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Research

Reduction of Plant Suitability for Corn Leaf Aphid (Hemiptera: Aphididae) Under Elevated Carbon Dioxide Condition

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Abstract

In the current context of global climate change, atmospheric carbon dioxide (CO_2) concentrations are continuously rising with potential influence on plant–herbivore interactions. The effect of elevated CO_2 (eCO_2) on feeding behavior of corn leaf aphid, *Rhopalosiphum maidis* (Fitch) on barley seedlings *Hordeum vulgare* L. was tracked using electrical penetration graph (EPG). The nutrient content of host plant and the developmental indexes of aphids under eCO_2 and ambient CO_2 (aCO_2) conditions were also investigated. Barley seedlings under eCO_2 concentration had lower contents of crude protein and amino acids. EPG analysis showed the plants cultivated under eCO_2 influenced the aphid feeding behavior, by prolonging the total pre-probation time of the aphids (wandering and locating the feeding site) and the ingestion of passive phloem sap. Moreover, fresh body weight, fecundity and intrinsic population growth rate of *R. maidis* was significantly decreased in eCO_2 in contrast to aCO_2 condition. Our findings suggested that changes in plant nutrition caused by eCO_2, mediated via the herbivore host could affect insect feeding behavior and population dynamics.

Key words: climate change, carbon dioxide, corn leaf aphid, feeding behavior, electrical penetration graph (EPG)

The world average carbon dioxide (CO_2) concentration in the atmosphere has steadily increased from pre-industrial values of approximately 280 ppm to 401 ppm (Mauna Loa Observatory: NOAA-ESRL). Concentrations are projected to increase to 550 ppm by 2050 and may surpass to 700 ppm by 2100 (Stocker et al. 2013). Elevated atmospheric CO_2 (eCO_2) has marked impact on plant growth and individual composition (Hartley et al. 2000, Bae and Sicher 2004, Seneweera and Conroy 2005), which indirectly affects the performance of herbivorous insect pests (Bale 2002, Zavala et al. 2013). Consequently, eCO_2 will in turn influence agro-ecosystem processes and crop productivity (Norby et al. 2005, Zvereva and Kozlov 2006, Lindroth 2010). CO_2 is the raw material for carbohydrate production for plants during photosynthesis. In general, the most significant changes in foliar chemistry are due to increase of the carbon:nitrogen (C:N) ratio in phloem sap while plants grow under eCO_2 (Hartley et al. 2000, Johns and Hughes 2002, Chen et al. 2005, Ainsworth and Rogers 2007), where nitrogen, mostly bound in amino acids and proteins, is a limiting factor for phloem sap feeding herbivorous insects (Mattson 1980). Subsequently, this will lead to less fitness of host plants and adverse effects on insects due to nutrition deficiency (Chen et al. 2005), which will result in heavier damage on the host plants (Marks and Lincoln 1996, Bezemer and Jones 1998, Sun et al. 2009a).

The phloem sap is sugar-rich with dominance of sucrose (Avigad and Dey 1997, Jensen et al. 2013) that is the most effective phagostimulant for insect herbivores (Srivastava and Auclair 1971). Aphids overcome the sugar barrier to phloem sap utilization through their possession in the gut of sucrose-transglucosidase activity (Ashford et al. 2000, Cristofoletti et al. 2003, Douglas 2006). Overall, a positive relationship between aphid performance and increasing sucrose concentration are mostly invariable (Srivastava and Auclair 1971, Kriegler 1972, Pescod et al. 2007, Puterka et al. 2017). However, the adapted range of sucrose concentrations by aphid is species-specific, which may link with its host range (Puterka et al. 2017). Oehme et al. (2013) reported that eCO_2 enhanced significant fructose and glucose levels in spring wheat foliage, which positively affected the relative growth rate of *R. padi*. However, the concentration of sucrose tends to increase at leaf development stage and to decrease at stem elongation stage due to eCO_2, but CO_2-induced these changes were not statistically significant.

Nitrogen is a limiting nutrient source for many herbivores and phloem sap feeding aphid in terms of quality (nutritional

components) and quantity (concentration of individual nutritional component) (Mattson 1980). Although aphid can overcome the nitrogen barrier in terms of essential amino acids in phloem sap by relying on their symbiotic bacteria (endosymbionts) including *Buchnera aphidicola* (Dadd 1985, Srivastava et al. 1985, Ohtaka and Ishikawa 1991, Douglas 1998, Spiteller et al. 2000, Nardi et al. 2002, Davis et al. 2006, Schloss et al. 2006, Crotti et al. 2010, Defossez et al. 2011). Histidine, isoleucine, and methionine are required dietary amino acids for *Myzus persicae* in the aphid-bacteria symbiosis (Mittler 1971).

Rising atmospheric CO_2 directly impacts the plant nitrogen concentration, is further transformed and affects the amino acid concentration (Docherty et al. 1997, Bertrand and Bigras 2006, Stiling and Cornelissen 2007, Sicher 2008, Sun et al. 2009a,b). Due to feeding solely on the phloem sap, aphid is one of the most sensitive insect responding to changes in quality and/or quantity of plants exposed to eCO_2 (Pritchard et al. 2007). Previous research suggests that the response of aphid to eCO_2-mediated alternation of foliar quality and/or quantity is species-specific (Hughes and Bazzaz 2001, Himanen et al. 2008, Oehme et al. 2013), i.e., being positively, negatively, or not significantly affected at both individual and population in terms of growth, development, fecundity, and abundance (Sandström and Pettersson 1994, Awmack et al. 1997, Docherty et al. 1997, Sandström 2000, Hughes and Bazzaz 2001, Holopainen 2002, Mondor et al. 2005, Oehme et al. 2013, Jiang et al. 2016). Previous reports indicated the changes in individual amino acid levels of phloem sap possibly alter the aphid behavior. Glutamine concentration from high to low levels for aphids *M. persicae* and *Macrosiphum euphorbiae* on potato plants could alter the fitness of host plant to aphids (Sandström 2000, Karley et al. 2002). This highlighted the importance of assessing the entire chemical profile rather than only total concentrations as commonly reported (Weibull 1987, Sandström and Pettersson 1994, Docherty et al. 1997, Sandström 2000).

