Effects of elevated CO_2 and transgenic Bt cotton on plant chemistry, performance, and feeding of an insect herbivore, the cotton bollworm

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Abstract

Effects of elevated atmospheric CO_2 (double-ambient CO_2) on the growth and metabolism of cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), fed on transgenic *Bacillus thuringiensis* (Berliner) (Bt) cotton [*Cry1A(c)*], grown in open-top chambers, were studied. Two levels of CO_2 (ambient and double-ambient) and two cotton cultivars (non-transgenic Simian-3 and transgenic GK-12) were deployed in a completely randomized design with four treatment combinations, and the cotton bollworm was reared on each treatment simultaneously. Plants of both cotton cultivars had lower nitrogen and higher total non-structural carbohydrates (TNC), TNC:Nitrogen ratio, condensed tannin, and gossypol under elevated CO_2. Elevated CO_2 further resulted in a significant decrease in Bt toxin level in GK-12. The changes in chemical components in the host plants due to increased CO_2 significantly affected the growth parameters of *H. armigera*. Both transgenic Bt cotton and elevated CO_2 resulted in a reduced body mass, lower fecundity, decreased relative growth rate (RGR), and decreased mean relative growth rate in the bollworms. Larval life-span was significantly longer for *H. armigera* fed transgenic Bt cotton. Significantly reduced larval, pupal, and adult moth weights were observed in the bollworms fed elevated CO_2-grown transgenic Bt cotton compared with those of bollworms reared on non-transgenic cotton, regardless of the CO_2 level. The efficiency of conversion of ingested food and of digested food of the bollworm were significantly reduced when fed transgenic Bt cotton, but there was no significant CO_2 or $CO_2 \times$ cotton cultivar interaction. Approximate digestibility of larvae reared on transgenic cotton grown in elevated CO_2 was higher compared to that of larvae fed non-transgenic cotton grown at ambient CO_2. The damage inflicted by cotton bollworm on cotton, regardless of the presence or absence of insecticidal genes, is predicted to be more serious under elevated CO_2 conditions because of individual compensatory feeding on host plants caused by nitrogen deficiency.

Introduction

The level of atmospheric CO_2 concentrations has risen from 280 ppmv to 360 ppmv following the industrial revolution, engendering a critical shift in global biogeochemical cycles. This level of CO_2 is anticipated to double by the end of this century (Watson et al., 1996; Houghton et al., 2001).

The rise in atmospheric CO_2 concentration may have a variety of direct and indirect effects on the relationships between host plants, their herbivores, and the herbivores' natural enemies (Stiling et al., 2002). The direct effects of elevated CO_2 on individual plant species have been well documented (e.g., Curtis & Wang, 1998; Luo et al., 1999). Elevated CO_2 tends to increase photosynthetic rates, growth, yield, and C:N ratios in most plant species, particularly in C_3 plants (e.g., Cure & Aycock, 1986; Bazzaz, 1990). As a result of increased photosynthetic rates and faster growth, C:N ratios in host plants increase, mainly due to the

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*Correspondence: Tel.: 08610 62548093; Fax: 08610 62565689; E-mail: gef@panda.ioz.ac.cn accumulation of non-structural carbohydrates (e.g., Williams et al., 1998). As nitrogen is an important limiting factor for phytophagous insects (Mattson, 1980), a reduction in percentage nitrogen alone may have potent effects on insect performance. Lincoln (1986) and Osbrink et al. (1987) reported that leaf-chewing insect herbivores exhibited compensatory increases in foliar consumption rate or a delay in development when reared on plants grown in elevated CO_2 environments. These effects may be accompanied by decreases in the efficiency of conversion to body mass, growth, and/or survival rates, and in insect population sizes (Masters et al., 1998; Williams et al., 1998).

Cotton, a C_3 plant, appears to be particularly responsive to CO_2. An elevation of CO_2 level from 330 to 660 ppmv led to a 95% yield increase in cotton compared with the average of 31% yield increase in many other plant species (Kimball, 1986). In China, the damage caused by the cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), has been greatly alleviated in recent years, due in part to the adoption of the transgenic Bt cotton cultivar GK-12 (Men et al., 2003), which represents a promising new technology (Forrester et al., 1993). When raised on transgenic Bt cotton, most lepidopteran pests suffered from higher larval mortality, lower development rate, and decreased pupal weight, fecundity, and reproductive rate (Henneberry et al., 2001; Liu et al., 2001; Stewart et al., 2001; Carrie et al., 2003). Despite the potentially significant consequences of higher future CO_2 levels for Bt technology, only limited research has been reported on the effects of elevated CO_2 on transgenic Bt cotton or the effects on bollworms fed on elevated CO_2-grown Bt cotton (Coviella et al., 2002).

