Effects of elevated CO_2 associated with maize on multiple generations of the cotton bollworm, *Helicoverpa armigera*

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Abstract

Under elevated environmental carbon dioxide (CO_2), leaf chewers tend to compensate for decreased leaf nutritional quality with increased consumption; mortality and development times also increase and cause a reduction in the fitness of leaf chewers. However, the effect of elevated CO_2 on multiple successive generations of these and other insects is not well understood. Furthermore, information about the direct effects of increased environmental CO_2 on developmental time and consumption of herbivores is lacking. In this paper, we tested the hypothesis that cascade effects of elevated CO_2 through plants, rather than the direct effects of elevated CO_2, are the main factors decreasing the fitness of cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae). We used two series of experiments to quantify the growth, development, and consumption of *H. armigera* fed on an artificial diet or C_4 plants (maize) grown under two CO_2 levels (ambient vs. double ambient). In the first series of experiments, elevated CO_2 had no effect on the population abundance or individual consumption for three successive generations of cotton bollworms fed on an artificial diet. In the second series of experiments, elevated CO_2 reduced population abundance of cotton bollworm larvae for two successive generations when they were fed maize milky grains. The specific effects were longer larval duration, lower fecundity, and decreased r_m of cotton bollworms. Furthermore, elevated CO_2 increased individual consumption when cotton bollworm was fed maize milky grains for two successive generations and decreased the population's total consumption in the first generation but increased it in the second generation. The results from this study indicate that: (1) The effects of elevated CO_2 on three successive generations of cotton bollworm fed on artificial diet were weak, or even non-existent, and (2) elevated CO_2 increased the consumption when cotton bollworm were fed maize. Our study also suggests that the damage inflicted by cotton bollworm on maize (a C_4 plant) will be seriously affected by the increases in atmospheric CO_2, which is unlike our previous results for spring wheat (a C_3 plant).

Introduction

The global atmospheric concentration of carbon dioxide (CO_2) has increased from a pre-industrial value of about 280–379 p.p.m. in 2005, and a value of 770 p.p.m. (double the current levels) is expected for 2100 (IPCC, 2007). Such an increase in CO_2 levels affects the biology of living organisms, including insects (Guerenstein & Hildebrand, 2008). Increased concentrations of CO_2 may influence the development of insect herbivores directly or indirectly through the effects of a CO_2-enriched environment on host plant chemistry. Elevated CO_2 generally increases photosynthesis rates, above-ground biomass, yield, and carbon:nitrogen (C:N) ratios and reduces N concentrations, thus impacting the production of plant nutrients (Cotrufo et al., 1998; Pritchard et al., 1999; Agrell et al., 2000;

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Hartley et al., 2000). In turn, lower foliar N and protein concentrations, which cause reductions in leaf nutritional quality (Mattson, 1980; Johns & Hugher, 2002), increase consumption rates, mortality, and development times, and thus decrease the fitness of insect herbivores (Stiling et al., 1999; Whittaker, 1999). Cascade effects of elevated CO$_2$ through plants are often considered to be responsible for the main impacts on the performance of herbivorous insects (Chen et al., 2005a, 2007; Wu et al., 2006). However, the direct effects of elevated CO$_2$ on multiple generations of herbivores, without taking into account the cascade effects of elevated CO$_2$ through plants, are not well known (Rasch & Rembold, 1994; Abrell et al., 2005). However, both direct and indirect effects of elevated CO$_2$ on herbivores should be studied, as such information will help to elucidate the interactions between host plants and phytophadus insects under future CO$_2$-enriched environments.

Cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), is a cosmopolitan phytophagous chewing insect (Zalucki et al., 1986; Zalucki & Furlong, 2005). In northern China, the first generation of cotton bollworm damages wheat, whereas successive generations alternate between maize and cotton (Ge et al., 2003, 2005). Wu et al. (2006) reported that, over three successive generations, consumption per cotton bollworm larva increased greatly after larvae fed on milky grains of spring wheat (a C$_3$ plant) grown under elevated CO$_2$. However, they found that the total consumption of the estimated potential population of cotton bollworm was reduced because of lower fecundity and population density under elevated CO$_2$. Maize [*Zea mays* L. (Poaceae)], a C$_4$ plant, is less sensitive to elevated CO$_2$ than are C$_3$ plants (Cure & Aycock, 1986; Lawlor & Mitchell, 1991; Barbehenn et al., 2004) and is an important host plant for the third and fourth generations of cotton bollworm in northern China (Ge et al., 2005). However, the ways in which elevated CO$_2$ and its cascade effects through maize affect the development and consumption patterns of *H. armigera* are largely unclear.

