CROP SCIENCE

Increased atmospheric CO_2 combined with local climatic variation affects phenolics and spider mite populations in coffee trees

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Abstract: Modelling studies on climate change predict continuous increases in atmospheric carbon dioxide concentration [CO_2] and increase in temperature. This may alter carbon-based phytochemicals such phenolics and modify plant interactions with herbivorous. We investigated the effects of enhanced [CO_2] and local climatic variation on young coffee plants, *Coffea arabica* L. cv Catuaí vermelho IAC-144 and Obatã vermelho IAC-1669-20, cultivated in the FACE (Free-Air Carbon Dioxide Enrichment) facility under two atmospheric [CO_2] conditions. Coffee leaves were evaluated for total soluble phenolics (TSP), chlorogenic (*5-CQA*) and caffeic (CAF) acids, diversity and population size of mites, along two dry and two rainy seasons. Elevated atmospheric CO_2 (e[CO_2]) significantly decreased 5-CQA in cv. Catuaí but did not affect cv. Obatã. Species richness and population size of mites in coffee leaves were not affected by e[CO_2] but were strongly related to the seasonal variability of coffee leaf phenolics. In general, high levels of phenolics were negatively correlated with population size while the mite species richness were negatively correlated with 5-CQA and TSP levels. Our findings show that [CO_2] enhancement affects phenolics in coffee plants differentially by cultivars, however seasonality is the key determinant of phenolics composition, mite species richness and population size.

Key words: *Coffea arabica*, climate change, free-air CO_2 enrichment (FACE), chlorogenic and caffeic acids, total phenolics, mites.

INTRODUCTION

Agricultural production faces the challenge to produce more food while constrained by a number of biotic and abiotic factors. Elevated atmospheric CO_2 (e[CO_2]) and temperature are altering the interactions between plants and insects with important implications for food security and natural ecosystems (DeLucia et al. 2012). The [CO_2] is estimated to continually increase from current the level of 400 ppm to between 750 and 1,300 ppm by the end of this century (IPCC 2014). The global mean surface air temperature is predicted to increase about 1.7–6.7 °C by the end of the 21st century in South America (Magrin et al. 2014). Although [CO_2] effects have been shown to be important for economic and food security impacts of climate change, no models currently account for all the interactions of [CO_2] with temperature, crop species, water status, and nitrogen availability, but these interrelationships are of similar importance as regional differences in climate effects and should be included in models (McGrath & Lobell 2013). Thus, even though global [CO_2] is increasing roughly uniformly, regional yield response to increased [CO_2] will vary due to differences in climate and the mixture of crops.

About three decades ago, free-air CO_2 enrichment (FACE) technology was developed that enabled the air above open-field plots to be enriched with CO_2 for entire growing seasons; since then an enormous amount has been learned about how plants respond to the projected future levels of $[CO_2]$ (Kimball 2016). Elevated $[CO_2]$ generally increases leaf mass per area, photosynthetic rate, foliar C/N ratio, and plant growth and yield (Ainsworth & Long 2005, Zhang et al. 2017). Moreover, $[CO_2]$ enhancement can lead to reallocation of carbon and nitrogen resources among plant organs, and change the secondary metabolites content of plant tissues (Salazar-Parra et al. 2015). These changes in primary and secondary metabolite under elevated $[CO_2]$ may lead to reduction in leaf damage by herbivores and their performance (Valkama et al. 2007). However, in combination with elevated temperature, elevated $[CO_2]$ decreases nitrogen content, thus lowering plant nutritional value (Saha et al. 2015, Zhang et al. 2017) and causing increased leaf consumption by herbivores to meet their nutritional needs (DeLucia et al. 2012). Part of the extra carbon assimilated as consequence of increased photosynthesis under CO_2-enriched atmospheric conditions is directed to the synthesis of phenolic compounds. These effects of elevated CO_2 on the concentration of phenolic compounds vary depending on the level and duration of exposure (Valkama et al. 2007) besides the management conditions, environmental factors and genetics also play a role (Ahmed et al. 2014). Phenolics can potentially be influenced by changes in carbon inputs (Johnson & Pregitzer 2007), and elevated CO_2 may influence the chemical pathways that regulate gene expression and synthesis of secondary compounds (Lindroth 2010). The shikimic acid pathway, known to produce phenolic compounds in trees, was found to be the most influenced pathway by

