Plant Physiology and Biochemistry 145 (2019) 84–94

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Research article

Effects of elevated CO_2 on foliar soluble nutrients and functional components of tea, and population dynamics of tea aphid, *Toxoptera aurantii*

Likun Li[a], Mengfei Wang[a], Sabin Saurav Pokharel[a], Chunxu Li[a], Megha N. Parajulee[b], Fajun Chen[a,**], Wanping Fang[c,*]

[a] Department of Entomology, Nanjing Agricultural University, Nanjing, 210095, China
[b] Texas A&M AgriLife Research and ExtensionCenter, Lubbock, TX79403, USA
[c] Department of Tea Science, College of Horticulture, Nanjing Agricultural University, China

ARTICLE INFO

Keywords:
Elevated CO_2
Tea plants
Toxoptera aurantii
Soluble nutrients
Functional components
Population dynamics
Climate change

ABSTRACT

The rising atmospheric CO_2 concentration has shown to affect plant physiology and chemistry by altering plant primary and secondary metabolisms. Nevertheless, the impacts of elevated CO_2 on plant nutrients and functional components of tea remain largely unknown, which will likely affect tea quality and taste under climate change scenario. Being sources of nutrients and secondary chemicals/metabolites for herbivorous insects, the variation in foliar soluble nutrients and functional components of tea plants resulting from CO_2 enrichment will further affect the herbivorous insects' occurrence and feeding ecology. In this study, the tea aphid, *Toxoptera aurantii* was selected as the phloem-feeding herbivore to study the effects of elevated CO_2 on foliar soluble nutrients and functional components of tea seedlings, and the population dynamics of *T. aurantii.* The results indicated that elevated CO_2 enhanced the photosynthetic ability and improved the plant growth of tea seedlings compared with ambient CO_2, with significant increases in net photosynthetic rate (+20%), intercellular CO_2 concentration (+15.74%), leaf biomass (+15.04%) and root-to-shoot ratio (+8.08%), and significant decreases in stomatal conductance (−5.52%) and transpiration rate (−9.40%) of tea seedlings. Moreover, elevated CO_2 significantly increased the foliar content of soluble sugars (+4.74%), theanine (+3.66%) and polyphenols (+12.01%) and reduced the foliar content of free amino acids (−9.09%) and caffeine (−3.38%) of tea seedlings compared with ambient CO_2. Furthermore, the relative transcript levels of the genes of theanine synthetase (+18.64%), phenylalanine ammonia lyase (+49.50%), s'-adenosine methionine synthetase (+143.03%) and chalcone synthase (+61.86%) were up-regulated, and that of caffeine synthase (−56.91%) was down-regulated for the tea seedlings grown under elevated CO_2 relative to ambient CO_2. In addition, the foliar contents of jasmonic acid (+98.6%) and salicylic acid (+155.6%) also increased for the tea seedlings grown under elevated CO_2 in contrast to ambient CO_2. Also, significant increases in the population abundance of *T. aurantii* (+4.24%–41.17%) were observed when they fed on tea seedlings grown under elevated CO_2 compared to ambient CO_2. It is presumed that the tea quality and taste will be improved owing to the enhanced foliar soluble nutrients and functional components of tea seedlings under the climate change scenario, especially on account of the rising atmospheric CO_2 concentration, while the climate change may exacerbate the occurrence of tea aphid, *T. aurantii,* despite the enhanced secondary defensive chemicals manifested by the CO_2 enrichment.

1. Introduction

The atmospheric carbon dioxide (CO_2) level has increased steadily from approximately 280 ppm before industrial revolution to 408 ppm at present (www.esrl.noaa.gov/gmd/ccgg/trends/), and it is predicted to rise up to about 800 ppm by 2100 (IPCC, 2014). As an important constituent for photosynthesis of plants (especially C_3 plants), such an increase of atmospheric CO_2 level directly affects plant photosynthesis and growth (Terashima et al., 2014), and also indirectly affects the growth and development of herbivorous insects and their physiological metabolism by altering plant biomass and quality, including changes in the composition and content of secondary defensive substances in their host plants (Stacey and Fellowes, 2002; Ge and Chen, 2006; Ge et al., 2010; Ge, 2011; Sun et al., 2011, 2016; Robinson et al., 2012).

---

[*] Corresponding author.
[**] Corresponding author.
E-mail addresses: fajunchen@njau.edu.cn (F. Chen), fangwp@njau.edu.cn (W. Fang).

https://doi.org/10.1016/j.plaphy.2019.10.023
Received 10 July 2019; Received in revised form 13 October 2019; Accepted 17 October 2019

Tea, *Camellia sinensis* (L.), is widely cultivated as small- and large-scale plantations within the tropics and subtropics between latitudes 42°N and 37°S (Carr and Stephen, 1992). It is grown in diverse agro-ecological conditions experiencing a wide range of climatic conditions, such as temperatures from 12 °C to 40 °C, annual rainfall from 938 to 6000 mm, radiation intensity from 0.3 to 0.8 $cal/cm^2/min$, and relative humidity from 30% to 90% in more than 34 countries across Asia, Africa, Latin America, and Oceania (Carr and Stephen, 1992; Deka et al., 2006). National economies of these countries are largely dependent on amount and quality of their tea production, whereas herbivorous insect pests are the most damaging constraint of tea production and cause an average 5%–55% yield lost (Sivapalan, 1999), and even 100% yield loss in some cases (Muraleedharan and Chen, 1997).

The domestication of crops quickly accompanied the plant pest problems and the same applied to tea cultivation (Muraleedharan and Chen, 1997). As a plantation crop, tea provides food supply for more than 1000 species of arthropods (Hazarika et al., 2009). Also, for being a C_3 crop, elevated CO_2 (800 $\mu mol\,mol^{-1}$ for 24 d) remarkably improved both the photosynthesis and the respiration in tea leaves, and increased the foliar concentrations of soluble sugar, starch, and total carbon (C), but decreased the foliar total nitrogen (N) concentration, resulting in an increased C: N ratio in tea leaves (Xu et al., 2016; Li et al., 2017). While the increased C: N ratio in plant tissues reduced the nutritional quality (Ainsworth et al., 2007), it induced the increased feeding by insect herbivores for compensatory nutritional intake (Stitt and Krapp, 1999). Niziolek et al. (2013) indicated that elevated CO_2 mediated variation in plants' nutritional qualities and defensive chemicals play a vital role in the feeding damage of insect herbivores. Among the functional components (also the secondary/defensive chemicals) of tea leaves, the polyphenol content increased and the caffeine content decreased after CO_2 enrichment, while the contents of individual catechins were altered differentially resulting in an increased total catechins content under elevated CO_2 (Xu et al., 2016; Li et al., 2017). However, the impacts of elevated CO_2 on the feeding ecology and population dynamics of tea insect pests mediated through CO_2 induced variation in foliar nutritional qualities and secondary metabolites remain unclear.

Phloem-feeding insects, such as aphids, whiteflies and planthoppers, have shown a consistently more positive response in population growth to elevated CO_2 than leaf-chewers and leaf-miners (Bezemer and Jones, 1998; Sun et al., 2016). Most leaf-chewing insects, e.g., *Orgyia leucostigma* (Lindroth et al., 2002), *Chilo suppressalis* (Chen et al., 2011), *Spodoptera exigua* (Coviella et al., 2002), and *Helicoverpa armigera* (Chen et al., 2005a,b, 2007, 2011; Wu et al., 2007a; Coll and Hughes, 2008), exhibit compensatory increases in food consumption and/or reduced growth, survival rates, and reduction in density, presumably because of the negative effects of rising atmospheric CO_2 concentrations on plant nutritional quality. However, most phloem-feeders, e.g., *Myzus persicae* (Hughes and Bazzaz, 2001; Stacey and Fellowers, 2002), *Rhopalo siphumpadi* (Zhang et al., 2003) and *Sitobion avenae* (Chen et al., 2004b), *Aphis gossypii* (Chen et al., 2004a, 2005c,d; Li et al., 2011; Jiang et al., 2016, 2018), *Bemisia tabaci* (Li et al., 2011), and *Nilaparvata lugens* (Chang et al., 2011), became much more serious threats under elevated CO_2, due to their shorter life span, increased body weight and fecundity, and population abundances. In this study, the tea aphid, *Toxoptera aurantii,* was selected as a model phloem-feeding insect pest to represent the phloem feeders. The objectives of this study were to: 1) study the effects of elevated CO_2 on foliar soluble nutrients and functional components of tea seedlings, 2) investigate the population dynamics of *T. aurantii*on fed on tea seedlings grown under elevated CO_2, and 3) measure the transcriptional expression levels of tea functional-component genes for theanine, polyphenol and caffeine of tea seedlings grown under elevated CO_2, in order to elucidate the molecular mechanism on the population occurrence of *Toxoptera aurantii* in response to rising atmospheric CO_2 levels.

2. Materials and methods

2.1. Setup of CO_2 levels

This study was conducted in the electronically controlled growth chambers (GDN-400D-4/CO_2; Ningbo Southeast Instrument CO., LTD., Ningbo, China) with a gas-tank system to supply 99.5%-purity CO_2 gas and maintain the desired levels of CO_2 concentration. Two levels of CO_2 concentrations were applied continuously: ambient CO_2 (399.5–415.3 μL/L; mean: 406.0 ± 6.7 μL/L), representing the current level of atmospheric CO_2 concentration, and elevated CO_2 (750.0–788.5 μL/L; mean: 770.5 ± 9.3 μL/L), simulating the predicted level of atmospheric CO_2 concentration at the end of this century (Mastrandrea et al., 2011). Three growth chambers were used for each CO_2 treatment as replicates (total six chambers). In these growth chambers, the environmental conditions were set at 27 °C and 65% RH during the day and 26.5 °C and 75% RH at night. The photoperiod was L14: D10, and the light intensity was 20,000 Lux in each growth chamber.