This study examined how elevated CO$_2$ and its cascade effects through maize influenced the growth, development, and consumption patterns of multiple generations of *H. armigera*. We tested the hypothesis that the cascade effect of elevated CO$_2$ through plants (rather than the direct effect of elevated CO$_2$) is the main factor that decreases the fitness of *H. armigera*. We therefore explored direct and indirect effects of increased environmental CO$_2$ on developmental times and consumption of cotton bollworms fed on artificial diets and on C$_4$ plants (maize) grown in elevated CO$_2$. Our specific objectives were to quantify: (1) direct effects on the growth, development,

and population parameters of three successive generations of *H. armigera* when reared on artificial diets under elevated CO$_2$ and (2) indirect effects on the growth, development, and population parameters of two successive generations of *H. armigera* when reared on elevated CO$_2$-grown maize under elevated CO$_2$.

Materials and methods

CO$_2$ concentration

Open-top chambers. This experiment was carried out using six octagonal open-top chambers (OTC), each 4.2 m in diameter, located at the Observation Station of the Global Change Biology Group, Institute of Zoology, Chinese Academy of Science (CAS) in Sanhe County, Hebei Province, China (35°57′N, 116°47′E). The atmospheric CO$_2$ concentration treatments were: treatment (1) current ambient CO$_2$ levels (375 μl l^{-1}) ('ambient CO$_2$'), and treatment (2) double the current ambient CO$_2$ levels (750 μl l^{-1}) ('elevated CO$_2$'). Three OTCs were used for each CO$_2$ concentration treatment. During the period from seedling emergence to harvesting for maize, CO$_2$ concentrations were monitored and adjusted with an infrared CO$_2$ analyser (Ventostat 8102; Telaire, Goleta, CA, USA) once every 20 min to maintain the CO$_2$ concentrations. The automatic-control system for adjusting the levels of CO$_2$ concentration, as well as specifications for the open-top chambers, are detailed in Chen et al. (2005a,b).

Closed-dynamics CO$_2$ chambers. Insects were reared in growth chambers (HPG280H; Orient Electronic, Haerbin City, China). Growth chamber conditions were maintained at 25 ± 1 °C, 60–70% r.h., L14:D10 photoperiod, and illumination measuring 9 000 lux (supplied by one 260-W fluorescent lamp in each chamber). Two atmospheric CO$_2$ concentrations consisting of the current ambient CO$_2$ levels (375 μl l^{-1}) and double the current ambient CO$_2$ levels (750 μl l^{-1}) were maintained, in order to match the conditions in the OTCs used for maize growth. Three chambers were used for each CO$_2$ treatment. As previously mentioned, CO$_2$ concentrations were automatically monitored and adjusted with an infrared CO$_2$ analyser (Ventostat 8102). A detailed explanation of the methodology employed by the automatic-control system for maintaining and adjusting the CO$_2$ concentrations is described in Chen & Ge (2004).

Maize variety and growth conditions

Maize (Kedan 8) seeds were sown in the six open-top chambers on 10 March 2005 in plastic pots (35 cm height, 45 cm diameter) with five seeds per pot. Fifteen pots were

placed in each OTC. Pot placement was re-randomised in each OTC weekly. Pure CO_2 mixed with ambient air was supplied to the chamber throughout maize development. The crop was irrigated sufficiently every other day using tap water. During the milky-grain stage, maize cobs were harvested from all six OTCs and were frozen at −20 °C until they were supplied to *H. armigera* as food.

