

Short communication

Impacts of early-season square abscission on the growth and yield of transgenic Bt cotton under elevated CO₂

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Abstract

A field study was carried out to quantify the compensation capacity of *Bacillus thuringiensis* (Bt)-transgenic cotton to simulated damage by manually removing squares during the early growing season in 2004 and 2005 in combination with CO₂ levels (ambient CO₂ and elevated CO₂). Treatments included: initial squares were wholly (100%) removed manually for 1 week (i.e., SR1 treatment) and for 2 consecutive weeks (i.e., SR2 treatment). Plant leaf area was measured every 2 weeks, and plant root, stem, leaf, shatters, boll dry weight and lint yield and maturity were measured at harvest. Significantly higher leaf area per plant was observed on each sampling date for SR1 and SR2 treatments compared with control (SR0) treatment in 2004 and 2005 under elevated CO₂. Significantly higher lint yield and maturity were observed for SR0, SR1 and SR2 treatments under elevated CO₂ in 2004 and 2005. CO₂ concentration and square removal significantly affected plant lint yield and maturity. Moreover, the interaction between CO₂ concentration × square removal had a significant effect on plant leaf dry weight, lint yield and maturity. Our results indicated that transgenic cotton plants can compensate for the manual removal of 100% of the initial squares for 1 and 2 weeks under ambient and elevated CO₂.

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Keywords: Elevated CO₂; Transgenic Bt cotton; Square removal; Maturity; Lint yield

1. Introduction

Current atmospheric carbon dioxide (CO₂) levels are expected to increase from about 350 ppmv to 650 ppmv by the year 2080 (Stacey and Fellowes, 2002), and is anticipated to double by the end of the 21st century. This is primarily the result of anthropogenic activities such as the burning of fossil fuels and changes in land use (Stacey and Fellowes, 2002).

The continuing rise in atmospheric carbon dioxide concentration has been projected to have widespread effects, particularly on plant productivity (Lincoln et al., 1984). The anticipated increase in plant growth and productivity derives from the involvement of carbon dioxide in photosynthesis and the limitation of photosynthesis by the current levels of CO₂ (Kramer, 1981). In general, elevated CO₂ reduces plant water and nitrogen content (Wu et al., 2006, 2007; Chen et al., 2005a) and

increases growth, leaf area, yield and C:N ratios (Oijen et al., 1999; Prtchard et al., 1999). Wang et al. (2001) reported that the biomass production of *Populus tremuloides* was significantly greater at elevated CO₂. Numerous authors also report reduction in leaf mineral concentration (dry weight basis) of rice, wheat, and cotton, grown in enriched CO₂ atmospheres (Aben et al., 1999; Rogers et al., 1993).

Many researchers have investigated the ability of cotton to compensate for square loss under ambient CO₂. However, cotton, as a C₃ plant, appears to be especially responsive to elevated CO₂. Wu et al. (2007) reported significantly higher plant height and leaf area per cotton were observed after cotton plants grown in elevated CO₂ compared with ambient CO₂ for 1, 2 and 3 months in both years' investigation. Yields of C₃ agricultural crops are estimated to increase by about 30% if CO₂ concentration doubles (Kimball, 1983). Determining the response of cotton to square loss under elevated CO₂ is important in developing strategies for future management of cotton ecosystems (Klironomos et al., 2005). Kimball et al. (2002) and Reddy et al. (2000) reported the growth and biomass of cotton responses to elevated CO₂. However, little is known regarding the interaction effects

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between CO₂ concentration and square removal on the yield and maturity of transgenic Bt cotton.

The objectives of this study were to evaluate: (1) the dynamic growth (plant leaf area) of transgenic cotton in response to elevated CO₂; (2) the interaction between atmospheric CO₂ and square removal on plant biomass, yield and maturity.

2. Materials and methods

2.1. Open-top chambers and setup of CO₂ levels

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 CO₂ concentration treatments were 370 μl/L and 750 μl/L (Chen and Ge, 2004). Three OTCs were used for each CO₂ level. During the period from seedling emergence to harvest, CO₂ concentrations were monitored 24-h day⁻¹ and adjusted with an infrared CO₂ analyzer (Ventostat 8102, Telaire Company, USA) once every 20 min to maintain the CO₂ concentrations (Chen et al., 2005b).

