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Impacts of elevated CO_2 on *Bemisia tabaci* infesting Bt cotton and its parasitoid *Encarsia formosa*

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Abstract

Atmospheric carbon dioxide concentration is expected to rise in the coming decades. Rising atmospheric CO_2 levels may alter plant-insect-parasitoid associations due to the indirect effects of CO_2 enrichment on phytochemicals important for herbivore and parasitoid nutrition. Tritrophic effects of elevated CO_2 on Bt cotton (GK-12) and non-transgenic (Simian-3, or S3) cotton [*Gossypium hirsutum* L. (Malvaceae)], *Bemisia tabaci* (Gennadius) biotype B (Hemiptera: Aleyrodidae), and its parasitoid *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), were examined in open-top chambers. Significantly, longer egg-adult developmental duration and higher mortality of nymphs were observed under elevated CO_2 concentrations on both cotton cultivars during three successive generations. However, no significant differences were found in adult longevity, offspring sex ratio, and the number of eggs laid per female adult of *B. tabaci* fed on transgenic (GK-12) or non-transgenic cotton (S3) grown under elevated CO_2. Abundance of *B. tabaci* adults increased from 10 to 120 per plant and then decreased to 40 per plant through the growing season, but no significant differences in density occurred between CO_2 treatments and between cultivar treatments. Similarly, no significant differences were found in the developmental duration, parasitization rate, and adult emergence rate of *E. formosa* after parasitizing *B. tabaci* for three successive generations. Our results showed that the effects of transgenic Bt cotton did not significantly affect the development, survivorship, life span, or fecundity of *B. tabaci* and its parasitoids. Moreover, interactions between *B. tabaci* and *E. formosa* were not significantly affected by elevated CO_2. These results suggest that the biological control of *B. tabaci* by *E. formosa* would not be influenced by transgenic Bt cotton and/or elevated CO_2, indicating that the current risk management strategy regarding *B. tabaci* outbreaks and biocontrol by *E. formosa* will remain effective if the atmospheric CO_2 level continues to rise.

Introduction

Agroecosystems are experiencing dynamic changes from external, internal, and endemic sources that must be understood better to inform management strategies that serve human needs. Carbon dioxide (CO_2) in the global atmosphere has increased from a pre-industrial value of about 280–379 p.p.m. in 2005, and 770 p.p.m. (double current levels) can be anticipated by 2100 (Intergovernmental Panel on Climate Change, 2007). Cotton [*Gossypium hirsutum* L. (Malvaceae)], and other transgenic Bt crops expressing a δ-endotoxin from *Bacillus thuringiensis* Berliner (Bt) for the control of lepidopteran pests, has proliferated widely over the past 2 decades (Flint et al., 1996; Coviella et al., 2000; Wu et al., 2008). Investigations of present and anticipated changes on crop plants and their arthropod complexes have shown that effects on production can vary from negative through neutral to positive depending on the conditions, crop, herbivore, and the natural enemy under study. Elevated CO_2 impacts plants, which affects herbivore insects that may then influence natural enemies in the food chain (Chen et al., 2007). A decreased quality of host plant due to an increase in the C:N ratio of foliage grown at elevated CO_2 levels can result in increased food consumption by some leaf-chewing insects

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(Bezemer & Jones, 1998; Coviella et al., 2000) that also exhibit reduced fitness compared to those reared on plants under ambient CO_2 (Williams et al., 1998; Stiling et al., 1999; Yin et al., 2009). Sap-feeding insects may or may not increase under elevated CO_2 (Bezemer et al., 1999; Whittaker, 1999; Newman, 2005; Gao et al., 2008). The effects of elevated CO_2 on interactions among cotton, whitefly, and parasitoids are unknown.

Altered plant nutrition under elevated CO_2 may directly influence the third trophic level (insect parasite and/or predator). Natural enemy performance may be increased (Stiling et al., 1999), decreased (Roth & Lindroth, 1995), or unaffected (Bezemer et al., 1998; Stacey & Fellowes, 2002) under elevated CO_2. Studies of the effects of transgenic crops on non-target organisms have shown variable results. Some studies reported no effect (Dogan et al., 1996; Pilcher et al., 1997; Naranjo, 2005), whereas others demonstrated impact of Bt-crops on non-target insects or natural enemies (Dutton et al., 2002; Wolfenbarger et al., 2008). Effects of Bt-toxin on parasitoids are postulated to occur through the tritrophic plant/herbivore/parasitoid interaction (Bernal et al., 2002; Liu et al., 2005). Further studies are needed including natural enemies that comprise the third trophic level (Lozzia et al., 1998; Bezemer et al., 1999; Stacey & Fellowes, 2002; Ozder & Saglam, 2003; Hoover & Newman, 2004).

The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) B-biotype, has inflicted heavy losses on cotton in China (Chu et al., 2007) and the USA (Williams, 2003). Although *Encarsia formosa* (Gahan) (Hymenoptera: Aphelinidae) is an important biological control agent of the whitefly (Abd-Rabou, 1999), there is no information on the interactions among cotton cultivars, whitefly, and its parasitoid as affected by elevated CO_2 levels. This study was designed to investigate effects of elevated CO_2 on the tritrophic interactions of cotton-herbivorous insects-natural enemies – i.e., transgenic Bt cotton (GK-12) and non-transgenic cotton (Simian-3, or S3), *B. tabaci* biotype B, and *E. formosa*. In open-top chambers, the effect of elevated CO_2 on *B. tabaci* biotype B and *E. formosa* was quantified and the impact of transgenic Bt cotton grown under elevated CO_2 was determined on *B. tabaci* and *E. formosa* life history parameters, including developmental time, fecundity, and population growth.

Materials and methods

Open-top chambers

This experiment was carried out using eight octagonal open-top chambers (OTCs) (4.2 m diameter, 2.4 m high) at the Observation Station of the Global Change Biology Group, Institute of Zoology, Chinese Academy of Science (CAS) in Xiao Tang Shan County, Beijing, China (40°11′N, 116°24′E). The atmospheric CO_2 concentration treatments were: (1) current ambient CO_2 levels (375 μl l^{-1}) ('ambient CO_2') and (2) double the current ambient CO_2 levels (750 μl l^{-1}) ('elevated CO_2'). Four blocks were used for the CO_2 treatment, and each block contained paired OTCs, one with ambient and one with elevated CO_2. During the experiment, CO_2 concentrations were monitored continuously and were adjusted using an infrared CO_2 analyzer (Ventostat 8102; Telaire, Goleta, CA, USA) once every 20 min to maintain the assigned CO_2 concentrations. The automatic control system for adjusting the levels of CO_2 concentration and specifications for the OTCs are described in detail in Chen et al. (2005b).