CO_2 treatment (Lindroth 2010, Kim et al. 2015). Most of the studies on the effects of e$[CO_2]$ have focused on the biochemical composition of plants, and very few studies have been carried out about the effects of e$[CO_2]$ on insect–host plant interactions (Zavala et al. 2013, Sharma et al. 2016). In general, phenolics are involved in the response to biotic and abiotic stresses mainly due to their antioxidant properties. In this way, they may play a role in adaptation to environmental change and in coevolution with pests and diseases (McElrone et al. 2010, Campa et al. 2012). Consequently, phenolics could be critical to understanding plant–animal interactions which are important in predicting the effects of climate change on expression and stability of host plant resistance to pest attack (Sharma et al. 2016).

High contents of natural phenolic acids and flavonoids are found in green tea, fruits, and vegetables, while lower concentrations of phenolics exist in coffee (Ghasemzadeh et al. 2010). Coffee is popular worldwide. The beverage production is mainly based on two plant species, *Coffea arabica* and *Coffea canephora*, also known as arabica and robusta coffees, respectively, which are cultivated in different countries around the world. Coffee is attractive for health benefits due to its antioxidant properties (Iwai et al. 2004, Sato et al. 2011, Tajik et al. 2017). Phenolic compounds, as major sources of antioxidant activity in coffee beans and have been receiving considerable attention as potentially protective factors against human chronic degenerative diseases, such as cancer and cardiovascular disease (Somporn et al. 2012, Ludwig et al. 2014). In coffee, phenolic compounds are present predominantly as a family of esters formed between certain hydroxycinnamic acids (caffeic acid, ferulic and p-coumaric) and quinic acid, collectively known as chlorogenic acids found in high concentrations in green

coffee seeds (Clifford 2000). The best-known conjugate is 5-caffeoylquinic acid, 5-CQA, commonly referred to as chlorogenic acid (CGA), which is naturally found in coffee leaves, as the caffeic acid. Chlorogenic acids have a marked influence in determining coffee quality and play an important role in formation of coffee flavor (Farah & Donangelo 2006, Somporn et al. 2012, Clemente et al. 2015).

In contrast to the considerable amount of research on coffee green beans, there are relatively few studies concerned with the metabolite content of the other parts of coffee plant, such as leaves (Campa et al. 2012). For example, the activity of some pathogens has been limited when phenolic compounds are expressed in some coffee leaves showing higher resistance to the leaf miner *Leucoptera coffeella*, Guérin-Méneville (Lepidoptera: Lyonetiidae) a serious coffee pest (Magalhães et al. 2010). Also Silva et al. (2006) described early accumulation of phenolic compounds in coffee associated with resistance to coffee rust disease caused by the fungus *Hemileia vastatrix* Berkeley & Broome (Uredinales).

Phytophagous mites are considered one of the most important pests causing significant damage to coffee plants in Brazil. Tetranychidae, Tenuipalpidae and Tarsonemidae families of mites are the key arthropod pests in coffee orchards, while the coffee red mite, *Oligonychus ilicis* (McGregor) (Acari: Tetranychidae) and the false spider mite *Brevipalpus* sp. (Acari: Tenuipalpidae), the latter as vector of the Coffee Ringspot Virus (CRV), are among the most important pests of the crop present in the main coffee producing areas in Brazil (Ajila et al. 2018). The coffee red spider mite *O. ilicis* is considered a key coffee pest in many producing countries because it feeds on the upper leaf surface, causing reduction of photosynthesis rate and premature leaf drop

as a consequence of infestation (Teodoro et al. 2009). The Phytoseiidae, predatory mites, is widely and commonly found in a range of different coffee management systems (Mineiro et al. 2008, Teodoro et al. 2009, Peixoto et al. 2017). Phytoseiid mites (Acari: Phytoseiidae) are efficient predators of phytophagous mites and are considered the most efficient natural enemies for biological control of pest mites (Reis et al. 2008, Toledo et al. 2013). The regular occurrence of pests including mite populations, year after year, reduces productivity and the quality of coffee and is known that the coffee management production interferes on mite population, being that more sustainable systems of production present smaller abundance of mites (Peixoto et al. 2017). However, the relationship between mites and coffee plants in a high CO_2 environments has not been investigated.