Insect stocks

Helicoverpa armigera egg masses were obtained from a laboratory colony maintained by the Insect Physiology Laboratory, Institute of Zoology, at CAS and reared in a growth chamber (HPG280H) using an artificial diet, as described by Wu & Gong (1997), or maize milky grains as food sources Wu et al., 2006; Yin et al., 2009). Conditions in each chamber were maintained at 25 ± 1 °C, 70 ± 10% r.h., and L14:D10 photoperiod.

Insect feeding

Helicoverpa armigera fed on artificial diet. In each CO_2 treatment, first instars obtained from the insect stocks were reared in closed-dynamics CO_2 chambers with 375 or 750 μl l^{-1} CO_2 concentration as described above and fed on artificial diets. Thirty insects were reared individually in a 75-mm-diameter Petri dish, with three replications per treatment (total of 90 insects per treatment). Feeding of fresh artificial diet occurred every other day, with unconsumed artificial diet collected and oven dried at 80 °C for 72 h. Individual consumption (measured in g per larva) was determined at each specific stage (every 2 days. Larval development times were recorded every day. Larval duration was calculated as the period from hatching until pupation and pupal duration was counted as the period from pupation until emergence. Newly-emerged *H. armigera* moths were placed in cages (30 × 30 × 40 cm) for 3 days, and then paired, one female with one male. Females were allowed to oviposit in plastic cups (9 cm in diameter, 15 cm height) covered with degreased cotton netting. On a daily basis, eggs were counted, cotton netting covers were replaced, and the number of hatched eggs per female was recorded. Adult longevity was counted as the period from emergence until death.

Similarly, first instars of second-generation cotton bollworms (obtained from the first generation) were reared individually in 75-mm-diameter Petri dishes with three replications (total of 90 insects per treatment) with the same CO_2 treatments. Life-history parameters of successive second and third generations of cotton bollworms were measured as in the first generation.

Helicoverpa armigera fed on maize grains. In each CO_2 rearing treatment, first instars from the first generation of *H. armigera* feeding on artificial diet were transferred to closed-dynamics CO_2 chambers with 375 or 750 μl l^{-1} CO_2 concentration and fed maize grains grown in an open-top chamber (OTC) with the same CO_2 levels. Feeding of fresh maize grains occurred every other day, with unconsumed maize grains collected and oven dried at 80 °C for 72 h. Life-history parameters and consumption of *H. armigera* moths fed on maize grains were measured daily as for those fed on artificial diets. Three replicates of 30 individuals each were studied for each treatment.

Similarly, first instars of second-generation cotton bollworms that fed on maize grains were reared individually in 75-mm-diameter Petri dishes with three replications (total of 90 insects per treatment) with the same CO_2 treatments. The life-history parameters and consumption of successive second generations of cotton bollworms fed on maize grains were measured as in the first generation described above.

Chemical composition of maize grains

Ears of maize were collected at harvest from the OTCs and frozen at −20 °C for later chemical composition assays. Thirty ears of maize were selected from each of the two CO_2 treatments, on three separate occasions, for a total of 90 ears. Water content, as proportion of fresh weight, was calculated after maize grains had been dried at 80 °C for 72 h. Protein concentration was determined by the Bradford (1976) assay. Total non-structural carbohydrates (mainly starch and sugar) were analyzed by acid hydrolysis using the method of Tissue & Wright (1995). Total amino acids were measured according to the reagent protocol (Nanjing Jiancheng, Nanjing, Jiangsu Province, China). Nitrogen content was assayed using the Kjeltec N analysis (Model 2100; Foss automated Kjeltec instruments, Hoganas, Sweden).

Estimation of intrinsic rate of increase

TWOSEX-MSChart groups the raw data and calculates a number of life-table parameters, for example, age-specific survival rates (l_x) and age-specific fecundity (m_x). The intrinsic rate of increase (r_m), as a composite index of growth, development, and fecundity of the whole population, was estimated by using the iterative bisection method from the Euler–Lotka formula:

$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

with age indexed from 0 (Goodman, 1982). TWOSEX-MSChart (Chi, 2005) is available at: http://140.120.197.173/Ecology/prod02.htm (Chung Hsing University)

and http://nhsbig.inhs.uiuc.edu/wes/chi.html (Illinois Natural History Survey).