In this study, we used the transgenic Bt cotton cultivar GK-12 [expressing *Cry1A(c)* genes from *Bacillus thurigiensis* Berliner var. *kurstaki*] and the non-transgenic cotton cultivar Simian-3 (from the same recurrent parent) to evaluate the influence of non-transgenic and transgenic Bt cotton on the growth, development, consumption, and digestibility of cotton bollworm in ambient and double-ambient CO_2.

Materials and methods

Open-top chambers

The experiment was carried out in two 4.2 m diameter open-top chambers in Sanhe County, Hebei Province, China (35°57′N, 116°47′E). Two levels of atmospheric CO_2 concentration were applied continuously, i.e., the current ambient level and double the ambient level (750 μl/l), representing the predicted level in about 100 years (Watson et al., 1996; Houghton et al., 2001). One open-top chamber was used for each CO_2 treatment. Concentrations were monitored and adjusted with an infrared CO_2 analyzer (Ventostat 8102, Telaire Company, USA) once every 20 min to ensure relatively stable CO_2 concentrations. Actual mean CO_2 concentration 24-h day^{-1} was 376 ± 22 μl/l in the ambient CO_2 chamber and 754 ± 33 μl/l in the double-ambient CO_2 chamber. Air temperature did not vary significantly (paired t-test: $P = 0.98$) between the two CO_2 chambers (26.8 ± 3.8 °C in the double-ambient CO_2 chamber and 26.5 ± 4.0 °C in the ambient CO_2 chamber) throughout the field experiment. Details of the automatic-control system of CO_2 and open-top chambers are provided in Chen et al. (2004) and Chen & Ge (2004).

Cotton cultivars

The two cotton cultivars used in the study included the transgenic Bt cultivar GK-12 and the non-transgenic cultivar Simian-3 from the same recurrent parental line. Both cultivars were planted in white plastic pots (35 cm diameter, 45 cm height) filled with 8 : 3 : 1 (by volume) loam:cow dung:earthworm frass. Thirty pots with two plants per pot for each cultivar were deployed randomly in each CO_2 treatment chamber and the pots were rotated within the chambers once weekly to minimize the effect of microclimate within the chamber. Both cultivars were planted on 18 May, and cotton bolls (not open, diameter > 2 cm) were harvested in October and stored in the refrigerator at 4 °C for the laboratory experiment. No chemical fertilizers or insecticides were used during the experiment. Plants were watered with 2 l tap water which was added to each pot once every 2 days. The open tops of the chambers were all covered with netting to prevent insect movement.

Insect stocks

Egg masses of *H. armigera* were obtained from a laboratory colony of the Insect Physiology Laboratory, Institute of Zoology, Chinese Academy of Sciences (CAS), and reared in a growth chamber (HPG280H, Orient Electronic Ltd Co., Haerbin City, 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) and the photoperiod was L14:D10 at 9000 lux, supplied by twelve 60 W fluorescent lamps in each chamber.

Insect feeding

Treatments. First instar larvae obtained from the insect stocks were reared on the stored immature cotton bolls (collected from the field experiment) from each of the four cultivar × CO_2 treatments in a growth chamber (the control conditions as described above). Treatments included: (1)

transgenic Bt cotton grown in ambient CO_2, (2) transgenic Bt cotton grown in double-ambient CO_2, (3) non-transgenic cotton in ambient CO_2, and (4) non-transgenic cotton grown in double-ambient CO_2. Thirty insects were reared individually in 75 mm diameter glass dishes per treatment, with three replications per treatment (total of 90 insects per treatment).

Rearing. A sample of cotton bolls (refrigerated bolls from the four treatments) were oven-dried at 80 °C for 72 h to calculate the proportion of dry matter and water content of the bolls immediately prior to the beginning of the insect rearing trials. One boll was dissected and offered to each test insect daily. After 24 h of feeding, the remaining portion of the boll and frass produced per larva were collected daily and oven-dried at 80 °C for 72 h. The fresh insect body weights were also measured every other day. Because cotton bollworm larvae tend to eat their sloughs, their ecdysis was not easily observed. Larval life-span was calculated as the time from egg hatch to pupation, pupal life-span as the time from pupation to eclosion, and moth life-span as the time from moth emergence to death. Larval weight was measured at the end of the larval development, before pupation; pupal weight was measured 12 h after pupation, and the moth weight was measured 12 h after eclosion. Fifteen pairs of moths (one male and one female) were randomly selected from each treatment and enclosed in a copulatory cage, with five pairs per cage in three cages. The number of eggs laid per moth was recorded and the eggs were removed every day until the last female in each cage died.

