

Long-term impacts of elevated carbon dioxide and transgenic Bt cotton on performance and feeding of three generations of cotton bollworm

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Abstract

The effects of elevated carbon dioxide (CO₂) on growth, metabolism, and performance of three generations of cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), were studied. The insects were continuously fed transgenic *Bacillus thuringiensis* (Berliner) (Bt) cotton [*CryIA(c)*] grown in open-top chambers. Two levels of CO₂ (ambient and 2× ambient) and two cotton cultivars (non-transgenic Simian-3 and transgenic Bt GK-12) were used and bollworm larvae were reared on all four treatment combinations for three generations. CO₂ level and cotton variety significantly affected the growth and food digestibility parameters of *H. armigera*, with the exception of larval consumption rate for cotton variety. Overall, elevated CO₂ and transgenic Bt cotton both increased larval lifespan, food consumption rate, relative consumption rate, and approximate digestibility, while decreasing pupal weight, survival rate, fecundity, frass output, relative and mean relative growth rates (RGR/MRGR), and the efficiency of conversion of ingested and digested food (ECI/ECD). Moreover, there were significant CO₂*variety interactions on pupal weight and ECD, and CO₂*generation interactions on pupal weight, frass output, and MRGR. Furthermore, transgenic Bt cotton significantly decreased the population-trend index compared to non-transgenic cotton for the three successive bollworm generations, especially at elevated CO₂. Damage inflicted by the cotton bollworm on cotton, irrespective of the presence of insecticidal genes, is predicted to be higher under elevated CO₂ conditions because of individual compensatory feeding on host plants. Conversely, population abundance is presumed to be lower under elevated CO₂ compared to that under ambient CO₂, particularly in combination with transgenic technologies.

Introduction

Most published studies on the response of insect herbivores to elevated carbon dioxide (CO₂) are short-term studies measuring growth, development, and consumption rates (Bezemer & Jones, 1998). Experiments conducted over multiple generations through the entire life cycle of the herbivore, or between feeding guilds (e.g., xylem-feeding

and leaf-chewing insects), however, reveal differences in the response of herbivores to changes in food quality owing to the effect of elevated CO₂ on the host plant (Fajer et al., 1991; Brooks & Whittaker, 1998, 1999; Wu et al., 2006). Despite the well-recognized need for additional long-term studies on this topic (Lindroth et al., 1995), there is a paucity of information on the response of multiple generations of insect herbivores to elevated CO₂. Brooks & Whittaker (1998) reported a marginal reduction in fecundity and egg size for the leaf beetle, *Gastrophysa viridula* (de Geer) (Coleoptera: Chrysomelidae) when reared through the second generation under elevated CO₂, but a significant reduction in those two parameters was found when the beetle was reared through three generations under similar conditions. Brooks &

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Whittaker (1999) indicated that elevated CO₂ significantly depressed the survival rate and slowed the development of a xylem-feeding insect, *Neophilaenus lineatus* (L.) (Homoptera: Cercopidae), relative to ambient CO₂ through three sequential generations, but they found no significant effect on fecundity. Thus, it is clear that long-term studies on the impacts of elevated CO₂ on the interactions between host plants and their herbivorous insects are needed in order to develop realistic predictions of long-term population dynamics of these herbivores (Williams et al., 1997; Wu et al., 2006).

Transgenic Bt cotton has been adopted worldwide to control lepidopteran insect pests. In China, the damage caused by cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), has been greatly alleviated in recent years due, in part, to the adoption of transgenic Bt cotton (Men et al., 2003). In northern China, *H. armigera* typically undergoes four complete generations per year, with the first generation on wheat and the remaining three generations on cotton (Li & Xue, 1981). Despite the potentially significant consequences of higher future CO₂ levels for Bt technology, only research involving short-term experiments has been reported on the effects of elevated CO₂ on transgenic Bt cotton or the effects on bollworms fed Bt cotton grown in an elevated CO₂ environment (Coviella et al., 2002; Chen et al., 2005b).

Direct effects of elevated CO₂ on herbivorous insects have been shown to be minimal, and the indirect impact of elevated CO₂ on herbivorous insects appears to be mediated through effects on host-plant quality/quantity (Chen et al., 2005c). This study examines the long-term influence of elevated CO₂ and transgenic Bt cotton on growth, development, consumption and digestibility, and population performance of cotton bollworm, *H. armigera*, fed cotton bolls over three successive generations.