Although some studies reported eCO_2-induced less fitness of host plant by altering foliar nutrient availability (Lincoln et al. 1993, Schädler et al. 2007, Cornelissen 2011), a recent research demonstrated that eCO_2-mediated increasing in foliar soluble sugars, free amino acids, and fatty acids could be favorable for ingesting cotton sap by *Aphis gossypii* (Glover), and consequently leading to increases in body weight, fecundity, and population abundance (Jiang et al. 2016). Srivastava et al. (1983) proved that eleven nonessential amino acids and amides play various roles in phagostimulation, growth and survival in *Acyrthosiphon pisum* (Harris). We suspected that changes in foliar compositions would alter the feeding behavior and therefore to change in suitability of corn leaf aphid under eCO_2.

Corn leaf aphid, *Rhopalosiphum maidis*, (Fitch) is a worldwide pest which often cause significant damage on cereal crops such as barley (*Hordeum vulgare* L.), corn (*Zea mays* L.), pearl millet (*Pennisetum glaucum*), wheat (*Triticum aestivum* L.), sorghum (*Sorghum bicolor* L. Moench), and broad bean (*Vicia faba* L.) (El-Ibrashy et al. 1972). Corn leaf aphid also a vector of plant viruses including sugarcane mosaic virus (SCMV), maize dwarf mosaic virus (MDMV), which often result in serious yield loss (Everly 1960, Foott and Timmins 1973, Bing et al. 1991, Blackman and Eastop 2000). Prior research reported that eCO_2 positively affected the fecundity of corn leaf aphid (Xie et al. 2014). The objective of this study was to assess eCO_2-mediated changes in the composition of phloem sap in barley and the resulting consequences for corn leaf aphid. Thus, we quantified eCO_2-mediated changes in leaf chemistry including crude protein, carbohydrates like glucose, fructose, sucrose and free amino acids in barley seedlings; examined the biological parameters of aphid developed on barley grown under ambient CO_2 (aCO_2) and eCO_2 conditions; and analyzed the effects of eCO_2 on aphid stylet ingestion on host plants by tracking corn leaf aphid feeding behavior with electrical penetration graph (EPG).

Materials and Methods

CO_2 Condition Chambers

Six transparent conditioned chambers (L 60 × W 50 × H 50 cm, PLEXIGLAS GS, clear 0F00 GT, 8 mm thick; Evonik Industries, Essen, Germany) were used for rearing plants and insects. In each chamber, a constant airflow (30 liters/min) was pushed using an air pump (Koi flow 30; Superfish, the Netherlands). Two levels of atmospheric CO_2 concentrations were applied, i.e., ambient level (aCO_2: 450 ± 50 ppm) and elevated level (eCO_2 = aCO_2 + 350 ppm). The $eCO2$ was achieved by using a CO_2 gas tank (>99% purity; Airliquide, Paris, France). Three chambers were used for each CO_2 level. These chambers were maintained at 23.0 ± 1.0°C and 60.0 ± 10.0% RH, with a 16 h light period under cool white light-emitting diode (LED) lights (77 lmol/sqm/s). CO_2 concentrations, temperature, and RH were continuously monitored in each chamber with MCH-383 SD data loggers (Lutron, Taipei, Taiwan).

Plant Material

Barley, *H. vulgare* L., was sown in black plastic pots (7.5 cm diameter, 9 cm high), with 30 seeds per pot, no chemical fertilizers or insecticides were used. After sowing, 25 pots/chamber were randomly transferred to aCO_2 and eCO_2 chambers. Two weeks old (14 d old), healthy barley seedlings were used for the experiment.

Aphid Rearing

A colony of corn leaf aphid, *R. maidis*, was originated collected from a corn field in Langfang City, Hebei Province, North China. Virus free aphid and barley seedlings were reared under aCO_2 and/or eCO_2 chambers more than 10 generations.

Crude Protein Analysis

For the Dumas method (Dumas 1831), fresh barley seedlings (0.2 g) were weighed in four replicates per CO_2 condition. Samples are wrapped in a tin foil, placed into an automated sample loader, and dropped into the induction furnace of an N analyzer. TruSpec CHNS Macro (Model CNS-2000, LECO, Inc.,St. Joseph, MI) instrument was used for the crude protein determination (Beljkaš et al. 2010). Crude protein content was calculated from the nitrogen content of the material, using a nitrogen conversion factor of 6.25 according to ISO/TS 16634-2 (2016).

Carbohydrate Contents Assay

Total soluble sugars were determined in four replicates per CO_2 condition based on the method of phenolsulfuric acid (Dubois et al. 1956). 0.2 g fresh barley seedlings was homogenized with deionized water and centrifuged at 4500 rpm for 10 min. Then, 1 ml of extract was treated with 0.125 ml 5% phenol and 2.5 ml 98% sulfuric acid. The mixture remained in the water bath at 30°C for 20 min. Absorbance was read at 490 nm using ultraviolet spectrophotometer (S 2100, Biochrom Ltd, England).

