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Response of successive three generations of cotton bollworm, *Helicoverpa armigera* (Hübner), fed on cotton bolls under elevated CO_2

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Abstract

The growth, development and consumption of successive three generations of cotton bollworm, *Helicoverpa armigera* (Hübner), fed on cotton bolls grown under elevated CO_2 (double-ambient vs. ambient) in open-top chambers were examined. Significant decreases in protein, total amino acid, water and nitrogen content and increases in free fatty acid were observed in cotton bolls. Changes in quality of cotton bolls affected the growth, development and food utilization of *H. armigera*. Significantly longer larval development duration in three successive generations and lower pupal weight of the second and third generations were observed in cotton bollworm fed on cotton bolls grown under elevated CO_2. Significantly lower fecundity was also found in successive three generations of *H. armigera* fed on cotton bolls grown under elevated CO_2. The consumption per larva occurred significant increase in successive three generations and frass per larva were also significantly increased during the second and third generations under elevated CO_2. Significantly lower relative growth rate, efficiency of conversion of ingested food and significant higher relative consumption rate in successive three generations were observed in cotton bollworm fed on cotton bolls grown under elevated CO_2. Significantly lower potential female fecundity, larval numbers and population consumption were found in the second and third generations of cotton bollworm fed on cotton bolls grown under elevated CO_2. The integrative effect of higher larval mortality rate and lower adult fecundity resulted in significant decreases in potential population consumption in the latter two generations. The results show that elevated CO_2 adversely affects cotton bolls quality, which indicates the potential population dynamics and potential population consumption of cotton bollworm will alleviate the harm to the plants in the future rising CO_2 atmosphere.

Key words: elevated CO_2; *Helicoverpa armigera*; growth; development; fecundity; cotton bolls; population consumption; successive generation

Introduction

Global atmospheric CO_2 concentration is currently increasing at a rate of approximately 1.5 μl/(L·a) largely owing to combustion of fossil fuels and the clearing of forests, especially in the tropics, and is expected to double within 21st century from its current level of 360 to 700 μl/L (Goverde *et al*., 1999; Wigley and Raper, 1992; Rossi *et al*., 2004).

Profound impacts of elevated CO_2 on terrestrial ecosystem (Dong *et al*., 2006; Yu *et al*., 2006), especially on chemical composition and nutrient quality of plants, are expected (Luo *et al*., 1999; Penuelas *et al*., 2002), that is, significant increases in photosynthesis, growth, water use efficiency, leaf area, yield and decreases in foliar nitrogen of plants, particularly C_3 plants, (Oechel and Strain, 1985; Cure and Aycock, 1986; Bazzaz, 1990; Prtchard *et al*., 1999; Oijen *et al*., 1999). As nitrogen is an important limiting factor for phytophagous insects (Mattson, 1980), and reduction in percent nitrogen alone may have potential effects on insect performance. Most leaf-chewing insects exhibit compensatory increases in food consumption and/or reduced growth, survival rates, and reduction in density, presumably due to increasing foliar C:N ratio (Scriber, 1982; Masters *et al*., 1998). For example, red-headed pine sawfly larvae increase nitrogen utilization efficiency in response to CO_2-mediated declines in foliar nitrogen in loblolly pine (Williams *et al*., 1994). Higher rates of insect mortality have been associated with nutritional deficiency that results from reduced foliar nitrogen concentrations under elevated CO_2 (Brooks and Whittaker, 1999; Stiling *et al*., 1999). 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.