2.2. Tea plants and aphid colony

The one-year old tea seedlings (cv. Longjing Changye) were supplied by the Department of Tea Science, College of Horticulture, Nanjing Agricultural University, and planted in plastic pots (4 cm diameter and 5 cm height) filled with nutrient-rich potting soil (Xingnong Organic Fertilizer CO., LTD., Zhenjiang, China). One seedling was planted per pot and these individual pots were placed in a larger plastic tray (40 cm width, 50 cm length and 7 cm height) accommodating 72 individual pots per tray. These trays were then placed in electronically controlled growth chambers that maintained ambient and elevated levels of CO_2 concentrations for 60 days. During the entire experimental period, the potted tea seedlings were watered moderately every other day, and no additional chemical fertilizers or insecticides were used during the entire experiment. The experiment consisted of 288 pots (i.e., 288 tea seedlings) arranged in four trays and placed in each growth chamber, with total 1728 tea plants (i.e., 288 plants per growth chamber × 3 growth chambers per CO_2 level × 2 CO_2 levels) for the whole experiment. Experimental pots (plants) in each growth chamber were re-randomized once a week to minimize position effects within the growth chamber.

The tea aphids used in this study were also provided by the Department of Tea Science, College of Horticulture, Nanjing Agricultural University. These aphids were reared on the tea seedlings for 2–3 generations in the laboratory to establish the aphid colony and then they were exposed to tea seedlings grown under ambient and elevated CO_2 conditions in electronically controlled growth chambers. Half of the experimental tea seedlings were inoculated with 5 adults per plant 30 days after the seedlings were transferred to CO_2 treatment chambers, and the aphid population abundances and dynamics (individuals per plant) were measured for 30 days after aphid inoculation at 5-day intervals. The other half of the experimental plants did not receive aphid inoculation and served as uninfested control plants.

2.3. Measurement of the biomass of tea seedlings grown under ambient and elevated CO_2

Sixty days after the transfer of potted plants into the CO_2 treatment chambers, the tea seedlings were uprooted by the wet excavation method, and the biomass of tea seedlings (g fresh weight) were measured. Fifteen tea seedlings (5 seedlings per growth chamber) were selected randomly from both the ambient and elevated CO_2 experimental treatments which were not inoculated with tea aphids, and then washed with distilled water. Each individual seedling was dissected by a sharp scalpel into root, stem and leaves, and then measured the fresh weight of each plant tissues separately using an electronic microbalance with an accuracy of ± 0.001g (Mettler Toledo XP6, Switzerland).

2.4. Measurement of photosynthesis of tea seedlings grown under ambient and elevated CO_2

Sixty days after the transfer of potted plants into the CO_2 treatment chambers, fifteen uninfested control tea seedlings (seedling with no aphid inoculation) were selected randomly from each growth chambers of the ambient and elevated CO_2 experimental treatments to individually measure leaf photosynthesis with a portable photosynthetic apparatus LI-6400XT (LI-COR Company, USA). The light intensity was set at $2000\ \mathrm{mol\ m^{-2}\ s^{-1}}$, and then leaf net photosynthetic rate (NPR), stomatal conductance (SmC), transpiration rate (TpR) and intercellular CO_2 concentration (ICC) were measured. A single mature leaf away from the terminal was randomly picked from each selected plant and tested thrice and the average value was recorded.

2.5. Measurement of foliar contents of soluble nutrients, functional components and secondary defensechemicals of tea seedlings grown under ambient and elevated CO_2

To study the biochemical changes of the tea seedlings grown under ambient and elevated CO_2 for 60 days, the foliar contents of soluble nutrients, functional components and secondary defense chemicals of 60-day-old tea seedlings without aphid inoculation were measured.

2.5.1. Foliar soluble nutrient contents

The foliar soluble components, such as soluble sugars (SSs), soluble proteins (SPs), free amino acids (FAAs), and free fatty acids (FFAs) were quantified. For the SS and FAA determinations, 1 g of leaf samples were cut into 5 ml of 80% ethanol and the mixture was boiled for 10 min and centrifuged at 13,000 rpm for 10 min. The supernatant was collected, and the pellet was re-extracted in 5 ml of 80% ethanol at 70 °C, then the supernatant was collected again (Jiang et al., 2016). The supernatants were pooled, and the foliar SS contents were estimated by an enzymatic analysis using phenol sulfuric acid (Dubois et al., 1956; Mohotti and Lawlor, 2002). The foliar FAA content was determined in the remaining supernatant according to the method of Moore and Stein (1954) using leucine as standard (Satyanarayana et al., 2011). The foliar SP content was measured according to the Bradford's method (1976), in which 5 ml of the protein reagent was added into 0.1 ml of the extraction and the contents were mixed on a vortex mixer. The absorbance was measured at 595 nm after 1 h. The foliar SP content was calculated from a constructed standard curve for bovine serum albumin. The estimation of the foliar FFA content was carried out following the extraction procedure of Garcia-Lopez et al. (1994), transformed to methyl esters using the method of Metcalfe et al. (1966), and then quantized FFA using gas chromatography (Cai et al., 2014; Shimadzu, Kyoto, Japan) equipped with an FID detector (Choudhary and Grover, 2013; Shoghi-Kalkhoran et al., 2013).

2.5.2. Determination of foliar functional contents

Amino acids content An automatic amino acids analyzer (Hitachi L-8900, Japan) was used to measure the foliar contents of amino acids (AAs), including theanine (The), aspartic acid (Asp), arginine (Arg), threonine (Thr), serine (Ser), alanine (Ala), glutamic (Glu), glycine (Gly), isoleucine (Ile), histidine (His) and lysine (Lys) of tea seedlings grown under ambient and elevated CO_2 for 60 days. AAs were measured by adding 5 mL of tea leaf extract with 5 mL of sulfo-salicylic acid and centrifuged the mixture at 13,000 rpm for 5 min to facilitate the reaction. The mixture was filtered through a 0.20 μm nylon filter membrane and run using the amino acid analyzer (Su et al., 2003; Wang et al., 2006).

Ploypheno1s content The phenolic content in tea leaf extract was determined by using the Folin-Ciocalteu colorimetric method (Singleton et al., 1999). All sample extracts were diluted 1:20 with distilled water to obtain readings within the standard curve ranges of 0.0–600.0, lg of gallic acid per mL. The tea leaf extracts were oxidized with the Folin-Ciocalteu reagent, and the reaction was neutralized with sodium carbonate. The absorbance was measured at 760 nm after 90 min at room temperature by an MRX II Dynex plate reader (Dynex Technologies, Inc., Chanilly, VA, USA). The absorbance values were then compared with those of standards with known gallic acid concentrations. All values were stated as the mean (mg gallic acid equivalents per g tea leaf extract) ± SD for three replications.

Caffeine content The tea leaf samples were dried at 80 °C for 24h. Then, caffeine was extracted and purified from dried tea leaves. The foliar caffeine content was quantified using an HPLC-based method (Kim and Sano, 2008).

2.5.3. Determination of foliar defense-chemicals

The contents of two major secondary defensive chemicals, i.e., jasmonic acid (JA) and salicylic acid (SA) were measured. The frozen leaf tissues of tea seedlings (about 100 mg) were homogenized in 1 mL of ethy1 acetate spiked with labeled internal standards. The homogenates were centrifuged at 13,000 rpm for 20 min at 4 °C and resulting supernatants were transferred to fresh 2-ml tubes. Then liquid portion was evaporated using a vacuum concentrator (Eppendorf). The residues were re-suspended in 0.5 ml of 70% methanol (vol/vol) and centrifuged at 13,000 rpm for 10 min at 4 °C. After pipetting the supernatants to glass vials, the foliar JA content was quantified by HPLC-MS/MS (Agilent Technologies Incorporated, US) following the method described by Wu et al. (2007b). In addition, HPLC-MS/MS was also used to determine the foliar SA content.

2.6. Bioassay of the transcript expression levels of synthetic genes of foliar functional components in tea seedlings grown under ambient and elevated CO_2

The transcript expression levels of five key synthetic genes of the foliar functional components in tea seedlings grown under ambient and elevated CO_2 for 60 days, including theanine synthetase (THS), phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), caffeine synthase (CAS) and s'-adenosine methionine synthetase (SaMS), were assayed through reverse transcription and real-time PCR analyses.

2.6.1. RNA preparation and reverse transcription

Sixty days after tea seedlings were grown in growth chambers under ambient and elevated CO_2, nine tea leaves were randomly collected from each growth chamber (one leaf per pot) and grouped into three samples for each chamber, each sample containing three randomly collected leaves; these three 3-leaf samples constituted as three biological replicates (i.e., total nine biological replicates for each CO_2 treatment). The total RNA was extracted from each replicate sample by using the TRIzol® reagent (Invitrogen). The concentration and quality of the samples were determined by NanoDrop™ spectrophotometer (Thermo Scientific) and 1.5% agarose gel electrophoresis. The 1st-strand complementary cDNA templates were synthesized with 100 ng total RNA by using the PrimeScript™ RT reagent Kit with gDNA Eraser (TaKaRa, Japan). Reverse transcriptase reactions were performed in a 20 μL final volume reaction.

2.6.2. Real-time PCR analysis

Each cDNA product was diluted twice from 20% to 1.25% solution using RNase-free dH_2O, in order to make the Ct value within the suitable range of 15–35 based on preliminary experiments. For the fluorescence-based quantitative real-time PCR (qRT-PCR), 2 μL cDNA dilution and 0.2 μM primer were used in 1 × SYBR®Premix Ex Taq™ (TaKaRa, Japan) with the 7500 Real-Time PCR Detection System (Applied Biosystems, Foster city, CA) following the supplier's instructions. The reactions were performed in a 20 μL final volume. Then, the

Table 1
Primers designed and used in measuring the transcript expression levels of the foliar functional components genes, including theanine synthetase (THS), phenylalanine ammonia lyase (PAL), chalcone synthase (CHS), caffeine synthase (CAS) and s'-adenosine methionine synthetase (SaMS) of tea seedlings in the qRT-PCR experiments.

