Response of amino acid changes in *Aphis gossypii* (Glover) to elevated CO_2 levels

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Abstract

Effects of elevated CO_2 levels on the amino acid constituents of cotton aphid, *Aphis gossypii* (Glover), fed on transgenic *Bacillus thuringiensis* (Berliner) (Bt) cotton [*Cryl A(c)*], grown in ambient and double-ambient CO_2 levels in closed-dynamics CO_2 chambers, were investigated. Lower amounts of amino acids were found in cotton phloem under elevated CO_2 than under ambient CO_2 levels. However, higher amounts of free amino acids were found in *A. gossypii* fed on elevated CO_2-grown cotton than those fed ambient CO_2-grown cotton, and the contents of amino acids in honeydew were not significantly affected by elevated CO_2 levels. A larger amount of honeydew was produced by cotton aphids feeding on leaves under elevated CO_2 treatment than those feeding on leaves under ambient CO_2 treatment, which indicates that *A. gossypii* ingests more cotton phloem because of the higher C:N ratio of cotton phloem under elevated CO_2 levels. Moreover, the amino acid composition was similar in bodies of aphids ingesting leaves under both CO_2 treatments, except for two alkaline amino acids, lysine and arginine. This suggests that the nutritional constitution of the phloem sap was important for *A. gossypii*. Our data suggest that more phloem sap will be ingested by *A. gossypii* to satisfy its nutritional requirement and balance the break-even point of amino acid in elevated CO_2. Larger amounts of honeydew produced by *A. gossypii* under elevated CO_2 will reduce the photosynthesis and result in the occurrence of some *Entomophthora* spp.

Introduction

Global atmospheric CO_2 concentration is predicted to increase from the current level of 350 ppm to about 650 ppm by the year 2080 (Houghton et al. 2001; Wigley and Raper 2001; Intergovernmental Panel on Climate Change 2007). Recent researches indicate that elevated CO_2 has profound effects on the physiological characteristics of plants (Poorter et al. 1997; Curtis and Wang 1998). Many reports have also documented the impacts of elevated CO_2 on plant–insect interactions (Hunter 2001; Holopainen 2002). Enriched atmospheric CO_2 can speed up plant growth and alter levels of leaf nutrients and defensive chemicals (Lindroth et al. 1995; Koricheva et al. 1998; Hartley et al. 2000). CO_2-induced changes in levels of foliar nutrients and secondary substances can alter host plant quality, which defines the suitability of host plants for the associated herbivores (Agrell et al. 2000; Goverde and Erhardt 2003). Moreover, it has long been recognized that the decrease in plant foliar nitrogen and increase in C:N ratio may influence the total amount of nitrogen (nitrogen quantity) and the composition of nitrogenous compounds (nitrogen quality), which may ultimately limit the growth and performance of many herbivorous insects (Sudderth et al. 2005). Furthermore, elevated CO_2 levels promote plant growth with consequent reallocation of resources, which markedly dilutes foliage nitrogen contents, and decreases its

availability for insects, modifying both the consumption rate and fitness of insects (Mattson 1980).

In general, chewing insects feeding on foliage grown under elevated CO_2 levels develop more slowly, suffer greater mortality, and have higher consumption rates (Chen et al. 2005a,b, 2007; Wu et al. 2006). Compared with leaf-chewers, phloem sap-suckers (e.g. aphids) respond differently to elevated CO_2 levels (Newman et al. 2003). Similar to many homopteran insects, aphids feeding exclusively on the phloem sap are known to be one of the most sensitive insect groups in terms of response to changes in quantity/quality of plants exposed to elevated CO_2 levels (Pritchard et al. 2007). Different from whole leaves, how does nutrient change in host plant phloem sap responding to elevated CO_2, was largely unknown. Furthermore, several recent studies have concluded that aphid responses are frequently 'species-specific' to elevated CO_2 levels (Bezemer and Jones 1998; Lesley and Fakhri 2001), including negative, positive or no significant effects (Awmack et al. 1997; Hughes and Bazzaz 2001; Holopainen 2002; Mondor et al. 2005).