There are few studies to measure the direct effects of

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elevated CO_2 on herbivorous insects development and food consumption. Wu *et al*. (2006a) reported that the life-span of *Helicoverpa armigera* Hübner was delayed and larvae fed more artificial diet and produced more frass under elevated CO_2 compared with those under ambient CO_2. Furthermore, elevated CO_2 marginally influenced the artificial diet-utilization efficiency of *H. armigera* larvae that decreased in relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI). Chen *et al*. (2005d) reported that the direct effects of elevated CO_2 on multiple generations of cotton aphid *Aphis gossypii* were weak, even no existing. Moreover, the impact of elevated CO_2 level on the growth, development and fecundity of *A. gossypii* was mainly indirect, even though the host plants growing under elevated CO_2 levels were directly affected. Most published studies of responses of herbivorous insects to CO_2 rising have been short-term experiments measuring consumption rates and development rates (Watt *et al*., 1995; Bezemer and Jones, 1998). However, experiments conducted over more than one generation reveal differences in responses between generations and also show that the outcome can depend on conditions of host-plant growth (Brooks and Whittaker, 1998, 1999). Wu *et al*. (2006b) reported that significantly longer larval life-span for the third generation and lower pupal weight for all generations were observed in *H. armigera* fed on milky grains of spring wheat grown in elevated CO_2. Moreover, the consumption, frass per *H. armigera* larvae and RCR significantly increased under elevated CO_2 compared to ambient CO_2. The leaf beetle, *Gastrophysa viridula*, was slightly affected by elevated CO_2 on performance after three consecutive generations fed on Rumex plants, despite measurable declines in indices of foliage quality and small eggs indicated at the end of the second generation which led to fewer and smaller larvae in the third generation (Brooks and Whittaker, 1998). Brooks and Whittaker (1999) reported reductions in the survival of spittlebugs nymphs in two sequential generations under elevated CO_2 and declines in the rate of development in consecutive years. Net damage, however, resulted from the interacting dynamics that occur among parameters like consumption per individual, developmental rate, mortality and fecundity, and their collective expression in the population through time is not clear (Wu *et al*., 2006b). Further studies are needed from multigenerational studies to understand integrative effects of elevated CO_2 at individual and population level to develop realistic predictions of long-term population dynamics (Williams *et al*., 1997).

Cotton, a C_3 plant, appears to be particularly responsive to CO_2. Elevated CO_2 (660 μl/L vs. 330 μl/L) led to a 95% yield increase in cotton compared with the average of 31% yield increase in many other plant species (Kimball, 1986). Cotton bollworm, *H. armigera*, is one of the most serious pests of cotton in North China. Despite the need for long-term studies being recognized (Lindroth *et al*., 1995), to date, few published studies were conducted for more than one generation to evaluate its population dynamics (Wu *et al*., 2006b). In this study, we reared three successive generations of cotton bollworm fed on cotton bolls grown under elevated CO_2 to evaluate: (1) the effect of elevated CO_2 on successive three generations of cotton bollworm in an attempt to determine the responses of *H. armigera* through different developmental stages and generations to elevated CO_2; (2) whether the potential population dynamics and potential population consumption alleviated harm to the cotton plant in the future rising CO_2 atmosphere.

1 Materials and methods

1.1 Open-top chamber

This experiment was carried out in six 4.2-m diameter octagonal open-top chambers (OTC) in Sanhe County, Hebei Province, China (35°57′N, 116°47′E). The atmospheric CO_2 concentration treatments were 370 μl/L and 750 μl/L (Chen *et al*., 2004). Three open-top chambers (OTCs) were used for each CO_2 level. During the period from seedling emergence to harvesting, CO_2 concentrations were monitored 24-h d^{-1} and adjusted with an infrared CO_2 analyzer (Ventostat 8102, Telaire Company, USA) once every 20 min to maintain the CO_2 concentrations. Details of the automatic-control system of CO_2 and open-top chambers are provided in Chen *et al*. (2005b) and Chen and Ge (2004).

1.2 Cotton variety and growth condition

Cotton cultivar Simian-3 was planted on May 3 in 2005 with white plastic pots (35 cm diameter and 45 cm height) filled with 8:3:1 (by volume) of loam:cow-dung:earthworm-frass. Eighteen pots were randomly placed in each OTC (total of 54 pots per treatment) and rerandomized once a week to minimize position effects. Pure CO_2 mixed with ambient air was supplied to each OTC for elevated CO_2 treatment from seedling emergence to harvest. The cotton bolls (no-open, diameter >2 cm) were harvested in September and stored in refrigerator at 4°C for the laboratory experiment. No chemical fertilizers or insecticides were used through the duration during the experiment. Open tops of the OTCs were all covered with netting to prevent insect movement.