Glucose/Fructose/Sucrose contents of barley seedlings grown under both CO_2 treatments were determined by a high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The chromatographic system was a Dionex

ICS-5000+ model (Sunnyvale, CA) equipped with an electrochemical detector, and an SP gradient pump. The column was a Dionex CarboPac PA-100 (250 mm × 4 mm i.d.) coupled to a CarboPac guard column (40 mm × 4 mm i.d.). The mobile phase consisted of 500 mM sodium hydroxide (A) and water (eluent B). The flow rate was 1 ml/min and the injection volume was 25 µl. Four replicates were conducted for each treatment.

Amino Acid Assay

In order to define the composition of amino acids, 0.2 g flours of barley seedlings were prepared after hydrolysis under nitrogen with 6M HCl at 110°C during 24 h. High-performance liquid chromatography (HPLC) analysis (Biochrom 20 Plus amino acid analyzer, Pharmacia, Cambridge, United Kingdom) was performed (Moore and Stein 1954) using norleucine as an internal standard. The amino acids were separated by elution with suitable buffers of increasing pH and were detected with ninhydrin in a continuous flow photometric analytical system. Amino acid standard solutions (AA-S-18 from Sigma Aldrich, Steinheim, Germany) (500 nM) containing norleucine were separately injected to calibrate the analyzer and to calculate the amount of amino acid in the samples (Dakia et al. 2007). Four replicates were maintained for each CO_2 level.

Feeding Behavior Test by EPG

Feeding behavior of corn leaf aphid on aCO_2 and eCO_2 barley seedlings was monitored by using the Giga-8 DC-EPG system (EPG systems, Wageningen, the Netherlands) at constant temperature 23.0 ± 1.0°C. Wingless adults reared under aCO_2 conditions were carefully collected by using a brush and then starved for about 30 min. A gold wire (diameter 20 µm, 3 cm length) was connected to the EPG amplifier with a copper wire attached to a copper nail. The other end of the gold wire was attached to the dorsum of the aphid with conductive water-based silver glue. Once a set of eight aphids was wired, the plant electrode was inserted into the soil pot. The recordings started in the morning at 10:00 a.m. and lasted for 6 h. Each aphid and each plant was used only once and 21 successful replicates for each treatment (aCO_2 and eCO_2) were obtained. Data were recorded with the Stylet+d software and analyzed with Stylet+a (EPG systems, Wageningen, The Netherlands) and the feeding activity phases were distinguished based on waveforms resulting from voltage variations (Fig. 1). EPG parameters, such as number or duration of waveform events, were automatically calculated using Excel workbook (Sarria et al. 2009).

Intrinsic Rate of Increase in Aphid Population Under aCO_2 or eCO_2 Conditions

In both aCO_2 and eCO_2 chambers, one newborn aphid (<6 h) was placed into a ventilated transparent plastic clip cages (2.7 cm diameter, 2.7 cm high) and restrained on one fresh leaf of a barley seedling. The edge of the clip cage was covered with a sponge to avoid mechanical wounds to the leaf. Development and survival of nymphs and adults were checked daily. New offspring and dead adults were removed after daily counting. The intrinsic rate of increase (r_m) for each system was calculated from the equation $r_m = 0.738 \times (\ln Md) / d$ where d is the period from the aphid birth to its first reproduction and Md is the number of progenies in a reproductive period equal to d (Wyatt and White 1977, Zhang et al. 2017). A total of 15 replicates per CO_2 condition were maintained.

Mean Relative Growth Rate of Aphid

Twenty newborn aphid nymphs were collected from either aCO_2 or eCO_2 chambers, then placed on 9-cm-diameter filter paper and weighed. All aphids were transferred back to aCO_2 or eCO_2 chambers on barley seedlings in clip cages as described above. After 7 d, all 20 aphids were collected and weighed again. A total of 10 replicates were performed for each CO_2 treatment. The mean relative growth rate (MRGR) of *R. maidis* was calculated with the equation: MRGR = (ln 7 d weight – ln birth weight) / 7 (Bruce et al. 2003).

Statistical Analysis

The nutrient contents of barley seedling (including crude protein, carbohydrates, and amino acid concentrations) and the biological parameters of aphid (including the development time, fecundity, r_m, body weight and MRGR) were examined by using *t* test. EPG parameters were analyzed by a Mann–Whitney *U* test. Since EPG waveforms data was non-Gaussian random variables, Spearson's correlation analysis was conducted to investigate the relationships among EPG waveforms, aphid biological parameters, and nutrient contents of barley seedlings. Pearson's correlation was used to analyze the relationship between aphid biological parameters and