Cotton treatments

A transgenic Bt cotton cultivar 'GK-12' and a non-Bt-transgenic cultivar 'Simian-3' (S3) from the same recurrent parent line were used. Four treatments associated with CO_2 and cotton cultivars were designed as follows: (1) transgenic Bt cotton grown in ambient CO_2; (2) transgenic Bt cotton grown in double-ambient CO_2; (3) non-Bt-transgenic cotton grown in ambient CO_2; and (4) non-Bt-transgenic cotton grown in double-ambient CO_2. Cotton seeds were sown in white plastic pots (22 cm diameter, 28 cm high) filled with loam (made of 75% soil and 25% cow dung) in the OTCs. The loam chemical composition was 391.2 mg kg^{-1} N, 279.8 mg kg^{-1} P, and 256.3 mg kg^{-1} K (Chen et al., 2004). Each of the eight OTCs contained 30 plants (15 pots of each cotton genotype × two plants per pot), 240 pots in total. Each week, pot placement was re-randomized within each OTC; the plants were used to assess the development of *B. tabaci* and *E. formosa*. Cotton plants were exposed to CO_2 treatment after seedling emergence. The plants were watered with 2 l tap water per pot once every 2 days. Compound fertilizers (5 g) were used once every 2 weeks and no insecticides were used.

Developmental time, fecundity, and adult longevity of *Bemisia tabaci* for three successive generations

Bemisia tabaci biotype B was collected on 5 May 2008 from cabbage at the Agriculture and Forest Academy of Beijing, China. The whitefly offspring were reared on transgenic Bt cultivar 'GK-12' or non-Bt-transgenic cultivar 'Simian-3' (S3) under ambient and elevated CO_2 for one generation. After growing for 8 weeks in each OTC, one pot (two cotton plants) was randomly selected from each cultivar in each OTC, and four leaves from each pot were inoculated with 10 pairs of 3- to 5-day-old whitefly adults (grown on the same cultivar and under the same CO_2 treatment) confined to the leaves by using clip cages (3.5 cm

diameter, 1.5 cm high). The adults were removed after 24 h of infestation and eggs were thinned to 10 eggs per leaf, this constituted the first generation cohort. The developmental time and mortality of *B. tabaci* were then recorded daily with the aid of a microscope until adult eclosion occurred. After adult eclosion, each pair of newly eclosed adults from each treatment was transferred to and confined on an uninfested leaf of the same cotton plant and in the same OTC using a clip cage. If a male died, another healthy male from the same treatment was added immediately. Adult longevity and fecundity for each individual whitefly were recorded daily.

Newly eclosed whitefly adults from the first generation were used to establish a cohort of eggs for the second generation maintained in the same OTC, using the same protocol. Egg cohorts of the previous generation were used to construct the life tables for the subsequent generation. The tops of the OTCs were covered with nylon netting to exclude other insects.

Population dynamics of *Bemisia tabaci* biotype B adult

To assess the density of the *B. tabaci* adults, a total of 20 pots with one plant in each pot for each cultivars were planted in each of the CO_2 treatments; five pots for each cotton cultivar were randomly placed in each OTC and re-randomized once a week to minimize position effects. Plants were grown for 10 weeks in each OTC and then 10 pairs of newly eclosed whitefly adults from the same cultivar and CO_2 treatment were inoculated on each plant in each pot, which was then covered with an air-permeable cellophane bag (25 × 45 cm). The number of adults on each plant was recorded weekly after 14 August, until the plants were dead.

Developmental time, parasitization rate, and emergence rate of *Encarsia formosa* reared on *Bemisia tabaci* for three successive generations

Encarsia formosa was collected on 17 June 2008, from *B. tabaci* biotype B fed on cabbage at the Observation Station in Xiao Tang Shan County, Beijing (40°11′N, 116°24′E). Offspring were reared for one generation on *B. tabaci* biotype B grown on cotton from the four treatments above. Cotton plants were grown for 20 weeks in each OTC. In this sampling section, two pots of each cultivar with two plants of each pot per OTC (16 plants in total for each CO_2 and variety treatments) were randomly selected and three leaves of each plant were inoculated with 10 pairs of whitefly adult, confined to each leaf by clip cages. When whiteflies had grown to the third instar, they were thinned to 20 nymphs per leaf. One pair of newly eclosed *E. formosa* adults reared was transferred to each leaf for a 24-h oviposition period and then the parasitoid was removed under ambient CO_2 (F_A) or elevated CO_2 condition (F_E) to compare their parasitizing ability. There were 48 pairs of newly eclosed *E. formosa* in each CO_2 treatment [4 OTCs × 4 plants (two pots per cultivar with two plants per pot) × 3 leaves per plant], to inoculate third-instar *B. tabaci* at a ratio of 1:20 (parasitoid: host). When the *E. formosa* pupated, the leaves were cut and placed on a plastic plate (9 cm diameter) in environmental chambers at 25 ± 1 °C, 70 ± 5% r.h., and L16:D8 photoperiod. Development, parasitization, and emergence of *E. formosa* reared on *B. tabaci* were checked daily, to calculate developmental time, parasitization rate, and emergence rate. Similarly, on the day of adult parasitoid emergence, one pair of newly eclosed *E. formosa* reared under ambient CO_2 (F_A) or elevated CO_2 conditions (F_E) from the first generation was transferred to each leaf with 20 third-instar *B. tabaci* grown in four treatments (see above) to obtain the successive generations.

Population parameters estimation

Net reproduction (R_0), mean generation time (T), and intrinsic rate of increase (r_m) were analyzed based on the age-stage, two-sex life table model developed by Chi & Liu (1985) and Chi (1988). Means and standard errors of population parameters were estimated using the jackknife method (Sokal & Rohlf, 1995). The computer program TWOSEX-MSChart (Chi, 1988) was developed for data analysis and jackknife estimation in Visual BASIC for the Windows operating system. This program is available at: http://140.120.197.173/Ecology/prod02.htm (Chung-Hsing-University), http://nhsbig.inhs.uiuc.edu/wes/chi.html (Illinois Natural History Survey) (Yin et al., 2009).