1.3 Insect stocks

The egg masses of cotton bollworm, *H. armigera*, were obtained from a laboratory colony of Insect Physiology Laboratory, Institute of Zoology, Chinese Academy of Sciences (CAS), and reared in a growth chamber (HPG280H, Orient Electronic Ltd. Co., Harbin, China) using standard artificial diet (Wu and 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 14 h light and 10 h dark at 9000 lx, supplied by twelve 60-W fluorescent lamps in each chamber.

1.4 Insect feeding

First instar larvae obtained from the insect stocks were reared on the stored cotton bolls in a growth chamber (the control conditions as described above). Thirty insects were reared individually in a 75-mm diameter glass dish with 3 replications per treatment (total of 90 insects per treatment).

A sample of cotton bolls (refrigerated bolls from the two CO_2 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 dried in oven at 80°C for 72 h. The fresh insect body weights were measured every other day. Because cotton bollworm larvae tend to eat their sloughs, their cast skins following ecdysis were not easily observed. Larval life-span was calculated as the period from hatch to pupation; pupal weight was measured about 12 h after pupation was noted. The rate of pupation was also recorded.

Adults were sexed upon emergence. The ratio of adult emergence and females were noted. Newly emerged moths were released in cages to mate for 3 d (cage size: 30 cm, 30 cm, and 40 cm in length, width, and height), then paired (one female and one male) and placed in a plastic cup with the size of 9 cm in diameter and 15 cm in height, closed with a net cover of degreased cotton yarn, to oviposit. The resulting eggs were counted and recorded daily. Cotton yarn covers were replaced daily. Hatched eggs paid per female were recorded every day.

First instar larvae of cotton bollworm obtained from 1st generation were reared individually in 75 mm diameter glass dishes with 3 replications (total of 90 insects per treatment) in same CO_2 treatment. The life history parameters of successive 2nd and 3rd generations of cotton bollworm were measured as in the 1st generation described above.

1.5 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 digestibility of cotton bollworm fed on the young bolls collected from the cotton cultivar Simian-3 grown under ambient CO_2 and double-ambient CO_2. Protein, total amino acid, and free fatty acid in cotton bolls were analyzed according to the reagent label directions (Nanjing Jiancheng Ltd. Co., Nanjing, Jiangsu Province, China). Nitrogen content was assayed using a CNH analyzer (Model ANCA-nt; Europa Elemental Instruments, Okehampton, UK).

1.6 Estimation of growth and development indices, and consumption rate

1.6.1 Growth and development indices

Four indices were used to measure the growth and development of cotton bollworms, including larval life-span, pupal weight, survival rate and fecundity.

1.6.2 Indices for larval consumption and utilization

The conventional, ratio-based nutritional indices, including mean relative growth rate (MRGR; Viskari *et al.*, 2000), RGR, RCR, ECI, efficiency of conversion of digested food (ECD), and approximate digestibility (AD), were determined gravimetrically following Waldbauer (1968) and Scriber and Slansky (1981). The amount (mg) of food ingested, frass produced, larval body weight gain were all calculated as dry weight. Equations for calculation of the indices measured are shown in Chen *et al*. (2005a).

1.6.3 Estimation of potential population dynamics and population consumption

Four parameters were used to estimate potential population dynamics and population consumption for the second and third generation of cotton bollworm as follows, including potential initial numbers of larval individuals for the second or third generation, potential population increase index for the second or third generation and potential total larval consumption for the second or third generation were calculated following the methods of Wu *et al*. (2006b).

1.7 Data analysis

T-test (SAS 6.12, SAS Institute Inc. USA, 1996) was used to analyze the effects of elevated CO_2 on the chemical compositions of cotton bolls. Difference between means was compared with least significant difference (LSD) test.

Population indices of the cotton bollworm (i.e. larval life-span, pupal weight, number of eggs laid per female and survival rate) and consumption and frass were analyzed using analysis of variance with CO_2 and generation as sources of variability, where the CO_2 level was main factor and bollworm generation was a sub-factor deployed in a split-plot design. Differences between means were determined using LSD test.