Thus, considering the importance of the coffee production and the lack of knowledge about the effects of e[CO_2] in coffee leaf phenolics and its correlation with mite populations, the purpose of this work was to assess whether [CO_2] combined with local climate variability affects the leaf levels of coffee phenolic compounds and, consequently, the mite populations. For this, two coffee cultivars (*Coffea arabica* cv. Catuaí Vermelho IAC 144 and cv. Obatã IAC 1669–20) were cultivated under ambient or elevated [CO_2] (390 and 550 ppm respectively) in free-air CO_2 enrichment (FACE) during two rainy and two dry seasons. The levels of total soluble phenols, chlorogenic and caffeic acids were assessed in mature coffee leaves and the relationship between those compounds and the abundance and diversity of mites was quantified. Our hypothesis were: (1) elevated [CO_2] increases phenolic compounds in coffee leaves reducing mite populations; (2) local climatic variability combined with different [CO_2] treatments can

change the levels of phenolic compounds modifying mite diversity and abundance in coffee leaves.

MATERIALS AND METHODS

Site description, CO_2 treatments, plant materials, and samplings

We carried out the experiment using the ClimapestFACE facility located in Jaguariúna municipality (22°43'10"S 47°01'16"W, 615 m above sea level), southeastern Brazil. The soil at the experimental area is a typical dystroferric red latosol. The climate is humid subtropical, a Cfa type according to the Köppen classification, with hot rainy summers and cold dry winters. Maximum and minimum mean monthly air temperature and precipitation were recorded during the experiment. To mimic coffee agroecosystems, the FACE system increased the ambient $[CO_2]$ in six 10 m diameter ring plots (elevated CO_2) within a continuous 7 ha coffee field. Six additional 10 m diameter ring plots served as controls, i.e. were left under ambient $[CO_2]$ conditions. Elevated and ambient-CO_2 plots were at least 70 m apart to minimize cross-plot contamination. Fumigation with CO_2 began on 25 August 2011. The average $[CO_2]$ at the beginning of the experiment was approximately 390 μmol mol⁻¹. The performance of the FACE system was adjusted so that the $[CO_2]$ as measured at the centre of the ring achieved target levels of 550 μmol mol⁻¹ of air. The plots were not enriched with CO_2 at night. Further details regarding the experimental site set-up and CO_2 control performance can be found in Ghini et al. (2015). Monthly the minimum and maximum mean of air temperature and precipitation were recorded during the experiment. Two coffee (*Coffea arabica* L.) cultivars, cv. Catuaí Vermelho IAC 144 and cv. Obata IAC 1669-20, were assessed. Plantlets with three to four pairs of leaves were

transplanted into the plots in March 2011. The cultivars were interspersed in rows that were 1.75 m apart, with 0.60 m between plants in the rows. The plants were submitted to routine agricultural practices for commercial coffee bean production, including applications of fungicides and insecticides. Each tree was fertilized annually with 46 g of N, 9 g of P and 23 g of K plus micronutrients. The crop was grown without supplemental irrigation.The youngest fully expanded leaves (the third or fourth leaf pair from the apex of the plagiotropic branches) in the upper third of three plants were collected for each biological sample. Sampling was carried out monthly in two contrasting periods of the coffee growth cycle: May to August (Dry season) and October to January (Rainy season) between 2012 and 2014. A total of two dry periods and two rainy ones were assessed. Immediately after harvesting, leaves were ground under liquid nitrogen with a mortar and pestle before being lyophilized for 36 hours (EC-Super Modulyo Model, Edwards, Crawley, UK) and stored at –20 ºC until analysis. Three replicates per biological sample were analyzed.