Materials and methods

Open-top chambers

The experiment was conducted in six open-top chambers, 2.5 m in height × 4.2 m in diameter, in Sanhe County, Hebei Province, China (35°57'N, 116°47'E). Two levels of atmospheric CO₂ concentration were applied continuously: the ambient level (375 μl l⁻¹) and 2× ambient level (750 μl l⁻¹). The 2× ambient concentration represents the predicted level of ambient CO₂ in about 100 years from now (Watson et al., 1996; Houghton et al., 2001). Three open-top chambers were used for each CO₂ treatment. Concentrations in each open-top chamber were separately monitored 24 h per day and were adjusted with an infrared CO₂ analyzer (Ventostat 8102, Telaire Company, Goleta, CA, USA). The open tops of these chambers were covered with plastic netting (mesh size: 0.15 mm × 0.15 mm) to prevent insect movement. Details of

the automatic control system for CO₂ and open-top chambers are provided in Chen & Ge (2004) and Chen et al. (2005a).

Cotton cultivars

The transgenic Bt cotton cultivar GK-12 [expressing *Cry1A(c)* genes from *Bacillus thuringiensis* Berliner var. *kurstaki*] and the non-transgenic cotton cultivar Simian-3 from the same recurrent parent were used in this study. Both cultivars were planted in white plastic pots (45 cm in height × 35 cm in diameter) filled with 8:3:1 (by volume) loam:cow dung:earthworm frass. Thirty pots with two plants per pot for each cultivar were placed randomly in each chamber and re-randomized every other day to minimize positional effects. Both cultivars were planted on 10 May 2004, and cotton bolls (unopened, diameter >2 cm) were harvested in October and were stored at -20 °C for the laboratory experiment. Pure CO₂ mixed with ambient air was supplied to the chambers throughout the period from seedling emergence to harvest. No chemical fertilizers or insecticides were applied to the crop plants during this study. Cotton plants were watered with 2 l tap water per pot once every 2 days.

Insects

Egg masses of *H. armigera* were obtained from a laboratory colony of the Insect Physiology Laboratory, Institute of Zoology, Chinese Academy of Sciences and then were 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) and 24 ± 1 °C (night) and photoperiod was L14:D10 at 9000 lux, supplied by 12 fluorescent lamps (60 W).

Insect feeding

In this study, the testing insect generations were not exposed directly to elevated CO₂; rather, they were fed cotton bolls from plants grown under controlled levels of atmospheric CO₂.

Treatments. Newly eclosed first instars obtained from the insect stocks were reared in growth chambers (see above) and were fed the stored immature cotton bolls collected from each of the four cultivar × CO₂ treatments. Treatments included (i) Bt cotton grown in ambient CO₂, (ii) Bt cotton grown in elevated CO₂, (iii) non-Bt cotton grown in ambient CO₂, and (iv) non-Bt cotton grown in elevated CO₂. The rearing temperature, photoperiod, and r.h. in the rearing test chambers were the same as for the insect stock.

Rearing. A sample of cotton bolls (refrigerated bolls from the four treatments) was 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 trial. The test bolls were dissected and provided daily to bollworm larvae to ensure that the amount and freshness of the boll sections were similar across test insects. The amount of food (sections of bolls) provided to each larva and the left-over food were weighed every day in order to construct the various dietetic indices. For each replicate of each treatment, 60 newly eclosed first instars were reared individually in glass dishes (75 mm in diameter) until pupation to determine life-history parameters, while 20 additional larvae were reared under the same conditions for each treatment, to provide an estimate of the dry matter content of the larvae. There were three replicates per treatment. Each day, the remaining portion of the boll and frass produced per larva were collected and oven dried at 80 °C for 72 h. The fresh insect body weight per larva was measured at the beginning of the rearing experiment and at the pupal stage.

Because cotton bollworm larvae tend to eat their exuviae following ecdysis, it was not feasible to record the duration of each instar. Larval lifespan was calculated as the period from hatch to pupation. Pupal weight was measured about 12 h after pupation. Survival rate was calculated as the number of moths emerged divided by the number of first instars used for each treatment. The percentage of females was calculated as the ratio of the number of female moths to the total number of moths. *Helicoverpa armigera* mostly frequently mates during the first 3 days of emergence, and during this period the female moths do not oviposit. Thus, newly emerged moths from each treatment were released into cages (45 × 30 × 50 cm) and were held under the same conditions as insect stocks for 3 days to allow mating. After mating, moths were transferred in pairs to plastic cups (20 cm in height × 15 cm in diameter; one pair per cup) covered with a net of degreased cotton yarn as an oviposition substrate. The cotton covers were replaced every day and the number of eggs in each oviposition cup was recorded daily until the death of all females. Eggs from each moth pair were held separately under the same conditions as for insect stock to allow hatching. The total number of eggs from each female was recorded as an estimation of lifetime fecundity, and the eclosion rate was calculated by dividing the number of newly eclosed first instars by the total number of eggs laid per moth.