Boll quality measurement

Some immature bolls were randomly selected and oven-dried at 80 °C for 72 h for nutrient quality analysis. The results were used to investigate the results of the growth, development, consumption, and digestion of cotton bollworm fed on the young bolls collected from cotton crops (GK-12 or Simian-3) grown in ambient and double-ambient CO_2. The bolls were assayed for nitrogen and carbon content using a CNH analyzer (Model ANCA-nt, Europa Elemental Instruments, UK). Non-structural carbohydrates (mainly starch and sugar) were analyzed by acid hydrolysis using the method of Tissue & Wright (1995). The condensed tannin contents of immature bolls collected from different CO_2 grown cotton plants was assayed by series High Pressure Liquid Chromatography (HPLC) analyses, following the method outlined by Zhang & Guo (2000): 50 mg powder from immature bolls was dissolved in 5 ml 70% methanol in an Eppendorf tube (5 ml), covered with film and gently vibrated for 20 min at 20 °C, then centrifuged at 25,000 ***g*** for 15 min. The upper liquid was used for the analysis of condensed tannin content in immature bolls by HPLC (HP-1100, ZORBAX SB-C18, 4.6 mm × 250 mm; washing liquid: A, double-distilled methanol; B, KH_2PO_3 buffer at pH 4.5; flow velocity = 260 nm s^{-1}).

Gossypol was measured using the method of Smith (1971). The Bt toxin protein was analyzed using ELISA (Chen et al., 1999). The chemical components of young bolls of non-transgenic and transgenic Bt cotton grown in ambient and double ambient CO_2 are shown in Figure 1.

Estimation of consumption rate and growth and development indices

Growth and development indices. Three indices were used to measure the growth and development of cotton bollworms, including life-span, body weight, and fecundity. The life-span index included larval duration, pupal duration, and adult longevity. The body weight index included larval weight, pupal weight, and moth weight.

Indices for larval consumption and digestibility. The conventional, ratio-based nutritional indices, including mean relative growth rate (MRGR; Viskari et al., 2000), relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and of digested food (ECD), and approximate digestibility (AD) were determined gravimetrically following the methods of Waldbauer (1968) and Scriber & Slansky (1981). The amount of food (mg boll) ingested, frass produced, larval body weight, and weight gain were all calculated as dry weights. Formulas for calculation of the indices measured are shown in Table 1.

Data analysis

All data were analyzed with a general linear model procedure (SAS Institute, 1996). Life history parameters of the cotton bollworm and the cotton boll quality parameters were analyzed using analysis of variance with CO_2 and cultivar as sources of variability, where the CO_2 level was a main factor and cotton variety was a subfactor deployed in a split-plot design. The data for growth indices were analyzed using an analysis of covariance (ANCOVA) with initial weight as a covariate for RCR, RGR, and MRGR, while food consumption was a covariate for ECI and AD to correct for the effect of variation in the growth and food assimilated on intake and growth (Raubenheimer & Simpson, 1992); food assimilated was also used as a covariate to analyze the ECD parameter (Hägele & Martin, 1999). The assumption of a parallel slope between covariate and dependent variable was satisfied for each analysis. Means were separated using the least significant difference (LSD).