Primers	Sequences (5′ to 3′)	Gene Bank Accession	Description
THS	Forward: ACCAACTACAGTACCAAGTCCAT Reverse: CCATAAGCAGCAATGTGTTCCT	DD410895	theanine-synthetase gene
PAL	Forward: GCTCATGTCATCTACCTACTTGG Reverse: ACTTGGCTTACTGTGTTCCTCA	D26596	phenylalanine-ammonia-lyase gene
CHS	Forward: GGCATCTCTGATTGGAACTCTT Reverse: GACATATTACCGTACTCACTTAGCA	AY656677	chalcone-synthase gene
CAS	Forward: ATCATACGCTCCGCTAAGTCTC Reverse: AGATATAGTGACGACGCCTAAGAG	AB031280	caffeine-synthase gene
SaMS	Forward: ATGATGAGATTGCTGCTGAT Reverse: AGAAGGGTTGAGGTGGAA	AB041534	s'-adenosine-methionine-synthetase gene
β-action	Forward: GCCATCTTTGATTGGAATGG Reverse: GGTGCCACAACCTTGATCTT	HQ420251	Housekeeping gene

specific primers were designed using Beacon Designer™ 7.9 software, and the housekeeping gene β-action (Etschmann et al., 2006; Ma et al., 2016) was used as the internal standard to analyze the transcript expression levels of five genes of foliar functional components in tea seedlings grown under ambient and elevated CO_2 for 60 days, including the synthetic genes of theanine synthetase (THS), phenylalanine ammonia lyase (PAL) and s'-adenosine methionine synthetase (SaMS), chalcone synthase (CHS), and caffeine synthase (CAS). All primers used for the qRT-PCR analysis were shown in Table 1. Quantification of the transcript expression levels of measured genes was conducted following the $2^{-\Delta\Delta Ct}$ normalization method (Livak and Schmittgen, 2001). The transcript expression levels of housekeeping (i.e., internal control) gene β-action were examined in every PCR plate to eliminate systematic errors. For each biological replicate (nine replicates per CO_2 treatment), three technical repeats were performed in the qRT-PCR analysis.

2.7. Data analysis

All experimental data were analyzed using IBM-SPSS v.20.0 (IBM, Armonk, NY, USA). The measured photosynthesis indices (including NPR, SmC, ICC and TpR), foliar soluble nutrients (including SSs, SPs and FFAs) and tea functional components (including theanine, TPs, caffeine and AAs), and the relative transcript expression levels of the measured five tea-functional-component genes (including THS, PAL, CHS, CAS and SaMS) of the tea seedlings grown under ambient and elevated CO_2 for 60 days were analyzed by one-way analysis of variance (ANOVA) with two CO_2 levels (ambient CO_2 vs. elevated CO_2) as sources of variability, and significant differences in the measured indices between treatments of ambient and elevated CO_2 were analyzed by the LSD test at $P < 0.05$. Moreover, one-way repeated-measures ANOVA was also used to analyze the effects of two CO_2 rearing conditions on the population dynamics of the tea aphidfed on tea seedlings after they were exposed to ambient and elevated CO_2 for 30 days, and significant differences between ambient and elevated CO_2 was analyzed by the paired-t test at $P < 0.05$. Furthermore, the Pearson test was performed by using R software (version R i386 3.4.2) to analyze the correlation between the population abundances of tea aphid after they were exposed to ambient and elevated CO_2 for 30 days and the foliar nutrients/functional components of tea seedlings grown under ambient and elevated CO_2 for 60 days.

3. Results

3.1. Biomass of tea seedlings grown under ambient and elevated CO_2

CO_2 level significantly affected the biomass of root ($F = 5.76$, $P = 0.023$), leaf ($F = 4.93$, $P = 0.035$) and total plant ($F = 6.95$, $P = 0.013$) of tea seedlings, but didn't significantly affect the biomass of stem ($F = 0.28$, $P = 0.60$) and shoot tissues ($F = 2.37$, $P = 0.14$), and the root-shoot ratio ($F = 0.41$, $P = 0.53$) of tea seedlings (Table 1). Significant increases were found in biomass of root (+22%), leaf (+15.04%) and total plant (+16.26%) of tea seedlings grown under elevated CO_2 in contrast to that in ambient CO_2 ($P < 0.05$; Fig. 1). In addition, some increases were also found in the biomass of stem (+8.97%), shoot tissues (+12.98%), and the root-shoot ratio (+8.08%) of tea seedlings grown under elevated CO_2 compared to that in ambient CO_2 ($P > 0.05$; Fig. 1).

3.2. Photosynthesis parameters of tea seedlings grown under ambient and elevated CO_2

CO_2 level significantly affected the net photosynthetic rate (NPR; $F = 5.93$, $P = 0.027$) and the intercellular CO_2 concentration (ICC; $F = 7.08$, $P = 0.017$) in tea seedlings, but didn't significantly altered stomatal conductance (SmC; $F = 0.32$, $P = 0.58$) and transpiration rate (TpR; $F = 1.69$, $P = 0.21$) in the seedlings (Table 1). Compared with ambient CO_2, elevated CO_2 significantly enhanced the values of NPR (+20%; $P < 0.05$) and ICC (+15.74%; $P < 0.05$), while decreased the values of SmC (−5.52%; $P > 0.05$) and TpR (−9.40%; $P > 0.05$) for the tea seedlings (Fig. 2).

3.3. Foliar soluble nutrients and functional components of tea seedlings grown under ambient and elevated CO_2

3.3.1. Foliar soluble nutrient components

CO_2 level significantly impacted the foliar contents of soluble sugars (SSs; $F = 25.52$, $P = 0.007$) and free fatty acids (FFAs; $F = 8.22$,

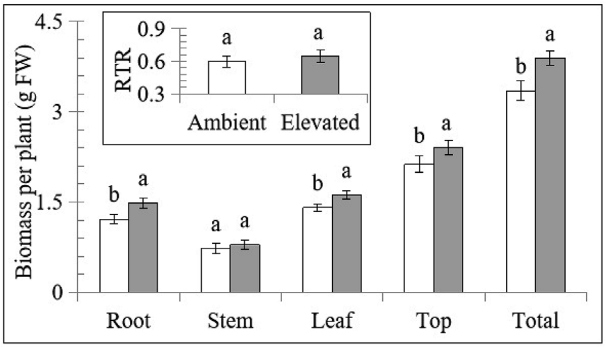

Fig. 1. Root, stem, leaf, and total plant biomass and root-shoot ratio (RSR) of 14-month old tea seedlings grown under ambient and elevated CO_2 for 60 days. (**Note:** Different lowercase letters indicated significant differences between ambient and elevated CO_2 for same measured index by the LSD test at $P < 0.05$; Same in the following figures).

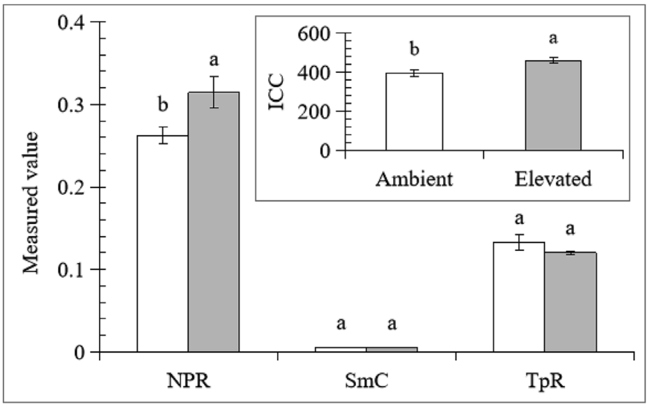

Fig. 2. Photosynthesis parameters of net photosynthetic rate (NPR; μmol CO_2 m^{-2} s^{-1}), stomatal conductance (SmC; mol H_2O m^{-2} s^{-1}), transpiration rate (TpR; mol H_2O m^{-2} s^{-1}) and intercellular CO_2 concentration (ICC; μmol CO_2 mol^{-1}) measured on 14-month old tea seedlings grown under ambient and elevated CO_2 for 60 days.

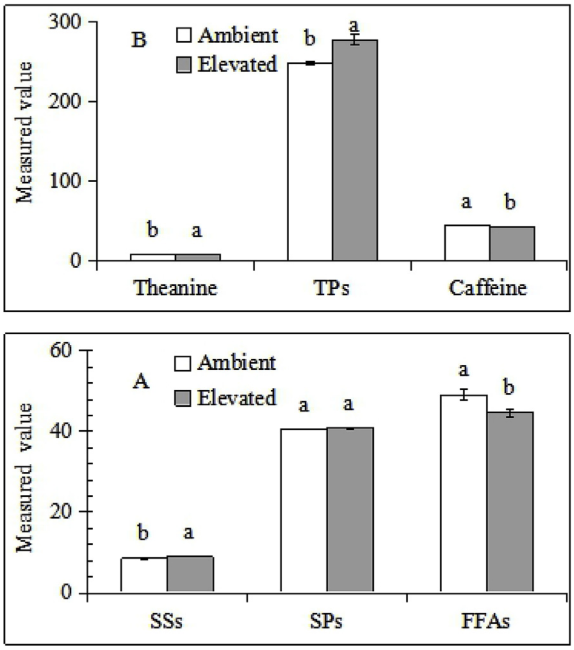

Fig. 3. Foliar contents of soluble nutrients (A; including soluble sugars (SSs; mg/g), soluble proteins (SPs; μg/g), free fatty acids (FFAs; μg/g)) and functional components (B; including theanine (mg/g), tea polyphenols (TPs; mg/g), caffeine (mg/g)) of 14-month old tea seedlings grown under ambient and elevated CO_2 for 60 days.

$P = 0.046$) of tea seedlings, but didn't significantly affect soluble proteins (SPs; $F = 3.70$, $P = 0.13$) (Table 1). Compared with ambient CO_2, elevated CO_2 significantly increased SSs (+4.74%; $P < 0.05$) and significantly reduced FFAs (−9.09%; $P < 0.05$), and slightly enhanced SPs (+0.67%; $P > 0.05$) for tea seedlings grown under ambient and elevated $CO_2$for 60 days (Fig. 3A).

3.3.2. *Foliar functional components*

CO_2 level significantly affected the foliar contents of functional components of tea seedlings ($F \geq 10.94$, $P \leq 0.030$; Table 1). Significant increases in the foliar content of theanine (+3.66%) and polyphenols (TPs; +12.01%), and significant decrease in the foliar content of caffeine (−3.38%) were found when tea seedlings were grown under elevated $CO_2$for 60 days in contrast to ambient CO_2 ($P < 0.05$; Fig. 3B).