Sixty newly eclosed first instars from each generation were followed through the complete life cycle for three successive generations following the same rearing protocol as for the first generation.

Development and consumption indices of *Helicoverpa armigera*

Life history and population indices. Four indices were used to measure the growth, development, fecundity, and survival

of cotton bollworms: life-span, pupal weight, survival rate, and fecundity (i.e., number of eggs laid per female). The population-trend index (I) was calculated following the method proposed by Cheng (2005):

$$I = N_1/N_0,$$

where N_0 = population abundance at the beginning of the rearing experiment for each test generation (i.e., 60 larvae per replicate), N_1 = population abundance at the end of the given generation, and $N_1 = N_0 \times$ survival rate \times percentage of females \times number of eggs laid per female \times eclosion rate of eggs.

Indices for larval consumption and digestibility. The ratio-based nutritional indices, mean relative growth rate (MRGR; Viskari et al., 2000), relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI), approximate digestibility (AD), and efficiency of conversion of digested food (ECD) were determined gravimetrically following the methods of Waldbauer (1968) and Scriber & Slansky (1981).

Data analysis

All data were analyzed using the general linear models procedure (SAS Institute, 1996). Life-history indices of the cotton bollworm (i.e., larval lifespan, pupal weight, survival rate, and number of eggs laid per female), consumption and frass per larva, and population-trend index (I) were analyzed by split plot analysis of variance (ANOVA) with CO₂ level and cotton cultivar as main factors and bollworm generation as the subfactor. The data for larval consumption and digestibility indices were analyzed using an ANOVA with initial weight as a covariate for RCR, RGR, and MRGR. Food consumption was used as 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 [i.e., bolls ingested (mg) – frass produced (mg)] was used as a covariate to analyze ECD (Hägele & Martin, 1999). The assumption of a parallel slope between covariate and dependent variables was satisfied for each analysis. As there was significant interaction between CO₂ level, cotton cultivar, and bollworm generation, the least significant difference (LSD) test was used to separate the means between treatments at $P < 0.05$.

Results

Life-history indices

The CO₂ level and cotton variety both significantly affected the four measured parameters ($P < 0.001$; Table 1). Overall, both elevated CO₂ and transgenic Bt cotton increased larval

Table 1 Effect of CO₂ level, cotton variety, and generation on life history parameters, food consumption, frass production, and population-trend index (P-value; ANOVA) and on growth, consumption, and digestion indices (P-value; ANCOVA) of *Helicoverpa armigera*

Indexes ¹	Covariate ²	CO ₂ ³	Variety ⁴	Generation ⁵	CO ₂ * variety	CO ₂ * generation	Variety* generation	CO ₂ * variety* generation
Larval lifespan (days)		0.0001	0.0001	0.0901	0.6972	0.1772	0.6972	0.6284
Pupal weight (g)		0.0001	0.0001	0.0919	0.0057	0.0352	0.7773	0.9813
Survival rate (%)		0.0003	0.0001	0.0697	0.6432	0.2338	0.9847	0.9498
Number of eggs laid per female		0.0001	0.0001	0.7179	1.0000	0.3867	0.2594	1.0000
Food consumption per larva (g)		0.0001	0.8676	0.5749	0.9121	0.8896	0.9966	0.8942
Frass produced per larva (g)		0.0001	0.0001	0.0990	0.5774	0.0457	0.5851	0.9133
Population-trend index (I-value)		0.4661	0.0001	0.0001	0.5392	0.0001	0.0124	0.0012
Percentage of females (%)		0.0001	0.0001	0.0034	0.0001	0.7833	0.4643	0.5379
Eclosion rate of eggs (%)		0.0001	0.0001	0.0001	0.0001	0.0044	0.0229	0.1707
MRGR	0.1456	0.0001	0.0001	0.0511	0.1915	0.0486	0.9424	0.4523
RGR (mg g ⁻¹ per day)	0.5321	0.0001	0.0001	0.4044	0.0824	0.1487	0.9543	0.9750
RCR (mg g ⁻¹ per day)	0.4533	0.0007	0.0001	0.1990	0.1504	0.4068	0.9397	0.9930
ECI (%)	0.4162	0.0001	0.0001	0.5100	0.1528	0.1617	0.9735	0.7890
AD (%)	0.3861	0.0002	0.0001	0.2687	0.2203	0.3999	0.9869	0.9688
ECD (%)	0.2332	0.0001	0.0001	0.1331	0.0498	0.1688	0.8658	0.9851

