' exposure time to herbivory as well as the physical environment affecting plant–insect associations (Braga *et al*., 2006). To date, however, little emphasis has been placed on how the environment affects the inducibility of plant chemical defenses. Bidart-Bouzat *et al*. (2005) were the first to report that herbivore induction of plant secondary chemicals (glucosinolates) can be affected by changes in climatic factors such as atmospheric CO_2 concentrations. Himanen *et al*. (2008) also found that inducibility of secondary metabolites can be altered by elevated CO_2. These previous studies show that variation in plant chemical induction can have significant ecological and evolutionary implications for plants and their interactions with insect herbivores.

In the present study, the allocation to defensive compounds of transgenic Bt cotton (cv. GK-12) and nontransgenic Bt cotton (cv. Simian-3) in response to injury by cotton bollworm *H. armigera* was studied in closed-dynamics CO_2 chambers (CDCC) with two CO_2 levels (elevated and ambient) aiming: (i) to evaluate the allocation pattern of defensive components (e.g. condensed tannin and Bt toxin protein) in cotton for comparison with the CNB hypothesis and (ii) to quantify the impacts of elevated CO_2 and cotton bollworm injury on allocation patterns of defensive compounds in cotton.

Materials and methods

Closed-dynamics CO_2 chamber

This experiment was performed in photoclimatic chambers (HPG280H; Orient Electronic, China). The chambers were maintained under an LD 14 : 10 h photocycle 28 ± 1 °C and 60–70% relative humidity, and at 9000 lux of active radiation, as supplied by 12 fluorescent lamps (60 W) in each chamber.

Two atmospheric CO_2 concentrations, 370 and 750 μL/L, representing the current ambient level and the predicted level in approximately 100 years (Houghton *et al*., 2001), respectively, were applied. Three chambers were used for each CO_2 treatment. CO_2 concentrations were automatically monitored and adjusted with an infrared CO_2 analyzer (Ventostat 8102; Telaire, Goleta, California). The detailed methodology of the automatic-control system for CO_2 as previously described by Chen and Ge (2004) was followed.

Cotton treatments

Two cotton cultivars were used: a transgenic Bt cultivar 'GK-12' and a nontransgenic cultivar 'Simian-3' from the same recurrent parent line were individually planted in white plastic pots (diameter 13 cm, height 15 cm) filled with 4 : 1 (by volume) loam: earthworm faeces. Cotton plants were exposed to the CO_2 treatments after seedling emergence; 20 pots (one plant/pot) for each cotton cultivar were randomly placed in each CDCC and re-randomized once a week to minimize position effects. Cotton treatments comprised: (i) transgenic Bt cotton grown in ambient CO_2; (ii) transgenic Bt cotton grown in double-ambient CO_2; (iii) nontransgenic cotton in ambient CO_2; and (iv) nontransgenic cotton grown in double-ambient CO_2.

Chemical testing of plants began after the seven-leaf stage (approximately 35–40 days after planting) in each study. Each plant tested was used only once and discarded. No chemical fertilizers or insecticides were applied during the experiment.

Insect stocks

Egg masses of cotton bollworm *H. armigera* were obtained from a laboratory colony maintained by the Insect Physiology Laboratory, Institute of Zoology, Chinese Academy of Sciences, and reared in a growth chamber (HPG280H, Orient Electronic Ltd Co., China) using a standard artificial diet (Wu & Gong,1997) for stock cultures. Relative humidity was maintained at 60% (day) and 70% (night). Temperature was maintained at 28 ± 1 °C (day)/24 ± 1 °C (night) under an LD 14 : 10 h photocycle at 9000 lux of active radiation supplied by 12 fluorescent lamps (60 W) in the growth chamber.

Insect feeding treatments

The induced defensive chemistry experiment was begun in the CDCC. Temperature was maintained at a constant 28 ± 1 °C under an LD 14 : 10 h photocycle at 9000 lux of active radiation supplied by 12 fluorescent lamps (60 W) in each CDCC.

Five pots per cultivar were randomly selected in each CDCC and covered with netting to prevent herbivory; these served as the control treatment to provide 15 control cotton pots for each cultivar × CO_2 treatment. Undamaged leaves were selected and stored at −20 °C to assay foliar chemical composition for comparison with the plants exposed to the bollworm larval treatment.