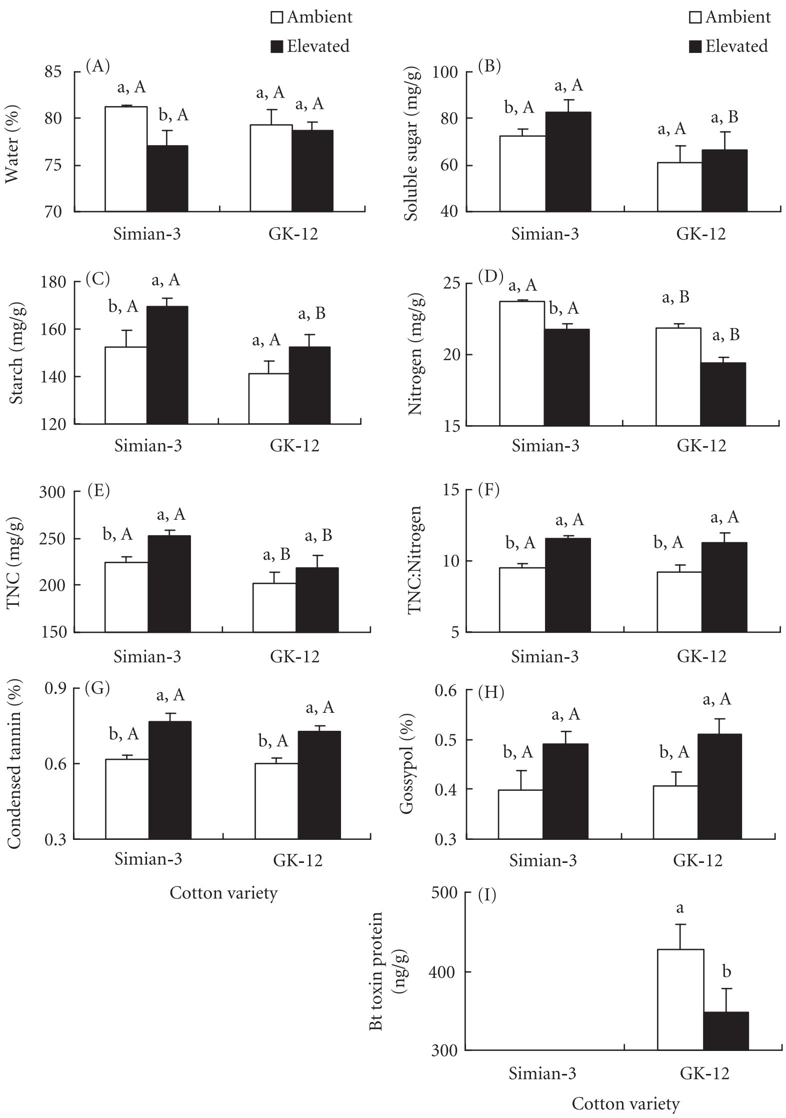

Figure 1 Average (+ SD) chemical components of young bolls (closed, diameter >2 cm) of non-transgenic and transgenic Bt cotton crops grown in ambient and elevated CO_2. Different lower case letters indicate significant differences between CO_2 treatments within cotton variety (LSD test: d.f. = 1,4, $P<0.05$); different upper case letters indicate significant differences between cotton variety within CO_2 level (LSD test: d.f. = 1,4, $P<0.05$).

Results

Quality of immature bolls

Increases in soluble sugar, starch, total non-structural carbohydrates (TNC), TNC:Nitrogen ratio, condensed tannin, and gossypol, and decreases in water content, nitrogen, and Bt toxin protein were observed in the young bolls from cotton plants grown under elevated CO_2 conditions compared with those in ambient CO_2-grown cotton for both transgenic Bt and non-transgenic cottons (Figure 1). For non-transgenic cotton, the increases and decreases in the chemical components of young bolls were all significant ($P<0.05$) for cotton plants grown in elevated CO_2 compared to those in ambient CO_2. For Bt cotton, only the increases in TNC:Nitrogen ratio, condensed tannin and gossypol, and decrease in Bt toxin protein of young bolls were significant ($P<0.05$) when the two CO_2 treatments were compared.

The CO_2 level significantly affected the contents of all measured chemical components of young bolls, but cotton variety only significantly affected nitrogen content, soluble sugar, starch, and TNC of young bolls (Table 2). Moreover, no CO_2 level × cotton variety interaction was observed on the measured physiological characteristics, except for the water content of the young bolls (Table 2).

Growth and development of cotton bollworms

CO_2 and cotton variety both significantly affected larval life-span (Table 3). Cotton variety also significantly influenced the number of eggs laid per moth, larval weight, pupal weight,

Table 1 Calculations for indices for larval consumption and digestibility. Two indices, RGR and MRGR, were used to measure the growth and development of the cotton bollworm, and four indices, RCR, ECI, ECD, and AD, were determined to study the consumption rate and digestibility of this pest insect on two cotton varieties at two CO_2 levels

Calculation formulae	References
Relative Growth Rate (RGR) = increase in mg larval body weight per average g insect body weight per day	Waldbauer (1968); Scriber & Slansky (1981)
Mean Relative Growth Rate (MRGR) = $(\log W_2 - \log W_1)/t$	Viskari et al. (2000)
Relative Consumption Rate (RCR) = mg bolls ingested/average g larval body weight per day	Waldbauer (1968); Scriber & Slansky (1981)
Efficiency of conversion of ingested food (ECI, %) = (mg larval body weight gained/mg bolls ingested) × 100	
Efficiency of conversion of digested food (ECD, %) = [mg larval body weight gained/(mg bolls ingested – mg frass produced)] × 100	
Approximate Digestibility (AD, %) = [(mg bolls ingested – mg frass produced)/mg bolls ingested] × 100	

W_1: The initial larval body weight in g; W_2: the final larval body weight in g; t: days from the initial to the final day.
The mg bolls ingested, mg frass produced, and larval body weight were all dry weights.

and moth weight (Table 3). However, no significant interaction between CO_2 and cotton variety was found (Table 3).