¹MRGR, mean relative growth rate; RGR, relative growth rate; RCR, relative consumption rate; ECI, efficiency of conversion of ingested food; AD, approximate digestibility; ECD, efficiency of conversion of digested food.

²Initial weight as a covariate for RCR, RGR, and MRGR, food consumption for ECI and AD, and food assimilated for ECD.

³Ambient CO₂ vs. double ambient CO₂ (i.e., elevated CO₂).

⁴Cotton type: transgenic Bt cotton (cv. GK-12) vs. non-transgenic cotton (cv. Simian-3).

⁵Three successive generations.

lifespan, decreased pupal weight, lowered survival rate, and reduced the number of eggs laid per female (Figure 1). In contrast, Bt cotton significantly lowered pupal weight at both CO₂ levels for each of the three successive generations compared with non-Bt cotton (P<0.05; Figure 1B); and elevated CO₂ significantly reduced pupal weight of the second (16.4% decrease) and third (20.8% decrease) generations for Bt cotton treatment (P<0.05; Figure 1B), but only significantly decreased pupal weight of the third generation for non-Bt cotton treatment (9.1% decrease; P<0.05; Figure 1B), contributing to the significant CO₂* variety (P<0.01) and CO₂*generation (P<0.05) interactions (Table 1). Moreover, pupal weight was significantly reduced by elevated CO₂ through three successive generations of bollworms fed Bt (10.0% decrease) or non-Bt (10.4% decrease) cotton (P<0.05; Figure 1B).

Population-trend index (I)

Cotton cultivar significantly affected the population-trend index (I) (P<0.001; Table 1), and the I-value consistently increased in the long-term experiment (Figure 2). In contrast, Bt cotton significantly decreased the I-value within a given CO₂ level compared with non-Bt cotton (P<0.05), except for the second generation at ambient CO₂ (Figure 2). Significant increases in I-value were observed through the three

generations at ambient CO₂ (P<0.05; Figure 2), whereas there was no significant difference between the first and third generations at elevated CO₂ regardless of the transgenic status of the cultivar (P<0.05; Figure 2).

Moreover, the main effects of CO₂, variety, and generation on the percentage of females, and eclosion rate of eggs were all significant (P<0.001; Table 1). Elevated CO₂ and transgenic Bt cotton both consistently enhanced the two parameters through the three successive generations (Table 2). In contrast, a significant increase in the percentage of females was observed by elevated CO₂ for non-Bt cotton or by Bt cotton at ambient CO₂ (P<0.05; Table 2), contributing to the significant interaction of CO₂ and variety (P<0.001; Table 1).

Larval consumption and digestibility

Consumption and frass. Elevated CO₂ significantly increased food consumption per larva regardless of the transgenic Bt gene in its diet for three successive generations (P<0.001; Table 1; Figure 3A). Simultaneously, CO₂ level and cotton cultivar both significantly affected the amount of frass produced per larva (P<0.001; Table 1). Overall, both elevated CO₂ and transgenic Bt cotton substantially decreased frass produced per larva (Figure 3B). In contrast, elevated CO₂ only significantly decreased frass produced per larva by 9.9%