Aphids, feeding on different plants, appear to have species-specific requirements for amino acids, because of the large variation in the proportion of essential amino acids in phloem saps (Wilkinson and Douglas 2003). Generally, only around 20% of the essential amino acids were found in the phloem sap, with a range from 15% to 48%, while the proportion of essential amino acids in aphid-body and optimal diets for herbivorous insects were approximately 50% (Sandström and Moran 1999). Thus these imbalanced diets are not supposed to match the aphids' needs. Free amino acids are needed and are transformed into other proteins (e.g. tyrosine for sclerotization in the cuticle after insect moulting), and are utilized as an energy resource and major respiratory substrates and especially for reproduction (Urich 1994; Febvay et al. 1995; Rhodes et al. 1996). Therefore, the amino acid content of aphids may be responsible for its performance and the honeydew excretion, which, in turn, affects the higher trophic level. With respect to nitrogen requirement, amino acid contents *in vivo* of aphids depend on both the composition of ingested phloem sap, as well as on the biosynthetic capabilities of the aphid and its intracellular symbionts (Buchner 1965). The changes in amino acid quality and quantity may influence the species-specific responses of aphids to elevated CO_2, which has been, to date, largely unexplored.

In this study, the response of the cotton aphid, *A. gossypii* feeding on transgenic Bt cotton exposed to elevated CO_2 was studied from the view of the amino acid budget. Our goals were to: (i) demonstrate changes in the amino acid content of phloem sap in transgenic Bt cotton under elevated CO_2; (ii) verify whether these cascade changes modified the amino acid budget of cotton aphid; and (iii) discuss the kind of strategy that cotton aphids adopt under elevated CO_2 levels.

Materials and Methods

Atmospheric CO_2 concentration treatments

This experiment was performed in six closed-dynamics CO_2 chambers (CDCC; Chen and Ge 2004). The chambers were maintained at 28 ± 1°C, 60–70% RH, and 14 : 10 h (light : dark) photoperiod at 9000 lx of active radiation supplied by 12, 60-w fluorescent lamps in each chamber.

Two CO_2 levels, 370 and 750 ppm, representing the current ambient level and double the current ambient level (i.e. the predicted level in about 100 years), respectively, were applied (Houghton et al. 2001). Three chambers were used for each CO_2 treatment. Double-ambient CO_2 concentrations were monitored and adjusted with an infrared CO_2 transmitter (Ventostat 8102; Telaire Company, Goleta, CA USA) once every 20 min to maintain relatively stable CO_2 concentrations. A detailed methodology of the automatic control system for CO_2 levels has been described in Chen et al. (2004, 2005a,b).

Host plants and cotton aphids

Cotton seeds of the transgenic Bt cultivar GK-12 (Seed Company of Shandong Province, Jinan City, China) were sown in pots (15 cm diameter by 17 cm high) filled with 4:1 (v/v) loam:earthworm faeces. Cotton plants were exposed to the CO_2 treatments after seedling emergence. Plants were randomly repositioned within each chamber weekly to minimize position effects.

On 6 September 2005, *A. gossypii* were collected from GK-12 crop fields and reared under ambient CO_2 in photoclimate chambers (HPG280; Orient Electronic, Haerbin, China) for five generations. Each of the nymphal instars from the same parthenogenetic female was inoculated on cotton leaves and was caged and kept in each chamber. The inoculation of *A. gossypii* was carried out on cotton plants, which were exposed to the two CO_2 levels from the seedling stage until the five- to seven-leaf stage (approximately 30–40 days). All *A. gossypii* used

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in the experiments were separately reared in each of the two CO_2 levels over 6 months before they were used in the experiments.

Cotton aphid growth and ingestion

Mean relative growth rate

Five first-instar aphids were randomly selected from each chamber and placed on the cotton plants after weighing with a Sartorius R200D automatic electro balance (Sartorius, Gottingen, Germany), and these aphids were weighed again after 5 days of the inoculation on the cotton plants. The mean relative growth rate (MRGR) of *A. gossypii* was calculated based on the method of Viskari et al. (2000): MRGR = $(\ln W_2 - \ln W_1)/t$, where W_1 is the initial weight (g), W_2 the final weight (g) and t the larval duration.