Overall, elevated CO_2 delayed the life-span (Figure 2A, C, E), decreased the body biomass (Figure 2B, D, F), and reduced moth fecundity (Figure 2G). Only larval weight decreased significantly in larvae fed on young bolls of elevated CO_2-grown GK-12 compared to bollworms fed on ambient CO_2-grown GK-12 ($P<0.05$; Figure 2B). On the other hand, significant decreases in larval weight ($P<0.05$; Figure 2B) and fecundity ($P<0.05$; Figure 2G) were observed in bollworms fed on ambient CO_2-grown GK-12 compared to those fed on ambient CO_2-grown Simian-3. At the same time, a significant increase in larval life-span ($P<0.05$; Figure 2A) and significant decreases in larval ($P<0.05$; Figure 2B), pupal ($P<0.05$; Figure 2D), and moth adult weight ($P<0.05$; Figure 2F) were observed when bollworms fed on elevated CO_2-grown GK-12 were compared to bollworms fed on elevated CO_2-grown Simian-3.

Table 2 Effects of CO_2, cotton variety, and CO_2 × cotton variety interaction on physiological characteristics of young cotton bolls (split-plot ANOVA)

Measured indices	CO_2	Variety	CO_2 × variety
Water content	0.0105*	0.9437	0.0399*
Nitrogen content	0.0001***	0.0001***	0. 2003
Soluble sugar	0.0390*	0.0020**	0.4745
Starch	0.0014**	0.0014**	0.3669
TNC	0.0034**	0.0008***	0.2410
TNC:Nitrogen	0.0001***	0.3375	0.9017
Condensed tannin	0.0001***	0.0892	0.5064
Gossypol	0.0006***	0.5057	0.7791
Bt toxin protein	0.0337*		

*$P<0.05$, **$P<0.01$, and ***$P<0.001$; d.f. = 1,4.

Larval consumption and digestibility

RGR and MRGR. Both RGR and MRGR were significantly lower in Bt cotton treatments regardless of CO_2 level ($P<0.05$; Figure 3). The elevated CO_2 did not significantly affect RGR of the bollworm fed GK-12 or Simian-3 ($P>0.05$; Figure 3A), but significantly affected MRGR ($P<0.05$; Figure 3B).

RCR, ECI, ECD, and AD. RCR increased significantly for larvae fed elevated CO_2-grown GK-12 compared to those larvae fed ambient CO_2-grown Simian-3 ($P<0.05$; Figure 4A). Significant decreases were shown in ECI ($P<0.05$; Figure 4B) and ECD ($P<0.05$; Figure 4C) for larvae fed GK-12 grown in ambient and elevated CO_2 compared to those bollworms fed Simian-3 grown in ambient CO_2. A significant decrease in AD ($P<0.05$; Figure 4D) was also observed when the larvae were fed ambient CO_2-grown Simian-3 compared to those fed with elevated CO_2-grown Simian-3, or with GK-12, regardless of the CO_2 level. Cotton variety showed a significant effect on RGR, MRGR, ECI, and ECD, and CO_2 only significantly affected MRGR ($P<0.05$; Table 3).

Discussion

Effects of elevated CO_2 on host plants

Elevated CO_2 caused a significant reduction in water content, nitrogen content, and Bt toxin protein, and a significant increase in soluble sugars, starch, TNC,

Table 3 Effect of CO_2, cotton variety, and $CO_2 \times$ cotton variety interaction on life history parameters (ANOVA) and growth indices (ANCOVA) of the cotton bollworm, *Helicoverpa armigera*

Measured indices		Covariate[a]	CO_2[b]	Variety[c]	$CO_2 \times$ variety
Life-span	Larvae		0.0135*	0.0039**	0.3086
	Pupae		0.2728	0.7176	0.9174
	Adult moths		0.1241	0.0472*	0.6519
Body weight	Larvae		0.2154	0.0035**	0.1522
	Pupae		0.3668	0.0040**	0.3364
	Adult moths		0.7275	0.0076**	0.8840
Number of eggs laid per female			0.0729	0.0097**	0.7831
Relative growth rate (RGR, mg g^{-1} day^{-1})		0.8265	0.5441	0.0091**	0.9038
Mean relative growth rate (MRGR)		0.1349	0.0102*	0.0322*	0.9336
Relative consumption rate (RCR, mg g^{-1} day^{-1})		0.1595	0.1327	0.6164	0.5270
Efficiency of conversion of ingested food (ECI, %)		0.5457	0.2173	0.0070**	0.7951
Efficiency of conversion of digested food (ECD, %)		0.7613	0.4568	0.0090**	0.9220
Approximate Digestibility (AD, %)		0.3382	0.1199	0.0586	0.2375

[a] Initial weight as a covariate for RCR, RGR, and MRGR, food consumption for ECI and AD, and food assimilated for ECD.