2.2. Cultural practice

Transgenic cotton cultivar GK-12 was used in this study and cottonseeds were potted (35 cm diameter: 45 cm height) in the six OTCs on 3 May, and harvested on 23 September in 2004 and 2005, respectively. These white plastic pots were filled with 8:3:1 (by volume) loam:cow dung:earthworm frass. Thirty pots, with one plant per pot, were randomly placed in each OTC and re-randomized daily to minimize position effects. From seedling emergence to harvest, pure CO₂ mixed with ambient air was supplied to each OTC of the elevated CO₂ treatment; the control OTCs received ambient air. No chemical fertilizers or insecticides were used and open tops of these OTCs were all covered with netting to prevent insect infestation.

2.3. Cotton square removal treatment

Hand removal of squares (diameter \geq 3 mm) began on 2 July in 2004 and 2005, respectively, e.g., during the second week after cotton squaring began. Squares that met the size criteria were grasped with index finger and thumb, and twisted until the peduncle snapped and disjoined (Bednarz and Roberts, 2001). Timing of a single manual removal of all squares was carried out on 2 July in 2004 and 2005, respectively, to form the SR1 treatment; squares were removed on 2 July and again on 9 July in 2004 and 2005, respectively, to form the SR2 treatment. All squares on a sympodial branch that met the size criteria, regardless of position, were wholly removed (Bednarz and Roberts, 2001). No squares were manually removed from the control (i.e., SR0 treatment).

2.4. Experimental treatment setup

Two CO₂ levels (ambient and double-ambient) and three types of manual removal of square (SR1 and SR2 versus SR0)

were deployed in a completely randomized design with six treatment combinations: (1) squares removed once in elevated CO₂; (2) squares removed once in ambient CO₂; (3) squares removed twice in elevated CO₂; (4) squares removed twice in ambient CO₂; (5) no square removal in elevated CO₂; (6) no square removal in ambient CO₂. Sample size was 10 pots per OTC with three OTCs for each CO₂ level \times manual removal treatment.

2.5. Data collection

From 9 July to harvest in 2004 and 2005, leaf area per plant was measured once every 15 day using a digital area meter (model CI-202 CID Inc., Camas, WA). At harvest (23 September in 2004 and 2005), cotton root, stem, leaves, lint, shatters (i.e., the leaves, buds and bolls withered and dropped from cotton plants) and bolls were separately collected from each cotton plant, and then oven dried at 80 °C for 48 h to constant weight (Pettigrew et al., 1992), and measured with the Cahn 20 automatic electrobalance (Cahn, St. Louis, MO). The open bolls per plant were collected to estimate cotton maturity (Gore et al., 2000).

2.6. Statistical analyses

All data were analyzed using the General Linear Models Procedure (SAS Institute, 1996). Three-way analysis of variance (ANOVA) was used to analyze the effects of CO₂ level (elevated versus ambient), square removal type (SR1 and SR2 versus SR0) and investigation year (2004/2005) on root, stem, leaf, lint, shatters, boll dry weight and harvested biomass per plant. Three-way analysis of variance (ANOVA) was also used to analyze the effects of CO₂ level, square removal type and investigation year on the leaf area per plant for each sampling date (9 and 24 July, 8 and 23 August and September 7), respectively. Differences among means were determined using a least significant difference (LSD) test at $P < 0.05$.

3. Results and discussion

3.1. Leaf area

In Table 1, the effects of CO₂ concentration and square removal treatment to plant leaf area were significantly influenced on every investigation date ($P < 0.001$). The effect of investigation year significantly affected plant leaf area on 8 August ($P < 0.01$), 23 August ($P < 0.01$) and other investigation dates ($P < 0.001$). The interaction between CO₂ atmosphere \times square removal treatment had a significant effect on plant leaf area for the dates of 24 July ($P < 0.05$), 8 August ($P < 0.05$) and 23 August ($P < 0.05$).