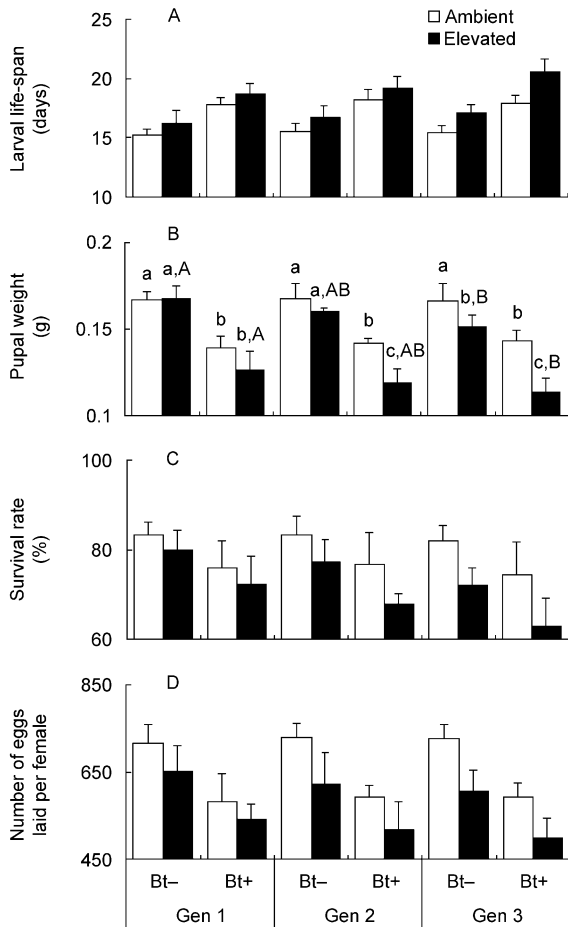


Figure 1 Mean (\pm SE) (A) larval life-span, (B) pupal weight, (C) survival rate, and (D) eggs laid per female of three generations (Generations 1–3) of *Helicoverpa armigera* fed non-transgenic (Bt–) Simian-3 and transgenic Bt (Bt+) GK-12 cotton grown in ambient and elevated CO₂ atmospheres at 28 \pm 1 °C (day)/24 \pm 1 °C (night). Different lowercase letters indicate significant differences between mean values across CO₂ \times cotton variety treatments within each bollworm generation [least significant difference (LSD) test: d.f. = 3,8, P<0.05]; Different uppercase letters indicate significant differences between three bollworm generations fed non-transgenic Simian-3 or transgenic GK-12 at elevated CO₂ (LSD test: d.f. = 2,6, P<0.05).

after bollworm larvae were fed non-Bt cotton for three generations (P<0.05; Figure 3B), thus contributing to a significant CO₂ \times generation interaction (P<0.05; Table 1).

MRGR and RGR. Both CO₂ level and cotton cultivar significantly affected the values of MRGR and RGR (P<0.001; Table 1). Overall, elevated CO₂ and transgenic Bt cotton decreased the values of MRGR and RGR for the three successive generations (Figure 4). Moreover, a significant

CO₂ \times generation interaction occurred affecting MRGR (P<0.05; Table 1), but only with a significant decrease for the third generation compared with that for the first generation of bollworms fed elevated CO₂-grown Bt cotton (14.2% decrease; P<0.05; Figure 4A).

RCR, ECI, AD, and ECD. The RCR, ECI, AD, and ECD values varied significantly with CO₂ and cotton cultivar treatments (P<0.001), while the interactions between CO₂ and cultivar was only significant for ECD (P<0.05; Table 1). Overall, both elevated CO₂ and transgenic Bt cotton increased the values of RCR and AD, and decreased the values of ECI and ECD for the three successive bollworm generations (Figure 5). In contrast, significant decreases were observed in ECD value for bollworm larvae fed Bt cotton compared to those fed non-Bt cotton at both levels of CO₂ in all three generations of this study (P<0.05; Figure 5C).

Discussion

The effects of rising CO₂ on interactions between plants and herbivorous insects have been a concern of the scientific community for over three decades (Bezemer & Jones, 1998; Fang & Zhuang, 2000; Hunter, 2001; Newman et al., 2003). Most herbivorous insects appear to be negatively affected by changes in plant chemistry resulting from elevated CO₂, especially decreases in foliar nitrogen (N) and increase in carbon:nitrogen (C:N) ratio (Bezemer & Jones, 1998). Alterations in plant chemistry due to changes in CO₂ level have been shown to lower growth rate, slow larval development, and increase compensatory feeding for herbivorous

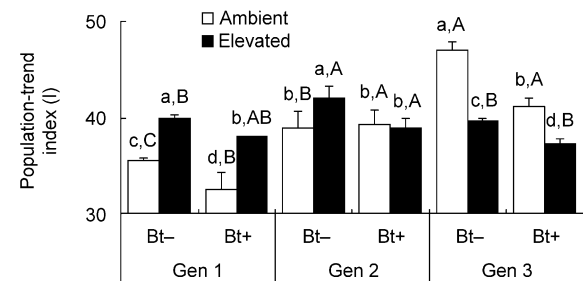


Figure 2 Population-trend index (I) of three generations (Generations 1–3) of *Helicoverpa armigera*, fed non-transgenic (Bt–) Simian-3 and transgenic Bt GK-12 cotton grown in ambient and elevated CO₂ at 28 \pm 1 °C (day)/24 \pm 1 °C (night). Different lowercase letters indicate significant differences between treatments within combination of CO₂ and cotton variety [least significant difference (LSD) test: d.f. = 3,8, P<0.05]; Different uppercase letters indicate significant differences between three bollworm generations fed same host plants (non-transgenic Simian-3 or transgenic GK-12) under same CO₂ level (ambient or elevated) (LSD test: d.f. = 2,6, P<0.05).