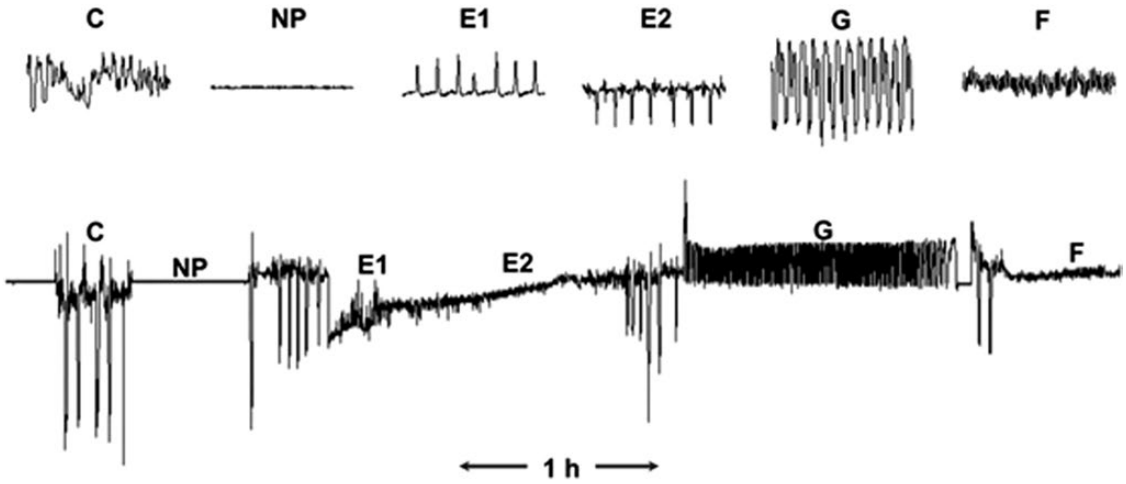

Fig. 1. Typical waveforms recorded by the EPG (Tjallingii and Hogen-Esch 1993). C: penetration of stylets into the plant between epidermis and mesophyll cells; np: wandering or stationary on the surface of the plant; E1: saliva transferred into phloem sieve elements; E2: passive sap ingestion from the phloem; G: active sap ingestion from the xylem; F: derailed stylet mechanics.

nutrient contents of barley seedlings. All analyses were performed with IBM SPSS Statistics 20.0 (IBM Corp, New York, NY, 2011).

Results

Nutrient Contents in aCO_2 and eCO_2 Barley Seedlings

Crude protein and total amino acid were significantly decreased in barley seedlings grown at eCO_2 when compared with aCO_2 condition. Though total soluble sugar was decreased at eCO_2 treatment, there was no statistically significant difference between aCO_2 and eCO_2 barley seedlings (Table 1). This indicated that eCO_2 resulted in a relative nutrient deficiency of barley seedlings.

CO_2 levels also have a major impact on the composition of individual amino acids in barley seedlings (Table 1). In the study, Glu and Asp were the predominant free amino acids in barley seedlings. Significant decrease was found for 13 basic amino acids including Asp (–13.9%), Thr (–23.5%), Ser (–26.7%), Glu (–20.0%), Pro (–18.8%), Gly (–16.7%), Ala (–8.3%), Cys (–50.0%), Iso (–20.0%), Leu (–15.4%), Tyr (–23.1%), Phe (–22.2%), and Arg (–20.0%) in eCO_2 barley seedlings ($P < 0.05$). However, there is no significant difference in glucose, fructose and sucrose levels.

Aphid Feeding Behavior on aCO_2 and eCO_2 Barley Seedlings

The plant CO_2 treatment had a significant impact on aphid feeding behavior. In the study, the total probing time of aphid was significant longer on eCO_2 barley seedlings. Aphid had a longer latency to start the first probe on eCO_2 barley seedling than on aCO_2 barley seedlings. The number and duration of np and of short probes were greater on aCO_2 barley seedlings than on eCO_2 barley seedlings. No significant difference was observed in total duration of C wave and pd between the two treatments. However, the passive sap ingestion (E2 waves) was significantly longer and more frequent in eCO_2 than in aCO_2. Despite the absence of significant difference in xylem ingestion phases between treatments, total duration of G waves in eCO_2 barley seedlings was about twice longer than on aCO_2 barley seedlings (Table 2).

Biological Parameters of Aphid

The fecundity, r_m and fresh body weight of 7 d *R. maidis* were significantly less under eCO_2 condition thanunder aCO_2 condition (Table 3). However, no significant differences were found in development time, weight of newborn nymphs, MRGR between aCO_2 and eCO_2 treatments.

Relationships Among EPG Waveforms of Aphid Feeding, Nutrient Contents of Barley Seedlings, and Biological Parameters of Aphid

The total probing time and duration of E2 were negatively correlated with the nutrient contents of barley seedlings and biological parameters of aphid (Table 4). Under aCO_2 and eCO_2 conditions, the total probing time was significantly correlated with the concentration of Ser, Ala, and Glu. The total duration of E2 was significantly correlated with the concentration of sucrose, Glu, Phe in both treatments. In addition, glucose was also significantly correlated under aCO_2 treatment.

The biological parameters we tested were positively correlated with the nutrient contents of barley seedling under both aCO_2 and eCO_2 treatments (Table 5). Furthermore, His, Lys, and Arg were significantly correlated with fecundity under aCO_2 treatment. R_m was significantly correlated with crude protein, Arg under aCO_2 treatment, while Gly, Ala, Ile, and Phe were significantly correlated under eCO_2 treatment. Fresh body weight of 7 d old aphid was significantly correlated with the concentration of total soluble sugar, glucose, fructose, sucrose, and some individual amino acids like Ile, Lys, Arg under aCO_2 treatment. Also, fructose, sucrose, Gly, Val,

Table 1. The concentration of crude protein, carbohydrates and amino acids in barley seedlings under aCO_2 and eCO_2 conditions