Data analysis

Multivariate analysis of variance (MANOVAs) tests (SPSS 10.0 for Windows; SPSS, Chicago, IL, USA) were used to analyze the effects of elevated CO_2 on the chemical composition of maize grains, population indices (mortality, fecundity, r_m), larval lifespan, pupal duration, and adult duration of cotton bollworm.

Repeated-measures ANOVAs were used to analyze the effects of CO_2 levels, generation, and the CO_2*generation interaction on the individual consumption at various developmental stages of *H. armigera* fed on maize grains grown in ambient and elevated CO_2. If variables or their interaction were significant, least significant difference (LSD) tests were used to separate the different levels.

Individual consumption of cotton bollworm on artificial diets or maize grains and total population consumption of cotton bollworm on maize grains through multiple successive generations were factors analyzed by two-way ANOVAs. The differences between means were determined using an LSD test.

Results

Helicoverpa armigera larvae fed on an artificial diet

Larval, pupal, and adult duration, fecundity, r_m, and individual consumption of artificial diet were not significantly influenced by elevated CO_2 levels or the CO_2*generation interaction on *H. armigera* fed on an artificial diet (Tables 1 and 2, Figure 1). However, elevated CO_2 significantly increased mortality of *H. armigera* larvae (Table 1).

Generation significantly influenced *H. armigera*'s adult duration and fecundity (Table 1). Fecundity was significantly lower in the second generation than in the first generation under elevated CO_2 ($F_{1,29} = 9.186$, $P = 0.003$). Adult duration of *H. armigera* larvae was longer in the third generation than in the second generation under ambient CO_2 ($F_{1,80} = 7.521$, $P = 0.008$).

Table 1 MANOVAs for the effects of CO_2 level, generation, and CO_2*generation interaction on life-history and population parameters of *Helicoverpa armigera* fed on an artificial diet

Parameter	CO_2 level		Generation		CO_2*generation	
	$F_{1,12}$	P	$F_{2,12}$	P	$F_{2,12}$	P
Larval period	0.704	0.418	0.472	0.635	0.261	0.775
Pupal period	2.51	0.139	1.756	0.214	0.222	0.804
Adult period	0.75	0.403	6.083	0.015*	0.25	0.783
Mortality	9.29	0.010*	1.355	0.295	0.774	0.483
Fecundity	4.018	0.068	7.84	0.007**	1.86	0.198
r_m	0.188	0.672	1.705	0.223	0.256	0.778

*P<0.05; **P<0.01.

Table 2 Life-history parameters (mean $\pm$ SE) of three successive generations (G1–3) of *Helicoverpa armigera* larvae fed on an artificial diet under two CO_2 levels

Parameter	Ambient CO_2			Elevated CO_2		
	G1	G2	G3	G1	G2	G3
Larval period (days)	10.7 ± 0.58aB	11.4 ± 0.31aB	15.4 ± 0.29aA	13.0 ± 0.49aB	14.6 ± 0.29aB	16.1 ± 0.38aA
Pupal period (days)	9.50 ± 0.16aA	9.46 ± 0.18aA	10.00 ± 0.12aA	9.92 ± 0.17aA	9.80 ± 0.17aA	9.93 ± 0.14aA
Adult period (days)	7.80 ± 0.38aB	7.33 ± 0.28aB	8.92 ± 0.44aA	7.85 ± 0.38aA	7.31 ± 0.34aA	8.23 ± 0.59aA
Mortality	0.4 ± 0.02aA	0.34 ± 0.01bA	0.32 ± 0.04aA	0.44 ± 0.02aA	0.48 ± 0.01aA	0.41 ± 0.07aA
Fecundity	661 ± 103aA	586 ± 91aA	565 ± 124aA	702 ± 93aA	386 ± 84aA	589 ± 159aA
r_m	0.153 ± 0.009aA	0.145 ± 0.01aA	0.134 ± 0.009aA	0.153 ± 0.09aA	0.146 ± 0.01aA	0.130 ± 0.011aA