Third-instar larvae were randomly collected from the stock colony and placed on the fourth leaf from the bottom with one

Table 1 Effects of CO_2 levels, cotton variety and the interactions between CO_2 levels × cotton variety on the foliar chemical compounds of nontransgenic cotton and transgenic Bt cotton without injury by cotton bollworm *Helicoverpa armigera* using analysis of variance

Source of variation	CO_2 level	Cotton variety	CO_2 level × cotton variety
Foliar nitrogen	$F = 103.49$; $P = 0.0001$	$F = 33.97$; $P = 0.001$	$F = 0.00$; $P = 1.00$
Carbon/nitrogen ratio	$F = 220.33$; $P = 0.0001$	$F = 0.00$; $P = 1.00$	$F = 0.12$; $P = 0.74$
Condensed tannin	$F = 22.14$; $P = 0.001$	$F = 17.49$; $P = 0.01$	$F = 0.47$; $P = 0.51$
Gossypol	$F = 79.49$; $P = 0.001$	$F = 44.96$; $P = 0.01$	$F = 0.10$; $P = 0.76$
Bt toxin protein	$F = 11.96$; $P = 0.026$	—	—

d.f. = 1.

larva per plant/pot; each plant was then covered with netting for each cultivar × CO_2 treatment. Five pots were randomly selected from the remaining 15 pots per cultivar in each CDCC for three treatments of *H. armigera* injury (i.e. feeding period of 1, 3 and 12 h, respectively). After *H. armigera* inoculation for 1, 3 and 12 h, the injured leaves were selected to assay for their foliar chemical composition.

Plant chemical composition assays

The cotton leaves with and without bollworm injury were collected separately from each cultivar × CO_2 treatment and placed in a super-cooling refrigerator at −65 °C. The sampled leaves were then lyophilized in a freeze-drier (Model: 7752030; Labconco Corp. Fort Scott, Kansas) at −60 °C and 0.20 mPa for 60 h, and then ground with liquid nitrogen using a mortar and pestle before analysis for foliar nitrogen and Bt toxin. Foliar nitrogen content was assayed using a Europa CHN analyzer (Model ANCA-nt; Europa Elemental Instruments, U.K.). The foliar Bt toxin of GK-12 was measured using an enzyme-linked immunosorbent assay (Chen *et al*., 2005b). For the condensed tannin and gossypol content analysis, leaf samples were dried at 38 °C for 72 h., and then ground with liquid nitrogen and quartz sand using a mortar and pestle; condensed tannin was measured in accordance with the method of Chen *et al*. (2005b), and gossypol content in accordance with the method of Xu and Zhang (2005).

Statistical analysis

All data were analyzed using split-plot analysis of variance (ANOVA) using SAS, version 6.12 (SAS Institute Inc., 1996). First, three-way ANOVAs were used to analyze the effects of CO_2 level (ambient versus elevated), cotton cultivar (transgenic Bt cotton GK-12 versus nontransgenic cotton Simian-3), infestation time (1, 3 and 12 h after inoculation) and their interactions on the foliar chemical composition (except Bt toxin) of cotton (GK-12 and Simian-3). Second, two-way ANOVA was used to analyze the impact of CO_2 level and cotton cultivar and their interaction on the foliar chemical composition (except Bt toxin) of cotton plants (GK-12 and Simian-3) protected from injury by cotton bollworm. Furthermore, two-way ANOVA was also used to analyze the impact of CO_2 level and infestation time and their interaction on the foliar Bt toxin content of transgenic Bt cotton (GK-12) injured by cotton bollworm. Differences between means were determined using a least significant difference test at $P < 0.05$.

Results

Effects of elevated CO_2 on foliar chemical compounds of nontransgenic and transgenic Bt cotton without H. armigera *injury*

CO_2 levels significantly affected all foliar chemical compounds (Table 1). Cotton cultivar significantly affected foliar nitrogen, condensed tannin and gossypol contents (Table 1).

A significantly lower foliar nitrogen content as well as a significantly higher carbon/nitrogen ratio and condensed tannin and gossypol contents was observed in both cotton cultivars under elevated CO_2 compared with ambient CO_2 (Table 2). Foliar Bt toxin was significantly lower in the leaves of transgenic Bt cotton under elevated CO_2 compared with ambient CO_2. Significantly lower foliar condensed tannin was observed in leaves of transgenic Bt cotton than nontransgenic cotton under elevated CO_2. Foliar gossypol content of transgenic Bt cotton was, however, significantly greater than that of nontransgenic cotton.