The data for larval consumption and digestibility indices were analyzed using an analysis of covariance (ANCOVA) with initial weight as a covariate for RCR, RGR, and MRGR; food consumption was a covariate for ECI to correct for the effect of variation in the growth and food assimilated on intake and growth (Raubenheimer and Simpson, 1992); and food assimilated was also used as a covariate to analyze the ECD parameter (Hägele and Martin, 1999). The assumption of a parallel slope between covariate and dependent variable was satisfied for each analysis. Means were separated using the LSD test.

2 Results and discussion

2.1 Cotton bolls quality

Significant decreases in water content ($P < 0.05$), nitrogen content ($P < 0.001$), protein ($P < 0.01$), total amino acid ($P < 0.05$) and increases in free fatty acid ($P < 0.001$) were observed in cotton bolls grown under elevated CO_2 (Table 1).

The direct physiological effects of enrich CO_2 atmosphere on individual plant species are becoming increasingly well documented (Luo *et al*., 1999; Chen *et al*.,

Table 1 Effects of elevated CO_2 on the chemical compositions (mean±SE) of cotton bolls grown under ambient CO_2 and elevated CO_2

Indices	CO_2 levels		T(*P*)
	Ambient	Elevated	
Water content (%)	87.9±1.6 a	80.6±2.3 b	4.56 (0.0103)
Nitrogen content (mg/g)	24.8±0.3 a	20.6±0.1 b	13.03 (0.0002)
Protein (g/L)	0.32±0.01	0.27±0.01	5.05 (0.0072)
Total amino acid (μmol/ml)	208±6	176±8	3.30 (0.0299)
Free fatty acid (μmol/ml)	114±1	132±1	14.49 (0.0001)

Means within a row indicated by different letters are significantly different (LSD test, $P < 0.05$).

2004). Elevated CO_2 concentration led to decreases in foliar N and increases in the biomass, C:N ratios, photosynthetic rate, whole plant growth, water use efficiency (WUE), light and nitrogen use efficiencies in the host plants (Masle, 2000; Lin and Wang, 2002). In our studies, the quality of cotton bolls was altered by elevated CO_2. Significant decreases in the protein, total amino acid, water and nitrogen content by 15.8%, 17.7%, 9.1% and 20.6% and increases in free fatty acid by 16.1% were observed in cotton bolls grown under elevated CO_2.

2.2 Growth and development of three generations of *H. armigera*

Table 2 shows that the larval life-span of *H. armigera* fed on cotton boll grown under elevated CO_2 treatment significantly varied among the three generations (F=5.91, df=2, 6, P=0.0382), and larval development duration of successive three generations were also significantly increased in elevated CO_2 treatment compared with ambient CO_2 treatment ($P < 0.05$). Significantly different survival rate values were noted among successive three generations of cotton bollworm for the elevated CO_2 treatment (F=7.8, df=2, 6, P=0.0214) but not for ambient CO_2 treatment (F=4.2, df=2, 6, P=0.0723). Significantly lower survival rate was observed in the second and third generations fed on cotton boll under elevated CO_2 treatment compared with ambient CO_2 treatment ($P < 0.05$).

Table 2 Life history parameters (mean±SE) of successively three generations of cotton bollworm

Generation	Life history parameter	CO_2 level	
		Ambient	Elevated
F1	Larval life-span (d)	10.9±0.1 bA	11.5±0.1 aB
	Pupal weight (g)	0.19±0.00 a	0.19±0.00 a
	Survival rate (%)	81.1±1.9 aA	75.6±1.9 bA
	Number eggs laid per female	416±9 aA	386±5 bA
F2	Larval life-span (d)	10.9±0.1 bA	11.6±0.1 aAB
	Pupal weight (g)	0.19±0.00 a	0.18±0.00 b
	Survival rate (%)	76.7±3.3 aAB	70±3.3 aB
	Number eggs laid per female	413±4 aA	361±9 bB
F3	Larval life-span (d)	10.8±0.1 bA	11.9±0.1 aA
	Pupal weight (g)	0.19±0.00 a	0.17±0.00 b
	Survival rate (%)	75.6±1.9 aB	67.8±1.9 bB
	Number eggs laid per female	405±2 aA	346±6 bB

F1, F2, and F3 are the first, second, and third generations; *H. armigera* fed on cotton bolls grown under ambient CO_2 and elevated CO_2 at 28±1°C (day)/24±1°C (night); means within a row indicated by different lowercase letters are significantly different; means of each life history parameter across three generations within a column indicated by different capital letters are significantly different (LSD test, $P < 0.05$).