[b] CO_2: ambient CO_2 vs. double ambient CO_2.

[c] Cotton type: transgenic Bt cotton vs. non-transgenic cotton. *P<0.05; **P<0.01.

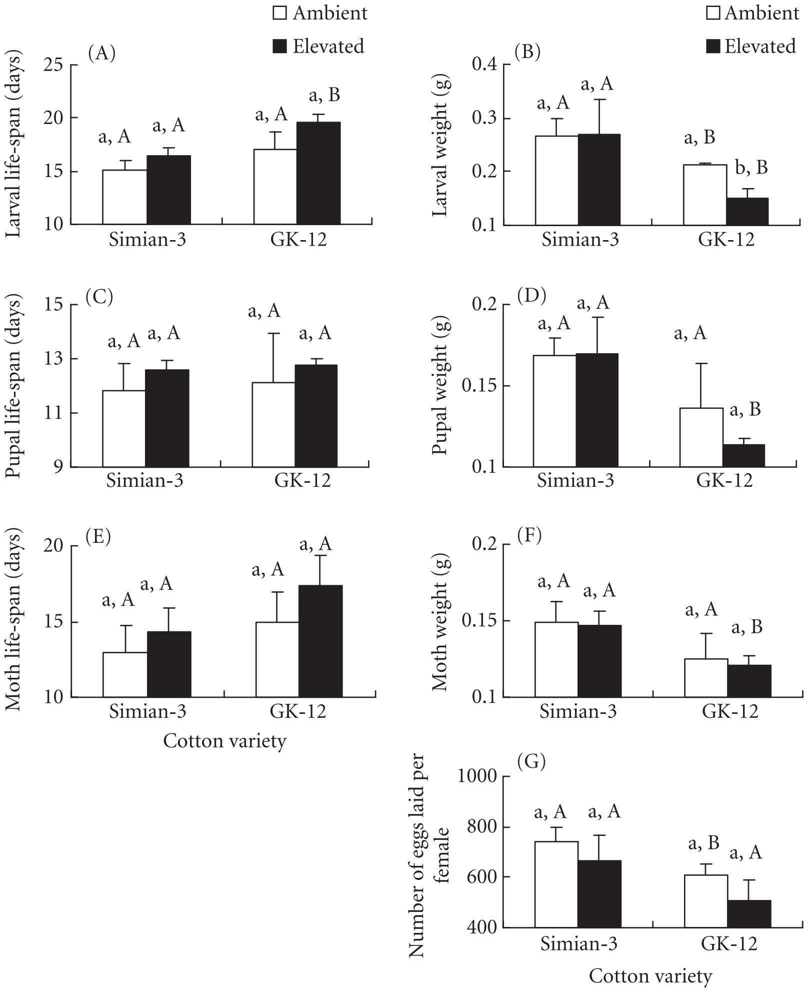

Figure 2 Average (+ SD) life-span, fresh body weight, and number of eggs laid per moth of the cotton bollworm, *Helicoverpa armigera*, fed on excised young bolls of non-transgenic and transgenic Bt cotton grown in ambient and double-ambient CO_2. Different lower case letters indicate significant differences between CO_2 treatments within cotton variety (LSD test: d.f. = 1,4, P<0.05); different upper case letters indicate significant differences between cotton variety treatments within a CO_2 level (LSD test: d.f. = 1,4, P<0.05).

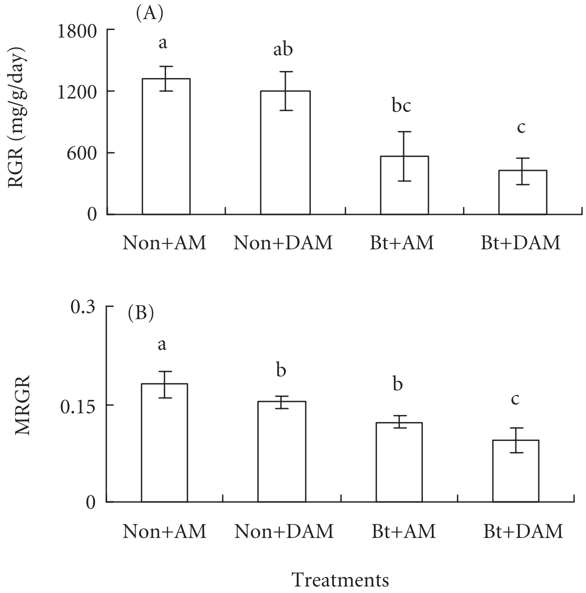

Figure 3 (A) Average (± SD) relative growth rate (RGR) and (B) mean relative growth rate (MRGR) of the cotton bollworm, *Helicoverpa armigera*, fed ingested excised young bolls of transgenic Bt (Bt) and non-transgenic (Non) cotton grown in ambient (AM) and double-ambient (DAM) CO_2. Different lower case letters indicate significant differences between treatments within combinations of CO_2 and cotton variety (LSD test: d.f. = 3,8, $P<0.05$).