Effects of elevated CO_2 on foliar chemical compounds of nontransgenic and transgenic Bt cotton with bollworm injury

CO_2 levels significantly affected foliar nitrogen, the carbon/nitrogen ratio, condensed tannin, gossypol and Bt toxin

Table 2 Changes in foliar chemical compounds (mean ± SE) of nontransgenic cotton (cv. Simian-3) and transgenic Bt cotton (cv. GK-12) without injury by cotton bollworm *Helicoverpa armigera* under ambient CO_2 and double-ambient CO_2

Cotton variety	Measured indices	CO_2 levels	
		370 μL/L	750 μL/L
Simian-3	Foliar nitrogen (%)	$4.23 \pm 0.03^{a,A}$	$3.91 \pm 0.04^{b,A}$
	Carbon/nitrogen ratio	$11.6 \pm 0.1^{b,A}$	$13.6 \pm 0.1^{a,A}$
	Condensed tannin (%)	$0.91 \pm 0.03^{b,A}$	$1.04 \pm 0.02^{a,A}$
	Gossypol (mg/kg)	$81.3 \pm 0.7^{b,B}$	$89.2 \pm 0.9^{a,B}$
GK - 12	Foliar nitrogen (%)	$4.08 \pm 0.05^{a,A}$	$3.72 \pm 0.02^{b,B}$
	Carbon/nitrogen ratio	$11.7 \pm 0.2^{b,A}$	$13.5 \pm 0.1^{a,A}$
	Condensed tannin (%)	$0.83 \pm 0.03^{b,A}$	$0.93 \pm 0.01^{a,B}$
	Gossypol (mg/kg)	$87.2 \pm 1.3^{b,A}$	$95.7 \pm 0.6^{a,A}$
	Bt toxin (ng/g)	488 ± 5^{a}	469 ± 3^{b}

Means within a row indicated by different superscript lowercase letters are significantly different; means of each measured parameter across different cotton variety within a column indicated by different superscript uppercase letters are significantly different (least significant difference test, $P < 0.05$, d.f = 1, 4).

Table 3 Three-way analysis of variance of the effects of CO_2 level, cotton variety, infestation time, and their interaction among CO_2 concentration, cotton variety and infestation time on foliar chemical compounds with *Helicoverpa armigera* injury

Source of variation	$F(P)^a_{CO_2}$	$F(P)^b_{variety}$	$F(P)^c_{time}$	$F(P)_{CO_2 \times variety}$	$F(P)_{CO_2 \times time}$	$F(P)_{variety \times time}$	$F(P)_{variety \times CO_2 \times time}$
Foliar nitrogen	209.8 (0.0001)	137.2 (0.0001)	82.7 (0.0001)	17.95 (0.0003)	0.13 (0.88)	0.10 (0.91)	0.10 (0.91)
Carbon/nitrogen ratio	1526 (0.0001)	1.63 (0.21)	5.73 (0.0093)	1.47 (0.237)	0.68 (0.514)	0.22 (0.8022)	0.57 (0.5738)
Condensed tannin	61.55 (0.0001)	19.53 (0.0002)	116 (0.0001)	0.00 (0.9730)	0.40 (0.673)	0.14 (0.8724)	0.04 (0.9567)
Gossypol	65.13 (0.0001)	38.87 (0.0001)	214 (0.0001)	0.02 (0.8699)	0.86 (0.4374)	0.02 (0.9846)	0.21 (0.8117)
Bt toxin protein	41.25 (0.0001)	—	64.3 (0.0001)	—	0.95 (0.4151)	—	—

[a]CO_2 levels (ambient and double-ambient CO_2).
[b]Cotton variety (Simian-3 and GK-12).
[c]Time (1, 3 and 12 h).

protein (Table 3). Cotton variety significantly affected foliar nitrogen, condensed tannin and gossypol. The infestation time also significantly affected foliar nitrogen, carbon/nitrogen ratio, condensed tannin, gossypol and Bt toxin protein. The interaction between CO_2 levels × cotton variety significantly affected foliar nitrogen.

Significantly lower foliar nitrogen content was found in nontransgenic cotton after bollworm feeding for 1, 3 and 12 h under elevated CO_2 compared with ambient CO_2 (Table 4). Foliar carbon/nitrogen ratio was significantly decreased in transgenic Bt cotton after bollworm feeding for 1, 3 and 12 h under elevated CO_2 compared with ambient CO_2. Significantly higher foliar condensed tannin was found in both cotton cultivars after *H. armigera* feeding for 1, 3 and 12 h under elevated CO_2 compared with ambient CO_2. Significantly higher foliar condensed tannin was found after bollworm injury for 12 h compared with foliage exposed for 1 and 3 h in nontransgenic cotton under two CO_2 levels, and in transgenic Bt cotton under ambient CO_2 and elevated CO_2. Foliar gossypol significantly increased in nontransgenic cotton after *H. armigera* feeding for 1, 3 and 12 h under elevated CO_2 compared with ambient CO_2. Significantly higher foliar gossypol was found after bollworm injury for 12 h compared with foliage exposed for 1 and 3 h in nontransgenic and transgenic Bt cotton under two CO_2 levels. Significantly lower foliar Bt toxin was found in transgenic Bt cotton after *H. armigera* feeding for 3 and 12 h under elevated CO_2 compared with ambient CO_2. Foliar Bt toxin significantly increased in transgenic Bt cotton after *H. armigera* injury for 12 h compared with foliage exposed for 1 and 3 h under ambient CO_2 and elevated CO_2.