Statistical analysis

SPSS 13.0 was used for statistical analysis of factors including CO_2 level and cultivar. Data were ln or arcsine transformed where appropriate to normalize variance. A split–split plot design was used to analyze the univariate responses of life history parameters (e.g., stage-specific developmental duration, mortality, and reproduction) of *B. tabaci* and *E. formosa*. In the following ANOVA model, CO_2 and block (a pair of OTCs with ambient and elevated CO_2) were the main effects, cotton cultivar was the subplot effect, and generation was the sub–subplot effect:

$$X_{ijklm} = \mu + C_i + B(C)_{j(i)} + G_k + CG_{ik} + GB(C)_{kj(i)} + H_l + CH_{il} + HB(C)_{lj(i)} + GHB(C)_{klj(i)} + e_{m(ijkl)}$$

where C is the CO_2 treatment (i = 2), B is the block (j = 4), G is the cotton cultivar genotype (k = 2), and H is the generation (l = 3). X_{ijklm} represents the error because

of the smaller scale differences between samples and variability within blocks (ANOVA; SAS institute, Cary, NC, USA). The effect of block and the interactive effects of block and other factors were not significant (P>0.05), and these are not presented so as to simplify the presentation. Tukey's multiple range tests were used to separate means when ANOVAs yielded significant effects.

Results

Developmental time of *Bemisia tabaci* for three successive generations

CO_2 level significantly affected the developmental time of egg ($F_{1,144}$ = 455.7, P<0.001), nymph ($F_{1,143}$ = 13.8, P<0.001), pupa ($F_{1,143}$ = 5.82, P = 0.017), and total immature stage of *B. tabaci* ($F_{1,143}$ = 12.7, P<0.001) (Table 1). Developmental time and longevity of *B. tabaci* did not differ between cotton cultivars (Table 1). Furthermore, generation significantly influenced the developmental time of egg ($F_{2,144}$ = 602.1), nymph ($F_{2,143}$ = 1434.2), pupa ($F_{2,143}$ = 249.5), total immature stage ($F_{2,143}$ = 1001.3), and longevity of adult females of *B. tabaci* ($F_{2,144}$ = 64.8, all P<0.001).

A significantly long development time was observed in egg ($F_{1,30}$ = 14.1, P<0.001), nymph ($F_{1,30}$ = 14.73, P<0.001), pupa ($F_{1,30}$ = 6.72, P = 0.02), and total immature stage ($F_{1,30}$ = 12.04, P<0.001) for *B. tabaci* fed cotton grown under elevated CO_2 (Table 2). Moreover, significantly long immature developmental time was observed in the first generation [Bt cotton (GK-12): $F_{1,30}$ = 10.74; non-Bt cotton (S3): $F_{1,30}$ = 7.32, both P<0.001], second generation (Bt cotton: $F_{1,30}$ = 13.6; non-Bt cotton: $F_{1,30}$ = 11.8, both P<0.001), and third generation (Bt cotton: $F_{1,30}$ = 12.95; non-Bt cotton: $F_{1,30}$ = 12.55, both P<0.001) for *B. tabaci* fed on cotton in elevated CO_2 compared with cotton in ambient CO_2 (Table 2).

Survivorship of *Bemisia tabaci* for three successive generations

Elevated CO_2 significantly decreased the survivorship of egg ($F_{1,144}$ = 18.30, P<0.001), nymph ($F_{1,144}$ = 6.05, P = 0.015), and egg-adult ($F_{1,144}$ = 29.9, P<0.001) of *B. tabaci*. Moreover, generation significantly influenced survivorship of nymph ($F_{2,144}$ = 70.6) and egg-adult ($F_{2,144}$ = 35.2, both P<0.001) of *B. tabaci*.

In the first generation, a significantly lower survivorship was observed for the egg stage of the *B. tabaci* fed on Bt cotton ($F_{1,30}$ = 9.65) and non-Bt cotton ($F_{1,30}$ = 12.5, both P<0.001), and the immature stage of the *B. tabaci* fed on Bt cotton ($F_{1,30}$ = 18.3) and non-Bt cotton ($F_{1,30}$ = 10.67, both P<0.001) under elevated CO_2. Similarly, the survivorship of eggs of whitefly fed on Bt cotton ($F_{1,30}$ = 10.89, P<0.001) and non-Bt cotton ($F_{1,30}$ = 8.67, P = 0.02) and the survivorship of immature fed on Bt cotton ($F_{1,30}$ = 13.82) and non-Bt cotton ($F_{1,30}$ = 13.26, both P<0.001) were significantly lower in the second generation under elevated CO_2. Also, the survivorship of eggs of whitefly fed on Bt cotton ($F_{1,30}$ = 14.78, P<0.001) and non-Bt cotton ($F_{1,30}$ = 7.64, P = 0.01), and immatures on Bt cotton ($F_{1,30}$ = 11.5) and on non-Bt cotton ($F_{1,30}$ = 10.04, both P<0.001) were significantly lower in the third generation under elevated CO_2 (Figure 1).

Density dynamics of *Bemisia tabaci* biotype B adult

Densities of *B. tabaci* increased from 10 to ca. 120 per plant and 266 then decreased to ca. 40 per plant during 17

Table 1 P values from three-way split-plot ANOVAs indicating the effects of CO_2, cotton cultivar, and their interactions on the developmental time, adult life span, and fecundity of *Bemisia tabaci* biotype B whiteflies for three successive generations

Life history parameter	CO_2[1]	Cultivar[2]	Generation[3]	CO_2*cultivar	CO_2*generation	Cultivar* generation	CO_2*cultivar* generation
Egg (days)	<0.001	0.1	<0.001	0.047	<0.001	0.022	0.50
Nymphal stage (days)	<0.001	0.69	<0.001	0.19	0.092	0.46	0.18
Pupa (days)	0.017	0.096	<0.001	0.76	0.004	0.19	0.14
Egg to adult stage (days)	<0.001	0.13	<0.001	0.13	<0.001	0.46	0.72
Longevity of adult female (days)	0.44	0.11	<0.001	0.50	0.001	0.32	0.33
Longevity of adult male (days)	0.69	0.43	0.071	0.75	0.081	0.70	0.47
Fecundity per female	0.014	0.30	<0.001	0.71	0.002	0.082	0.88
r_m (per day)	0.18	0.65	0.001	0.85	0.084	0.47	0.54
Generation time, T (days)	0.79	0.55	<0.001	0.80	0.001	0.97	0.80
Net reproductive rate per generation, R_o	0.50	0.89	<0.001	0.93	0.38	0.64	0.075

[1]CO_2 levels: ambient vs. double-ambient.
[2]Cultivar: GK-12 vs. S3.
[3]Generation: G1, G2, and G3.