Water loss and respiration of aphid

Fifteen fourth-instar aphids were randomly collected from cotton plants of each CO_2 treatments and isolated in a Petri dish for 30 min to produce honeydew. Thereafter, the aphids were transferred into a small vial (length × width × height = 29 × 29 × 19 mm) (28 ± 1°C and 60–70% RH), and weighed four times after 0, 1, 2 and 3 h to calculate the weight loss due to water loss and respiration (mg losses per mg aphid per day) according to the method described by Sandström and Moran (2001).

Honeydew collection

Fifteen fourth-instar aphids inoculated on cotton plants grown under ambient and elevated CO_2 levels were each removed from the plants and weighed with a Sartorius R200D automatic electro balance, and then transferred to new plants for 12 h. These aphids were inoculated on back side of leaves, and the honeydew was collected under *n*-hexadecane (Van Helden et al. 1994) on tinfoil in a square collection cage (2.5 cm length) for 24 h to assay amino acids (Wilkinson and Douglas 1995). Finally, the aphids were weighed again. The tinfoil was weighed before and after honeydew collection. According to the method described by Sandström and Moran (2001), the honeydew samples were collected dry to avoid possible breakdown of amino acids during collection, and the dry honeydew was dissolved in 50 μl of 80% methanol, and free amino acid contents were analysed in the same manner as described for the phloem sampling. The amounts of individual amino acids were divided by the mean of the two aphid weight measurements (nmol amino acids/mg aphid/day). Honeydew production was calculated by dividing the honeydew weight by the mean of the two weights of fresh aphid measurements (mg honeydew/mg aphid/day).

Phloem sap ingested

The amount of phloem sap ingested by *A. gossypii* was estimated by exuvial and honeydew productions in conjunction with water loss and respiration (Sandström and Moran 2001). In the estimates of ingestion rate, the density of phloem sap was approximated to 1.07 (Lalonde et al. 2003). This is the density of a 500-mM sucrose solution, which corresponds approximately to phloem sap.

Amino acid of cotton phloem sap and aphids

Cotton phloem sap sampling

An ethylenediaminetetraacetic acid (EDTA) exudation method (King and Zeevaart 1974; Wilkinson and Douglas 2003) was followed to obtain phloem sap. The method was modified by Douglas (1993) to avoid generalized damage to plant tissues and non-specific amino acid release caused by high EDTA concentrations or long incubation periods. Excised leaf petiole was immediately put into an EDTA solution of chelated calcium ions to prevent sieve element sealing (King and Zeevaart 1974). For each CO_2 treatment, the fifth leaf above the cotyledon of 30-day-old cotton plants was excised using clean sharp scissors from the leaf petiole, and immediately immersed into 600 μl of 5 mM Na_2EDTA, and then the petiole was cut again in the buffer to prevent air contact with the phloem. The sampling was carried out in a light-proof box at 25°C for 90 min with a saturated solution of KH_2PO_4 to maintain high humidity. The leaf was discarded and the phloem exudates in the EDTA solution were stored at −20°C for amino acid analysis.

Sampling and assaying of amino acids in aphids

Fourth-instar aphids reared in ambient and elevated CO_2 chambers were randomly selected, weighed and transferred to a microfuge tube, and then frozen at −80°C. The tube was frozen in liquid nitrogen, and the aphids were crushed with a pestle and further homogenized after addition of 1.0 ml of ice-cold buffer (50 mM phosphate, pH 7.0). After centrifugation, 1.0 ml of 12 M HCl was added to the supernatant and the air in the tube was removed with a nitrogen stream. The tube was capped tightly and heated at 110°C for 22 h, and then cooled at room temperature. The acid hydrolysates were neutralized to pH 4–6, made up to 5 ml volume with ultra-pure water, filtered through a 0.45-μm membrane (Millipore

Corporation, Billerica, MA, USA) and kept frozen until analysis. Samples for obtaining free amino acid contents of aphids were crushed as above, but these aphids were homogenized in 1.0 ml of ice-cold 80% methanol. After centrifugation, the supernatant was used for analyses without hydrolysis. Free amino acid and total amino acid (proteins, peptides and free amino acids) analyses were performed using high-performance liquid chromatography (HPLC) with fluorescent detection. All analyses were performed on four to six replications.