Chemicals and reagents

Chemical reagents such as tannic (TA), chlorogenic (*5-O-caffeoilquinic acid*,5-CQA) and caffeic acids (3,4-dihydroxycinnamic acid, CAF) were 99.0; 98.0 and 99.5% analytical purity, respectively. Commercial standards were purchased from Sigma–Aldrich Brazil Ltda, São Paulo. Solvents and phosphoric acid were HPLC-grade, from J.T. Baker, and the water used was ultra-purified by a Milli-Q© system (Millipore, Brazil). Samples were filtered by cellulose ester membrane 0.45 μm (Millipore, Brazil). The total concentration of soluble phenolic compounds was determined using Folin–Ciocalteu reagent according to Spanos & Wrolstad (1990) and the absorbance was measured at 765 nm by spectrophotometry

(UV-Vis, Lambda 20 model, Perkin Elmer). The total soluble phenolic contents (TSP) of the samples were estimated in milligrams of tannic acid equivalents (TAeq) and expressed by mg TAeq g^{-1} SP. Extractions of CAF and 5-CQA acids were adapted from Ky et al. (1997) and Hinneburg & Neubert (2005). Coffee sample (0.030g) was extracted with methanol:water (70:30, v/v, 3 mL) and heat at 60°C for 30 min in water bath (B480 model, Büchi). After cooling, extracts were filtered and its volume was completed to 10 mL with methanol:water (70:30, v/v). 2 mL of extract was filtered (0.45 μm pore size) and analyzed using a HPLC system (Agilent, 1100 Series). CAF and 5-CQA separation and quantification were adapted from Pellati et al. (2005) and the HPLC analysis was carried out using a UV-visible detector that operated at 325 nm, injection volume 10 μL, C-18 Partisil 5 ODS-2 column; reversed phase, 4,6 x 250 mm, with mobile phase of ultra-purified water with phosphoric acid 0.1% (solvent A) and acetonitrile (solvent B) at flow rate of 0.9 mL min^{-1} stabilized with 90% solvent A and 10% solvent B (time zero). Identification was performed by comparing spectra and retention times with commercial standards by extract fortification; results were expressed in mg g^{-1}.

Mite collection and identification

The analysis of mite fauna in coffee plants was performed on eighteen leaves of each cultivar (from six plants/cultivar/plot) that were collected monthly within each ring of the FACE (three leaves per plant, one from each third of vertical profile- upper, middle and lower.) totaling 108 leaves assessed of each cultivar per [CO_2] treatment. Each sample was stored separately in a labelled paper bag and stored cold during transit until the lab. The leaves of each plant were immersed for 10 min in alcohol solution (70%), and these solutions were slightly shaken to remove the mites from the leaves. The alcohol containing the mites was passed through a sieve of 400 mesh (wire mesh opening: 0.038 mm). The mites retained on the screen were kept in 70% ethanol (Mineiro et al. 2009). Mite populations were quantified and the species were identified.

Statistical analysis

The experiment was set up in a 2x2 factorial design with two levels of [CO_2], ambient and elevated, and two seasons, dry and rainy, with 24 replicates. Data collected for each response variable were subjected to analysis of variance (ANOVA) and Tukey's test was performed after significant Anova, to compare means employing the SAS GLM procedure (SAS 2008). Finally, Pearson Correlation was performed within all response variables utilizing the CORR procedure of SAS (SAS 2008).

RESULTS AND DISCUSSION

Weather conditions over two years of the experiment (Figure 1) were characterized by low average air temperatures (±18°C) and precipitation (5-10mm) from May to August period (dry season) and high average temperatures (±26 °C) and precipitation (80-130mm) from October to January (rainy season).