Table 2 Mean (± SE; n = 4) developmental time, adult life span, fecundity, and life-table parameters of *Bemisia tabaci* biotype B whiteflies fed for three successive generations on Bt (GK-12) and non-Bt (S3) cotton cultivars in ambient (375 μl l^{-1}) and elevated CO_2 (750 μl l^{-1})

		Ambient		Elevated	
Generation	Life history parameter	S3	GK-12	S3	GK12
G1	Egg (days)	7.76 ± 0.052aB	7.65 ± 0.036aB	7.98 ± 0.037aA	8.05 ± 0.043aA
	Nymph (days)	10.72 ± 0.124aB	10.69 ± 0.081aB	11.09 ± 0.073aA	10.94 ± 0.100aA
	Pupa (days)	5.117 ± 0.139aA	5.190 ± 0.097aA	4.89 ± 0.129 aA	5.11 ± 0.0689aA
	Egg to adult (days)	23.60 ± 0.067aB	23.54 ± 0.075aB	23.96 ± 0.057aA	24.09 ± 0.067aA
	Longevity of adult female (days)	13.85 ± 0.47aA	13.34 ± 0.41aA	13.43 ± 0.45aA	13.56 ± 0.35aA
	Longevity of adult male (days)	7.74 ± 0.32aA	8.075 ± 0.29aA	7.93 ± 0.29aA	7.83 ± 0.29aA
	Fecundity (no. eggs)	57.16 ± 1.90aA	58.66 ± 1.54aA	58.91 ± 2.40aA	60.56 ± 1.76aA
	r_m	0.1168 ± 0.0051aA	0.1179 ± 0.0051aA	0.1153 ± 0.005aA	0.1161 ± 0.0046aA
	R_0	31.27 ± 4.46aA	31.94 ± 4.35aA	30.06 ± 4.14aA	29.90 ± 4.1aA
	T	29.58 ± 0.35aA	28.97 ± 0.33aA	29.56 ± 0.49aA	30.39 ± 0.35aA
G2	Egg (days)	6.55 ± 0.060aB	6.55 ± 0.041aB	7.02 ± 0.540aA	7.04 ± 0.062aA
	Nymph (days)	10.58 ± 0.097aA	10.59 ± 0.078aA	10.60 ± 0.124aA	10.73 ± 0.093aA
	Pupa (days)	6.37 ± 0.010aA	6.31 ± 0.105aA	6.63 ± 0.104aA	6.61 ± 0.168aA
	Egg to adult (days)	23.50 ± 0.121aB	23.42 ± 0.085aB	24.25 ± 0.091aA	24.37 ± 0.102aA
	Longevity of adult female (days)	11.21 ± 0.35bA	12.29 ± 0.33aA	10.83 ± 0.40aB	11.24 ± 0.42aA
	Longevity of adult male (days)	8.11 ± 0.24aA	8.05 ± 0.26aA	8.19 ± 0.25aA	8.46 ± 0.39aA
	Fecundity (no. eggs)	70.91 ± 2.01aA	65.38 ± 1.83aB	70.41 ± 1.58aA	72.00 ± 1.82aA
	r_m	0.1247 ± 0.0043aA	0.1240 ± 0.0049aA	0.1239 ± 0.005aA	0.1236 ± 0.0045aA
	R_0	40.87 ± 4.97aA	40.44 ± 5.23aA	34.55 ± 4.8aB	36.88 ± 5.09aA
	T	29.81 ± 0.3aA	29.61 ± 0.26aA	29.99 ± 0.31aA	29.19 ± 0.25aA
G3	Egg (days)	7.21 ± 0.534aB	7.05 ± 0.066aB	7.63 ± 0.058aA	7.69 ± 0.068aA
	Nymph (days)	13.54 ± 0.076aB	13.47 ± 0.132aB	13.91 ± 0.092aA	13.94 ± 0.096aA
	Pupa (days)	4.70 ± 0.129aB	4.97 ± 0.152aA	5.11 ± 0.089aA	5.22 ± 0.130aA
	Egg to adult (days)	25.30 ± 0.054aB	25.49 ± 0.079aB	26.65 ± 0.084aA	26.78 ± 0.074aA
	Longevity of adult female (days)	13.85 ± 0.41aA	13.86 ± 0.42aB	14.83 ± 0.45aA	15.79 ± 0.54aA
	Longevity of adult male (days)	7.80 ± 0.36aA	8.10 ± 0.38aA	7.26 ± 0.32aA	7.75 ± 0.32aA
	Fecundity (no. eggs)	77.22 ± 1.73aA	77.94 ± 1.60aA	74.00 ± 1.52bA	80.41 ± 2.00aA
	r_m	0.1163 ± 0.0045aA	0.1191 ± 0.0043aA	0.1137 ± 0.0042aB	0.1153 ± 0.0048aB
	R_0	40.27 ± 5.55aA	44.31 ± 5.62aA	40.49 ± 5.85aA	40.65 ± 5.38aA
	T	31.9 ± 0.37aA	31.85 ± 0.22aA	32.66 ± 0.19aA	32.19 ± 0.29aA

Different upper case letters following means within a row indicate significant differences between CO_2 levels; different lower case letters following means within a row indicate significant differences between cotton cultivars (Tukey test: $P<0.05$).

August to 8 October 2008, but no significant differences in adult density were observed among CO_2 treatments and cultivar treatments (Figure 2).

Developmental time, parasitization rate, and emergence rate of *Encarsia formosa* reared on *Bemisia tabaci* for three successive generations

No significant differences were observed in the developmental time, parasitization rate, and emergence rate of *E. formosa* reared under ambient (Table 3) and elevated CO_2 (Table 4) during the three generations of *B. tabaci* fed on Bt cotton (GK-12) and non-Bt cotton (S3) in ambient and elevated CO_2. The developmental time, parasitization rate, and emergence rate of *E. formosa* did not significantly change with cotton cultivars grown in ambient CO_2 and elevated CO_2 treatments during these three generations. Moreover, CO_2 level and cotton cultivar had no significant main and interaction effect on developmental time, parasitization rate, and emergence rate of *E. formosa* (Table 5).