Means within the same generation but under different CO_2 level followed by different lowercase letters are significantly different; means of each stage duration or life-history parameter across three generations within a CO_2 level followed by different uppercase letters are significantly different (LSD test: P<0.05).
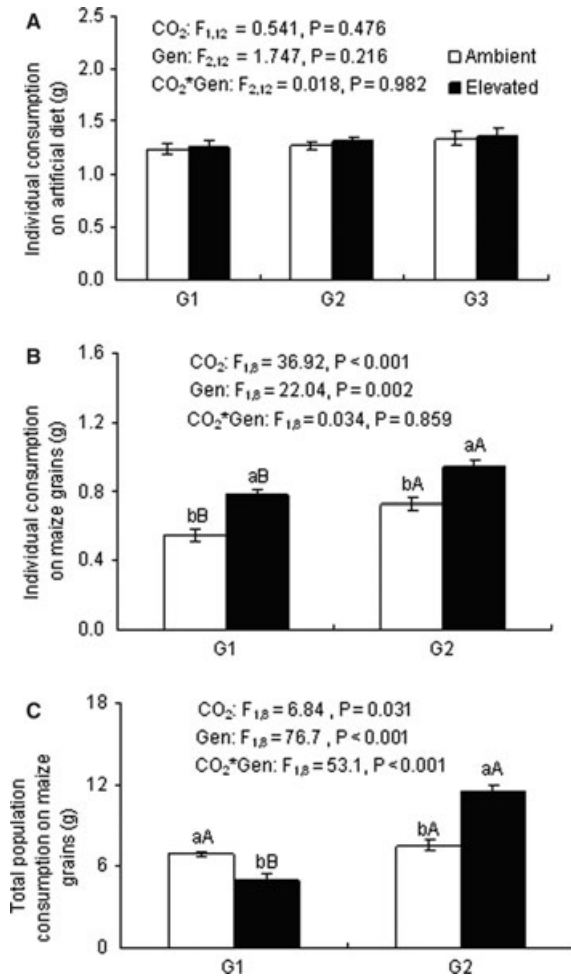
Figure 1 Mean (± SE) individual consumption of *Helicoverpa armigera* (A) for three successive generations (G1–3) fed on an artificial diet in ambient and elevated (750 μl l^{-1}) CO_2 at 29 ± 1 °C, and (B) for two successive generations (G1–2) fed on maize grains grown under the same conditions. (C) Total mean (± SE) population consumption of maize grains (g). Different lowercase letters indicate significant differences between CO_2 treatments and different uppercase letters indicate significant differences across generations (LSD test: P<0.05).

Maize grain quality

Nitrogen content, protein, and total amino acids significantly decreased by 13.8, 18.0, and 55.5%, respectively, but water content and TNC:protein ratio significantly increased by 41 and 33.9%, respectively, in grains of maize plants grown under elevated CO_2 relative to grains grown under ambient conditions (Table 3).

Helicoverpa armigera larvae fed on milky maize grains

Developmental times. CO_2 significantly slowed the development of sixth instar *H. armigera* ($F_{1,277} =$ 42.833, P<0.001) (Table 4). Generation significantly influenced the developmental time of the fourth, fifth, and sixth instar (Table 4). Moreover, elevated CO_2 increased the developmental time of the third-sixth instars and total larval duration of *H. armigera* for the first generation, and also of the sixth instars and total larval duration in the second generation (Table 5). Furthermore, treatment of elevated CO_2 increased the developmental time of the sixth instar and the larval stage with 15% ($F_{1,126} = 125.761$, P<0.001) and 18% ($F_{1,155} = 20.04$, P<0.001), respectively, in the second generation compared with the first.

Population parameters. CO_2 and generation significantly affected mortality of *H. armigera* (Table 4). Elevated CO_2 increased the mortality of two successive generations (first: $F_{1,4} = 48.08$, P = 0.002; second: $F_{1,4} = 62.5$, P = 0.001) (Table 5). Generation increased mortality under both CO_2 levels (ambient: $F_{1,4} = 220.5$, P<0.001; elevated: $F_{1,4} = 21.0$, P = 0.01). Increased mortality and reduced fecundity ($F_{1,4} = 8.729$, P = 0.042) resulted in a decrease of r_m ($F_{1,4} = 30.04$, P = 0.005) of the first generation of *H. armigera*.