Amino acid analyses

The amino acids in individual exudate samples were analysed by reverse-phase HPLC with pre-column derivatization using *o*-phthaldialdehyde (Jones et al. 1981). The analysis was performed using Agilent 1100 HPLC systems (Agilent Technologies, Palo Alto, CA, USA). A reverse-phase Agilent Zorbax Eclipse C18 column AAA (4.6 × 150 mm, 3.5 μm) and fluorescence detector were used for chromatographic separation. Chemstation Plus Family for LC software was used for data acquisition and analysis. Amino acid concentrations were quantified by comparison of sample peak areas to a three-level calibration plot of the reference amino acid mixture (Sigma Chemical Co., St Louis, MO, USA). All amino acids, the component of protein, except proline and cysteine, could be detected by this method at a detection limit of approximately 0.5 pmol.

Statistical analyses

Statistical analyses were performed with SAS software (SAS Institute, 2002; Cary, NC, USA). Differences in individual amino acid of the phloem sap, and in growth rate, honeydew production, water loss, respiration rate, exuvial production and body weight of *A. gossypii* were analysed by independent-group t-test at $P < 0.05$. The effects of CO_2 treatment on individual amino acid in the amino acid budget were also analysed with independent-group t-test at $P < 0.05$. Furthermore, the changes in composition and contents of amino acids from phloem sap, honeydew and aphid body were analysed by paired-group t-test at $P < 0.05$ (Goulden 1956).

Results

Cotton aphid growth and ingestion

Significantly more amount of honeydew ($t = -2.31$, d.f. = 1, 12, $P = 0.039$) was produced by *A. gossypii* fed on elevated CO_2-grown cotton plants as compared with those fed on plants exposed to ambient CO_2 levels (table 1). However, the growth rate of aphids feeding on plants grown under elevated CO_2 treatment was not different from that of aphids feeding on plants grown under ambient CO_2 treatment ($t = 0.78$, d.f. = 1, 12, $P = 0.45$). Moreover, water loss and respiration were not significantly different between *A. gossypii* fed plants grown under elevated CO_2 and ambient CO_2 ($t = -1.49$, d.f. = 1, 12, $P = 0.16$). *A. gossypii* ingested more phloem sap under elevated CO_2 relative to ambient CO_2 ($t = -2.64$, d.f. = 1, 12, $P = 0.022$; table 1).

Amino acids of phloem sap and *A. gossypii*

Amino acids of phloem sap

Significantly lower content of aspartic acid ($t = 2.70$, d.f. = 1, 10, $P = 0.022$), histidine ($t = 2.98$, d.f. = 1, 10, $P = 0.017$), glycine ($t = 9.18$, d.f. = 1, 10, $P < 0.001$), alanine ($t = 7.14$, d.f. = 1, 10, $P < 0.001$), methionine ($t = 5.05$, d.f. = 1, 10, $P = 0.001$), tryptophan ($t = 2.94$, d.f. = 1, 8, $P = 0.022$), lysine ($t = 2.89$, d.f. = 1, 10, $P = 0.018$) and threonine ($t = 7.06$, d.f. = 1, 10, $P < 0.001$) was observed in cotton phloem sap grown in elevated CO_2 compared with that of ambient CO_2 (fig. 1a). Elevated CO_2 significantly decreased the amino acid content in the phloem sap of cotton leaves (paired t-test: n = 15, $t = 3.192$, $P = 0.006$).

Table 1 Growth, honeydew, water loss and respiration, exuvial production and total phloem saps ingested by the nymphs of *Aphis gossypii*, fed on transgenic Bt cotton GK-12 under ambient CO_2 and elevated CO_2

Measured indices	Ambient CO_2 (mean ± SE)	Elevated CO_2 (mean ± SE)
Growth (mg/mg/day)	0.158 ± 0.003 a	0.154 ± 0.004 a
Honeydew per aphid (mg/mg/day)	1.036 ± 0.042 b	1.291 ± 0.102 a
Water loss and respiration per aphid (mg/mg/day)	0.318 ± 0.009 a	0.341 ± 0.013 a
Exuvial production per aphid (mg/mg/day)	0.067 ± 0.005 a	0.076 ± 0.006 a
Total phloem sap ingestion per aphid (μl/mg/day)	1.689 ± 0.045 b	1.994 ± 0.106 a

mg/mg/day = mg measured indices weight/mg aphid weight/day. Total phloem sap ingestion per aphid (μl/mg/day) = (aphid body weight + honeydew + water loss and respiration + exuvial production) (mg/mg/day) × 1.07.