Population parameters of three successive generations of *Bemisia tabaci* biotype B

CO_2 level significantly affected the fecundity ($F_{1,336} = 6.16$, $P = 0.014$) of *B. tabaci*, but was not significant for the population parameters including R_0, r_m, and T (Tables 1 and 2). Generation significantly influenced all the population parameters of *B. tabaci*. The interaction of CO_2 and generation significantly altered average fecundity ($F_{2,336} = 6.18$, $P = 0.002$) (Table 1). Females laid

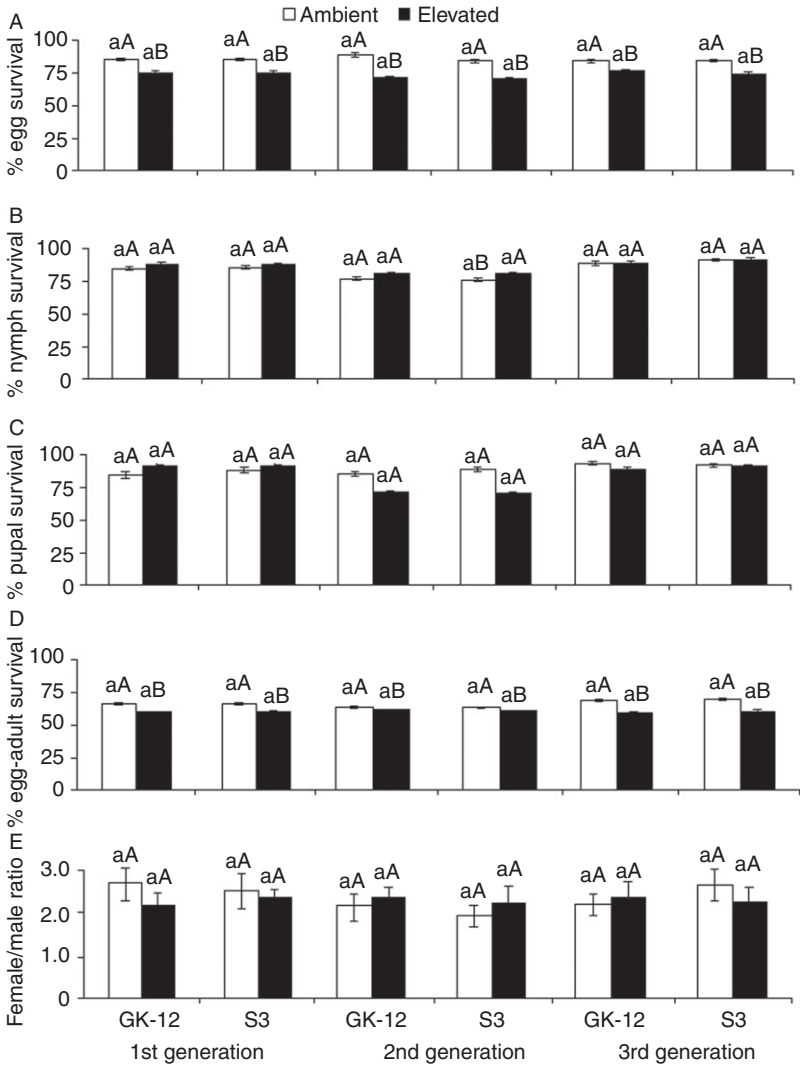

Figure 1 Mean (± SE; n = 4) survivorship of (A) eggs, (B) nymphs, (C) pupae, (D) total egg-adult stage, and (E) female-to-male offspring ratio of *Bemisia tabaci* biotype B whiteflies reared for three successive generations on Bt (GK-12) and non-Bt (S3) cotton cultivars under ambient and elevated CO_2. Different uppercase letters indicate significant differences between CO_2 treatments within cotton cultivars; different lowercase letters indicate significant differences between cotton cultivars within CO_2 level (Tukey test: $P<0.05$).

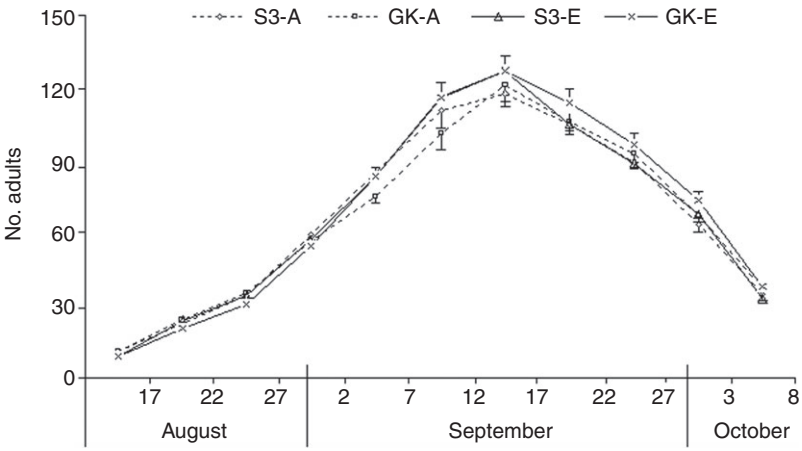

Figure 2 Mean (± SE; n = 4) numbers per plant of *Bemisia tabaci* biotype B whiteflies fed on GK-12 (GK) and Simian-3 (S3) growing under ambient (A; 375 µl l^{-1}) and elevated (E; 750 µl l^{-1}) CO_2.

significantly fewer eggs in the non-Bt cotton (S3) under elevated CO_2 (Table 2).

Discussion

Elevated CO_2 can affect plant quality by inducing changes in allocation to primary and secondary metabolites, which affects tritrophic interactions. Hartley et al. (2000) found that changes in phenolic biosynthesis in response to elevated CO_2 was species-specific (four plant species were studied) and that the responses changed markedly between generations. Variation in plant quality under elevated CO_2 can have a profound impact on herbivore populations by changes in survival, movement, and mortality due to natural enemies. Chewing insects typically have long developmental time and low survivorship, low adult weight, and low fecundity under elevated CO_2 (Williams et al., 1998; Stiling et al., 1999). The performance of sap-feeding aphids may increase, decrease, or be unaffected with elevated CO_2 (Hughes & Bazzaz, 2001; Newman et al., 2003; Chen et al., 2005a; Sudderth et al., 2005; Gao et al., 2008). Whiteflies also differ from aphids in that once the neonate

Table 3 Mean (± SE; n = 4) developmental time, parasitization rate, and emergence rate of *Encarsia formosa* reared under ambient CO_2 for three successive generations on *Bemisia tabaci* biotype B whiteflies fed on Bt (GK-12) or non-Bt (S3) cotton cultivars, in ambient (375 μl l^{-1}) and elevated CO_2 (750 μl l^{-1})