Nutrient contents	aCO_2	eCO_2	P
Crude protein (%)	4.0 ± 0.1	3.5 ± 0.0	0.016*
Total soluble sugar (mg/100 ml)	23.5 .10.9	22.2 . 1.4	0.625
Total amino acid (in g amino acid/100 g of dry material, g/100 D.F.)	30.6 .id.0	25.7 . 2.6	0.043*
Carbohydrates (mg/100 ml)			
Glucose	7.6 uco.4	6.7 ± 0.1	0.165
Fructose	0.5 uct.0	0.6 ± 0.0	0.101
Sucrose	1.5 cro.2	1.2 ± 0.2	0.354
Amino acid concentrations (g/100 D.F.)			
Aspartic acid (Asp)	3.6 ± 0.4	3.1 ± 0.1	0.047*
Threonine (Thr)	1.7 ± 0.3	1.3 ± 0.0	0.016*
Serine (Ser)	1.5 ± 0.3	1.1 ± 0.1	0.045*
Glutamic acid (Glu)	4.0 uta.6	3.2 ± 0.1	0.013*
Proline (Pro)	1.6 ± 0.4	1.3 ± 0.0	0.036*
Glycine (Gly)	1.8 yci.3	1.5 ± 0.0	0.034*
Alanine (Ala)	2.4 ± 0.3	2.2 ± 0.1	0.028*
Cystine (Cys)	0.2 ± 0.1	0.1 ± 0.0	0.042*
Valine (Val)	1.7 ± 0.2	1.5 ± 0.1	0.203
Isoleucine (Ile)	1.5 ele.3	1.2 ± 0.0	0.036*
Leucine (Leu)	2.6 uci.4	2.2 ± 0.1	0.046*
Tyrosine (Tyr)	1.3 ± 0.3	1.0 ± 0.0	0.033*
Phenylalanine (Phe)	1.8 eny.4	1.4 ± 0.0	0.037*
Histidine (His)	0.7 ± 0.1	0.8 ± 0.0	0.068
Lysine (Lys)	2.2 sin.3	1.8 ± 0.1	0.306
Arginine (Arg)	2.0 ± 0.4	1.6 ± 0.0	0.042*

Each value is the mean ± SE of four replicates for both aCO_2 and eCO_2 barley seedlings. Asterisk indicates significant differences between treatments ($P < 0.05$).

Table 2. EPG parameters of *Rhopalosiphum maidis* on aCO_2 and eCO_2 barley seedlings

EPG parameters[a]	aCO_2	eCO_2	*P*
General probing behavior			
Number of probes	12.6 ± 1.5	6.9 ± 1.1	0.004*
Total probing time (h)	4.1 ± 0.3	5.4 ± 0.1	0.000*
Time to first probe from start of EPG (min)	2.1 ± 0.5	7.1 ± 1.3	0.002*
Number of short probes (C < 3 min)	6.0 ± 0.9	2.6 ± 0.6	0.004*
Number of np	12.7 ± 1.5	6.9 ± 1.1	0.003*
Total duration of np (h)	1.8 ± 0.3	0.6 ± 0.1	0.000*
Pathway phase			
Number of C	16.0 ± 1.7	10.1 ± 1.4	0.007*
Total duration of C (h)	2.1 ± 0.3	1.5 ± 0.2	0.061
Mean duration of C (h)	0.1 ± 0.0	0.2 ± 0.0	0.753
Number of pd	65.0 ± 11.4	67.7 ± 10.7	0.696
Total duration of pd (s)	296.9 ± 53.8	283.0 ± 43.7	0.811
Mean duration of pd (s)	4.5 ± 0.2	4.3 ± 0.1	0.385
Phloem phase			
Number of E1	3.8 ± 0.7	3.6 ± 0.6	0.919
Total duration of E1 (min)	20.1 ± 4.8	9.8 ± 3.2	0.068
Mean duration of E1 (min)	5.6 ± 1.3	3.4 ± 1.4	0.092
Number of single E1	2.2 ± 0.5	0.9 ± 0.3	0.054
Total duration of single E1 (min)	5.2 ± 1.5	2.1 ± 0.9	0.052
Number of probes to the first E1	4.9 ± 1.1	3.8 ± 0.6	0.970
Number of E2	1.3 ± 0.2	2.5 ± 0.5	0.019*
Total duration of E2 (min)	80.1 ± 18.6	187.9 ± 21.3	0.001*
Mean duration of E2 (min)	56.4 ± 13.9	103.8 ± 18.6	0.044*
Time from start of EPG to first sustained E2 (10 min) (h)	3.5 ± 0.4	2.3 ± 0.3	0.054
Duration of the longest E2 (min)	75.5 ± 18.7	168.4 ± 21.5	0.002*
Number of sustained E2 (>10 min)	0.9 ± 0.1	1.4 ± 0.2	0.029*
Other phases			
Number of G	0.3 ± 0.1	0.6 ± 0.2	0.223
Duration of G (min)	9.5 ± 3.6	21.3 ± 8.5	0.315
Number of F	0.2 ± 0.1	0.3 ± 0.1	0.461
Total duration of F (min)	7.0 ± 4.0	16.9 ± 10.2	0.628

[a]All parameters were calculated for the whole 6-h recording. Each value is the mean ± SE of 21 replicates for both aCO_2 and eCO_2 barley seedlings. Asterisk indicates significant differences between treatments ($P < 0.05$).