Table 2
One-way ANOVAs for the effects of CO_2 levels (ambient vs. elevated) on biomass, root-top ratio, photosynthesis indexes, foliar soluble nutrients and functional components, and the relative transcript levels of respective synthetic genes of foliar functional components of tea seedlings grown under ambient and elevated CO_2 for 60 days, and one-way repeated-measures ANOVA for the impacts of CO_2 levels on the population dynamics of tea aphid, *Toxoptera aurantii* fed on tea seedlings grown under ambient and elevated CO_2 during the 30-day exposure period (*F*/*P* values).

Measured indexes		*F* value	*P* value
Biomass per seedling (g FW)	Root (i.e., belowground)	5.76	0.023*
	Stem	0.28	0.60
	Leaf	4.93	0.035*
	Shoot (i.e., aboveground)	2.37	0.14
	Total plant	6.95	0.013*
Root-shoot ratio (RSR)		0.41	0.53
Photosynthesis indexes	NPR (μmol CO_2 m^{-2} s^{-1})	5.93	0.027*
	SmC (mol H_2O m^{-2} s^{-1})	0.32	0.58
	ICC (μmol CO_2 mol^{-1})	7.08	0.017*
	TpR (mol H_2O m^{-2} s^{-1})	1.69	0.21
Leaf soluble nutrients	SSs (mg/g.FW)	25.52	0.007**
	SPs (μg/g.FW)	3.70	0.13
	FFAs (μg/g.FW)	8.22	0.046*
Leaf functional components	Theanine (mg/g.FW)	97.78	< 0.001***
	TPs (mg/g.FW)	137.21	< 0.001***
	Caffeine (mg/g.FW)	10.94	0.030*
	JA (μg/kg.FW)	112.90	< 0.001***
	SA (μg/kg.FW)	41.50	0.003**
Synthetic genes of leaf functional components	*THS*	12.00	0.003**
	PAL	8.10	0.012*
	CHS	75.61	< 0.001***
	CAS	60.11	< 0.001***
	SaMS	188.98	< 0.001***
Population dynamics of tea aphids		28.42	< 0.001***

Note: $^*P < 0.05$,$^{**}P < 0.01$, $^{***}P < 0.001$.

Moreover, Table 2 indicated that CO_2 level significantly affected several essential amino acids in tea seedling leaves, including aspartic acid (Asp) ($F = 1596.2$, $P < 0.001$), glutamic acid (Glu) ($F = 708.6$, $P < 0.001$), Serineacid (Ser) (F = 33.98, P = 0.0043), histidine acid (His) (F = 19.85, P = 0.011), and arginine acid (Arg) ($F = 52.95$, $P = 0.0019$), while other amino acids were not significantly affected ($F \leq 3.85$, $P \geq 0.12$). Compared with ambient CO_2, elevated CO_2 significantly decreased the foliar contents of Asp (−75.04%) and Glu (−46.10%), and significantly increased the foliar contents of Ser (+31.85), His (+53.69), and Arg (+70.99) content ($P < 0.05$; Fig. 4).

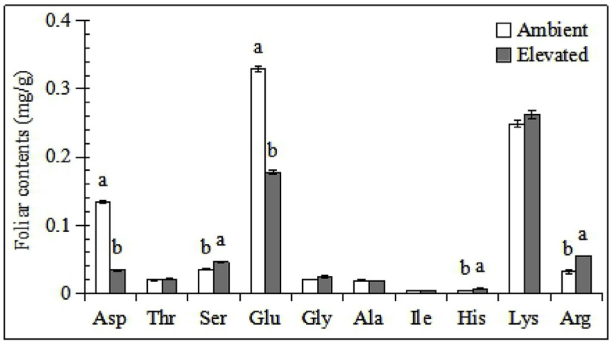

Fig. 4. The foliar contents of 10 types of essential amino acids in the leaves of 14-month old tea seedlings grown under ambient and elevated CO_2 for 60 days.

Table 3
One-way ANOVAs for the effects of CO_2 levels (ambient vs. elevated) on the foliar contents of 10 types of essential amino acids in the leaves of tea seedlings grown under ambient and elevated CO_2 for 60 days (*F*/*P* values).

Amino acids (mg/g)	*F* value	*P* value
Aspartic acid (Asp)	1596.2	< 0.001***
Threonine (Thr)	1.30	0.32
Serine (Ser)	33.98	0.0043**
Glutamic acid (Glu)	708.6	< 0.001***
Glycine (Gly)	3.85	0.12
Alanine (Ala)	0.03	0.88
Isoleucine (Ile)	2.40	0.20
Histidine (His)	19.85	0.011*
Lysine (Lys)	3.17	0.15
Arginine (Arg)	52.95	0.0019**

Note: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

3.4. Foliar jasmonic acid (JA) and salicylic acid (SA) content of tea seedlings grown under ambient and elevated CO_2

CO_2 level significantly influenced the foliar contents of JA ($F = 112.90$, $P = 0.0004$) and SA ($F = 41.50$, $P = 0.003$) of tea seedlings (Table 3). Compared with ambient CO_2, elevated CO_2 significantly increased the foliar contents of JA (+107.94%) and SA (+94.28%) of tea seedlings ($P < 0.05$; Fig. 5).

3.5. Relative expression levels of synthetic genes of foliar functional components of tea seedlings grown under ambient and elevated CO_2

The relative transcript expression levels of measured five genes of foliar functional components in tea seedlings were significantly affected by CO_2 levels ($F \geq 8.10$, $P \leq 0.012$; Table 1). The relative transcript expression levels of the genes of theanine synthetase (THS; +18.64%), Phenylalanine ammonia lyase (PAL; +49.50%),s'-adenosine methionine synthetase (SaMS; +143.03%), and chalcone synthase (CHS; +61.86%) were up-regulated, and that of the gene of caffeine synthase (CAS; −56.91%) was down-regulated for the tea seedlings grown under elevated CO_2 ($P < 0.05$; Fig. 6).

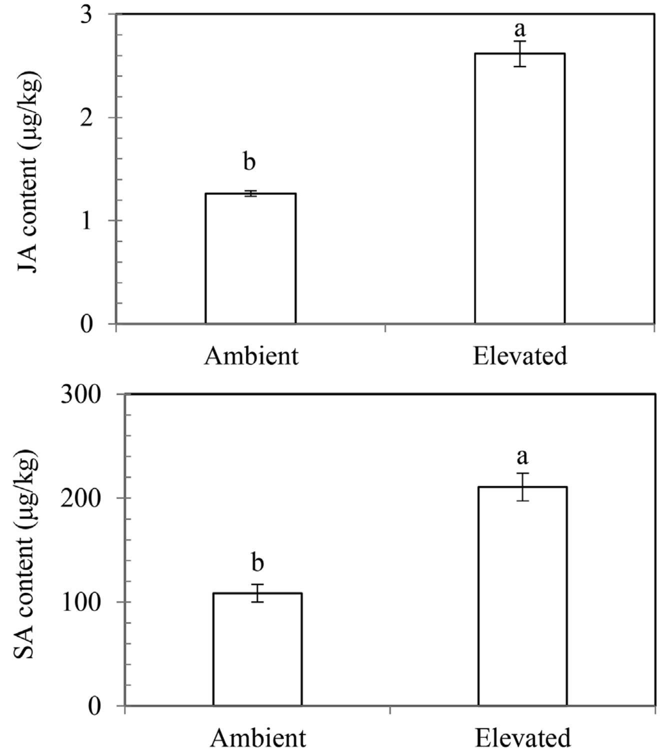

Fig. 5. Foliar contents of jasmonic acid (JA) and salicylic acid (SA) of 14-month old tea seedlings grown under ambient and elevated CO_2 for 60 days.

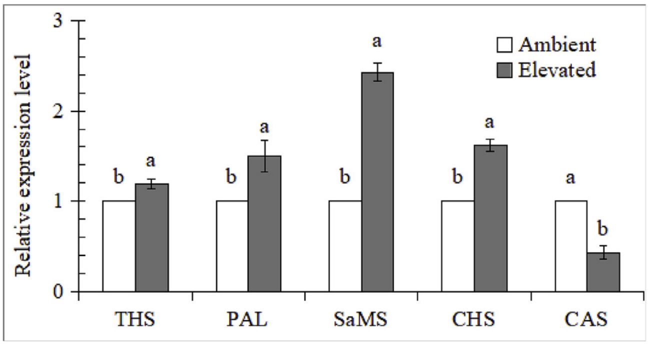

Fig. 6. Relative transcript expression levels of the measured genes (including theanine synthetase (THS), phenylalanine ammonia lyase (PAL), s'-adenosine methionine synthetase (SaMS), chalcone synthase (CHS) and caffeine synthase (CAS)) to regulate foliar functional components of 14-month old tea seedlings grown under ambient and elevated CO_2 for 60 days.

3.6. Population dynamics of tea aphids fed on tea seedlings after exposure to ambient and elevated CO_2 for 30 days

Elevated CO_2 significantly affected the foliar contents of soluble nutrients (including SSs, SPS and FFAs) and functional components (including theanine, polyphenols, caffeine and some essential amino acids) of tea seedlings under enriched CO_2 condition, which would, in turn, affect the population occurrence of tea aphid, *T. aurantii*. Significant effects of CO_2 levels were observed on the population dynamics of *T. aurantii* fed on tea seedlings grown under ambient and elevated CO_2 during the 30-day exposure after aphid inoculation ($F = 28.42$, $P < 0.001$; Table 1). Significant increases (+4.24%–41.17%) in the population abundance of *T. aurantii* were found when they fed on tea seedlings grown under elevated CO_2 compared to ambient CO_2 ($P < 0.05$; Fig. 7).