TNC:Nitrogen ratio, condensed tannin, and gossypol in both non-transgenic and transgenic Bt cotton, confirming the results of other studies (e.g., Bazzaz, 1990; Williams et al., 1998). In general, plants (especially C_3 plants) respond to elevated CO_2 with increased photosynthesis and growth rates (Wong, 1979; Rodgers et al., 1983), increasing the C:N ratio and water-use efficiency in foliage and decreasing foliar N, primarily due to the accumulation of non-structural carbohydrates (Kimball et al., 1994; Lindroth et al., 1995). Our results are consistent with the Carbon Nutrient Balance (CNB) hypothesis (Bryant et al., 1983); i.e., increases in the relative availability of carbon and nutrients should cause increases in carbon-based defences (condensed tannin and gossypol in this study), and a reduction in N-based defences (here, Bt toxin protein). At elevated CO_2 levels, transgenic Bt cotton had significantly lower nitrogen, soluble sugars, starch, and TNC than non-transgenic cotton. Similarly, a significant decrease in Bt toxin was found in GK-12 grown in elevated CO_2 compared to that in ambient CO_2.

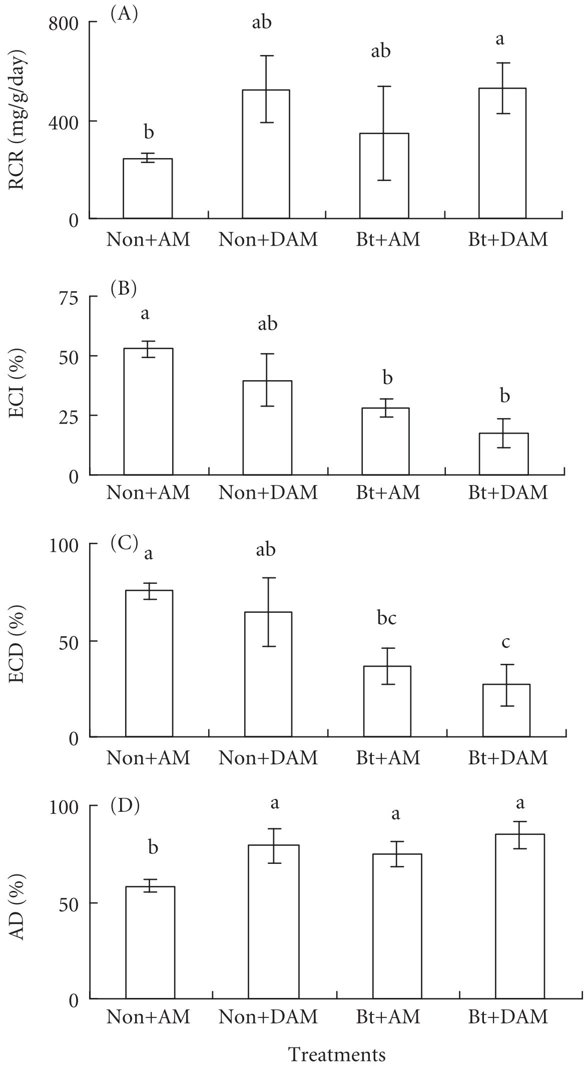

Figure 4 (A) Average (± SD) relative consumption rate (RCR), (B) growth efficiency (ECI), (C) conversion efficiency (ECD), and (D) approximate digestibility (AD) of the cotton bollworm, *Helicoverpa armigera*, fed excised young bolls of transgenic Bt (Bt) and non-transgenic (Non) cotton grown in ambient (AM) and double-ambient (DAM) CO_2. Different lower case letters indicate significant differences between treatments within combinations of CO_2 and cotton variety (LSD test: d.f. = 3,8, $P<0.05$).

Effects of transgenic Bt cotton and elevated CO_2 on insect growth and development

Over 100 studies have been reported thus far on the effects of elevated CO_2 on plant–herbivore interactions (see reviews by Watt et al., 1995; Bezemer & Jones, 1998; Coviella & Trumble, 1999; Whittaker, 1999, 2001). Most herbivorous insects appear to be negatively affected by elevated CO_2 because of the reduction in foliar N and the increase in C:N ratio, with the exception of phloem-feeding insects (Watt et al., 1995; Bezemer & Jones, 1998). In most herbivorous insect diets, nitrogen content limits insect growth and development. Because nitrogen is the single most important limiting resource for phytophagous insects (Mattson, 1980), a decrease in foliar N of host plants could affect the population dynamics of herbivores and the extent of damage to their associated host plants.