Specific-stage individual consumption. CO_2 level ($F_{1,8} = 47.2$, P<0.001) and generation ($F_{1,8} = 22.793$, P = 0.001) significantly influenced the day-specific individual consumption by cotton bollworms every other day (Figure 2). Greater individual consumption was observed on the 13th -14th days of the two successive generations of *H. armigera* under elevated CO_2, than under ambient CO_2 (first generation: $F_{1,4} = 89.47$, P = 0.001; second: $F_{1,4} = 29.26$, P = 0.006) (Figure 2).

Individual consumption. CO_2 level ($F_{1,8} = 46.725$, P<0.001) and generation ($F_{1,8} = 24.505$, P = 0.001) significantly affected the individual consumption for two successive generations of *H. armigera* (Figure 1).

Discussion

The primary objective of this investigation was to analyze the direct and indirect effects of elevated CO_2 on developmental time and consumption of cotton bollworm. Our results clearly show that the direct effect of elevated CO_2 was not significant for the performance of cotton bollworm. Moreover, regardless of CO_2 level, steady population abundance of cotton bollworm and unchanged individual consumption resulted in stable population dynamics of *H. armigera* fed on an artificial diet. By contrast, the indirect effect of elevated CO_2 through the plants

Table 3 Chemical composition (mean $\pm$ SD) of maize grains grown under ambient and elevated CO_2

| Measured indices[1] | CO_2 level | | $F_{1,4}$ | P |
	Ambient	Elevated		
Water (%)	68.9 $\pm$ 0.3b	71.72 $\pm$ 0.6a	17.02	0.015*
Nitrogen (mg g^{-1})	1.52 $\pm$ 0.03a	1.31 $\pm$ 0.01b	41.81	0.003**
TNC[1] (mg g^{-1})	167.5 $\pm$ 2.7b	174.8 $\pm$ 1.64a	5.35	0.082
Protein (g l^{-1})	0.50 $\pm$ 0.01a	0.41 $\pm$ 0.01b	81.63	0.001**
TNC:protein ratio	334.5 $\pm$ 2.6b	448.0 $\pm$ 8.5a	117.65	<0.001***

Means within a row followed by different letters are significantly different (LSD test: P<0.05; after MANOVA: *P<0.05, **P<0.01, ***P<0.001).
[1]Total non-structural carbohydrates.

Table 4 MANOVAs for the effect of CO_2 level, generation, and CO_2*generation interaction on life-history and population parameters of *Helicoverpa armigera* fed on maize grains grown in ambient and elevated CO_2

| Parameter | CO_2 level | | Generation | | CO_2*generation | |
	F	P	F	P	F	P
First instar	0.615	0.434	0.014	0.907	0.479	0.490
Second instar	0.117	0.733	0.045	0.832	0.211	0.647
Third instar	3.15	0.077	0.138	0.711	3.062	0.082
Fourth instar	0.395	0.530	4.823	0.029*	10.567	0.001**
Fifth instar	2.205	0.139	4.632	0.033*	24.012	<0.001***
Sixth instar	34.58	<0.001***	63.13	<0.001***	98.782	<0.001***
Larval period	1.116	0.292	17.44	<0.001***	15.457	0.001**
Pupal period	0.045	0.832	0.427	0.514	0.132	0.717
Adult period	0.194	0.660	2.367	0.125	0.13	0.719
Mortality	113.09	<0.001***	73.09	<0.001***	0.043	0.840
Fecundity	0.488	0.505	1.365	0.276	1.735	0.224
r_m	20.275	0.002**	1.137	0.317	0.543	0.482

*P<0.05; **P<0.01; ***P<0.001.
d.f.: First instar – adult period: 1,210; mortality – r_m: 1,8.

was the most dominant factor affecting the fitness of cotton bollworm.