3.7. Correlation analysis between population abundances of tea aphids and foliar soluble nutrients/functional components of tea seedlings grown under ambient and elevated CO_2

The population dynamics of tea aphid were markedly affected by the foliar contents of soluble nutrients and functional components of tea seedlings. The contents of Asp (+0.87), Glu (+0.84), FFA (+0.57), and Caffeine (+0.63) in tea leaves were positively correlated with the population abundance of tea aphids. Among them, there were high

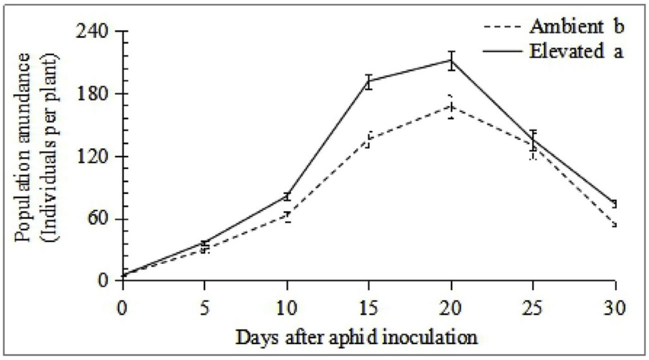

Fig. 7. Population dynamics of the tea aphid, *Toxoptera aurantii* fed on tea seedlings after they were exposed to ambient and elevated CO_2 for 30 days after aphid inoculation. (**Note:** Different lowercase letters indicated significant differences between ambient and elevated CO_2 by paired-T test at $P < 0.05$).

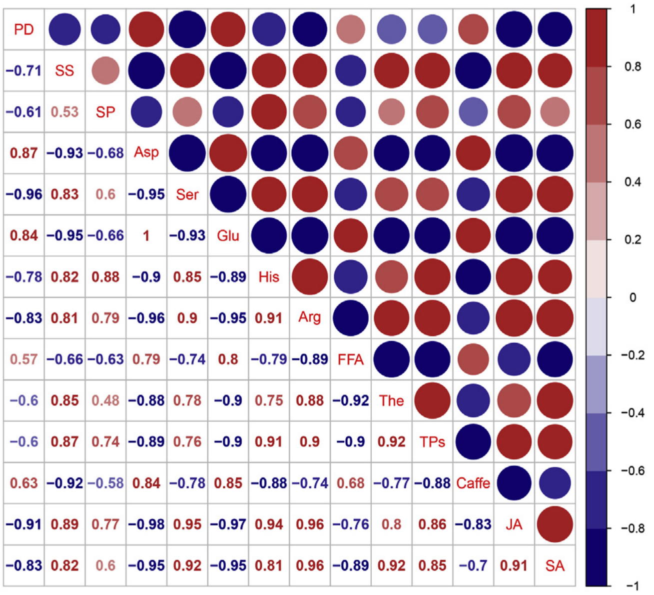

Fig. 8. Correlation analysis of foliar soluble nutrients and functional components of tea seedlings and population abundance of tea aphid, *T. aurantii.* (**Note:** Red indicates positive correlation and blue indicates negative correlation. The darker the color, the stronger the correlation). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

positive correlations between population abundances of *T. aurantii* and foliar contents of Asp and Glu. In contrast, the foliar contents of SS (−0.71), SP (−0.61), Ser (−0.96), His (−0.78), Arg (−0.83), The (−0.60), TPs (−0.60), JA (−0.91) and SA (−0.83) were negatively correlated with the population abundance of *T. aurantii* (Fig. 8).

4. Discussion

4.1. Impacts of elevated CO_2 on the biomass and foliar soluble nutrients of tea seedlings

Being one of the substrates essential for the photosynthesis of plants, CO_2 promotes the growth and development of vascular plants. It is expected that the CO_2 concentration of atmosphere will rise to 800 ppm by the end of this century (IPCC, 2014). In this study, the influence of high CO_2 concentration on tea seedlings and tea aphid was studied, and the CO_2 concentration was maintained at 800 ppm. The results indicated that elevated CO_2 enhanced photosynthesis and improved plant growth in 14 month old tea seedlings exposed for 60 days, with significant increases in net photosynthetic rate (+20%), intercellular CO_2 concentration (+15.74%), leaf biomass (+15.04%) and root-shoot ratio (+8.08%), and significantly decreased stomatal conductance (−5.52%) and transpiration rate (−9.40%) of tea seedlings grown under elevated CO_2 in contrast to ambient CO_2, showing similar results as described by Li et al. (2017). Elevated CO_2 increased photosynthetic rate of rice, but stomatal conductance and transpiration rate were decreased (Jing et al., 2019). Soybean grown under elevated CO_2 exhibited increased photosynthesis that resulted in a higher biomass, plant height, and leaf area (Bencke-Malato et al., 2019; Habermann et al., 2019). Furthermore, elevated CO_2 increased the foliar contents of soluble sugar, starch, and total carbon in tea plants (Li et al., 2017). Elevated CO_2 raised the CO_2 assimilation rate in tea plants, resulting in increased sugar and starch accumulation (Li et al., 2017). They reported that 24-day exposure of tea plants to 800 μmol mol^{-1} CO_2 triggered a remarkable increase in soluble sugar, sucrose, and starch contents in Longjing tea leaves. Elevated CO_2 significantly increased leaf area index, leaf numbers, and branches (Huang et al., 2007; Kimball, 2016). Moreover, elevated CO_2 has shown to increase the root-shoot ratio (Huang et al., 2007). Li et al. (2017) found that elevated CO_2 (800 μmol mol^{-1} for 24 days) could increase tea plant height, shoot dry weight, and root dry weight by 13.46%, 24.68%, and 67.80%, respectively. Our current study found 9.51%, 11.34%, and 36.33% increase in plant height, shoot dry weight, and root dry weight, respectively. Additionally, Li et al. (2017) reported that elevated CO_2-induced promotion in both shoot and root biomass accumulation ultimately improved the root-shoot ratio by 27.66% compared with that in ambient CO_2, which is much higher than 8% increase in root-shoot ratio observed in our study. According to Wijeratne et al. (2017), a study conducted in SriLanka to assess the impact of climate change on tea production showed that elevation of atmospheric CO_2 from 370 ppm to 600 ppm will likely increase the tea yield by approximately 33–37%. Meanwhile, Li et al. (2017) found that tea plants grown under 800 μmol mol^{-1}of elevated CO_2 for 24 days showed an increase in amino acid content compared with that in ambient CO_2. Elevated CO_2 induced variations on amino acid biosynthesis during CO_2 exposureand acted as a possible catalyst for the associated acclimation responses (Li et al., 2017). Hence, we can speculate that the rise in atmospheric CO_2 level will likely increase the tea yield.

4.2. Impacts of elevated CO_2 on the foliar soluble functional components and relative expression levels of synthetic genes of tea seedlings

The expression levels of five genes of foliar functional components in 14-month-old tea seedlings grown under ambient and elevated CO_2 for 60 days were significantly affected by CO_2 levels. The relative transcript levels of the genes of theanine synthetase (THS), phenylalanine ammonia lyase (PAL), 's-adenosine methionine synthetase (SaMS), and chalcone synthase (CHS) were up-regulated, and that of the gene of caffeine synthase (CAS) were down-regulated for the tea seedlings grown under elevated CO_2 for 60 days. Caffeine (purine alkaloid) is an important secondary metabolite found in *Camellia sinensis* (Ashihara et al., 2008). Recently, Li et al. (2017) found that decreased caffeine biosynthesis under elevated CO_2 condition increased plant susceptibility to *Colletotrichum gloeosporioides,* a significant phyto-pathogen causing anthracnose disease in tea. Even a short period of CO_2 enrichment (800 μmol mol^{-1} for 24 days) caused a significant reduction in caffeine content in tea leaves (Li et al., 2017). In plants, caffeine synthesis is dependent on several key genes, such as inosine 5′-monophosphate dehydrogenase (TIDH), S-adenosyl-L-methionine-synthase (sAMS), and tea caffeine synthase 1 (TCS1). Li et al. (2017) showed that the exposure to elevated CO_2 could substantially suppress the transcript levels of TIDH, sAMS, and TCS1 in tea leaves, indicating that elevated CO_2-induced down-regulations of caffeine biosynthetic genes eventually decrease caffeine content in tea leaves under elevated CO_2 condition. This phenomenon is consistent with the results of our present study.

Theanine is the major tea amino acids accounting for about 50% of total free amino acids in tea leaves and is responsible for umami flavor of tea (Cheng et al., 2017). Paucity of information is available on the effects of elevated CO_2 on theanine concentration in tea leaves. Jiang et al. (2018) reported that exposure of tea plants to 500 and 750 μmol mol^{-1} CO_2 treatments for 6 months resulted in a decreased theanine content in spring tea compared to that in the ambient air CO_2 (350 μmol mol^{-1}) concentration. However, Li et al. (2017) found that tea plants grown under 800 μmol mol^{-1} CO_2 for 24 days accumulated an increased theanine content compared with that in plants grown under 400 μmol mol^{-1} CO_2. Results from this study are in agreement with Li et al. (2017) wherein both studies had tea plants exposed to elevated CO_2 for a shorter duration (up to 60 days), in contrast to Jiang et al. (2018) study with substantially longer exposure. It is plausible that the tea plant responses to elevated CO_2 altering foliar theanine content is saturated when plants are exposed to elevated CO_2 for a longer duration. Additional research is warranted to elucidate these relationships. It is not uncommon to find that CO_2 concentration can change the foliar content of tea functional substances. The two major functional substances, polyphenols and theanine, determine the quality of tea. Tea polyphenols have a variety of biological activities, including anti-radiation, anti-inflammation and bacteriostasis, and have potential effects in the prevention of neurodegenerative diseases, metabolic diseases, and cancerous cell growth (Yang et al., 2018). Theanine is the main flavoring substance in tea that increases the 'umami' and sweetness in the tea (Juneja et al., 1999). Our study suggests that these vital functional substances in tea can be improved by way of manipulating the crop growth environment via exposure to elevated CO_2 during the early growth stage.