The pupal weight was observed significantly different among the successive three generations of cotton bollworm for the elevated CO_2 treatment (F=24.79, df=2, 6, P=0.0013). Significantly lower pupal weight of *H. armigera* was also found in the second (F=21.22, df=1, 4, P=0.01) and third (F=79.4, df=1, 4, P=0.0009) generations fed on cotton boll under elevated CO_2 treatment compared with ambient CO_2 treatment (Table 2).

Number eggs laid per female was significantly reduced in the first (F=8.49, df=1, 4, P=0.0435), second (F=27.73, df=1, 4, P=0.0062) and third (F=85.53, df=1, 4, P=0.0008) generations of cotton bollworm fed on cotton boll grown under elevated CO_2. Significantly different fecundity occurred among successively three generations of cotton bollworm in elevated CO_2 treatment (F=9.14, df=2, 6, P=0.0151). And significantly lower fecundity was found in the second and third generations than in the first generation under elevated CO_2 treatment ($P < 0.05$; Table 2). Plant nitrogen is known to be an important element for insect success (Mattson, 1980) and the reduction in food protein and nitrogen content often leads to poorer insect performance behavioral or physiological adaptation (Scriber and Slansky, 1981). 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 and Jones, 1998). In most herbivorous insect diets, nitrogen content limits insect growth and development (Chen *et al*., 2005c). Insects feeding on CO_2 enriched plant foliage exhibit reduced larval growth (Roth and Lindroth, 1994, 1995), increased leaf consumption (Williams *et al*., 1994), prolonged larval development (Lindroth *et al*., 1997) and reduced female fecundity (Traw *et al*., 1996). The ability of insects to compensate for CO_2-mediated reductions in foliage quality is key to understanding long-term effects on herbivore population (Hunter, 2001). However, most of these published studies focused on response of herbivorous insects to elevated CO_2 in short-term experiments. The emergence of a combination of short- and long-term experiments has provided a clearer picture of insect population dynamics in response to elevated CO_2 (Brooks and Whittaker, 1998). In our studies, CO_2 mediated changes in cotton bolls quality also affected the growth and development of *H. armigera*. The results showed that larval life-span of *H. armigera* increased by 5.49% in first generation, 7.02% in second generation and 10.26% in the third generation, and larval survival rate decreased by 7.35% in first generation, 9.52% in second generation and 11.48% in the third generation under elevated CO_2 compared with ambient CO_2.

Nitrogen and nitrogenous compounds are important for insect fecundity. Buse *et al*. (1998) found that winter Moth *Operophtera brumata* reproduced more eggs when reared on oak *Quercus Robur* leaves grown under elevated CO_2. Other studies reported no effects of elevated CO_2 on fecundity of herbivorous insects (Fajer *et al*., 1989; Brooks and Whittaker, 1998; Marcel and Andreas, 2003). However, our results clearly indicated the fecundity of *H. armigera* decreased by 7.74% in the first generation, 14.23% in the second generation and 16.85% in the third generation, significantly lower fecundity capacity of *H. armigera* was found in the second and third generations under elevated CO_2 compared with ambient CO_2. It is suspected that the reduction of fecundity capacity of *H. armigera* is due to cumulative effects of elevated CO_2, the fecundity capacity is likely to be reduced even further in the next generation. Because precise nutritive requirements for success of most arthropod herbivores (including *H. armigera*) are not known (Heagle *et al.*, 2002), it is possible only to guess which nutritive changes may have affected the fecundity capacity of *H. armigera* in the current study. Thus, to date, no clear pattern of the interaction between insect fecundity and CO_2 enrichment has been found. Fecundity capacity responses appear to be species-specific and may also depend on other unknown factors (Marcel and Andreas, 2003).