Table 2 Percentage of females and eclosion rate of eggs (mean \pm SE) in three successive generations of *Helicoverpa armigera* fed on non-transgenic Simian-3 and transgenic Bt GK-12 cotton grown in ambient and elevated CO₂ at 28 \pm 1 °C (day)/24 \pm 1 °C (night)

Generation	Measured parameters (%)	Simian-3		GK-12	
		Ambient CO ₂	Elevated CO ₂	Ambient CO ₂	Elevated CO ₂
First	Percentage of females	47.8 \pm 0.2b	53.6 \pm 1.7a	54.9 \pm 1.8a	56.0 \pm 0.1a
	Eclosion rate of eggs	12.5 \pm 0.5c,B	14.3 \pm 0.7b,C	13.5 \pm 0.3bc,C	17.3 \pm 0.5a,C
Second	Percentage of females	49.6 \pm 0.4b	55.7 \pm 0.5a	56.7 \pm 0.6a	56.5 \pm 1.3a
	Eclosion rate of eggs	13.0 \pm 0.2c,B	15.6 \pm 0.3b,B	15.4 \pm 0.4b,B	19.6 \pm 0.1a,B
Third	Percentage of females	51.9 \pm 1.6b	56.1 \pm 1.8a	56.7 \pm 0.7a	57.2 \pm 2.6a
	Eclosion rate of eggs	15.3 \pm 0.3c,A	16.3 \pm 0.4b,A	16.5 \pm 0.1b,A	20.5 \pm 0.4a,A

Means within a row followed by different lowercase letters are significantly different [least significant difference (LSD) test: d.f. = 3,8, P<0.05]; means for each of the measured parameters within a column followed by different uppercase letters are significantly different across the three bollworm generations (LSD test: d.f. = 2,6, P<0.05).

insects (Fajer et al., 1989; Lincoln et al., 1993). It is also important to measure changes in plant chemistry in response to elevated CO₂ rather than to measure only foliar nitrogen and C:N ratio (Peñuelas & Estiarte, 1998; Ward & Strain, 1999). Some noted specific changes in plant chemistry due to elevated CO₂ include an increase in carbon-based secondary compounds (e.g., starch, condensed tannins, gossypol, and terpenoid aldehydes) and a decrease in nitrogen-based compounds (e.g., protein and Bt toxin for transgenic Bt cotton) (Kinney et al., 1997; Coviella et al., 2002; Chen et al., 2005b). CO₂-mediated changes in host-plant quality, in turn,

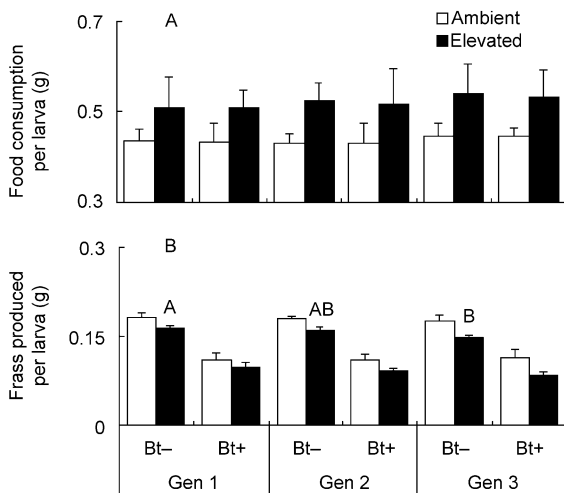


Figure 3 Mean (+SE) food consumption (A) and frass produced (B) per larva of three generations of *Helicoverpa armigera* fed non-transgenic (Bt-) Simian-3 and transgenic Bt (Bt+) GK-12 cotton grown in ambient and elevated CO₂ atmospheres at 28 \pm 1 °C (day)/24 \pm 1 °C (night). Different uppercase letters indicate significant differences between three bollworm generations fed non-transgenic Simian-3 cotton at elevated CO₂ [least significant difference (LSD) test: d.f. = 2,6, P<0.05].