Caffeine, one of the most abundant compounds in brewed tea, is also a major contributor to the bitter taste of tea and is an important determinant of tea quality (Zhang et al., 2017). Additionally, caffeine may impact tea flavor in more complex ways since bitterness can affect the sensory perception of some volatiles (Drewnowski, 2001). There are also other classes of compounds that directly and indirectly impact the quality of tea, but volatiles, polyphenols, methylxanthines, and amino acids are primary contributors to the flavor, aroma, and health benefits of tea. Elevated CO_2 appears to impact these plant constituents in a way to determine the tea quality for marketability and consumption.

4.3. Impacts of foliar soluble components on the population dynamics of tea aphid and the quality of tea

Plants have the ability to produce numbers of metabolites that have diverse functions with respect to structural and functional significances. Primary metabolites which comprise only a small portion of total plant metabolites are of paramount importance for plant growth and development; greater portion of the total plant metabolites are the secondary metabolites which are critical for other diverse functions, including plant defenses against biotic and abiotic stressors (Zhao et al., 2013). Also, robust sets of secondary metabolisms contribute well to the enhancement of the nutraceutical quality in plants (Pérez-López et al., 2018). Studies have shown that elevated CO_2 augments the rate of photosynthesis and also enhances the content of high energy compounds, such as adenosine triphosphate (ATP) and nicotinamide adenide dinucleotide phosphate (NADPH). Carbohydrate abundance is increased following the increased photosynthetic rates, resulting in enhanced state for the production of secondary metabolites in plants. Caffeine is thought as a repellant and toxicant to insects and other herbivorous pests (Hollingsworth et al., 2003). Variation in caffeine content has been associated with pest resistance in tea. Additionally, caffeine may have antimicrobial activity in tea. Li et al. (2016) found that a reduction in caffeine concentration in tea leaves grown under elevated CO_2 was associated with a sharp increase in susceptibility to brown blight disease. In tea, caffeine is generally more concentrated in younger leaves compared to older leaves, possibly because younger leaves are more susceptible to herbivores and diseases (Lin et al., 2003).

The primary free amino acid in tea is L-theanine (The), a glutamate derivative, which accounts for 1–2% of the dry weight of tea (Nobre et al., 2007). Theanine is synthesized in tea roots from glutamate and transported to leaves via phloem where, among other functions, it serves as a precursor for catechins. While L-theanine itself has no known anti-herbivore properties, its content in tea leaves is known to be affected by environmental factors including elevation, shade, and temperature (Saijo, 1980; Li et al., 2015). Aphids, belonging to the family Hemiptera with their piercing-sucking mouthparts, penetrate plant tissues and suck the cell saps from phloem, xylem, or other cell contents. Aphid feeding frequently activates the salicylic acid (SA) pathway in plants in addition to the JA pathway. Going further, infestation by tea aphids leads to increased emission of benzaldehyde and E-hexenal from tea shoots compared to that in control treatment (Han and Chen, 2002).

Elevated CO_2 generally increases the food intake of insects in many field crops and horticultural crops (Leslie, 2004). However, it is believed that Eastern Beauty Oolong tea is only produced when tea leaves are infested by tea green leafhoppers (*Empoasca vitis*) and a distinct flavor is manifested due to the volatile emissions from insect feeding. The merits of so-called 'bug bitten tea' might not be limited to East Asia only as cases are also seen in tea growing regions of India, Darjeeling. Mechanical damages inflicted by *E. vitis* leads to the emission of constitutive volatiles in live tea plants (linalool, hotrienol, methyl salicylate, phenylacetaldehyde, 2-phenylethanol, E-β-ocimene, DMNT, and (E, E) - α - farnesene) which attract predators such as jumping spiders and this leads to tea aroma (Cai et al., 2014). In addition, tea attacked by leafhoppers and thrips showed up-regulation of alcohol dehydrogenase, geraniol synthase, several linalool synthases, and other enzymes related to volatile secondary metabolite production during the enzymatic browning step of Darjeeling black tea processing relative to undamaged leaves. This resulted in a significant increase in volatile concentration in processed tea. Therefore, cell-rupture feeders may improve tea quality through a combination of induction of precursor metabolites (such as linalool) and priming (up-regulation of metabolic pathways), which, when combined with mechanical damage during tea processing, results in a greater concentration and unique blend of volatiles in the processed tea (Cho et al., 2007). However, the density of leaf hoppers and the amount of damage required to produce this unique blend of aroma has not been well understood.

We found that elevated CO_2 significantly affected the foliar contents of soluble nutrients (including SSs, SPS and FFAs) and functional components (including theanine, polyphenols and caffeine) of tea seedlings, which would, in turn, affect the population occurrence of tea aphid, *T. aurantii*. Significant increases in the population abundance of *T. aurantii* were found when they fed on tea seedlings grown under elevated CO_2 compared to ambient CO_2 during the 30-day exposure after the aphid inoculation 30 days after the tea seedlings were exposed to CO_2 treatment chambers. In plants, caffeine acts as a natural deterrent that paralyzes and kills many insects feeding upon them. Hence, we can postulate that the severity of infestation of tea aphid under elevated CO_2 will be enhanced since there is reduction of caffeine content and also since elevated CO_2 down-regulated the relative transcript level of gene of caffeine synthase (CAS) for the tea seedlings, which is the precursor for caffeine production. However, significant increases in the foliar contents of theanine and tea polyphenol, and the up-regulation of the levels of the genes of theanine synthetase (THS), phenylalanine ammonia lyase (PAL), s'-adenosine methionine synthetase (SaMS), and chalcone synthase (CHS) under the elevated CO_2 demands more research upon the tea aphids response. Additionally, significant increase of SSs and marginal increase of SPs under the rising CO_2 concentration, but no significant reduction of the foliar contents of FFAs and no significant effects upon the foliar content of soluble proteins (SPs) of tea seedlings grown under elevated CO_2 pose additional curiosity into the response of tea aphids under these conditions. The population abundance of tea aphid increased under elevated CO_2 concentration, and the pest may improve the quality of tea. Therefore, the interaction between tea aphid and plants under the elevated CO_2 condition is worthy of further study. One of the things that should be noted is that the interaction is determined by not only the plants but also the insect pests. Herbivorous insects can adapt to changes in host plants or other environments (Manel et al., 2009). The tea aphid may be affected by elevated CO_2 and may also be able to adapt to it quickly. The results observed in this study may have been different if we had used adapted generation of insects to study the effect of elevated CO_2 on the interaction between plant and insect. Therefore, additional study comparing elevated CO_2 adapted versus non-adapted aphids may elucidate further insights into the effect of elevated CO_2 on aphid-plant interactions.

5. Summary

Rise in the atmospheric CO_2 level is affecting plant physiology and

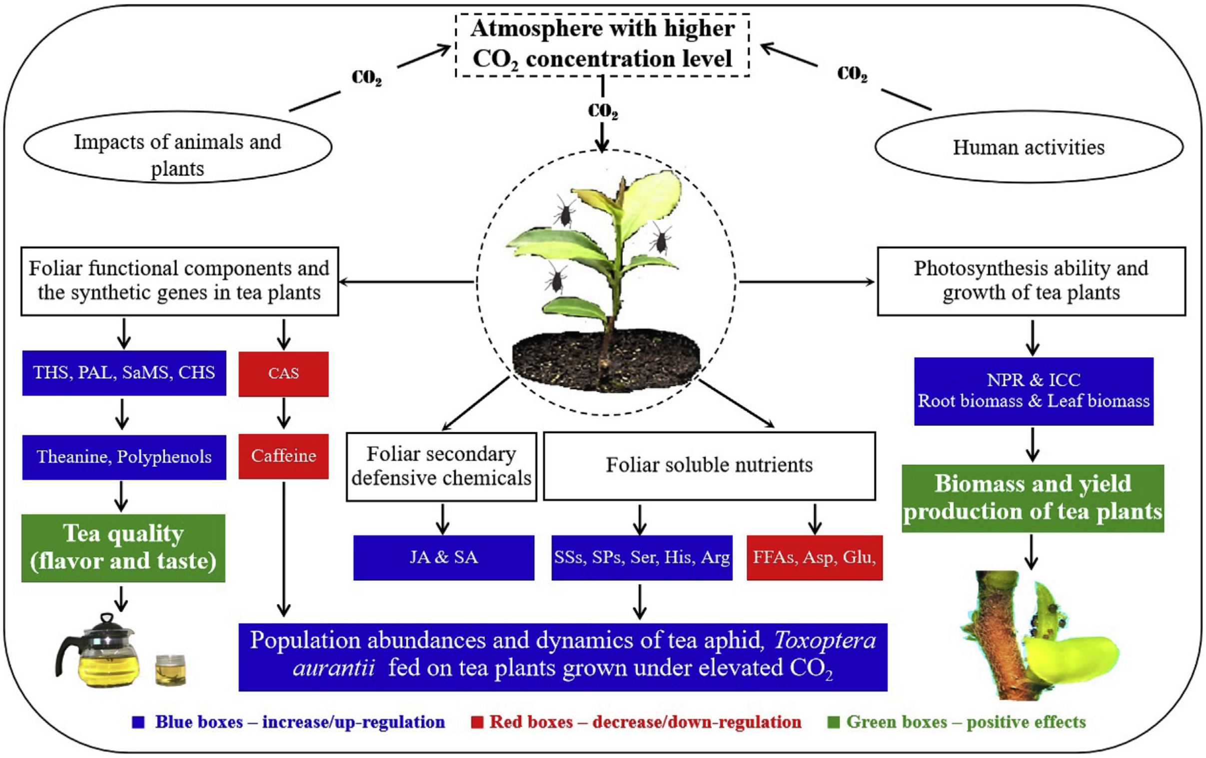

Fig. 9. A schematic model of insect-plant interactions involving herbivores (here, tea aphid, *T. aurantii*) and tea plantation under a climate change scenario of atmospheric CO$_2$ concentration level arising that may result in improved tea production enterprise.

biochemical properties of plants by altering plant primary and secondary metabolisms. In this study, the results indicated that elevated CO$_2$ enhanced the photosynthesis and improved plant growth and development of tea seedlings, with significant increases in net photosynthetic rate, intercellular CO$_2$ concentration, and leaf biomass and root-shoot ratio. As a result, the tea yield is expected toincrease. Moreover, the relative transcript levels of the genes of theanine synthetase, phenylalanine ammonia lyase, s'-adenosine methionine synthetase, and chalcone synthase were all up-regulated, and elevated CO$_2$ significantly increased the foliar content of soluble sugars, theanine and tea polyphenols of tea seedlings. In addition, the foliar content of caffeine decreased, and the foliar contents of jasmonic acid (JA) and salicylic acid (SA) increased. The positive effect of leaf nutrition on tea aphid, *T. aurantii,* was greater than the negative effect of secondary defense substances constituted by tea plants. Therefore, the population abundance of tea aphid increased under an elevated CO$_2$ production condition. While further investigation is warranted to characterize the complex plant-insect interactions influenced by increased level of CO$_2$, the effect of climate change on tea production and its cascading effect on tea aphid population dynamics will potentially result in improved tea quality, measured by tea aroma and taste. We propose a schematic model of insect-plant interactions involving herbivores and tea plantation under a climate change scenario (Fig. 9) that may result in improved tea production enterprise. Further research may elucidate these complex interactions.