2.3 Larval consumption of three generations of *H. armigera*

Consumption per larva of cotton bollworm fed on cotton bolls grown under elevated CO_2 treatment occurred significant increase for the first (F=9.19, df=1, 4, P=0.0387), second (F=42.22, df=1, 4, P=0.0029) and third (F=108.36, df=1, 4, P=0.0005) generations (Fig.1a). Similarly, frass produced per larva were also significantly increased during the second (F=65.00, df=1, 4, P=0.0013) and third (F=55.77, df=1, 4, P=0.0017) generations under elevated CO_2 (Fig.1b).

Most leaf-chewing insects exhibit compensatory increases in food consumption (Scriber, 1982; Masters *et al.*, 1998). These results are generally explained as a response of herbivorous insects to reduced forage quality, especially the reduction in forage nitrogen (Wu *et al.*, 2006a, b). Most of the initial published studies focused on food compensate response of herbivorous insects to elevated CO_2 in short-term experiments. However, the emergence of a combination of short- and long-term experiments has provided a clearer picture of the dynamics in response to elevated CO_2 (Brooks and Whittaker, 1998). In our studies, consumption per larva of *H. armigera* fed on cotton bolls grown under elevated CO_2 treatment increased by 10.4% in the first generation, 10.9% in the second generation and 9.6% in the third generation. These insects seem to compensate for the lower cotton bolls nitrogen concentration by compensatory food consumption under elevated CO_2.

2.4 Indices for larval consumption and utilization of three generations of *H. armigera*

In Table 3, the effects of CO_2 concentration were seen in MRGR for the first (F=33.16, df=1, 4, P=0.0045), second (F=33.25, df=1, 4, P=0.0045) and third (F=90.21, df=1, 4, P=0.0007) generations, with significant decreases under elevated CO_2 treatment compared with ambient CO_2 treatment (P < 0.05), and significant decrease also occurred from the first and second generations to the third generation under elevated CO_2 treatment. Significantly lower RGR was observed in the first (F=18.15, df=1, 4, P=0.0131), second (F=58.70, df=1, 4, P=0.0016) and third (F=103.83, df=1, 4, P=0.0005) generations under elevated CO_2 treatment compared with that under ambient treatment.

RCR was significantly higher under elevated CO_2 treatment compared with that in ambient treatment for the three generations (P < 0.05; Table 3), and the effect of bollworm generation was more pronounced in elevated

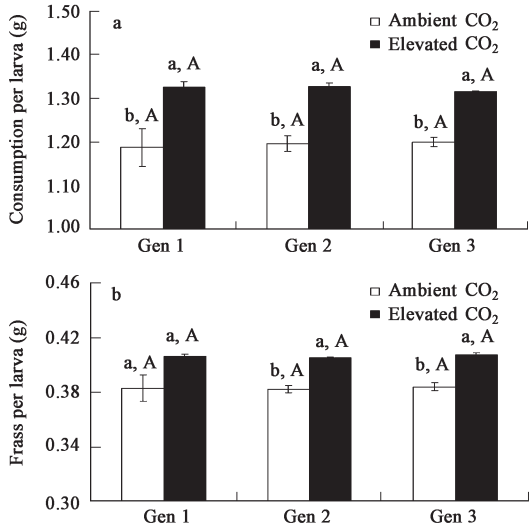

Fig. 1 Consumption (a) and frass (b) of successivel three generations of cotton bolloworm, *H. armigera* fed on cotton bolls grown under ambient CO_2 and elevated CO_2 at 28±1°C (day)/24±1°C (night). Gen 1: the first generation; Gen 2: the second generation; Gen 3: the third generation, different lowercases show significant difference between CO_2 treatments and different capitals indicate significant difference across three generations by LSD test at P < 0.05.

Table 3 Growth indices (mean±SE) of successive three generations of *H. armigera*