In Table 2, on 9 July, significant differences occurred among square removal treatments under ambient CO₂ ($P < 0.05$) in 2004 and both levels of atmospheric CO₂ concentrations ($P < 0.01$) in 2005. On 24 July, significant differences occurred among square removal treatments under ambient CO₂ ($P < 0.05$) and elevated CO₂ ($P < 0.01$) in 2004 and elevated

Table 1

Three-way ANOVAs of the effects of CO₂ level, square removal treatment, investigation year and their interaction among CO₂ level, square removal treatment and investigation year on transgenic cotton leaf area (*P*-value)

| Sampling date | CO ₂ ^a | Removal ^b | Year ^c | CO ₂ × removal | CO ₂ × year | Removal × year | CO ₂ × removal × year |
|---------------|------------------------------|----------------------|-------------------|---------------------------|------------------------|----------------|----------------------------------|
| 9 July | <0.001*** | <0.001*** | <0.001*** | 0.7381 | 0.1314 | 0.0770 | 0.3090 |
| 24 July | <0.001*** | <0.001*** | <0.001*** | 0.0135* | 0.1034 | 0.2599 | 0.4355 |
| 8 August | <0.001*** | <0.001*** | 0.0014** | 0.0417* | 0.2082 | 0.8881 | 0.6071 |
| 23 August | <0.001*** | <0.001*** | 0.0057** | 0.0348* | 0.9419 | 0.8699 | 0.1490 |
| 7 September | <0.001*** | <0.001*** | <0.001*** | 0.4033 | 0.7965 | 0.2656 | 0.7371 |

^a CO₂ levels (ambient and double-ambient).

^b Square removal treatments (SR0, SR1 and SR2).

^c Investigation years (2004 and 2005).

* *P* < 0.05.

** *P* < 0.01.

*** *P* < 0.001.

CO₂ (*P* < 0.01) in 2005. Significant differences occurred among square removal treatments in both CO₂ treatments on 8 August (*P* < 0.01), 23 August (*P* < 0.01) and 7 September (*P* < 0.05) in 2004 and 2005.

Elevated CO₂ generally results in increased leaf-area index (Dermody et al., 2006) and increased rates of growth (Saxe et al., 1998). Stacey and Fellowes (2002) reported that Brussels sprouts (*Brassica oleracea* L.) grown in elevated CO₂ had more leaves per plant and larger leaf area than plants grown in ambient CO₂. In our studies, significantly higher plant leaf areas were observed on every sampling date in successive years under elevated CO₂ compared with ambient CO₂. However, we also found that plant leaf area exhibited a dynamic change through time under elevated CO₂. For example, plant leaf area showed significant increases at the early and medium time-dependent sampling dates (e.g., from 9 July to 23 August) while decreasing by the later sampling dates (e.g., 7 September), compared with intermediate sampling dates (e.g., 23 August) under elevated CO₂. Additionally, significantly higher plant leaf area was observed in the 100% square removal for 1-week and 2-week treatments, compared with no square removal treatment on every sampling date in successive years under

elevated CO₂, which indicates the compensatory capacity of plant leaf area may depend on the square removal time under elevated CO₂.

3.2. Plant biomass, yield and maturity

In Table 3, the main effects of CO₂ concentration and square removal were significant for all measured indices except the effect of square removal on root biomass. The interaction between CO₂ concentration × square removal treatment had a significant effect on the plant leaf dry weight (*P* < 0.01), lint yield (*P* < 0.05) and boll maturity (*P* < 0.05). The interaction between CO₂ concentration × investigation year had a significant effect on the plant leaf dry weight (*P* < 0.05).

In Table 4, plant root dry weight showed significant differences among square removal treatments under elevated CO₂ (*P* < 0.05) in 2004 and 2005 and under ambient CO₂ in 2005. Significant differences in plant stem dry weight were found among square removal treatments under ambient CO₂ (*P* < 0.01) and elevated CO₂ (*P* < 0.05) in 2004 and both CO₂ levels (*P* < 0.01) in 2005. Plant leaf dry weight was significantly different among square removal treatments under

Table 2

The leaf area per plant (mean ± S.E.) of square removal investigations conducted on every sampling date in ambient and elevated CO₂ during the two successive years of study