Values with different lowercase letters show significant difference between CO_2 treatments by independent-group t-test at $P < 0.05$.

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Free amino acids of A. gossypii

Significantly higher content of aspartic acid ($t = -5.75$, d.f. = 1, 10, $P < 0.001$), serine ($t = -4.20$, d.f. = 1, 10, $P = 0.002$), histidine ($t = -7.68$, d.f. = 1, 10, $P < 0.001$), alanine ($t = -2.78$, d.f. = 1, 10, $P = 0.021$), phenylalanine ($t = -2.70$, d.f. = 1, 10, $P = 0.024$) and isoleucine ($t = -3.10$, d.f. = 1, 10, $P = 0.013$) was found in the body of *A. gossypii* fed cotton leaves under elevated CO_2 when compared with ambient CO_2 (fig. 1b). Moreover, the content of free amino acids significantly increased in aphids fed cotton plants grown in elevated CO_2 compared with that in aphids fed cotton plants grown in ambient CO_2 (paired t-test, n = 15, $t = -2.72$, $P = 0.016$).

Total hydrolysis of amino acids of A. gossypii

There was no significant difference in nearly all amino acid levels between aphids fed on elevated CO_2-grown cotton and those fed on ambient CO_2-grown cotton

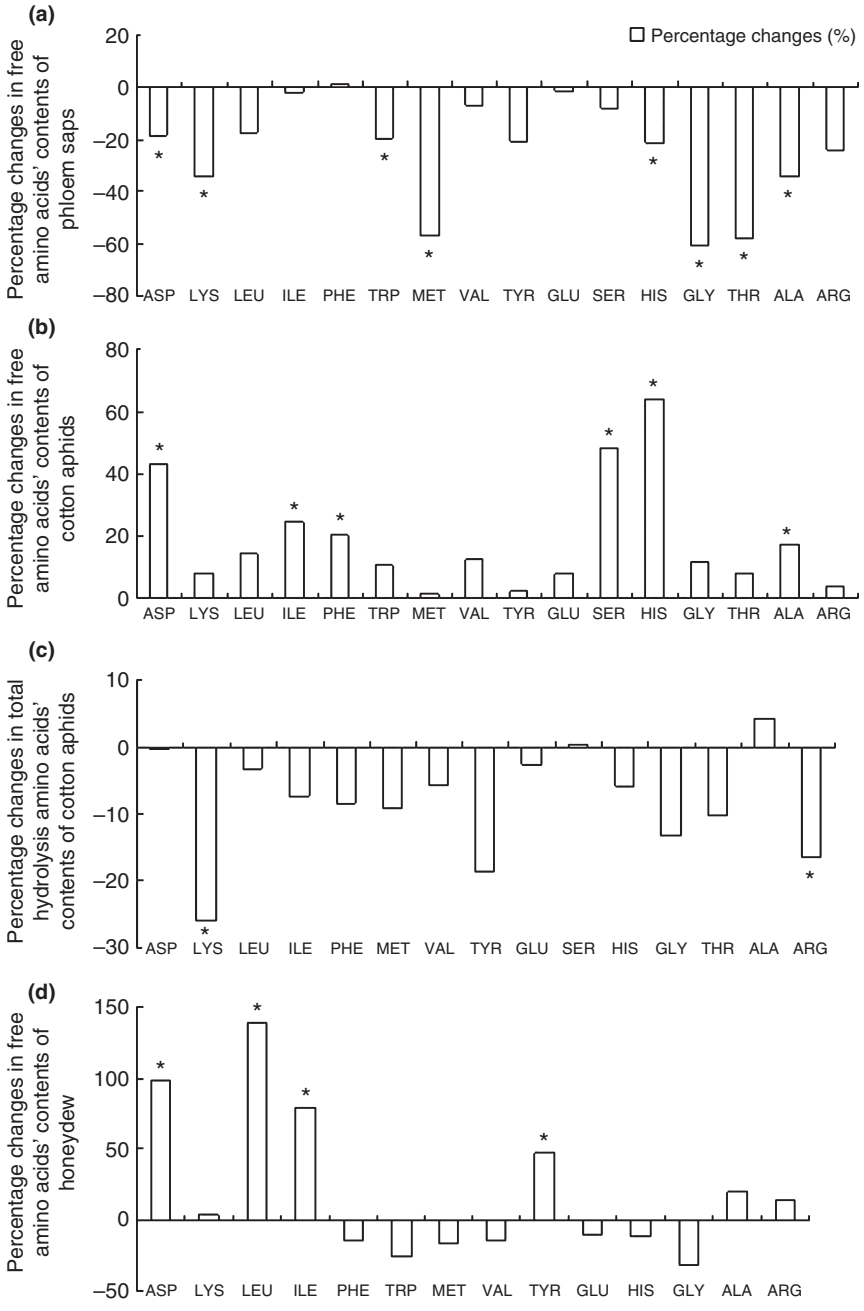

Fig. 1 Percentage changes in (a) free amino acid contents of phloem saps of transgenic Bt cotton GK-12 grown in ambient and elevated CO_2; (b) free amino acid contents of *Aphis gossypii* fed transgenic Bt cotton GK-12 grown in ambient and elevated CO_2; (c) total hydrolysis amino acid contents of *A. gossypii* fed transgenic Bt cotton GK-12 grown in ambient and elevated CO_2; and (d) free amino acid contents of aphid honeydew produced by *A. gossypii* fed transgenic Bt cotton GK-12 grown in ambient and elevated CO_2. Percentage change value (%) = (elevated − ambient)/ambient × 100, *$P < 0.05$.