Several studies have been conducted to determine the influence of transgenic Bt cotton on target and non-target arthropods (Fitt, 1994; Huang et al., 1999). The direct goal of the presence of the Bt toxin on cotton bollworms was to increase the mortality and reduce development (Fitt, 1994). Most lepidopteran pests have been found to be negatively affected by transgenic Bt cotton, with higher larval mortality, lower pupal weight, lower fecundity and reproductive rate, and longer life-span to pupation for both males and females (Carrie et al., 2003; Goverde & Erhardt, 2003). Although transgenic Bt cotton plants have been grown over a wide geographical range, little is thus far known on their long-term effects on agriculture and environment, especially the effect of rising CO_2 on bollworm population dynamics in the future. In this study, transgenic Bt cotton and elevated CO_2 both delayed the development of bollworms and led to a reduction in body weight and larval relative growth rate (RGR) and mean relative growth rate (MRGR). Transgenic Bt cotton delayed larval life-span, reduced body weight and fecundity, and significantly reduced larval RGR and MRGR. In contrast, elevated CO_2 did not significantly affect the growth and development of cotton bollworms with respect to cotton variety. Thus, the influence of a transgenic gene in cotton far outweighed the influence of altered plant physiology under elevated CO_2 conditions. However, the effects of transgenic Bt cotton on the growth and development of cotton bollworm were enhanced when grown under elevated CO_2 conditions.

Effects of transgenic Bt cotton and elevated CO_2 on insect consumption and digestibility

Scriber & Slansky (1981) reported that water content in the host plant was an important factor towards the food's digestibility in herbivorous insects. The palatability of a plant for herbivorous insects is mainly determined by a combination of plant nitrogen and water content. Leaf-chewing herbivores respond to elevated CO_2 by increasing their food consumption and reducing their food conversion efficiency (Roth & Lindroth, 1995; Brooks & Whittaker, 1998; Williams et al., 1998). Our results indicated that larval ECI and ECD were both significantly reduced and RCR increased for cotton bollworms fed transgenic Bt cotton plants grown in elevated CO_2, which implies that feeding on transgenic Bt cotton crops grown in elevated CO_2 increased larval food consumption and reduced food conversion efficiency. Furthermore, changes in allelochemical concentrations and other secondary chemicals may affect the feeding behaviour of insect herbivores (Scriber & Slansky, 1981). In this study, the *Cry1A(c)* toxin of transgenic Bt cotton (GK-12) and increases in condensed tannin and gossypol in young bolls of elevated CO_2-grown cotton affected cotton bollworm feeding, but the decrease in Bt toxin of elevated CO_2-grown GK-12 may alleviate the adverse effect on bollworms. So the transgenic Bt cotton (GK-12) places a new demand on plant defence allocation, increases in condensed tannin and gossypol and decreases in Bt toxin, when grown under elevated CO_2.

Transgenic Bt cotton and elevated CO_2 level both increased larval approximate digestibility (AD). While AD is the index of food digestibility, this index does not directly describe the amount of food ingested or the amount of frass produced by herbivorous insects. In this study, larval AD increased significantly when bollworms were reared on elevated CO_2-grown transgenic Bt cotton. In agreement with the above indices, it is clear that the cotton bollworm increased the amount of food digested and exhibited reduced food conversion efficiency when fed elevated CO_2-grown transgenic Bt cotton. Therefore, more of the energy obtained from the digested food coming from the elevated CO_2-grown transgenic Bt cotton was consumed compared to that from the ambient CO_2-grown non-transgenic cotton, with the increased cost most likely coming from compensatory feeding and from digesting the increased amount of food (Stearns, 1989; Slansky, 1993).

This study exemplifies the complexities of predicting herbivore responses to future climate conditions, particularly in combination with transgenic technologies. While atmospheric conditions are important in modifying several aspects of bollworm response to transgenic Bt cotton, these modifications appear to be overwhelmed by differences between transgenic and non-transgenic plants. Thus, global change appears likely to either not change or only slightly enhance the efficacy of Bt technology against cotton bollworms. However, further research will be needed to elucidate the effects of a decrease in Bt toxin expressed in elevated CO_2-grown GK-12 on cotton bollworm physiology, and to further quantify the changes in both plant and insect physiologies in response to global change, particularly over multiple generations and for a broader range of insects, including non-target herbivores.

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