| Date | Ambient CO ₂ | | | Elevated CO ₂ | | |
|-------------|-------------------------|------------------------|------------------------|--------------------------|------------------------|------------------------|
| | SR0 (cm ²) | SR1 (cm ²) | SR2 (cm ²) | SR0 (cm ²) | SR1 (cm ²) | SR2 (cm ²) |
| 2004 | | | | | | |
| 9 July | 39.1 ± 0.9 bB | 43.4 ± 0.8 aA | 40.6 ± 0.7 bB | 42.9 ± 0.9 bA | 45.5 ± 0.4 aA | 44.4 ± 0.7 abA |
| 24 July | 63.1 ± 0.5 bB | 65.1 ± 0.2 aB | 65.6 ± 0.7 aB | 67.7 ± 0.3 bA | 70.1 ± 0.7 aA | 71.4 ± 0.3 aA |
| 8 August | 90.7 ± 1.0 bB | 94.2 ± 0.2 aB | 95.5 ± 0.5 aB | 95.7 ± 0.2 bA | 98.9 ± 0.2 aA | 99.2 ± 0.3 aA |
| 23 August | 110 ± 1 bB | 113 ± 0 aB | 114 ± 0 aB | 116 ± 0 bA | 119 ± 0 aA | 120 ± 0 aA |
| 7 September | 83.5 ± 0.5 bB | 88.9 ± 1.0 aB | 87.9 ± 1.1 aB | 89.2 ± 0.7 bA | 93.2 ± 0.7 aA | 91.7 ± 0.6 aA |
| 2005 | | | | | | |
| 9 July | 41.0 ± 0.7 bB | 43.0 ± 0.2 aB | 43.2 ± 0.8 aB | 45.0 ± 0.3 bA | 47.7 ± 0.7 aA | 47.5 ± 1.1 aA |
| 24 July | 64.6 ± 0.8 aB | 65.4 ± 0.4 aB | 65.4 ± 0.5 aB | 69.4 ± 0.5 cA | 71.2 ± 0.7 bA | 72.9 ± 1.0 aA |
| 8 August | 89.4 ± 0.2 bB | 93.9 ± 0.6 aB | 95.1 ± 0.4 aB | 94.5 ± 0.5 bA | 97.5 ± 0.7 aA | 97.5 ± 0.5 aA |
| 23 August | 110 ± 0 bB | 112 ± 0 aB | 113 ± 0 aB | 115 ± 0 cB | 118 ± 0 bB | 120 ± 0 aB |
| 7 September | 79.8 ± 1.1 bB | 83.4 ± 0.4 aB | 82.6 ± 0.4 aB | 85.2 ± 0.4 bA | 87.6 ± 0.5 aA | 87.6 ± 0.7 aA |

SR0: no square removal treatment; SR1: 100% square removal for 1-week treatment; SR2: 100% square removal for 2-week treatment. Within a row, means indicated by different lowercase letters are significantly different at same CO₂ level (LSD test, *P* < 0.05, d.f. = 2, 6); means indicated by different uppercase letters are significantly different in double-ambient CO₂ compared with ambient CO₂ (LSD test, *P* < 0.05, d.f. = 1, 4).

Table 3
Three-way ANOVAs of the effects of CO₂ level, square removal treatment, investigation year and their interaction among CO₂ level, square removal treatment and investigation year on plant root, stem, leaf, shatters, lint yield dry weight and boll maturity at harvest (*P*-value)

| Measured indexes | CO ₂ ^a | Removal ^b | Year ^c | CO ₂ × removal | CO ₂ × year | Removal × year | CO ₂ × removal × year |
|------------------|------------------------------|-----------------------|-------------------|---------------------------|------------------------|----------------|----------------------------------|
| Root | <0.001 ^{***} | 0.06 | 0.14 | 0.84 | 0.58 | 0.85 | 0.72 |
| Stem | <0.001 ^{***} | <0.001 ^{***} | 0.35 | 0.67 | 0.38 | 0.83 | 0.59 |
| Leaf | <0.001 ^{***} | <0.001 ^{***} | 0.25 | 0.002 ^{**} | 0.03 [*] | 0.13 | 0.76 |
| Shatters | <0.001 ^{***} | <0.001 ^{***} | 0.03 [*] | 0.25 | 0.89 | 0.66 | 0.95 |
| Lint yield | <0.001 ^{***} | <0.001 ^{***} | 0.21 | 0.01 [*] | 0.36 | 0.78 | 0.90 |
| Maturity | <0.001 ^{***} | <0.001 ^{***} | 0.90 | 0.03 [*] | 0.35 | 0.60 | 0.35 |

^a CO₂ levels (ambient and double-ambient).

^b Square removal treatments (SR0, SR1 and SR2).

^c Investigation years (2004 and 2005).

* *P* < 0.05.