Table 3. Biological parameters (mean ± SE) of *Rhopalosiphum maidis* on aCO_2 and eCO_2 barley seedlings

Biological parameters	aCO_2	eCO_2	*t*	*P*
Development time (d)	7.0 vel.2	7.5 ± 0.2	−1.916	0.067
Fecundity	4.7 cun.2	3.3 ± 0.1	6.866	<0.001*
Intrinsic rate of increase (r_m)[a]	0.4 tri.0	0.3 ± 0.0	5.514	<0.001*
W_{nymph}[b]	0.0 ± 0.0	0.0 ± 0.0	1.948	0.067
W_{adult}[c]	0.5 ult.0	0.4 ± 0.0	4.944	<0.001*
Mean relative growth rate (MRGR)[d]	0.4 GR .01	0.4 1 0.0	1.043	0.311

[a]$r_m = 0.738 \times (\ln Md) / d$, where d is the period from the aphid birth to its first reproduction and Md is the number of progenies in a reproductive period equal to d. Asterisks indicate significant differences between treatments ($P < 0.05$).

[b]W_{nymph} means fresh body weight of newborn nymphs (<6 h) (mg).

[c]W_{adult} means fresh body weight of 7 d adults (mg).

[d]MRGR = (ln 7 d weight − ln birth weight) / 7.

Phe, His, Lys, and Arg were significantly correlated under eCO_2 treatment.

Discussion

Elevated CO_2 was found to affect the feeding behavior via the change of the plant nutrient contents and exerting an influence on the herbivore performance. Rearing in eCO_2 condition, barley seedlings significantly decreased the concentration of crude protein, total amino acids, and 13 individual amino acids. The corn leaf aphid prolonged the total probing time and sustained ingestion on eCO_2 barley seedlings but was lower in fecundity, r_m and fresh body weight which negatively impacted population abundance of aphid.

Rising atmospheric CO_2 is likely to decrease the protein concentration of many crops, including wheat (Jablonski et al. 2002, Loladze 2002), potato (Fangmeier et al. 2002), and soybean (Ainsworth et al. 2002). The reduced concentration of plant protein under eCO_2 condition mainly due to accumulation of nonstructural carbohydrates

Table 4. Coefficients of Spearman's correlation between EPG waveforms, nutrient contents of barley seedlings, biological parameters of *Rhopalosiphum maidis* under aCO_2 and eCO_2 conditions

Components	aCO_2								eCO_2							
	Total probing time (min)	Time to first probe from start of EPG (min)	Total duration of E1 (min)	Total duration of E2 (min)	Number of probes	Number of np	Number of C	Number of E2	Total probing time (min)	Time to first probe from start of EPG (min)	Total duration of E1 (min)	Total duration of E2 (min)	Number of probes	Number of np	Number of C	Number of E2
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Crude protein	−0.400	0.400	0.400	−0.800	0.200	0.400	0.400	0.400	−0.800	0.800	0.400	−0.800	0.200	0.632	0.400	0.211
Total soluble sugar	−0.800	0.200	0.200	−0.800	0.400	0.800	0.200	0.200	−0.800	0.800	0.400	−0.200	0.800	0.949	0.600	0.949
Total amino acid	−0.800	0.200	0.200	−0.400	0.400	0.400	0.200	0.200	−0.400	0.400	0.800	−0.600	0.400	0.316	0.200	0.105
Carbohydrates																
Glucose	−0.800	0.400	0.400	−0.960*	0.600	0.200	0.400	0.400	−0.800	0.800	0.400	−0.800	0.200	0.632	0.400	0.211
Fructose	−0.800	0.200	0.400	−0.800	0.200	0.400	0.600	0.200	−0.800	0.600	0.400	−0.800	0.400	0.632	0.400	0.056
Sucrose	−0.600	0.400	0.600	−0.990*	0.200	0.200	0.400	0.600	−0.800	0.800	0.400	−0.965*	0.400	0.632	0.400	0.211
Amino acid concentrations																
Asp	−0.800	0.400	0.400	−0.800	0.200	0.400	0.400	0.400	−0.800	0.800	0.400	−0.800	0.200	0.632	0.400	0.778
Thr	−0.800	0.400	0.200	−0.800	0.400	0.400	0.400	0.600	−0.800	0.800	0.400	−0.800	0.400	0.389	0.400	0.211
Ser	−0.970*	0.600	0.200	−0.800	0.200	0.200	0.400	0.400	−0.800	0.800	0.400	−0.800	0.200	0.632	0.400	0.211
Glu	−0.800	0.600	0.400	−0.985*	0.600	0.200	0.800	0.600	−0.990*	0.800	0.400	−0.990*	0.400	0.389	0.400	0.056
Pro	−0.400	0.200	0.400	−0.800	0.200	0.200	0.400	0.400	−0.800	0.632	0.632	−0.738	0.200	0.632	0.400	0.211
Gly	−0.600	0.400	0.600	−0.800	0.400	0.200	0.600	0.400	−0.632	0.800	0.400	−0.800	0.316	0.632	0.105	0.211
Ala	−0.955*	0.600	0.600	−0.800	0.200	0.400	0.400	0.600	−0.800	0.800	0.400	−0.949	0.200	0.389	0.400	0.056
Cys	−0.400	0.400	0.200	−0.800	0.400	0.400	0.600	0.400	−0.800	0.632	0.211	−0.800	0.200	0.632	0.400	0.211
Val	−0.400	0.400	0.400	−0.800	0.200	0.200	0.400	0.400	−0.800	0.800	0.400	−0.800	0.400	0.632	0.316	0.211
Ile	−0.600	0.200	0.200	−0.800	0.200	0.600	0.400	0.600	−0.800	0.600	0.400	−0.800	0.400	0.632	0.400	0.056
Leu	−0.800	0.400	0.400	−0.800	0.400	0.400	0.200	0.400	−0.800	0.800	0.400	−0.975*	0.200	0.576	0.400	0.211
Tyr	−0.400	0.200	0.200	−0.800	0.200	0.400	0.600	0.400	0.949	0.949	0.316	−0.800	0.200	0.833	0.632	0.778
Phe	−0.800	0.600	0.400	−0.955*	0.400	0.600	0.400	0.600	0.800	0.800	0.400	−0.965*	0.316	0.632	0.400	0.056
His	−0.400	0.400	0.200	−0.800	0.200	0.400	0.400	0.400	−0.632	0.632	0.632	−0.738	0.200	0.500	0.105	0.211
Lys	−0.600	0.200	0.600	−0.800	0.600	0.600	0.200	0.200	−0.800	0.800	0.400	−0.800	0.316	0.632	0.400	0.211
Arg	−0.800	0.200	0.400	−0.960*	0.200	0.400	0.600	0.400	−0.800	0.800	0.400	−0.800	0.200	0.632	0.400	0.211
Biological parameters																
Development time	−0.832	0.211	0.211	−0.738	0.105	0.316	0.211	0.211	−0.949	0.800	0.105	−0.316	0.211	0.889	0.949	0.778
Fecundity	−0.800	0.400	0.400	−0.800	0.200	0.600	0.400	0.400	−0.775	0.775	0.258	−0.775	0.258	0.544	0.775	0.272
r_m	−0.832	0.211	0.211	−0.738	0.105	0.211	0.211	0.211	−0.632	0.632	0.211	−0.949	0.105	0.389	0.316	0.056
W_{nymph} [a]	−0.816	0.316	0318	−0.632	0.316	0.316	0.316	0.316	−0.800	0.800	0.400	−0.800	0.200	0.632	0.400	0.211
W_{adult} [b]	−0.800	0.400	0.400	−0.800	0.200	0.400	0.400	0.400	−0.800	0.800	0.400	−0.800	0.200	0.632	0.400	0.211
MRGR	−0.800	0.400	0.400	−0.800	0.200	0.400	0.600	0.400	−0.400	0.400	0.400	−0.800	0.400	0.105	0.200	0.316