with the exception of lysine (t = 5.463, d.f. = 1, 6, P = 0.002) and arginine (t = 2.789, d.f. = 1, 6, P = 0.032) (fig. 1C) in the aphids under elevated CO_2, and significantly less total amino acid content was found in aphids fed on cotton leaves under elevated CO_2 compared with ambient CO_2 (paired t-test: n = 14, t = 3.062, P = 0.008).

Free amino acids in honeydew of A. gossypii

There was significantly higher amount of aspartic acid (t = −9.72, d.f. = 1, 4, P = 0.001), tyrosine (t = −3.53, d.f. = 1, 4, P = 0.024), isoleucine (t = −4.34, d.f. = 1, 4, P = 0.012) and leucine (t = −6.97, d.f. = 1, 4, P = 0.002) in honeydew of *A. gossypii* fed cotton grown in elevated CO_2 compared with ambient CO_2 (fig. 1d). Moreover, free amino acid content in honeydew was not affected by elevated CO_2 (paired t-test: n = 13, t = 2.79, P = 0.44). Furthermore, serine and threonine were not detected in honeydew of aphids grown under elevated CO_2, and serine was not detected in honeydew of aphids grown under ambient CO_2.

Discussion

It is widely recognized that elevated CO_2 can reduce foliar nitrogen in most plants, which in turn, results in limiting the resource for phytophagous insects (Mattson 1980). However, the nutritional quality of phloem sap may be the important limiting resource for the growth, development and performance of aphids because of their phloem-sucking behaviour (Bezemer and Jones 1998). Docherty et al. (1997) reported that the total amino acid concentration in beech foliage phloem was unaffected by elevated CO_2, while the total amino acid concentration of sycamore foliage was 31% lower in elevated CO_2, compared with that in ambient CO_2. The host plant 'species-specific' response to elevated CO_2 (Holopainen 2002; Awmack et al. 2004) indicates that *A. gossypii* fed on transgenic Bt cotton may display different nutritional strategies under ambient CO_2 and elevated CO_2.