Most of the significant treatment effects on the variables analysed were observed for the seasonality factor (dry/rainy) rather than for [CO_2] factor in both coffee cultivars (Catuaí and Obatã). In cv. Catuaí the [CO_2] factor (ambient/ elevated) had a significant effect only in the 5-CQA contents (p=0.002) and an interactive trend of [CO_2] x seasonality was observed for this variable (p=0.087) (Table I). Additionally, seasonality had a highly significant effect on TSP amounts (p<0.0001), 5-CQA (p<0.0001), CAF (p=0.0019) and *sp*mites (p=0.034). No
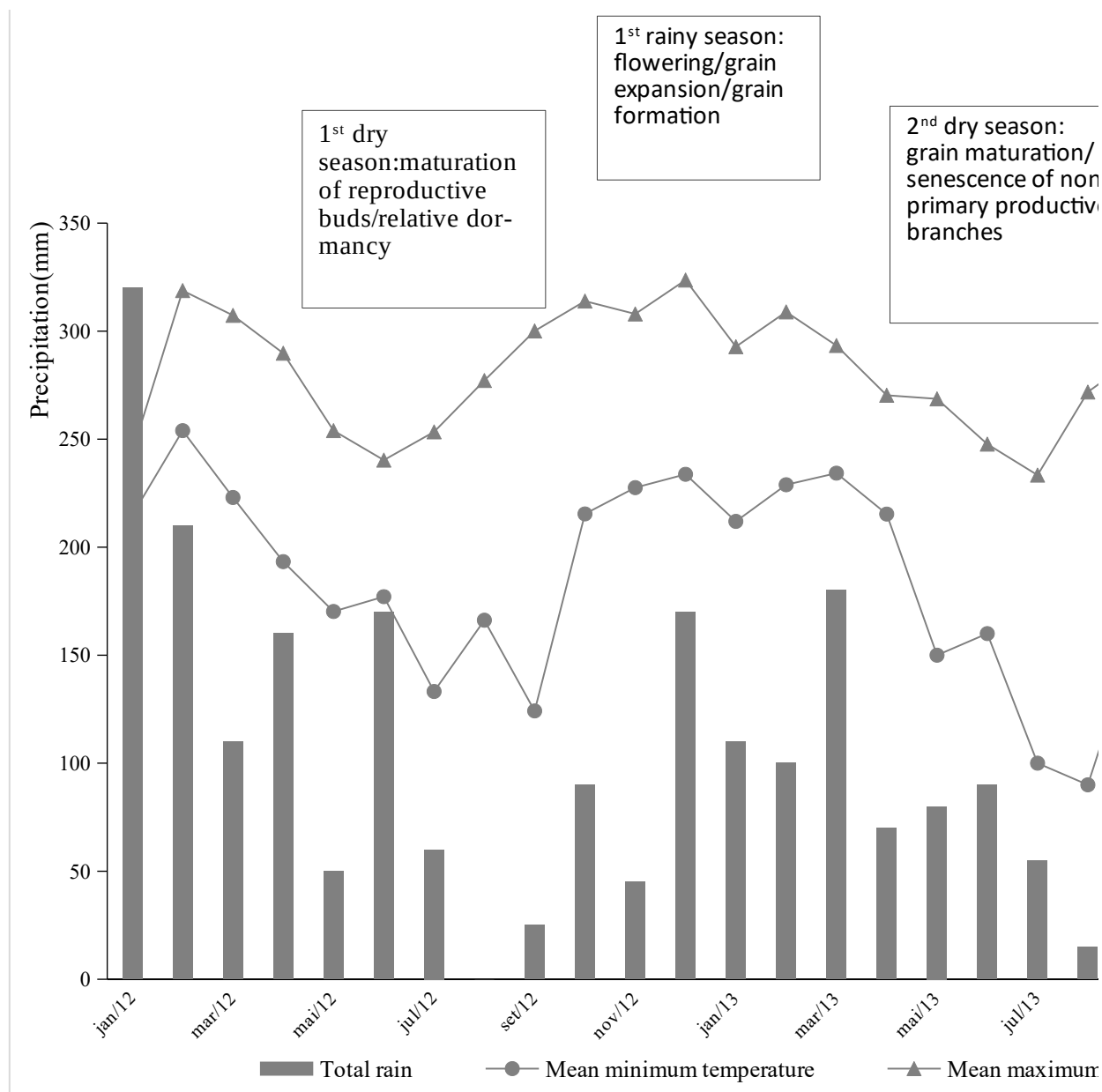
Figure 1. Monthly minimum and maximum mean air temperatures, rainfall distribution and phenological stage of coffee plants in FACE octagons.

significant effects of $[CO_2]$ was observed in cv. Obatã but seasonality had significant effect on TSP (p<0.0001), 5-CQA (p<0.0001) and *sp*mites (p=0.032) (Table I).