CO_2 level	Cultivar	Generation	Nymphal stage (days)	Pupal stage (days)	Egg to adult stage (days)	Parasitized (%)	Emergence (%)
Ambient	GK-12	G1	9.17 ± 5.44	7.69 ± 0.160	16.86 ± 0.145	29.68 ± 2.01	55.32 ± 4.42
		G2	8.67 ± 0.187	8.13 ± 0.242	16.81 ± 0.093	26.00 ± 1.12	54.20 ± 3.08
		G3	8.94 ± 0.060	7.85 ± 0.114	16.79 ± 0.012	24.06 ± 1.23	52.22 ± 3.76
	S3	G1	9.18 ± 4.63	7.50 ± 8.64	16.67 ± 0.086	35.31 ± 2.68	45.22 ± 3.18
		G2	8.84 ± 0.181	8.22 ± 0.221	17.05 ± 0.145	24.69 ± 1.40	49.12 ± 3.50
		G3	8.95 ± 0.051	7.83 ± 0.129	16.78 ± 0.122	25.63 ± 1.64	58.62 ± 4.25
Elevated	GK-12	G1	9.15 ± 0.05	7.97 ± 0.135	17.12 ± 0.120	30.00 ± 1.88	52.90 ± 5.37
		G2	8.99 ± 0.189	8.07 ± 0.275	17.06 ± 0.119	23.13 ± 1.20	56.67 ± 4.32
		G3	8.83 ± 0.098	8.05 ± 0.144	16.89 ± 0.145	22.82 ± 1.44	50.60 ± 3.29
	S3	G1	9.11 ± 6.36	7.88 ± 0.180	16.99 ± 0.162	28.75 ± 2.39	55.92 ± 5.31
		G2	8.70 ± 0.227	8.12 ± 0.256	16.82 ± 0.134	24.38 ± 1.01	55.52 ± 4.37
		G3	8.87 ± 0.091	8.18 ± 0.112	17.05 ± 0.101	24.69 ± 1.33	52.81 ± 1.44

Means within a column were compared between CO_2 levels and between cotton cultivars, but no significant differences were found (Tukey test: all $P>0.05$).

Table 4 Mean (± SE; n = 4) developmental time, parasitization rate, and emergence rate of *Encarsia formosa* reared under elevated CO_2 for three successive generations on *Bemisia tabaci* biotype B whiteflies fed on Bt (GK-12) or non-Bt (S3) cotton cultivars, in ambient (375 μl l^{-1}) and elevated CO_2 (750 μl l^{-1})

CO_2 level	Cultivar	Generation	Nymphal stage (days)	Pupal stage (days)	Egg to adult stage (days)	Parasitized (%)	Emergence (%)
Ambient	GK-12	G1	9.00 ± 0.037	7.69 ± 0.11	16.69 ± 0.11	25.94 ± 1.31	52.38 ± 4.47
		G2	8.74 ± 0.960	8.18 ± 0.146	16.92 ± 0.136	24.38 ± 1.43	52.38 ± 4.7
		G3	8.53 ± 0.064	8.17 ± 0.159	16.71 ± 0.124	25.00 ± 1.44	51.10 ± 2.66
	S3	G1	8.98 ± 0.034	7.90 ± 0.073	17.09 ± 0.11	27.5 ± 1.88	51.22 ± 3.95
		G2	8.59 ± 0.769	8.22 ± 0.143	16.81 ± 0.013	23.75 ± 1.16	51.22 ± 4.0
		G3	8.71 ± 0.072	7.97 ± 0.015	16.68 ± 0.011	23.44 ± 9.92	50.10 ± 3.49
Elevated	GK-12	G1	9.02 ± 0.060	7.99 ± 0.15	17.02 ± 0.160	25.94 ± 2.10	49.90 ± 2.71
		G2	8.51 ± 0.093	8.39 ± 0.135	16.91 ± 0.188	22.81 ± 1.12	49.90 ± 2.7
		G3	8.64 ± 0.081	8.31 ± 0.162	16.95 ± 0.118	23.44 ± 1.27	55.91 ± 4.40
	S3	G1	9.00 ± 0.042	7.83 ± 0.167	16.83 ± 0.153	24.06 ± 1.78	53.97 ± 3.37
		G2	8.64 ± 0.093	8.30 ± 0.187	16.94 ± 0.156	23.13 ± 1.28	53.97 ± 3.4

Means within a column were compared between CO_2 levels and between cotton cultivars, but no significant differences were found (Tukey test: all $P>0.05$).

Table 5 P values from split-plot ANOVAs indicating the effects of CO_2, cotton cultivar, and their interactions on parasitism parameters of *Encarsia formosa* grown on *Bemisia tabaci* biotype B whiteflies for three successive generations, fed on Bt (GK-12) or non-Bt (S3) cotton cultivars, under ambient (F_A) or elevated CO_2 (F_E)

Parameters	CO_2[1]	Cultivar[2]	Generation[3]	CO_2*cultivar	CO_2* generation	Cultivar* generation	CO_2*cultivar* generation
F_A							
Pre-pupal stage (days)	0.042	0.78	0.82	0.59	0.32	0.44	0.17
Pupal stage (days)	0.10	0.96	0.017	0.74	0.25	0.68	0.94
Egg to adult stage (days)	0.042	0.78	0.82	0.59	0.32	0.44	0.17
Parasitized (%)	0.16	0.41	<0.001	0.15	0.72	0.61	0.22
Emergence (%)	0.65	0.061	0.009	0.40	0.26	0.002	0.23
F_E							
Pre-pupal stage (days)	0.18	0.71	0.49	0.35	0.84	0.75	0.33
Pupal stage (days)	0.11	0.50	<0.001	0.40	0.97	0.66	0.61
Egg to adult stage (days)	0.18	0.71	0.49	0.35	0.84	0.75	0.33
Parasitized (%)	0.20	0.72	0.066	0.90	0.88		0.41
Emergence (%)	0.91	0.55	0.71	0.71	0.92	0.87	0.62

[1]CO_2 levels: ambient vs. double-ambient.
[2]Cultivar: GK-12 vs. S3.
[3]Generation: G1, G2, and G3.

has settled subsequent instars remain in place except for slight movements during their molt into the next instar. Our results showed that CO_2 levels significantly affected the developmental time and survivorship of *B. tabaci*. Elevated CO_2 delayed nymphal developmental time in three successive generations of *B. tabaci* reared on cotton and the pupal developmental time in the second generation, as well as decreased survivorship in the three successive generations of *B. tabaci* feeding on Bt and non-Bt cotton, respectively. As a whole, however, elevated CO_2 did not significantly affect the female-to-male offspring ratio, life span, fecundity, net reproductive rate, mean generation time, and innate capacity for increase. These results suggest that elevated CO_2 exerted very little, if any, impact on whitefly.