Discussion

Atmospheric CO_2 is the basic source of carbon for plants (Vurro *et al*., 2009), and an excess of carbon at certain nutrient level leads to changes in the production of defensive secondary chemicals in plants (Hartley *et al*., 2000; Braga *et al*., 2006). The CNB hypothesis predicts that plants grown under elevated CO_2 will have more carbon-based defensive compounds than those grown under ambient CO_2 conditions (Bazin *et al*., 2002). When nitrogen limits plant growth, the CNB hypothesis predicts that carbohydrates will accumulate

Table 4 Changes in foliar chemical compounds (mean ± SE) of nontransgenic cotton (cv. Simian-3) and transgenic Bt cotton (cv. GK-12) separately ingested by cotton bollworm *Helicoverpa armigera* for different periods under ambient CO_2 and elevated CO_2

		Simian-3		GK-12	
Sampling time	Measured indices	370 μL/L	750 μL/L	370 μL/L	750 μL/L
1 h after ingestion	Foliar nitrogen (mg/g)	$4.38 \pm 0.03^{a,B}$	$4.15 \pm 0.02^{b,C}$	$4.21 \pm 0.01^{b,C}$	$3.83 \pm 0.03^{c,B}$
	Carbon/nitrogen ratio	$11.7 \pm 0.1^{b,A}$	$13.6 \pm 0.1^{a,A}$	$11.7 \pm 0.1^{b,A}$	$13.7 \pm 0.1^{a,A}$
	Condensed tannin (%)	$1.03 \pm 0.03^{bc,C}$	$1.17 \pm 0.03^{a,C}$	$0.96 \pm 0.05^{c,C}$	$1.11 \pm 0.02^{ab,C}$
	Gossypol (mg/kg)	$92.6 \pm 1.1^{c,C}$	$98.7 \pm 1.5^{b,C}$	$98.1 \pm 1.9^{b,C}$	$105 \pm 2^{a,C}$
	Bt toxin (ng/g)	—	—	$514 \pm 3^{a,C}$	$498 \pm 5^{a,C}$
3 h after ingestion	Foliar nitrogen (mg/g)	$4.47 \pm 0.03^{a,B}$	$4.28 \pm 0.04^{b,B}$	$4.33 \pm 0.02^{b,B}$	$3.95 \pm 0.07^{c,B}$
	Carbon/nitrogen ratio	$11.8 \pm 0.1^{b,A}$	$13.7 \pm 0.1^{a,A}$	$11.7 \pm 0.1^{b,A}$	$13.9 \pm 0.1^{a,A}$
	Condensed tannin (%)	$1.18 \pm 0.03^{b,B}$	$1.29 \pm 0.02^{a,B}$	$1.11 \pm 0.01^{c,B}$	$1.21 \pm 0.03^{b,B}$
	Gossypol (mg/kg)	$101 \pm 1^{c,B}$	$111 \pm 1^{b,B}$	$108 \pm 1^{b,B}$	$116 \pm 2^{a,B}$
	Bt toxin (ng/g)	—	—	$534 \pm 4^{a,B}$	$516 \pm 3^{b,B}$
12 h after ingestion	Foliar nitrogen (mg/g)	$4.66 \pm 0.03^{a,A}$	$4.46 \pm 0.05^{b,A}$	$4.52 \pm 0.02^{b,A}$	$4.16 \pm 0.04^{c,A}$
	Carbon/nitrogen ratio	$11.9 \pm 0.1^{b,A}$	$13.8 \pm 0.1^{a,A}$	$11.9 \pm 0.1^{b,A}$	$14.0 \pm 0.1^{a,A}$
	Condensed tannin (%)	$1.35 \pm 0.02^{bc,A}$	$1.47 \pm 0.03^{a,A}$	$1.27 \pm 0.03^{c,A}$	$1.39 \pm 0.02^{ab,A}$
	Gossypol (mg/kg)	$117 \pm 2^{c,A}$	$124 \pm 1^{ab,A}$	$123 \pm 1^{bc,A}$	$130 \pm 3^{a,A}$
	Bt toxin (ng/g)	—	—	$563 \pm 3^{a,A}$	$537 \pm 4^{b,A}$