Generation	Life history parameter	CO_2 level	
		Ambient	Elevated
F1	MRGR	0.17±0.00aA	0.16±0.00bA
	RGR (mg/(g·d))	90.9±0.5aA	85.9±1.1bA
	RCR (mg/(g·d))	471±6bA	527±6aB
	ECI (%)	19.5±0.1aA	16.4±0.3bA
	ECD (%)	29.0±0.3aA	23.8±0.5bA
	AD (%)	67.9±0.4aA	69.1±0.1aA
F2	MRGR	0.17±0.00aA	0.16±0.00bA
	RGR (mg/(g·d))	90.6±0.2aA	85.9±0.6bA
	RCR (mg/(g·d))	479±1bA	546±3aA
	ECI (%)	19.0±0.1aA	15.8±0.1bA
	ECD (%)	28.0±0.1aA	22.8±0.2bA
	AD (%)	68.1±0.3bA	69.3±0.2aA
F3	MRGR	0.18±0.00aA	0.15±0.00bB
	RGR (mg/(g·d))	91.7±0.8aA	83.3±0.3bB
	RCR (mg/(g·d))	482±6bA	520±6aB
	ECI (%)	19.1±0.3aA	16.0±0.1bA
	ECD (%)	28.2±0.6aA	23.3±0.1bA
	AD (%)	69.8±0.5aA	68.9±0.1aA

F1, F2, and F3 are the same meaning as that in Table 2; *H. armigera* fed on cotton bolls grown under ambient CO_2 and elevated CO_2 at 28±1°C (day)/24±1°C (night); MRGR: 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. Means within a row indicated by different lowercase letters are significantly different; means of each life history parameter across three generations within a column indicated by different capital letters are significantly different (LSD test, P < 0.05).

CO_2 treatment (F=6.71, df=2, 6, P=0.0295) compared with that in ambient CO_2 treatment (F= 92.098, df=2, 6, P=0.3652). The effects of CO_2 concentrations were seen in ECI and ECD for the first (F=101.31, df=1, 4, P=0.0005 and F=78.59, df=1, 4, P=0.0009, respectively), second (F=894.59, df=1, 4, P=0.0001 and F=981.12, df=1, 4, P=0.0001, respectively) and third (F=113.94, df=1, 4, P=0.0004 and F=74.08, df=1, 4, P=0.001, respectively) generations, with significant decrease under elevated CO_2 treatment compared with ambient CO_2 treatment (P < 0.01). However, significantly higher AD was observed in the second (F=10.83, df=1, 4, P=0.0302) generation under elevated CO_2 treatment compared with that under ambient treatment (Table 3).

Scriber and Slansky (1981) reported that water content in host plant was an important factor for the food's digestibility in herbivorous insects, and the reduction in food protein, water and nitrogen content often lead to a poorer insect performance or behavioral or physiological adaptations. The palatability of a plant for herbivorous insects is mainly determined by combination of plant nitrogen and water content. Leaf-chewing herbivores respond to elevated CO_2 by increasing their food consumption and reducing food conversion efficiency (Roth and Lindroth, 1995; Brooks and Whittaker, 1998; Williams *et al*., 1998; Chen *et al*., 2005a). ECI and RCR are two important indices to determine food utilization of insect. Brooks and Whittaker (1998) reported that the efficiency with which leaf beetle *Gastrophysa viridula* convert ingested food into growth (ECI) was significantly reduced by elevated CO_2, but this was only in the third generation for first instar larvae and in the first two generations for third instars. In our studies, for compensation response to low nitrogen food, the ECI of cotton bollworm fed on cotton bolls grown under elevated CO_2 was decreased by 19.0% in the first generation, 20.2% in the second generation and 18.8% in the third generation. However, the RCR of cotton bollworm fed on cotton bolls grown under elevated CO_2 was significantly increased by 11.8% in the first generation, 14.0% in the second generation and 7.9% in the third generation.

2.5 Potential population dynamics and population consumption

In Table 4, significantly lower potential female fecundity, larval numbers and population consumption were found in the second and third generations of cotton bollworm fed on cotton bolls grown under elevated CO_2 treatment compared with ambient CO_2 treatment (P < 0.01). The potential female fecundity, larval numbers and consumption of cotton bollworm fed on cotton bolls grown under elevated CO_2 was reduced by 34.8%, 18.1% and 18.1% in the second generation, and 78.0%, 52.2% and 55.6% in the third generation, respectively (Table 4). Potential population increase index were 115 and 98.9 in the second generation, 113 and 85.8 in the third generation under elevated CO_2 treatment and ambient CO_2 treatment, respectively.