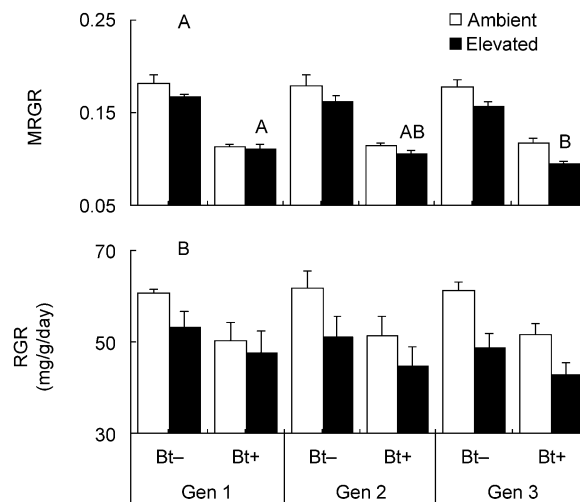


Figure 4 (A) Average (+SE) mean relative growth rate (MRGR) and (B) relative growth rate (RGR) of three generations of *Helicoverpa armigera* successively fed non-transgenic (Bt-) Simian-3 and transgenic Bt (Bt+) GK-12 cotton grown in ambient and elevated CO₂ atmospheres at 28 \pm 1 °C (day)/24 \pm 1 °C (night). Different uppercase letters indicate significant differences between three bollworm generations fed transgenic Bt GK-12 cotton at elevated CO₂ [least significant difference (LSD) test: d.f. = 2,6, P<0.05].

affect growth, development, and food digestibility of insect herbivores. However, most of the initial studies focused on the responses of insect herbivores to elevated CO₂ in single-generation experiments (e.g., Lindroth et al., 1993; Masters et al., 1998), and in combination with transgenic technologies (e.g., Coviella et al., 2002; Chen et al., 2005b).

Single-generation experiments have indicated that elevated CO₂ modifies several biological attributes of the cotton bollworm fed transgenic Bt or non-transgenic

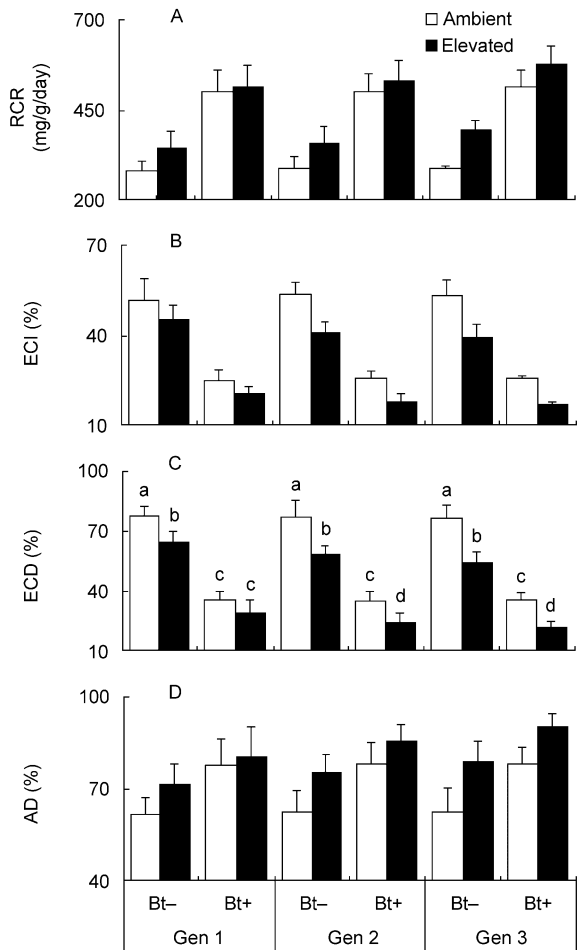


Figure 5 (A) Average (+SE) relative consumption rate (RCR), (B) efficiency of conversion of ingested food, (C) approximate digestibility, and (D) efficiency of conversion of digested food (ECD) of three generations of *Helicoverpa armigera* successively fed non-transgenic (Bt-) Simian-3 and transgenic Bt GK-12 cotton grown in ambient and elevated CO₂ atmospheres at 28 ± 1 °C (day)/24 ± 1 °C (night). Different lowercase letters indicate significant differences between CO₂ × cotton variety treatments within bollworm generations [least significant difference (LSD) test: d.f. = 3,8, P<0.05].