In this study, elevated CO_2 reduced the amino acid contents of cotton phloem saps. However, little difference in MRGR performance was shown under elevated CO_2. This nutrient imbalance suggests that, in order to match their needs, aphids may modify their feeding and metabolism (i.e. change the feeding rate and alter the feeding place). Dixon et al. (1993) found that the feeding rates and ingestion efficiency of aphids increased as amino acid contents of the host plants were lower. This leads to the prediction that elevated atmospheric CO_2 levels can enhance aphid feeding activities and result in heavier ingestion of sap of transgenic Bt cotton. Moreover, in contrast to free amino acid susceptibility, total content of each amino acid (tissue hydrolysates) was more stable to elevated CO_2, with only two alkaline amino acids, lysine and arginine, significantly affected by different CO_2 levels. Previous studies found that the free amino acid pool in aphid body can be quite variable from one species to another (Febvay et al. 1999; Wilkinson and Ishikawa 1999; Sandström and Moran 2001). Furthermore, our results showed an increase in free amino acid content in *A. gossypii* body in elevated CO_2 compared with that in ambient CO_2. More amino acid resource in aphid tissue would be advantageous for protein synthesis under elevated CO_2 levels. In contrast, the amino acid contents of aphid body significantly decreased under elevated CO_2 levels, which suggests that aphid predators have to consume more aphids to maintain their nutrient requirement under elevated CO_2 levels compared with that under ambient CO_2 levels.

Although this study indicated lower contents of amino acids in the phloem sap, and higher contents of amino acids as well as more amount of aphid honeydew in elevated CO_2 conditions, *A. gossypii* can satisfy growth requirements with compensatory feeding and possibly transforming some amino acids into aspartic acid. However, different from aspartic acid, essential amino acids (e.g. histidine) cannot be synthesized, and under this condition, some endosymbionts (e.g. *Buchnera*) would contribute to the supply of these amino acids (Dixon et al. 1993). Moreover, elevated CO_2 can increase the nutrient supply of *A. gossypii*, with significantly higher MRGR after feeding transgenic Bt cotton grown under elevated CO_2 for three successive generations (Chen et al. 2004). On the other hand, the amino acid composition of aphid honeydew, modified by the impacts of endosymbionts (e.g. *Buchnera*), is distinctly different from the phloem sap ingested (Woodring et al. 2004). Moreover, when *A. gossypii* was exposed to elevated CO_2 levels, more leucine was found in aphid honeydew. This indicates that some endosymbionts may help alleviate the poorer nutritional quality of host plants (Douglas and Prosser 1992) with higher amplification of Leu biosynthetic gene (Thao et al. 1998). Moreover, higher amount of aphid honeydew was produced under elevated CO_2 conditions, leading to much more intensive response by mutualistic ants (Völkl et al. 1999; Mailleaux et al. 2000), which suggests that

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aphids get more benefits from this mutualism primarily by reduced predation and a reduced rate of fungal infection under elevated CO_2 conditions (Völkl 1992). Of course, all these problems should be ascertained by further intensive experiments.

Thus far, the mechanism involved in species-specific aphid responses to elevated CO_2 remains unknown. In our study, the amino acid budget of aphid was considered to be involved in this interaction. Furthermore, three possible explanations were proposed. First, the plants respond differently to elevated CO_2 levels (Wang and Nobel 1995; Pritchard et al. 2007) – better, worse, and no difference in phloem sap quality of plant leaves. Secondly, as the amino acid concentration of aphid honeydew is strongly affected by elevated CO_2, it is tempting to speculate that aphids may adopt some strategies to sustain the balance of amino acid input/output when plant phloem sap quality gets worse under elevated CO_2. Moreover, some endosymbionts, which can synthesize some essential amino acids, were also involved in this physiological metabolism changes (Baumann et al. 1995; Douglas 1998; Shigenobu et al. 2000). Finally, compensatory feeding may act to satisfy aphid growth requirements under elevated CO_2 levels (Awmack et al. 1997), e.g. the growth of *Acyrthosiphon pisum* was compensated by changes in the feeding location, metabolism and ingestion rates (Abisgold et al. 1994). Increases in phloem-sap pressure and flow rates, leaf toughness and stylet penetration frequency also contributed to aphid compensatory feeding (Girousse and Bournoville 1994; Watling et al. 2000).

The response of transgenic Bt cotton to elevated CO_2 will modify *A. gossypii* feeding with higher compensatory ingestion. This may lead to heavier damage on host plants by *A. gossypii* by the ingestion of larger quantities of phloem sap. Furthermore, the changes in the amino acid contents of aphid body under elevated CO_2 conditions will modify the nutrient intake and performance of its natural enemies (Stacey and Fellowes 2002). Moreover, genome studies of biosynthetic genes coding the amino acids of some endosymbionts (especially some essential amino acids) may provide necessary information that helps to explain the individual aphid responses to elevated CO_2 levels.

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