Herbivorous insects are affected by certain changes in the chemical components of their host plants. Several studies have suggested that elevated CO_2 decreased the protein level but increased the carbohydrate content of some plants (Barbehenn et al., 2004; Taub et al., 2008). Both protein and carbohydrate are two macronutrients that are most important to herbivorous insects. More nutritious host plants have higher levels of protein and non-structural carbohydrates (Mattson, 1980; Lee et al., 2002; Barbehenn et al., 2004). In this study, elevated CO_2 reduced protein level but increased the TNC:protein ratio of maize grains. Thus, our results suggest that elevated CO_2 decreased the nutritional quality of maize grains.

Depending on the nutritional quality of plants, individual species of insects interacting with their host plant have varying responses to elevated CO_2 levels (Hunter, 2001;

Hattenschwiler & Schafellner, 2004; Barbehenn, 2005). In some cases, herbivorous insects exhibit reduced growth, lower survival rates and density, and/or compensatory increases in food consumption resulting from those changes in their host plant (Bezemer et al., 1998; Coviella et al., 2002; Knepp et al., 2005). Goverde & Erhardt (2003) found that elevated CO_2 prolonged the developmental time of *Coenonympha pamphilus* (L.) reared on four species of host plants. Conversely, the difference in chemical composition of the host plant does not always alter growth and development of insects. For example, elevated CO_2 was not significant for larvae of the leaf beetle *Gastrophysa viridula* (De Geer) over three generations when reared on *Rumex obtusifolius* L. (Brooks & Whittaker, 1998). In our second series of experiments, larval duration and mortality increased through two successive generations under elevated CO_2. However, the intrinsic rate of increase was significantly decreased. The results presented

Table 5 Mean (± SE) duration (days) of two successive generations (G1–2) of *Helicoverpa armigera* larvae fed on maize grains grown under ambient and elevated CO_2

	Ambient CO_2		Elevated CO_2	
Parameter	G1	G2	G1	G2
First instar	2.08 ± 0.03aA	2.09 ± 0.03aA	2.09 ± 0.03aA	2.09 ± 0.03aA
Second instar	2.08 ± 0.03aA	2.11 ± 0.03aA	2.11 ± 0.03aA	2.12 ± 0.03aA
Third instar	2.10 ± 0.04bB	2.26 ± 0.06aA	2.53 ± 0.07aA	2.30 ± 0.06aA
Fourth instar	1.92 ± 0.03bB	2.51 ± 0.06aA	2.43 ± 0.06aA	2.36 ± 0.05aA
Fifth instar	1.83 ± 0.04bB	2.88 ± 0.06bA	2.87 ± 0.07aA	2.25 ± 0.05aA
Sixth instar	4.15 ± 0.06bA	3.85 ± 0.10bB	4.53 ± 0.13aB	5.21 ± 0.20 aA
Larval period	14.0 ± 0.12aA	14.1 ± 0.28bA	13.3 ± 0.3aB	15.7 ± 0.34aA
Pupal period	9.44 ± 0.14aA	9.48 ± 0.13aA	9.29 ± 0.12aA	9.41 ± 0.14aA
Adult period	9.71 ± 0.33aA	9.31 ± 0.28aA	9.68 ± 0.36aA	9.03 ± 0.38aA
Mortality	0.09 ± 0.01bB	0.32 ± 0.01bA	0.37 ± 0.04aB	0.6 ± 0.03aA
Fecundity	668 ± 93aA	391 ± 65aB	391 ± 106bA	414 ± 93aA
r_m	0.163 ± 0.006aA	0.136 ± 0.008aB	0.131 ± 0.012bA	0.113 ± 0.012aA

Means within the same generation but under different CO_2 level followed by different lowercase letters are significantly different; means of each stage duration or life-history parameter across two generations within a CO_2 level indicated by different uppercase letters are different (LSD test: $P<0.05$).

here thus indicate that the integrative effect of longer larval duration, higher mortality, and decreased r_m resulted in a decrease in population abundance in cotton bollworm fed on maize through two successive generations.