Means comparisons (Tukey's test, 5%) indicated reduction of 5-CQA levels in cv. Catuaí growing under e$[CO_2]$ and significantly lower TSP, 5-CQA and CAF contents in dry season

comparing with the rainy one. Instead, *sp*mites was significantly higher during dry season than rainy season (Table II). In cv. Obatã TSP and 5-CQA contents were reduced significantly in dry season, whereas an increase was observed for *sp*mites. No significant effects of $[CO_2]$ treatments were detected for Obatã plants (Table II).

Our results showed that elevated CO_2 only affected concentrations of 5-CQA in cv. Catuaí coffee leaves. Like green tea and green coffee beans, the main phenolic constituent of coffee leaves is 5-CQA that is significantly correlated with antioxidant and anti-inflammatory activities in human health (Campa et al. 2012, Somporn et al. 2012, Vagiri et al. 2017, Chen et al. 2018). In coffee beans chlorogenic acids (CGA) such as 5-CQA, are important determinant of coffee flavor contributing to the astringency and beverage bitterness (Clifford et al. 2017). Additionally, CGA synthesis in the coffee plant may contribute to the control of seed germination and cell growth, through regulations of the levels of indolacetic acid, a plant growth hormone of physiological significance during the formation of the beans (Farah & Donangelo 2006). Considering its

relevance to coffee plants, reduction of 5-CQA levels could be an undesirable effect of e[CO_2].

Contrary to the Carbon–Nutrient Balance Hypothesis that predicts an increase of carbon-based defence compounds as a result of the 'excess' C under e[CO_2] (Robinson et al. 2012) we found relatively low changes of phenolic concentration in coffee leaves. However, such alterations are in accordance with several studies demonstrating little or no effect of e[CO_2] on phenolics, or even decreases in their levels (Robinson et al. 2012, Goufo et al. 2014, Saha et al. 2015, AbdElgawad et al. 2016). Noteworthy alterations of phenolic compounds were observed in white and brown rice (Goufo et al. 2014) and in two varieties of ginger (Ghasemzadeh et al. 2010). Some explanation to inconsistency on antioxidant responses to

Table I. Variance analysis of TSP, CAF, 5-CQA, mite diversity (spmites) and mite population (nmites), in two coffee cultivars (Catuaí and Obatã), with two levels of [CO_2] (elevated/ambient), in two seasons (dry/rainy) under factorial arrange.

Variable	Factor	Catuaí		Obatã	
		F[1]	P[2]≥|F|	F	P[2]≥|F|
Total Soluble Phenolics (TSP)	CO_2	0.74	0.3934	0.47	0.4938
	Season	16.72	<0.0001	17.82	<0.0001
	CO_2xSeason	1.12	0.2921	3.29	0.0732
Caffeic acid (CAF)	CO_2	0.70	0.4049	1.34	0.2505
	Season	10.21	0.0019	2.07	0.1539
	CO_2xSeason	0.00	0.9744	0.01	0.9039
Chlorogenic acid (5-CQA)	CO_2	10.04	0.0021	0.09	0.7691
	Season	101.38	<0.0001	143.35	<0.0001
	CO_2xSeason	2.99	0.0874	0.12	0.7313
#spmites	CO_2	3.82	0.0538	0.03	0.8673
	Season	4.62	0.0342	4.74	0.0320
	CO_2xSeason	0.00	1.0000	0.11	0.7383
##nmites	CO_2	0.98	0.3248	0.39	0.5344
	Season	1.32	0.2544	3.50	0.0645
	CO_2xSeason	0.89	0.3486	0.24	0.6288

[1] df = 1; [2] nominal significance level of F-test; Values in bold indicate statistical significance by ANOVA; in all cases, df of: model = 3; error = 92; corrected total = 95; # number of mite species identified; ## total number of mites collected.

e[CO_2] is that in FACE experiments the effects of CO_2 on growth are less pronounced than in growth cabinet experiments (Ainsworth et al. 2008). Other authors have suggested that the relatively large variety in antioxidant responses to e[CO_2] may not directly correlate to the stress and CO_2 treatment only, but there are an integrated response of changes in various metabolic processes (AbdElgawad et al. 2016).