Elevated CO_2 effects on plant-herbivore interaction may further influence the biological parameters of parasitoids at the third trophic level. Many studies reported that the effect of elevated CO_2 on growth, development and parasitization rate was either weak or none (Stiling et al., 1999; Stacey & Fellowes, 2002). Also, our previous study indicated that the responses of natural enemies to elevated CO_2 are species-specific. For example, remarkably higher mean relative growth rates were observed in *Harmonia axyridis* (Pallas) ladybeetle larvae under elevated CO_2 treatments (Chen et al., 2005a). No significant differences in survival and lifetime fecundity of the ladybeetle *Propylea japonica* (Thunberg) were seen between cultivars and CO_2 concentration treatments (Gao et al., 2008). The growth and development of *H. axyridis* was weak, whereas the abundance of the parasitoid *Aphidius picipes* (Nees) showed a great increase in 550 (12.5%) and 750 (19.6%) $\mu l\ l^{-1}$ CO_2 compared to ambient CO_2, respectively (Chen et al., 2007). We also observed no effects of elevated CO_2 on the population relationship between the noctuid cotton bollworm, *Helicoverpa armigera* Hübner, and its braconid parasitoid, *Microplitis mediator* Haliday (Yin et al., 2009). Our present study further indicates that the impact of elevated CO_2 on the development, parasitism rate, and eclosion rate of *E. formosa* after parasitizing *B. tabaci* is weak, suggesting the interaction between *B. tabaci* and *E. formosa* may not be affected in the future under elevated CO_2. Tritrophic interactions are complex, and further studies are needed to clarify the impact of elevated CO_2 on tritrophic interactions.

Transgenic Bt cotton appears to be a promising new technology to manage cotton bollworm (Forrester et al., 1993; Wu et al., 2008). It also offers the potential to reduce the total use of broad-spectrum chemical insecticides to control lepidopterous pests (Gary & Fitt, 1994), and may have fewer side effects on non-target organisms (Meeusen & Warren, 1989). The impact on non-target species may be positive due to the reductions of disruptive pesticides (Romeis et al., 2006), or negative due to the effective removal of a lepidopterous host insect in the case of parasitoids or prey in the case of predators (Fitt, 1994). Wilson et al. (1992) suggested that population densities of whitefly on Bt cotton are higher than on the

control cultivars as a consequence of reduced leaf feeding damage by lepidopterous insects. However, Naranjo (2005) in assessing the long-term impact of Bt cotton on 22 taxa of arthropods through a 6-year field study, indicated that *B. tabaci* and natural enemies were unaffected by Bt cotton. Our previous studies showed that Bt cotton had no effect on the natural enemy's community abundance (Men et al., 2003) and population build-up of *P. japonica* (Zhu et al., 2006). In this study, no significant effect of transgenic Bt cotton was observed on the growth, development, fecundity, or density for three successive generations of *B. tabaci* fed on Bt cotton (GK-12). Similarly, the developmental time, parasitization rate, and rate of adult emergence of *E. formosa* did not change, whether its host *B. tabaci* fed on Bt or non-Bt cotton (S3). Our results suggested that transgenic Bt cotton may not have any effects on survival, development, and fecundity of *B. tabaci* biotype B and its parasitoid, *E. formosa* through the food chain. Moreover, these effects may not change in the future under elevated CO_2. These results will help develop strategies for the use of crop plant resistance in integrated pest management under future CO_2-enriched environments.

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References

Abd-Rabou S (1999) Biological control of the cotton whitefly, *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). Egypt Shashpa 6: 53–57.

Bernal CC, Aguda RM & Cohen MB (2002) Effect of rice lines transformed with *Bacillus thuringiensis* toxin genes on the brown planthopper and its predator *Cyrtorhinus lividipennis*. Entomologia Experimentalis et Applicata 102: 21–28.

Bezemer TM & Jones TH (1998) Plant-insect herbivore interactions in elevated atmospheric CO_2: quantitative analyses and guild effects. Oikos 82: 212–222.

Bezemer TM, Jones TH & Knight KJ (1998) Long-term effects of elevated CO_2 and temperature on populations of the peach potato aphid *Myzus persicae and its parasitoid Aphidius matricariae*. Oecologia 116: 128–135.

Bezemer TM, Jones TH & Knight KJ (1999) Long-term effects of elevated CO_2 and temperature on populations of the peach potato aphid *Myzus persicae* and its parasitoid *Aphidius matricariae*. Oecologia 116: 128–135.

Chen FJ, Wu G & Ge F (2004) Impacts of elevated CO_2 on the population abundance and reproductive activity of aphid *Sitobion avenae* Fabricius feeding on spring wheat. Journal of Applied Entomology 128: 723–730.

Chen FJ, Ge F & Parajulee MN (2005a) Impact of elevated CO_2 on tritrophic interaction of *Gossypium hirsutum*, *Aphis gossypii* and *Leis axyridis*. Environmental Entomology 34: 37–46.

Chen FJ, Ge F & Su JW (2005b) An improved open-top chamber for research on the effects of elevated CO_2 on agricultural pests in field. Chinese Journal of Ecology 24: 585–590.

Chen FJ, Wu G, Parajulee MN & Ge F (2007) Impact of elevated CO_2 on the third trophic level: a predator *Harmonia axyridis* (Pallas) and a parasitoid *Aphidius picipes* (Nees). Biocontrol Science and Technology 17: 313–324.

Chi (1988) Life-table analysis incorporating both sexes and variable development rate among individuals. Environmental Entomology 17: 26–34.

Chi H & Liu H (1985) Two new methods for the study of insect population ecology. Bulletin of the Institute of Zoology, Academia Sinica 24: 225–240.

Chu D, Liu GX, Zhong FX & Li TY (2007) Genetic differentiation of different geographical populations of *Bemisia tabaci* (Gennadius) complex. Agricultural Sciences in China 6: 696–705.

Coviella CE, Morgan DJW & Trumble JT (2000) Interactions of elevated CO_2 and nitrogen fertilization: effects on the production of *Bacillus thuringiensis* toxins in transgenic plants. Environmental Entomology 29: 781–787.

Dogan EB, Berry RE, Reed GL & Rossignol PA (1996) Biological parameters of convergent lady beetle (Coleoptera: Coccinellidae) feeding on aphids (Homoptera: Aphididae) on transgenic potato. Journal of Economic Entomology 89: 1105–1108.

Dutton A, Klein H, Romeis J & Bigler F (2002) Prey-mediated effects of *Bacillus thuringiensis* spray on the predator *Chrysoperla carnea* in maize. Biological Control 26: 209–215.

Fitt GP (1994) Field evaluation of transgenic cottons in Australia: environmental environmental considerations and consequences of expanding trial size. Proceedings of the Third International Symposium on Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Monterey, CA, USA 11: 37–48.