Most leaf-chewing insects exhibit compensatory increases in food consumption when restricted to suboptimal diets (Masters et al., 1998; Lee et al., 2002). Insects fed on elevated CO_2-grown plants were shown to increase their individual consumption due to the poor food quality of these plants (Watt et al., 1995; Bezemer et al., 1998; Coviella et al., 2000; Hunter, 2001). For example, grasshoppers (Johnson & Lincoln, 1990, 1991) and caterpillar larvae (Lindroth et al., 1993, 1995) generally consume more leaf area when they feed on elevated CO_2-grown plants. Such effects would induce greater crop damage by
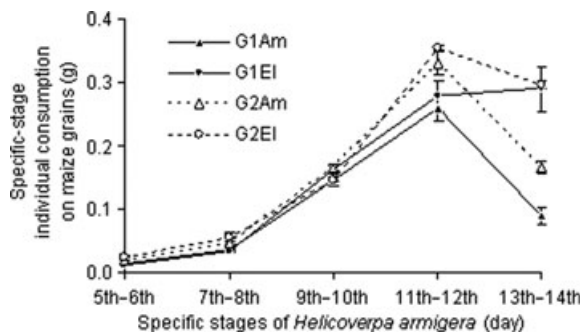

Figure 2 Mean (± SE) individual consumption for specific stages of *Helicoverpa armigera* for two successive generations (G1–2) fed on maize grains grown in ambient (Am: 375 µl l^{-1}) and elevated (El: 750 µl l^{-1}) CO_2 at 29 ± 1 °C.

the pest under elevated CO_2 conditions (Lincoln et al., 1984). One of our previous studies pointed out that elevated CO_2 adversely affects wheat grain quality, which results in an increase in consumption of *H. armigera* larvae; however, the potential population consumption by cotton bollworm was significantly reduced in the latter two generations under elevated CO_2 because of increased mortality and reduced fecundity of *H. armigera* (Wu et al., 2006). These results suggest that net damage of cotton bollworm on wheat will be less under elevated CO_2. In the present study, significantly increased individual consumption was found in two successive generations of *H. armigera* larvae fed on maize grains under elevated CO_2. However, significantly higher mortality (more than four times) and lower fecundity and r_m resulted in significantly lower population abundance in the first generation of cotton bollworm larvae fed on maize grains grown under elevated CO_2. In the second generation, the mortality of cotton bollworm larvae appeared to increase (less than two-fold), while no significant difference was observed in fecundity and r_m of cotton bollworm on maize grains grown under elevated CO_2. Although individual consumption increased significantly in the two successive generations, seriously reduced population abundance resulted in decreased population consumption in the first generation. By contrast, relatively steady population abundance led to increased population consumption by cotton bollworm in the second generation.

C_3 plants have higher nutritional quality than C_4 plants because of their higher nitrogen, protein, and TNC content and lower TNC:protein ratio (Barbehenn et al., 2004).

In our previous study on wheat (a C3 plant; Wu et al., 2006), we indicated that maize (a C$_4$ plant) would continue to be less nutritious than wheat under future atmospheric conditions, as plants with higher protein content and lower TNC:protein ratio are more nutritious for insects (Lincoln et al., 1993; Barbehenn et al., 2004). Meanwhile, the opposite trend of population consumption through multiple successive generations was shown between cotton bollworm larvae fed on wheat and maize. Thus, we speculate that higher consumption by cotton bollworm larvae fed on maize grains through successive generations, compared with that of larvae fed on wheat grains, is presumably due to lower nutritional quality of the host plant.

In this study, we tested the hypothesis that the cascade effect of elevated CO$_2$ through plants is the dominant factor that decreases the fitness of *H. armigera* (rather than the direct effects of elevated CO$_2$) and that the cascade effect of elevated CO$_2$ through C$_4$ plants on *H. armigera* is less than that through C$_3$ plants. The direct effects of elevated CO$_2$ through three successive generations of cotton bollworm fed on artificial diet were weak, or even nonexistent. The indirect effects of elevated CO$_2$ on cotton bollworm fed on maize grains were strong. Under elevated CO$_2$, lower nutritional quality of plants resulted in higher mortality, lower r_m, and unusually higher individual consumption of cotton bollworms. Based on the results of this study, we conclude that population consumption by cotton bollworm on maize will be significantly increased under elevated CO$_2$ in the future.

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