The integrative effect of higher larval mortality rate and lower adult fecundity resulted in potential population decrease in cotton bollworm by 18.1% in the second generation and 52.2% in the third generation under elevated CO_2. Potential population increase index were reduced by 16.8% in the second generation 31.8% in the third generation under elevated CO_2. Ultimately, the effects of increases in atmospheric on crop damage by herbivorous insects will depend upon changes in herbivore performance at the insects potential population levels. The potential population consumption or damage ability of *H. armigera* to compensate for CO_2-mediated reductions in cotton bolls quality is key to understanding long-term effects on cotton bollworm potential population dynamics and the potential injure that will be inflicted upon cotton of economic importance. In accordance to our hypothesis, the potential population consumption of cotton bollworm decreased by 18.0% in the second generation and 55.6% in the third generation because of lower fecundity and potential population numbers under elevated CO_2 compared with ambient CO_2. Thus, the potential population dynamics and potential population consumption of cotton bollworm will alleviate the harm to the plants in the future rising CO_2 atmosphere. The potential population damage mechanisms by cotton bollworm may compensate for CO_2-mediated changes in plant quality are opposite to most of the initial published studies.

3 Conclusions

In this study, significant decreases in protein, total amino acid, water and nitrogen scontent and increases in free fatty

Table 4 Estimations (mean±SE) of potential population dynamics and consumptions of the second (F2) and third generation (F3) of cotton bollworm

Generation	Parameter	CO_2 level	
		Ambient	Elevated
F2	Potential initial numbers of larval individuals	3508±78a	2972±34b
	Potential total eggs laid by all females	480631±15597a	356434±12509b
	Potential population increase index	116±1a	99±2b
	Potential total larval consumption (g)	3256±58a	2759±36b
F3	Potential initial numbers of larval individuals	437779±14206a	287724±10098b
	Potential total eggs laid by all females	54351295±1923875a	30528173±559477b
	Potential population increase index	113±1a	86±2b
	Potential total larval consumption (g)	317015±9110a	185558±6080b

H. armigera fed on cotton bolls grown under two CO_2 atmospheric concentrations; means within a row indicated by different letters are significantly different (LSD test, P < 0.05).

acid were observed in cotton bolls grown under elevated CO_2 compared with ambient CO_2. Nitrogen and nitrogenous compounds are important for herbivorous insects. Changes in nitrogen content of cotton bolls affected the growth, development and food utilization of *H. armigera*. In this present experiment, significantly longer larval development duration in three successive generations and lower pupal weight of the second and third generations were observed in cotton bollworm fed on cotton bolls grown under elevated CO_2. Significantly lower fecundity was also found in successive three generations of *H. armigera* fed on cotton bolls grown under elevated CO_2. The consumption per larva occurred significant increase in successive three generations and frass per larva were also significantly increased during the second and third generations under elevated CO_2.

Changes in nitrogen and water content of cotton bolls also affected the diet-utilization efficiency of herbivorous insects. The effect of CO_2 atmosphere on herbivore food utilization was different along with the insect species and insect stages. For example, the first leaf beetle *G. viridula* larvae relative consumption rate (RCR) was not affected by elevated CO_2, but was significantly increased in the third generation, while the third instars showed the opposite response with RCR being increased by elevated CO_2 in the first two generations but unaffected in the third generation (Brooks and Whittaker, 1998). In this study, significantly lower relative growth rate (RGR), efficiency of conversion of ingested food (ECI) and significant higher relative consumption rate (RCR) in successive three generations were observed in cotton bollworm fed on cotton bolls grown under elevated CO_2. Significantly lower potential female fecundity, larval numbers and population consumption were found in the second and third generations of cotton bollworm fed on cotton bolls grown under elevated CO_2. Consequently it is not surprising that the results obtained using different insect and plant species do not all show the same response to elevated CO_2 (Wu *et al*., 2006b).

This study exemplifies the effect of elevated CO_2 on successive three generations of cotton bollworm in an attempt to determine the responses of *H. armigera* through different developmental stages and generations to elevated CO_2. Measuring the development and food utilization of *H. armigera* over more than one generation provide more meaningful predictions of potential population dynamics and potential population consumption of cotton bollworm on the harm to the plants in the future rising CO_2 atmosphere.

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