r = correlation coefficient. Asterisks indicate level of significance: $P < 0.05$.

[a]W_{nymph} means fresh body weight of newborn nymph (< 6h) (mg).

[b]W_{adult} means fresh body weight of 7-d adult (mg).

Table 5. Coefficients of Pearson's correlation between nutrient contents of barley seedlings and biological parameters of *Rhopalosiphum maidis* under aCO_2 and eCO_2 conditions

Components	aCO_2						eCO_2					
	Development time	Fecundity	r_m	W_{nymph} [a]	W_{adult} [b]	MRGR	Development time	Fecundity	r_m	W_{nymph}	W_{adults}	MRGR
	r	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*	*r*
Crude protein	0.904	**0.979***	**0.954***	0.921	0.890	0.904	0.884	**0.989***	0.896	0.746	0.825	0.808
Total soluble sugar	0.946	**0.892**	0.936	0.898	**0.970***	0.911	0.597	0.932	0.892	0.877	0.938	0.836
Total amino acid	0.917	0.855	0.902	0.925	0.928	0.932	0.803	0.876	0.939	0.892	0.863	0.824
Carbohydrates												
Glucose	0.924	0.937	0.893	0.904	**0.972***	0.946	0.85	0.872	0.934	0.811	0.879	0.862
Fructose	0.944	0.892	0.946	0.949	**0.998***	0.914	0.759	0.915	0.880	0.904	**0.953***	0.939
Sucrose	0.937	0.949	0.945	0.795	**0.966***	0.873	0.585	0.725	0.941	0.877	**0.99***	0.945
Amino acid concentrations												
Asp	0.742	0.794	0.945	0.942	0.762	0.867	0.804	0.920	0.859	0.841	0.909	0.898
Thr	0.879	0.866	0.781	0.929	0.837	0.928	0.649	0.552	0.725	0.877	0.815	0.735
Ser	0.903	0.744	0.891	0.85	0.804	0.848	0.76	0.702	0.812	0.896	0.865	0.795
Glu	0.852	0.917	0.909	0.841	0.092	0.90	0.703	0.839	0.875	0.873	0.781	0.938
Pro	0.909	0.795	0.898	0.901	0.842	0.895	0.472	0.503	0.799	0.938	0.897	0.845
Gly	0.913	0.751	0.902	0.846	0.812	0.849	0.673	0.801	**0.966***	0.925	**0.985***	0.678
Ala	0.919	0.763	0.908	0.855	0.823	0.858	0.451	0.706	**0.971***	0.874	0.912	0.937
Cys	0.932	0.833	0.922	0.918	0.876	0.919	0.636	0.87	0.921	0.92	0.875	0.929
Val	0.943	0.879	0.936	0.946	0.914	0.941	0.362	0.605	0.929	0.786	**0.976***	0.897
Ile	0.926	0.93	0.936	0.759	**0.955***	0.843	0.61	0.769	**0.971***	0.917	0.932	0.921
Leu	0.892	0.890	0.788	0.887	0.938	0.921	0.783	0.737	0.831	0.896	0.875	0.808
Tyr	0.783	0.737	0.831	0.896	0.875	0.808	0.802	0.728	0.800	0.896	0.844	0.772
Phe	0.909	0.905	0.92	0.715	0.927	0.806	0.724	0.882	**0.985***	0.934	**0.972***	0.926
His	0.904	**0.992***	0.909	0.931	0.922	0.87	0.455	0.522	0.83	0.922	**0.975***	0.878
Lys	0.901	**0.996***	0.908	0.894	**0.988***	0.945	0.634	0.675	0.881	0.942	**0.971***	0.893
Arg	0.946	**0.986***	**0.952***	0.882	**0.995***	0.95	0.6	0.743	0.928	0.932	**0.954***	0.928

r = correlation coefficient. Asterisks indicate level of significance: $P < 0.05$. Bold numbers corresponded to significant results at $P < 0.05$.