conventional cotton cultivars. However, these modifications appear to be overwhelmed by the differences between transgenic and non-transgenic plants (e.g., Chen et al., 2005b). In contrast, Brooks & Whittaker (1998, 1999) demonstrated that differences in the response of herbivorous insects to changes in food quality owing to the effect of rising CO₂ on host plant were observed between generations of different feeding guilds. Wu et al. (2006) indicated that net damage of cotton bollworm on wheat will be less under elevated atmospheric CO₂ levels because increased consumption

is offset by slower development and reduced fecundity of *H. armigera* after they are successively reared on elevated CO₂-grown wheat for three generations. In this study, elevated CO₂ and transgenic Bt cotton both increased larval lifespan, food consumption rate, RCR and AD, and decreased pupal weight, survival rate, fecundity, frass output, MRGR, RGR, ECI, and ECD for all three successive generations. These results indicate that CO₂ level within a cotton cultivar appeared to have a lesser impact on most of the measured parameters, while cotton variety within a CO₂ level substantially affected most of these parameters. However, the effect of elevated CO₂ on those parameters (i.e., pupal weight, larval frass, population-trend index, eclosion rate, and MRGR) intensified through successive generations regardless of the cultivar. These data suggest that the effects of elevated CO₂ on cotton bollworm physiology and population dynamics may intensify through successive generations. These findings are in general agreement with those of Brook & Whittaker (1998, 1999), who showed a significant reduction in fecundity and egg size in the third generation of *G. viridula* reared under an elevated CO₂ regime, and a significant decrease in the survival rate and a significantly longer development time of *N. lineatus* after two sequential generations at elevated CO₂ compared to that in ambient CO₂.

In this study, the population-trend index (I) significantly decreased in transgenic Bt GK-12 compared to that in non-transgenic Simian-3 for the three generations of cotton bollworms. Also, elevated CO₂ substantially suppressed the population-trend index, with a marginal decrease in I-value after bollworm larvae were fed non-Bt cotton (0.5%) or Bt cotton (1.9%) for three generations. Besides survival rate and adult fecundity, the population-trend index incorporates two additional variables, the percentage of females and the eclosion rate of eggs in the cohort. Elevated CO₂ and transgenic Bt cotton both consistently enhanced the percentage of females and eclosion rate through three successive generations. Therefore, it appears that both elevated CO₂ and transgenic Bt cotton are adverse environmental factors for cotton bollworm long-term population growth, and the combination of the two factors may intensify their adverse impact on the population performance of *H. armigera*.

Most herbivorous insects reared on foliage grown in elevated CO₂ have frequently been shown to increase consumption rates (e.g., Watt et al., 1995; Chen et al., 2004, 2005b; Wu et al., 2006), which is consistent with the results of this study. The results of this study clearly suggest that the adaptation/plasticity of the *H. armigera* is accomplished by consuming (i.e., increasing RCR) and digesting (i.e., increasing AD) more food to obtain enough energy in response to adverse effects (here, elevated CO₂ and transgenic Bt cotton) (Scriber & Slansky, 1981; Stearns, 1989; Slansky, 1993). Moreover, a decrease in Bt toxin was observed in elevated

CO₂-grown transgenic Bt cotton (Coviella et al., 2002; Chen et al., 2005b), which, in turn, would lower the resistance of host plants to cotton bollworms. However, our data indicated that a significant decrease in ECD was observed for bollworm larvae fed Bt cotton compared to those fed non-Bt cotton regardless of the CO₂ level, which resulted in lighter pupal weight. Moreover, the extent of decrease in pupal weight or ECD value due to elevated CO₂ consistently increased with bollworm generations for transgenic Bt cotton, suggesting that the rising CO₂ appears likely to enhance the efficacy of transgenic Bt cotton against cotton bollworms through multiple generations. This may be attributable to an increase in carbon-based secondary compounds (e.g., condensed tannins, gossypol, and terpenoid aldehydes) (Coviella et al., 2002; Chen et al., 2005b), having long-term effects on growth and metabolism of insect herbivores (e.g., *H. armigera*) (Peñuelas & Estiarte, 1998). Therefore, further research is needed to investigate the effects of changes in plant chemistry (especially the secondary defensive compounds) of transgenic Bt cotton grown in elevated CO₂ (e.g., decrease in Bt toxin and increase in carbon-based secondary compounds) on cotton bollworm physiology, and to further quantify the changes in both plant and insect physiology in response to global change for a broader range of insects, including non-target herbivores.

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