** *P* < 0.01.

*** *P* < 0.001.

ambient CO₂ (*P* < 0.01) and elevated CO₂ (*P* < 0.05) in 2004 and 2005. Significant differences for plant shatters dry weight were found among square removal treatments under both CO₂ levels (*P* < 0.05) in 2004 and 2005.

Significant differences in plant lint yield were observed among square removal treatments under ambient CO₂ (*P* < 0.01) and elevated CO₂ (*P* < 0.05) in 2004 and 2005. Also, significant differences in plant boll maturity among square removal treatments were observed under ambient CO₂ (*P* < 0.05) and elevated CO₂ (*P* < 0.01) treatments in 2004 and under both CO₂ concentrations (*P* < 0.01) in 2005 (Table 4).

The interaction between cotton square removal treatments (SR0 and SR1) and CO₂ concentrations (ambient CO₂ and elevated CO₂) had a significant effect on plant lint yield, while no interaction was observed between cotton square removal treatments (SR0 and SR2) and CO₂ levels (ambient CO₂ and elevated CO₂) on plant lint yield. The results indicate that early time-dependent square removal had a significant effect on plant lint yield under elevated CO₂. Nevertheless, medium or later time-dependent square injury (100% square removal for

2-week treatment) had no significant effect on plant lint yield under elevated CO₂. Compensation capacity of cotton for square removal may depend on the removal time under elevated CO₂.

Crop maturity is an important factor in making management decisions. The interaction between cotton square removal treatments (SR0, SR1 and SR2) and CO₂ levels (ambient CO₂ and elevated CO₂) had no significant effect on plant boll maturity. However, a significant interaction between cotton square removal treatments (SR0 and SR2) and CO₂ levels was observed for cotton maturity. From this experiment, the interaction between cotton square removal treatments (SR0 and SR1) and CO₂ levels had no significant effect on maturity of plant bolls, which indicates that medium or later time-dependent square removal (SR2) can significantly delay the plant maturity compared with the early time-dependent square removal (SR1) under elevated CO₂. It is anticipated that square removal later than SR2 can even further delay maturity under elevated CO₂. This may have a significant impact on management of early-season pests if rising atmospheric CO₂ concentrations continue.

Table 4
Average (±S.E.) plant root, stem, leaf, shatters, lint yield dry weight and boll maturity of transgenic cotton treated by manual removal of square under ambient and elevated CO₂ in 2004 and 2005

| Year | Measured indexes | Ambient CO ₂ | | | Elevated CO ₂ | | |
|------|------------------|-------------------------|---------------|----------------|--------------------------|----------------|----------------|
| | | SR0 | SR1 | SR2 | SR0 | SR1 | SR2 |
| 2004 | Root (g) | 16.9 ± 0.7 aA | 18.2 ± 0.9 aA | 18.1 ± 0.3 aA | 17.6 ± 0.2 bA | 18.8 ± 0.3 aA | 18.6 ± 0.2 aA |
| | Stem (g) | 46.0 ± 0.4 bB | 47.9 ± 0.2 aB | 47.9 ± 0.2 aB | 47.8 ± 0.2 bA | 49.5 ± 0.3 aA | 49.5 ± 0.5 aA |
| | Leaf (g) | 12.2 ± 0.1 bB | 12.9 ± 0.2 aB | 13.1 ± 0.1 aB | 13.9 ± 0.1 bA | 14.2 ± 0.1 abA | 14.3 ± 0.0 aA |
| | Shatters (g) | 9.7 ± 0.1 aB | 9.4 ± 0.1 abB | 9.2 ± 0.1 bB | 11.0 ± 0.1 aA | 10.7 ± 0.1 abA | 10.3 ± 0.2 bA |
| | Lint yield (g) | 15.8 ± 0.1 bB | 17.4 ± 0.1 aB | 17.1 ± 0.3 aB | 17.8 ± 0.2 bA | 18.7 ± 0.0 aA | 18.5 ± 0.2 aA |
| | Maturity (%) | 83.7 ± 0.3 aB | 81.4 ± 1.0 bB | 79.8 ± 0.2 bB | 89.3 ± 0.2 aA | 86.7 ± 0.5 bA | 83.7 ± 0.5 cA |
| 2005 | Root (g) | 16.8 ± 0.2 bA | 17.9 ± 0.2 aA | 17.7 ± 0.4 abA | 17.1 ± 0.3 bA | 17.9 ± 0.3 abA | 18.4 ± 0.3 aA |
| | Stem (g) | 46.1 ± 0.2 bB | 47.8 ± 0.3 aB | 47.9 ± 0.2 aB | 48.0 ± 0.1 bA | 50.2 ± 0.3 aA | 49.5 ± 0.3 aA |
| | Leaf (g) | 12.1 ± 0.1 cB | 12.8 ± 0.1 bB | 13.4 ± 0.1 aB | 13.6 ± 0.2 bA | 13.9 ± 0.1 abA | 14.2 ± 0.1 aA |
| | Shatters (g) | 9.9 ± 0.1 aB | 9.6 ± 0.1 abB | 9.3 ± 0.1 bB | 11.3 ± 0.3 aA | 10.9 ± 0.2 abA | 10.4 ± 0.0 bA |
| | Lint yield (g) | 15.9 ± 0.3 bB | 17.5 ± 0.1 aB | 17.0 ± 0.2 aB | 18.2 ± 0.1 bA | 18.9 ± 0.1 aA | 18.6 ± 0.2 abA |
| | Maturity (%) | 84.0 ± 0.6 aB | 82.1 ± 0.6 aB | 79.8 ± 0.5 bB | 89.7 ± 0.3 aA | 87.9 ± 0.3 bA | 83.0 ± 0.6 cA |