Means within a row indicated by different superscript lowercase letters are significantly different (least significant difference test, $P < 0.05$, d.f = 3, 8); means of each measured parameter across three sampling times within a column indicated by different superscript uppercase letters are significantly different (least significant difference test, $P < 0.05$, d.f = 2, 6).

in plant tissues (Hamilton *et al*., 2001). Lindroth *et al*. (1995) reported that condensed tannin concentrations in paper birch (*Betula papyrifera*) increased two-fold when trees were grown in elevated CO_2. Similar increases in phenols and tannins have been found in studies conducted under elevated CO_2 (Lincoln *et al*., 1993; Lindroth, 1996). Other studies suggest that further work is needed to clarify the CNB hypothesis in plants (Lerdau & Coley, 2002; Nitao *et al*., 2002) and Hamilton *et al*. (2001) concluded the CNB hypothesis did not apply as a general explanatory theory. Coviella *et al*. (2002) observed a strong CO_2 effect on the nitrogen content of cotton plants. The present studies showed a significant increase in carbon-based secondary metabolites (condensed tannins) and a significant decrease in nitrogen-based compounds (foliar nitrogen and Bt toxin protein) in the leaves of nontransgenic and transgenic Bt cotton without cotton bollworm injury under elevated CO_2 treatment compared with ambient CO_2 treatment. Furthermore, the results obtained in the present study showed an increase in the C : N ratio under elevated CO_2. The C : N ratio results were similar to those reported previously by Coviella *et al*. (2002).

The secondary metabolites present in plants provide protection against invaders because of their antimicrobial activity (Kamra *et al*., 2006). Elevated CO_2 leads to plants allocating more carbohydrate resources to their secondary metabolism (Agrell *et al*., 2004; Casteel *et al*., 2008), which may generate higher concentrations of defensive compounds that are toxic against herbivorous insects (Coviella & Trumble, 1999). Goverde *et al*. (2004) found that the condensed tannin concentrations of the legume *Lotus corniculatus* increased by 10.4% when injured by larvae of *Polyommatus icarus*. In the present study, foliar condensed tannin and gossypol significantly increased in nontransgenic cotton and transgenic Bt cotton exposed to *H. armigera* for 12 h compared with those exposed for 1 and 3 h under elevated CO_2. Foliar condensed tannin and gossypol content increased by 12.2% and 10.6% in nontransgenic cotton and 16.2% and 10.2% in transgenic Bt cotton after exposure to larvae for 1 h compared with foliage not infested by *H. armigera* under elevated CO_2. Foliar condensed tannin and gossypol contents increased by 24.4% and 24.5% in nontransgenic cotton and by 30.0% and 21.7% in transgenic Bt cotton after feeding by *H. armigera* for 3 h under elevated CO_2. However, foliar Bt toxin content in transgenic Bt cotton decreased by 3–5% with or without bollworm injury treatment under elevated CO_2 compared with ambient CO_2, demonstrating that CO_2 concentration had a greater effect than bollworm injury on foliar Bt toxin content in transgenic Bt cotton. Although CO_2 level, cotton variety and infestation time significantly affected the concentrations of most foliar chemical compounds measured, the condensed tannin content of cotton plants showed a limited response to treatment: only the interaction between CO_2 level × cotton variety show a significant effect on tannin concentration.

In conclusion, the present study demonstrated that CO_2 levels and infestation time significantly affected foliar condensed tannin, gossypol and Bt toxin. Significantly higher foliar condensed tannin was observed in both cotton cultivars after *H. armigera* feeding for 1, 3 and 12 h under elevated CO_2 compared with ambient CO_2. Significantly lower foliar Bt toxin was, however, found in transgenic Bt cotton after *H. armigera* feeding for 3 and 12 h under elevated CO_2 compared with ambient CO_2. The findings of the present study show that the patterns of plant allocation to carbon-based secondary metabolites (condensed tannins) and nitrogen-based compounds (foliar nitrogen and Bt toxin protein), partially supported the CNB hypothesis as demonstrated by the excess carbon in cotton plants in response to elevated CO_2. In other words, elevated CO_2 led to an increased allocation to plant defensive compounds (i.e. condensed tannin and gossypol). The present study also highlighted the complexities involved with respect to predicting plant allocation to defensive compounds and herbivorous insect responses to future climate conditions, particularly in combination with transgenic technologies.

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