Our study showed significant effects of seasonality in phenolic levels of coffee leaves indicating low levels in dry seasons and higher contents in the rainy season. Coffee plants are more affected by low temperatures than by water restriction (Silva et al. 2004, Amaral et al. 2006) in dry seasons. In the same experimental plots of this study, Ghini et al. (2015) verified in both, Catuaí and Obatã coffee plants, that photosynthesis was stimulated by e[CO_2] but the carbon assimilation was limited by diffusive constrains in leaf mesophyll observed in dry season. This physiological limitation of coffee plants could explain low carbon availability to investments in carbon-based phenolic compounds resulting in low levels of these secondary metabolites in coffee leaves in dry

season, contrary to the rainy ones, when both photosynthesis (Ghini et al. 2015) and phenolic leaf levels were higher. To corroborate this assumption, Salgado et al. (2008) also verified in ambient [CO_2] that phenolic levels of coffee leaves were conditioned by competition for carbohydrates between the primary and secondary metabolism along phenological phases.

No effects of e[CO_2] were observed on mite abundance and diversity on coffee leaves, however, seasonality had a significant effect on diversity of mites in both cultivars. The higher diversity of mites during the dry seasons is probably associated with the favorable conditions for the population increase of several species of phytophagous (e.g., Tetranychidae, Tenuipalpidae) and predatory (e.g., Stigmaeidae) mites, besides lower predation rates by phytoseiid mites (Acari: Phytoseiidae), which are negatively affected by the low humidity (Gerson et al. 2003, Matioli & Oliveira 2007, Mineiro et al. 2008). The phytoseiid mites (Acari: Phytoseiidae) are considered the most important natural enemies for biological control of pest mites in high abundance periods (Reis et al. 2008,

Table II. Average content of TSP, CAF and 5-CQA; mite diversity (*sp*mites) and mite population (*n*mites), in coffee cultivars Catuaí and Obatã, in a factorial experiment with two levels of [CO_2] (elevated/ambient) and leaves collected in two seasons (dry/rainy).

Coffee cv.	Treatments		TSP	CAF	5-CQA	*sp*mites	*n*mites
Catuaí	[CO_2]	Ambient	47.6±6.8	0.55±0.08	25.1 A	0.85+0.90	2.12+5.87
		Elevated	46.7±5.0	0.54±0.10	23.1 B	1.27+1.20	14.21+84.46
	Season	Dry	44.8 B	0.51 B	21.0 B	1.29 A	1517+84.50
		Rainy	49.4 A	0.57 A	27.2 A	0.83 B	1.17+1.72
Obatã	[CO_2]	Ambient	47.3±8.0	0.51±0.14	26.2±5.9	1.23±1.28	2.33±3.28
		Elevated	48.3±7.7	0.47±0.13	26.0±6.2	1.19±1.20	1.96±2.63
	Season	Dry	44.7 B	0.47±0.12	21.4 B	1.48 A	2.71±3.61
		Rainy	50.9 A	0.51±0.16	30.8 A	0.94 B	1.58±2.02

* Means followed by capital letters indicate statistical difference between [CO_2] or season treatments (5%, Tukey's test); means with no statistical difference are followed by standard deviation.

Toledo et al. 2013, Castilho et al. 2015), however they also may compete with predatory mites of other families (Sato et al. 2001, Mineiro et al. 2008). The lower abundance of mites observed during rainy seasons when compared to dry seasons may be also due to abiotic factors such as temperature and relative air humidity. Gherlenda et al. (2016) verified significant effects of rainfall-driven leaf phenology and no effect of $e[CO_2]$ on leaf consumption or preference of insects herbivores in mature *Eucalyptus* woodland canopy after two years of fumigation in FACE. Castilho et al. (2015) correlated heavy rainfall to population decrease of predatory mites while rainfall affected the number of predatory (Phytoseiidae and Stigmaeidae) and phytophagous mites (Tenuipalpidae and Tetranychidae) in a range of crop management systems (Neto et al. 2010). Negative correlations between mite densities and temperature were observed for *Euseius concordis* (Chant) (Acari: Phytoseiidae) on leaf surfaces and branches, and for *Zetzellia malvinae* Matioli, Ueckermann & Oliveira (Acari: Stigmaeidae) in domatia, that are minute structures found on the underside of the leaves. A positive correlation between the number of mites (per plant) and temperature was detected for *Brevipalpus* sp. on fruits, in a coffee plantation (*C. arabica* cv Mundo Novo) in the State of São Paulo (Mineiro et al. 2008). Additionally, Abreu et al. (2014) verified negative correlation between total number of mites and precipitation levels in Arabic coffee cv. Paraiso.