Submit statements

Respected editorial department: We solemnly declare that we have full copyright on the manuscript (Effects of elevated CO2 on foliar soluble nutrients and functional components of tea, and population dynamics of tea aphid, *Toxoptera aurantii* (PLAPHY-D-19-01277R2)), do not infringe on the rights of others. This manuscript has not been submitted to any other magazine. And that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder.

Author's contribution

Likun Li - Conception and design, execution of experiment, analysis and interpretation of the data, drafting of the article, and critical revision of the article for important intellectual content.

Fajun Chen - Execution of experiment and drafting of the article.

Mengfei Wang, Sabin Saurav Pokharel, Chunxu Li, Wanping Fang - Execution of experiment.

Megha N Parajulee - Revise the grammar of the manuscript.

Declaration of competing interest

None.

Acknowledgement

This research was funded by the earmarked fund for China Agricultural Research System (CARS-19), China; the National Nature Science Foundations of China (NSFC) (31871963 and 31272051), China; the National Key Research and Development Program of China (2017YFD0200400), China; the Fundamental Research Funds for the Central Universities (KYZ201818), China and the Qing-Lan Project of Jiangsu Province of China.

References

Ainsworth, E.A., Rogers, A., Leakey, A.D.B., Heady, L.E., Gibon, Y., Stitt, M., Schurret, U., 2007. Does elevated atmospheric [CO$_2$] alter diurnal C uptake and the balance of C and N metabolites in growing and fully expanded soybean leaves? J. Exp. Bot. 58 (3), 579–591.

Ashihara, H., Sano, H., Crozier, A., 2008. Caffeine and related purine alkaloids: biosynthesis, catabolism, function and genetic engineering. Phytochemistry (Oxf.) 69, 841–856.

Bencke-Malato, M., De Souza, A.P., Ribeiro-Alves, M., Schmitz, J.F., Buckeridge, M.S., Alves-Ferreira, M., 2019. Short-term responses of soybean roots to individual and combinatorial effects of elevated CO_2 and water deficit. Plant Sci. 280, 283–296.

Bezemer, T.M., Jones, T.H., 1998. Plant-insect herbivore interactions in elevated atmospheric CO_2: quantitative analysis and guild effects. Oikos 82, 212–222.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Chem. 72, 248–254.

Cai, X.M., Sun, X.L., Dong, W.X., Wang, G.C., Chen, Z.M., 2014. Herbivore species, infestation time, and herbivore density affect induced volatiles in tea plants. Chemoecology 24, 1–14.

Carr, M.K.V., Stephens, W., 1992. Climate, weather and the yield of tea. In: Wilson, K.C., Clifford, M.N. (Eds.), Tea: Cultivation to Consumption. Chapman and Hall, London, pp. 87–135.

Chang, X.N., Wei, H., Xiao, N.W., Li, J.S., Han, L.Z., Chen, F.J., 2011. Effects of elevated CO_2 and transgenic Bt rice on yeast-like endosymbiote and its host brown planthopper. J. Appl. Entomol. 135, 333–342.

Chen, F.J., Wu, G., Ge, F., 2004a. Impacts of elevated CO_2 on the population abundance and reproductive activity of aphid *Sitobion avenae* Fabricius feeding on spring wheat. J. Appl. Entomol. 128, 723–730.

Chen, F.J., Ge, F., Liu, X.H., 2004b. Responses of cotton to elevated CO_2 and the effects on cotton aphid occurrences. Acta Ecol. Sin. 24, 991–996.

Chen, F.J., Wu, G., Ge, F., Parajulee, M.N., Shrestha, R.B., 2005a. Effects of elevated CO_2 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., Wu, G., Lü, J., Ge, F., 2005b. Effects of elevated CO_2 on the foraging behavior of cotton bollworm, *Helicoverpa armigera* Hübner. Insect Sci. 12, 359–365.

Chen, F.J., Ge, F., Parajulee, M.N., 2005c. Impact of elevated CO_2 on tri-trophic interaction of *Gossypium hirsutum, Aphis gossypii*, and *Leis axyridis*. Environ. Entomol. 34, 37–46.

Chen, F.J., Wu, G., Ge, F., 2005d. Impacts of elevated CO_2 on the growth, development and reproduction of cotton aphid *Aphis gossypii* (Glover). Acta Ecol. Sin. 25, 2601–2607.

Chen, F.J., Wu, G., Parajulee, M.N., Ge, F., 2007. Impacts of elevated CO_2 and transgenic Bt cotton on performance and feeding of three generations of cotton bollworm in a long-term experiment. Entomol. Exp. Appl. 124, 27–35.

Chen, F.J., Wu, G., Ge, F., Parajulee, M.N., 2011. Relationships between exogenous-toxin quantity and increased biomass of transgenic Bt crops under elevated carbon dioxide. Ecotoxicol. Environ. Saf. 74, 1074–1080.

Cheng, S., Fu, X., Wang, X., Liao, Y., Zeng, L., Dong, F., Yang, Z., 2017. Studies on the biochemical formation pathway of the amino acid l-theanine in tea (*Camellia sinensis*) and other plants. J. Agric. Food Chem. 65, 7210–7216.

Cho, J.Y., Mizutani, M., Shimizu, B., Kinoshita, T., Ogura, M., Tokoro, K., Lin, M.L., Sakata, K., 2007. Chemical profiling and gene expression profiling during the manufacturing process of Taiwan Oolong Tea "Oriental Beauty". Biosci. Biotechnol. Biochem. 71, 1476–1486.

Choudhary, M., Grover, K., 2013. Evaluation of fatty acid composition and oxidative stability of blended rice bran and olive oil. Asian J. Dairy Food Res. 32, 290–297.

Coll, M., Hughes, L., 2008. Effects of elevated CO_2 on an insect omnivore: a test for nutritional effects mediated by host plants and prey. Agric. Ecosyst. Environ. 123, 271–279.

Coviella, C.E., Stipanovie, R.D., Trumble, J.T., 2002. Plant allocation to defensive compounds: interactions between elevated CO_2 and nitrogen in transgenic cotton plants. J. Exp. Bot. 53, 323–331.

Deka, A., Deka, P.C., Mondal, T.K., 2006. Tea. In: Parthasarathy, V.A., Chattopadhyay, P.K., Bose, T.K. (Eds.), Plantation Crops. Naya Udyog, Kolkata, pp. 1–147.

Drewnowski, A., 2001. The science and complexity of bitter taste. Nutr. Rev. 59, 163–169.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28, 350–356.

Etschmann, B., Wilcken, B., Stoevesand, K., von der Schulenburg, A., Sterner-Kock, A., 2006. Selection of reference genes for quantitative real-time PCR analysis in canine mammary tumors using the GeNorm algorithm. Vet. Pathol. 43, 934–942.

Garcia-Lopez, S., Echeverria, E., Tsui, I., Balch, B., 1994. Changes in the content of conjugated linoleic acid (CLA) in processed cheese during processing. Food Res. Int. 27, 61–64.

Ge, F., 2011. Challenges facing entomologists in a changing global climate. Chinese J Applied Entomology 48 (5), 1117–1122.

Ge, F., Chen, F.J., 2006. Impacts of elevated CO_2 on insects. Acta Ecol. Sin. 26, 935–944.

Ge, F., Chen, F.J., Wu, G., Sun, Y.C., 2010. Research advance on the response of insects to elevated CO_2 in China. Chinese Bulletin of Entomology 47, 229–235.

Habermann, E., San Martin, J.A.B., Contin, D.R., Bossan, V.P., Barboza, A., Braga, M.R., Groppo, M., Martinez, C.A., 2019. Increasing atmospheric CO_2 and canopy temperature induces anatomical and physiological changes in leaves of the C_4 forage species *Panicum maximum*. PLoS One 14 (2), e0212506.

Han, B.Y., Chen, Z.M., 2002. Secreting rhythm and components of tea aphid honeydew and its attracting activity to nine species of natural enemies. Proc. Int. Conf. O-cha (tea) Cult. Sci., Shiuoka. 86–89.

Hazarika, L.K., Bhuyan, M., Hazarika, B.N., 2009. Insect pests of tea and their management. Annu. Rev. Entomol. 54 (1), 267–284.

Hollingsworth, R.G., Armstrong, J.W., Campbell, E., 2003. Caffeine as a novel toxicant for slugs and snails. Ann. Appl. Biol. 142, 91–97.

Huang, J.G., Bergeron, Y., Denneler, B., Berninger, F., Tardif, J., 2007. Response of forest trees to increased atmospheric CO_2. Crit. Rev. Plant Sci. 26, 265–283.

Hughes, L., Bazzaz, F.A., 2001. Effects of elevated CO_2 on five plant-aphid interactions. Entomol. Exp. Appl. 99, 87–96.

IPCC, 2014. Climate Change, 2014. Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, Switzerland.

Jiang, S.L., Liu, T.J., Yu, F.L., Parajulee, M.N., Chen, F.J., 2016. Feeding behavioral response of cotton aphid, *Aphis gossypii* (Glover), to elevated CO_2: EPG test with leaf microstructure and leaf chemistry. Entomol. Exp. Appl. 160, 219–228.