SR0: no square removal treatment; SR1: 100% square removal for 1-week treatment; SR2: 100% square removal for 2-week treatment. Within a row, means indicated by different lowercase letters are significantly different at same CO₂ level (LSD test, *P* < 0.05, d.f. = 2, 6); means indicated by different uppercase letters are significantly different in double-ambient CO₂ compared with ambient CO₂ (LSD test, *P* < 0.05, d.f. = 1, 4).

Pre-bloom square loss is mostly caused by insect injury (Stewart et al., 2001). Cotton bollworm, *H. armigera* is a damaging pest of cotton throughout China. Our results showed that significant increases in transgenic cotton lint yield and boll weight are observed when squares were manually removed for 1-week (SR1 treatment) relative to no square removal (SR0) under elevated CO₂, while no difference occurred between no square removal (SR0) and 100% square removal for 2-week (SR2) under elevated CO₂. The results suggest that transgenic cotton can tolerate at least modest levels of early-season square injury without yield reduction under elevated CO₂. Transgenic cotton had the ability to overcompensate for early-season square removal by cotton bollworm damage under elevated CO₂. Thus, there are negative effects that may result from early-season insecticide applications made to “eliminate” this loss. The economic thresholds in early-season cotton are based largely on square retention and the potential for plant recovery to injury (Mi et al., 1998). This study indicates the development of a dynamic treatment threshold for early-season squares attacked by cotton bollworm is needed in a rising CO₂ environment. Modifying pest management approaches will reduce the risk of transgenic cotton yield reduction, pest resistance and environmental contamination in Northern China.