Flint HM, Antilla L, Leggett JE & Parks NJ (1996) Seasonal infestation by pink bollworm *Pectinophora gossypiella* (Saunders) of transgenic cotton containing the Bollgard gene, planted in commercial fields in central Arizona. Southwest Entomology 21: 229–235.

Forrester NW, Cahill M, Bird LJ & Layland JK (1993) Management of pyrethroid and endosulfan resistance in *Helicoverpa armigera* (Hübner) in Australia. Bulletin of Entomological Research Supplement Series 1: 1–132.

Gao F, Zhu SR, Du L, Parajulee MN, Kang L & Ge F (2008) Interactive effect of elevated CO_2 and cotton cultivar on tritrophic interaction of *Gossypium hirsutum*, *Aphis gossypii*, and *Propylaea japonica*. Environmental Entomology 37: 29–37.

Gary P & Fitt GP (1994) Field evaluation and potential ecological impact of transgenic cottons (*Gossypium hirsutum*) in Australia. Biocontrol Science and Technology 4: 535–548.

Hartley SE, Jones CG, Couper GC & Jones T (2000) Biosynthesis of plant phenolic compounds in elevated atmospheric CO_2. Global Change Biology 6: 497–506.

Hoover JK & Newman JA (2004) Tritrophic interactions in the context of climate change: a model of grasses, cereal aphids and their parasitoids. Global Change Biology 10: 1197–1208.

Hughes L & Bazzaz FA (2001) Effects of elevated CO_2 on five plant-aphid interactions. Entomologia Experimentalis et Applicata 99: 87–96.

Intergovernmental Panel on Climate Change (2007) Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (ed. by S Solomon, D Qin, M Manning, Z Chen, M Marquis, KB Averyt, M Tignor & Miller HL). Cambridge University Press, Cambridge, UK.

Liu X, Zhang Q, Zhao JZ, Cai Q, Xu H & Li J (2005) Effects of the Cry1Ac toxin of *Bacillus thuringiensis* on *Microplitis mediator*, a parasitoid of the cotton bollworm, *Helicoverpa armigera*. Entomologia Experimentalis et Applicata 114: 205–213.

Lozzia GC, Furlanis C, Manachini B & Rigamonti IE (1998) Effects of Bt corn on *Rhopalosiphum padi* L. (Rhynchota: Aphididae) and on its predator *Chrysoperla carnea* Stephen (Neuroptera: Chrysopidae). Bolletino di Zoologia Agraria e di Bachicoltura 30: 153–164.

Meeusen RL & Warren G (1989) Insect control with genetically engineered crops. Annual Review of Entomology 34: 373–382.

Men XY, Ge F, Liu XH & Yardim EN (2003) Diversity of arthropod communities in transgenic Bt cotton and nontransgenic cotton agroecosystems. Environmental Entomology 32: 270–275.

Naranjo S (2005) Long-term assessment of the effects of transgenic Bt cotton on the abundance of nontarget arthropod natural enemies. Environmental Entomology 34: 1193–1210.

Newman JA (2005) Climate change and the fate of cereal aphids in Southern Britain. Global Change Biology 11: 940–944.

Newman JA, Gibson DJ & Parsons AJ (2003) How predictable are aphid population responses to elevated CO_2. Journal of Animal Ecology 72: 556–566.

Ozder N & Saglam O (2003) Effects of aphid prey on larval development and mortality of *Adalia bipunctata* and *Coccinella septempunctata* (Coleoptera: Coccinellidae). Biocontrol Science and Technology 13: 449–453.

Pilcher SD, Obrycki JJ, Rice ME & Lewis LC (1997) Preimaginal development, survival, and field abundance of insect predators on transgenic *Bacillus thuringiensis* corn. Environmental Entomology 26: 446–454.

Romeis J, Meissle M & Bigler F (2006) Transgenic crops expressing *Bacillus thuringiensis* toxins and biological control. Nature Biotechnology 24: 63–71.

Roth SK & Lindroth RL (1995) Elevated atmospheric CO_2: effects on phytochemistry, insect performance and insect-parasitoid interactions. Global Change Biology 1: 173–182.

Sokal RR & Rohlf FJ (1995) Biometry, 3rd edn. WH Freeman, New York, NY, USA.

Stacey DA & Fellowes ME (2002) Influence of elevated CO_2 on interspecfic interactions at higher trophic levels. Global Change Biology 8: 668–678.

Stiling P, Rossi AM, Hungate B, Dijkstra P, Hinkle CR et al. (1999) Decreased leafminer abundance in elevated CO_2: reduced leaf quality and increased parasitoid attack. Ecological Applications 9: 240–244.

Sudderth EA, Stinson KA & Bazzaz FA (2005) Host-specific aphid population responses to elevated CO_2 and increased N availability. Global Change Biology 11: 1997–2008.

Whittaker JB (1999) Impacts and responses at population level of herbivorous insects to elevated CO_2. European Journal of Entomology 96: 149–156.

Williams MR (2003) Cotton Insect Losses, 2002. Proceedings of the Beltwide Cotton Conferences, Nashville, TN, USA 2003: 6–10.

Williams RS, Lincoln DE & Norby RJ (1998) Leaf age effects of elevated CO_2-grown white oak leaves on spring-feeding lepidopterans. Global Change Biology 4: 235–246.

Wilson FD, Flint HM, Deaton WR, Fischhof DA, Perlak FJ et al. (1992) Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to pink bollworm and other insects. Journal of Economic Entomology 85: 1516–1521.

Wolfenbarger LL, Naranjo SE, Lundgren JG & Bitzer RJ (2008) Bt crop effects on functional guilds of non-target arthropods: a meta-analysis. PLoS ONE 3: 2118.

Wu KM, Lu YH, Feng HQ, Jiang YY & Zhao JZ (2008) Suppression of cotton bollworm in multiple crops in China in areas with Bt toxin-containing cotton. Science 321: 1676–1678.

Yin J, Sun YC, Wu G, Parajulee MN & Ge F (2009) No effects of elevated CO_2 on the population relationship between cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), and its parasitoid, *Microplitis mediator* Haliday (Hymenoptera: Braconidae). Agriculture Ecosystems and Environment 132: 267–275.

Zhu SR, Su JW, Liu XH, Du L, Yardim EN & Ge F (2006) Development and reproduction of *Propylaea japonica* (Coleoptera: Coccinellidae) fed on *Aphis gossypii* (Homoptera: Aphididae) on transgenic cotton. Zoological Studies 45: 98–103.