[a] W_{nymph} means fresh body weight of newborn nymphs (<6 h) (mg).

[b] W_{aduit} means fresh body weight of 7-d-old adults (mg).

dilutes the concentration of proteins (Kimball et al. 1994, Williams et al. 1998, Gifford et al. 2000). In this study, eCO_2 level significantly decreased the concentration of 13 basic amino acids of barley seedlings. In accordance, Wang and Nobel (1995) reported doubled CO_2 concentrations led to 17% less amino acids in phloem of *Opuntia ficus-indica* (L.). The amino acid concentrations were lower in needles of black spruce (*Picea mariana* Mill. B.S.P.) (Bertrand and Bigras 2006) and in phloem of cotton (Sun et al. 2009a) at eCO_2 condition. Decreased amino acid concentration in plant under eCO_2 condition may as a result of 'nitrogen dilution' (Docherty et al. 1997).

The eCO_2 significantly increased the concentration of sucrose of soybean (Ainsworth et al. 2007) and broccoli (Krumbein et al. 2010). However, there was no significant effect on the concentration of sucrose in leaves of spring wheat under CO_2 enrichment (Högy and Fangmeier 2008) and the concentrations of sucrose, fructose and glucose in maize leaves remained unchanged under eCO_2 (Leakey et al. 2006). In our study, we also did not find any significant difference in total soluble sugar, glucose, fructose, sucrose between aCO_2 and eCO_2 barley seedlings. The carbohydrates synthesis of young cereals may be less affected by eCO_2 (Havelka et al. 1984, Cure and Acock 1986, Ryle et al. 1992), probably because they do not convert increased photosynthetic capacity in an increased level of extractable carbohydrates (Ingvardsen and Veierskov 1994).

The analysis of EPG recordings revealed that the cultivation of barley in different concentration of CO_2 greatly affected the feeding behavior of *R. maidis*. The total probing time of *R. maidis* was significantly longer on eCO_2 barley seedlings. In the study, the total duration of E2 was the main component of total probing time and which was also significant longer in eCO_2 condition. The total duration of E2 was negatively correlated with nutrient contents and biological parameters of aphid which indicates that the decrease in plant nutrient contents in eCO_2 barley seedlings may increase the time spending in passive phloem feeding which were unfavorable for its reproductive and population abundance.

In contrast with eCO_2 barley seedlings, aphid feeding on aCO_2 barley seedlings had a higher frequency of probes, which suggested the absence of negative factors in epidermis that might have caused the withdrawal of stylets (Dancewicz et al. 2016). Long np phase observed of aphid on aCO_2 barley seedlings would have indicated the presence of barriers during stylets insertion in plant tissues (Alvarez et al. 2006), and vice versa, aphid feeding on eCO_2 barley seedlings may have more obstacles on the surface of epidermis and less barriers during stylets insertion in plant tissues. *Rhopalosiphum maidis* had a longer latency to begin its first probe also implied the epidermal obstacles on eCO_2 barley seedlings, in addition, the result analysis pointed that the time

to first probe was less affected by carbohydrate or amino acids content of plant.

The number of C wave refers to the ease at which aphid moved the stylets from the mesophyll to the phloem (Benatto et al. 2018). Feeding on eCO_2 barley seedlings, aphid had a lower frequency of C phase suggested that aphid may reach easier to deep tissues of eCO_2 barley seedlings by penetrating the meristematic tissues, which also supported the view that the aphid has less barriers during stylets insertion into the tissues of eCO_2 barley seedlings. EPG results in our experiment showed that, aphid feeding on eCO_2 barley seedlings may have obstacles in initial probing on the surface of the epidermis, but they were successfully penetrated into the deeper tissues of plant and sustained ingestion was significant longer when compared to the aCO_2 barley seedlings.

The biological parameters were positively correlated with nutrient contents of barley seedlings, especially fecundity, r_m, weight of 7 d old adults. Barley seedlings cultivation in eCO_2 condition had a significantly lower concentration in crude protein, total amino acids, and 13 individual amino acids. Aphid performance on plants could not only be affected by the overall amino acid concentration, but also by the relative concentration of different amino acids (Mittler 1967). According to Dadd (1985), essential amino acids for aphid are His, Thr, Trp, Met, Val, Phe, Ile, and Lys. However, the demand of individual amino acids differs with the aphid species (Sandström and Moran 2001). While *M. persicae* needs Met and g-amino butyric acid, the amino acids Thr, His and Ala are important for *R. padi* Koch (Kazemi and Van Emden 1992).

Herbivore insects respond to the poor nutritional quality of the host plant by increasing their food consumption, prolonging development time, and reducing growth rates (Lincoln et al. 1993, Roth and Lindroth 1995, Brooks and Whittaker 1998, Williams et al. 2000, Tuchman et al. 2002, Hale et al. 2003). Our results support most of these predictions. *R. maidis* feeding on eCO_2 barley seedlings showed significantly decreased body weight, fecundity, and r_m, which may result in decreased of population abundance under elevated atmospheric CO_2 environment. Further studies will be required to determine the defense mechanisms including epidermal integrity, defense proteins, and secondary metabolites of plant which might also hinder the penetration of stylets.

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