Jiang, S.L., Dai, Y., Lu, Y.Q., Fan, S.Q., Liu, Y.M., Bodlah, M.A., Parajulee, M.N., Chen, F.J., 2018. Molecular evidence for the fitness of cotton aphid, *Aphis gossypii* in response to elevated CO_2 from the perspective of feeding behavior analysis. Front. Physiol. 9, 1444.

Jing, L.Q., Hu, S.W., Lu, Q., Niu, X.C., Wang, Y.X., Zhu, J.G., Wang, Y.L., Yang, L.X., 2019. Effects of elevated atmospheric CO_2 concentration on photosynthesis of hybrid rice varieties in cloudy and sunny days: a FACE study. Chin. J. Appl. Ecol. 30, 884–892.

Juneja, L.R., Chu, D.C., Okubo, T., 1999. L-theanine a unique amino acid of green tea and its relaxation effect in humans. Trends Food Sci. Technol. 10, 199–204.

Kim, Y.S., Sano, H., 2008. Pathogen resistance of transgenic tobacco plants producing caffeine. Phytochemistry 69, 882–888.

Kimball, B.A., 2016. Crop responses to elevated CO_2 and interactions with H_2O, N, and temperature. Curr. Opin. Plant Biol. 31, 36–43.

Leslie, B., 2004. Global warming and insect pheromones. Front. Ecol. Environ. 2 455-455.

Li, Z.Y., Liu, T.J., Xiao, N.W., Li, J.S., Chen, F.J., 2011. Effects of elevated CO_2 on the interspecific competition between two sympatric species of *Aphis gossypii* and *Bemisia tabaci* fed on transgenic Bt cotton. Insect Sci. 18, 426–434.

Li, X., Ahammed, G.J., Zhang, Y.Q., Zhang, G.Q., Sun, Z.H., Zhou, J., Zhou, Y.H., Xia, X.J., Yu, J.Q., Shi, K., 2015. Carbon dioxide enrichment alleviates heat stress by improving cellular redox homeostasis through an ABA-independent process in tomato plants. Plant Biol. 17, 81–89.

Li, X., Ahammed, G.J., Li, Z.X., Zhang, L., Wei, J.P., Shen, C., Yan, P., Zhang, L.P., Han, W.Y., 2016. Brassinosteroids improve quality of summer tea (*Camellia sinensis* L.) by balancing biosynthesis of polyphenols and amino acids. Front. Plant Sci. 30, 1304.

Li, X., Zhang, L., Ahammed, G.J., Li, Z.X., Wei, J.P., Shen, C., Yan, P., Zhang, L.P., Han, W.Y., 2017. Stimulation in primary and secondary metabolism by elevated carbon dioxide alters green tea quality in Camellia sinensis L. Sci. Rep. 7, 7937.

Lin, Y.S., Tsai, Y.J., Tsay, J.S., Lin, J.K., 2003. Factors affecting the levels of tea polyphenols and caffeine in tea leaves. J. Agric. Food Chem. 51, 1864–1873.

Lindroth, R.L., Wood, S.A., Kopper, B.J., 2002. Response of quaking aspen genotypes to enriched CO_2: foliar chemistry and tussock moth performance. Agric. For. Entomol. 4, 315–323.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.

Ma, K.S., Li, F., Liang, P.Z., Chen, X.W., Liu, Y., Gao, X.W., 2016. Identification and validation of reference genes for the normalization of gene expression data in qRTPCR analysis in *Aphis gossypii* (Hemiptera: Aphididae). J. Insect Sci. 16, 1–9.

Manel, S., Conord, C., Després, L., 2009. Genome scan to assess the respective role of host-plant and environmental constraints on the adaptation of a widespread. BMC Evol. Biol. 9, 288.

Mastrandrea, M.D., Mach, K.J., Plattner, G.K., Edenhofer, O., Stocker, T.F., Field, C.B., 2011. The IPCC AR5 guidance note on consistent treatment of uncertainties: a common approach across the working groups. Clim. Change 108, 675.

Metcalfe, L.D., Schmitz, A.A., Pelka, J.R., 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38, 514–515.

Mohotti, A.J., Lawlor, D.W., 2002. Diurnal variation of photosynthesis and photoinhibition in tea: effects of irradiance and nitrogen supply during growth in the field. J. Exp. Bot. 53, 313–322.

Moore, S., Stein, W.H., 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. 211, 907–913.

Muraleedharan, N., Chen, Z.M., 1997. Pests and diseases of tea and their management. J. Plant. Crops 25, 15–43.

Niziolek, O.K., Berenbaum, M.R., DeLucia, E.H., 2013. Impact of elevated CO_2 and increased temperature on Japanese beetle herbivory. Insect Sci. 20, 513–523.

Nobre JR., H.V., Maia, F.D., de Oliveiraa, R.A., Bandeira, M.A.M., do Ó Pessoa, C., Moraesa, M.O., 2007. Neuroprotective actions of tannins from *Myracrodruon urundeuva* on 6-hydroxydopamine-induced neuronal cell death. J. Herbs, Spices, Med. Plants 13, 41–57.

Pérez-López, U., Sgherri, C., Miranda-Apodaca, J., Micaelli, F., Lacuesta, M., Mena-Petite, A., Quartacci, M.F., Muñoz-Rueda, A., 2018. Concentration of phenolic compounds is increased in lettuce grown under high light intensity and elevated CO_2. Plant Physiol. Biochem. 123, 233–241.

Robinson, E.A., Ryan, G.D., Newman, J.A., 2012. A meta-analytical review of the effects of elevated CO_2 on plant-arthropod interactions highlights the importance of interacting environmental and biological variables. New Phytol. 194, 321–336.

Saijo, R., 1980. Effect of shade treatment on biosynthesis of catechins in tea plants. Plant Cell Physiol. 21, 989–998.

Satyanarayana, B., Devi, P.S., Arundathi, A., 2011. Biochemical changes during seed germination of *Sterculia urens* Roxb. Not. Sci. Biol. 3, 105–108.

Shoghi-Kalkhoran, S., Ghalavand, A., Modarres-Sanavy, S.A.M., Mokhtassi-Bidgoli, A., Akbari, P., 2013. Integrated fertilization systems enhance quality and yield of sunflower (Helianthus annuus L.). J. Agric. Sci. Technol. 15, 1343–1352.

Singleton, V.L., Orthofer, R., Lamuela Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152–178.

Sivapalan, P., 1999. Pest management in tea. In: Jain, N.K. (Ed.), Global Advances in Tea

Science. Aravali Books, New Delhi, pp. 625–646.

Stacey, D.A., Fellowers, M.D.E., 2002. Influence of elevated CO_2 on interspecific interactions at higher trophic levels. Glob. Chang. Biol. 8, 668–678.

Stitt, M., Krapp, A., 1999. The interaction between elevated carbon dioxide and nitrogen nutrition: the physiological and molecular background. Plant Cell Environ. 22, 583–621.

Su, Y.L., Leung, L.K., Huang, Y., Chen, Z.Y., 2003. Stability of tea theaflavins and catechins. Food Chem. 83, 189–195.

Sun, Y.C., Guo, H.J., Liu, Z.Y., Ge, F., 2011. The mechanisms by which elevated CO_2 affects the interaction between herbivorous insects and their host plants. Chinese J Appl Entomol 48, 1123–1129.

Sun, Y.C., Guo, H.J., Ge, F., 2016. Plant-aphid interactions under elevated CO_2: some cues from aphid feeding behavior. Front. Plant Sci. 7, 202.

Terashima, I., Yanagisawa, S., Sakakibara, H., 2014. Plant responses to CO_2: background and perspectives. Plant Cell Physiol. 55, 237–240.

Wang, H.F., Tsai, Y.S., Lin, M.L., Ou, A.S.M., 2006. Comparison of bioactive components in GABA tea and green tea produced in Taiwan. Food Chem. 96, 648–653.

Wijeratne, M.A., Anandacoomaraswamy, A., Amarathunga, M.K.S.L.D., Janaka, Ratnasiri, Basnayake, B.R.S.B., Kalra, N., 2017. Assessment of impact of climate change on productivity of tea (Camellia sinensis L.) plantations in Sri Lanka. J. Natl. Sci. Found. Sri Lanka 35, 119–126.

Wu, G., Chen, F.J., Ge, F., 2007a. Elevated atmospheric CO_2 lower chewing herbivore damage: evidence from the population consumption of multiple generations of cotton bollworm, *Helicoverpa armigera* Hübner fed on cotton bolls. J. Environ. Sci. 19, 1318–1325.

Wu, J., Hettenhausen, C., Meldau, S., Baldwin, I.T., 2007b. Herbivory rapidly activates MAPK signaling in attacked and unattached leaf region but not between leaves of *Nicotiana attenuata*. Plant cell 19, 1096–1122.

Xu, H., Li, L., Li, Q.H., Zhou, L., Zhu, X.J., Chen, F.J., Wang, Y.H., Fang, W.P., 2016. Effects of elevated atmospheric CO_2 concentration and temperature on photosynthesis system and quality components in tea plant. J. Nanjing Agric. Univ. 39, 550–556.

Yang, C.S., Wang, H., Sheridan, Z.P., 2018. Studies on prevention of obesity, metabolic syndrome, diabetes, cardiovascular diseases and cancer by tea. J. Food Drug Anal. 26, 1–13.

Zhang, J., Xing, G.M., Liao, J.X., Hou, Z.D., Wang, G.X., Wang, Y.F., 2003. Effects of different atmospheric CO_2 concentrations and soil moistures on the populations of bird cherry-oat aphid (*Rhopalosiphum padi*) feeding on spring wheat. Eur. J. Entomol. 100, 521–530.

Zhang, Q.F., Liu, M., Ruan, J.Y., 2017. Metabolomics analysis reveals the metabolic and functional roles of flavonoids in light-sensitive tea leaves. BMC Plant Biol. 17, 64.

Zhao, N., Wang, G., Norris, A., Chen, X., Chen, F., 2013. Studying plant secondary metabolism in the age of genomics. CRC Rev Plant Sci 32, 369–382.