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References

- Aben, S.K., Seneweera, S.P., Ghannoum, O., Conroy, J.P., 1999. Nitrogen requirements for maximum growth and photosynthesis of rice, *Oryza sativa* L. cv. Jarrah grown at 36 and 70 Pa CO₂. *Aust. J. Plant Physiol.* 26, 759–766.
- Bednarz, C.W., Roberts, P.M., 2001. Spatial yield distribution in cotton following early-season floral bud removal. *Crop Sci.* 41, 1800–1808.
- Chen, F.J., Ge, F., 2004. An experimental instrument to study the effects of changes in CO₂ concentrations on the interactions between plants and insects—CDCC-1 chamber. *Entomol. Knowl.* 41, 37–40 (in Chinese).
- Chen, F.J., Wu, G., Ge, F., Parajulee, M.N., Shrestha, R.B., 2005a. Effects of elevated CO₂ and transgenic Bt cotton on plant chemistry, performance, and feeding of an insect herbivore, the cotton bollworm. *Entomol. Exp. Appl.* 115, 341–350.
- Chen, F.J., Ge, F., Su, J.W., 2005b. An improved top-open chamber for research on the effects of elevated CO₂ on agricultural pests in field—improved open-top chamber. *Chin. J. Ecol.* 24 (5), 585–590 (in Chinese).
- Dermody, O., Long, S.P., Delucia, E., 2006. How does elevated CO₂ or ozone affect the leaf-area index of soybean when applied independently? *New Phytol.* 169, 145–155.
- Gore, J., Leonard, B.R., Burris, E., Cook, D.R., Fife, J.H., 2000. Maturity and yield response of non-transgenic and transgenic Bt cotton to simulated bollworm injury. *J. Cot. Sci.* 4, 152–160.
- Kimball, B.A., 1983. Carbon dioxide and agricultural yield: an assemblage and analysis of 430 prior observations. *Agron. J.* 75, 779–788.
- Kimball, B.A., Kobayashi, K., Bindi, M., 2002. Responses of agricultural crops to free-air CO₂ enrichment. *Adv. Agron.* 77, 293–368.
- Klironomos, J.N., Michael, F.A., Matthias, C.R., Jeff, P., Scokouh, M.N., Benjamin, E.W., Jeff, R.P., 2005. Abrupt rise in atmospheric CO₂ overestimates community response in a model plant-soil system. *Nature* 43, 621–624.
- Kramer, P.J., 1981. Carbon dioxide concentration, photosynthesis and dry matter production. *Bioscience* 31, 29–33.
- Lincoln, D.E., Sionit, N., Strain, B.R., 1984. Growth and feeding response of *Pesudoplusia includens* (Lepidoptera: Noctuidae) to host plants grown in controlled carbon dioxide atmospheres. *Environ. Entomol.* 13, 1527–1530.
- Mi, S., Danforth, D.M., Tugwell, N.P., Cochran, M.J., 1998. Plant-based economic injury level for assessing economic threshold in early-season cotton. *J. Cot. Sci.* 2, 35–52.
- Oijen, M.V., Schapendonk, A.H.C.M., Jansen, M.J.H., Pot, C.S., Maciorowski, R., 1999. Do open-top chambers overestimate the effects of rising CO₂ on plants. An analysis using spring wheat? *Glob. Change Biol.* 5, 411–421.
- Pettigrew, W.T., Heitholt, J.J., Meredith, W.R., 1992. Early-season floral bud removal and cotton growth, yield and fiber quality. *Agron. J.* 84, 209–214.
- Prtchard, S.G., Rogers, H.H., Prior, S.A., Peterson, C.M., 1999. Elevated CO₂ and plant structure: a review. *Glob Change Biol.* 5, 807–837.
- Reddy, K.R., Hodges, H.F., Kimball, B.A., 2000. Crop ecosystem responses to global change: cotton. In: Reddy, K.R., Hodges, H.F. (Eds.), *Climate Change and Global Crop Productivity*. CABI Publishing, New York, pp. 161–187.
- Rogers, G.S., Payne, L., Milham, P., Conroy, J., 1993. Nitrogen and phosphorus requirements of cotton and wheat under changing atmospheric CO₂ concentrations. *Plant soil.* 155/156, 231–234.
- SAS Institute Inc., 1996. SAS/STAT Software: Changes and Enhancements Through Release 6.12. SAS Institute Inc., Cary, NC.
- Saxe, H., Ellsworth, D.S., Heath, J., 1998. Tree and forest functioning in an enriched CO₂ atmosphere. *New Phytol.* 139, 393–436.
- Stacey, D.A., Fellowes, M.D.E., 2002. Influence of elevated CO₂ on interspecific interactions at higher trophic levels. *Glob. Change Biol.* 8, 668–678.
- Stewart, S.D., Layton, M.B., Williams, M.R., Ingram, D., Maily, W., 2001. Response of cotton to prebloom square loss. *J. Econ. Entomol.* 94 (2), 388–396.
- Wang, X.Z., Curtis, P.S., Vogel, C.S., 2001. Effects of soil fertility and atmospheric CO₂ enrichment on leaf, stem and root dark respiration of *Populus tremuloides*. *Pedosphere* 11 (3), 199–208.
- Wu, G., Chen, F.J., Ge, F., 2006. Response of multiple generations of cotton bollworm *Helicoverpa armigera* Hübner, feeding on spring wheat, to elevated CO₂. *J. Appl. Entomol.* 130 (1), 2–9.
- Wu, G., Chen, F.J., Ge, F., Sun, Y.C., 2007. Effects of elevated CO₂ on the growth and foliar chemistry of transgenic Bt cotton. *J. Integr. Plant Biol.*, in press.