Besides the climatic factors, food resources availability determines abundance and diversity of mites in coffee plantations. For example, *Ricoseius loxocheles* (De Leon) (Acari: Phytoseiidae) is often found in coffee crops and is known to feed on coffee leaf rust, *H. vastatrix* (Ajila et al. 2018). Populations densities of red spider mites, *O. ilicis*, were positively correlated with populations densities of

coffee leaf miner, *Leucoptera coffeella* Guérin-Méneville (Lepidoptera: Lyonetiidae) and leaf rust in the field (Teodoro et al. 2009). In the same experiment of this study, Ghini et al. (2015) related a peak of leaf miner incidence on the second dry season of this study, when weather conditions were favorable to pest infestation. In this study, the diversity of mites in coffee plants was related to phytochemical profile of leaves that presented higher levels of phenolic compounds in rainy season. Phenolics inhibit the digestion of proteins in various herbivores and thus commonly act as plant defenses (Ballhorn et al. 2011) and these effects may also have been responsible for the low levels of mite diversity on coffee leaves during rainy seasons. Insect performance usually correlates positively with nitrogen concentration and negatively with the concentration of carbon-based compounds (Kuokkanen et al. 2003, Lindroth 2010) but these relationships were not described for mites up to date. In our study, mite diversity in cv. Catuaí presented a negative correlation with 5-CQA, CAF and TSP levels while abundance of mites was negatively correlated only with 5-CQA level (Table III). In cv. Obatã mite diversity was negatively correlated with 5-CQA and TSP while mite abundance was negatively correlated with TSP only (Table III). During dry and rainy seasons, mite diversity was negatively correlated with 5-CQA and TSP and with CAF levels only in the rainy season (Table III). In all treatment levels (cultivar, season and $[CO_2]$) mite diversity was negatively correlated to 5-CQA and TSP levels indicating the importance of these compounds in mite-coffee leaves interrelationships. Interestingly, abundance but not diversity of mites, always correlated negatively with all phenolic compounds only under $e[CO_2]$ (Table III).

Magalhães et al. (2010) showed no correlation between resistance to *L. coffeella*

and the leaf levels of alkaloids and phenolics, however, infestation by leaf miners led to a nearly four-fold decline in the leaf levels of chlorogenic acid promoting infestation by generalist insects, such as *Coccus viridis (Hemiptera: Sternorrhyncha: Coccidae).* Ramiro et al. (2006) investigating 5-CQA participation on coffee resistance to *L. coffeella,* suggest that phenol content apparently does not play a central role but, conversely, the reduction of soluble phenols in leaves is a general plant response to feeding damage making proteins less available for assimilation by the digestive tract of the insects.

This is the first study about phenolic compounds interactions with mite infestations in coffee growing under FACE system. Our assumption is that in dry season the lower levels of TSP, 5-CQA and CAF resulted from low carbon availability to the synthesis of phenolic compounds contributing for higher mite diversity in coffee leaves. Additionally, e[CO_2] intensified this effect in cv. Catuaí resulting in

lower levels of 5-CQA, which could indicate a higher susceptibility of that cultivar to attack of pests and diseases when subjected to an increase of [CO_2] in atmosphere.

CONCLUSIONS

The interaction between e[CO_2] and natural climatic variability, besides its effects on plant chemistry and insect herbivores needs to be further investigated. Here we analyzed alterations in leaf phenolic compounds of young coffee plants growing in FACE and the respective relationships with abundance and diversity of mites. Contrary to our hypothesis, e[CO_2] did not elevate coffee leaf phenolics, but reduced concentration of chlorogenic acid (5-CQA) of *C. arabica* cv Catuaí in the dry season. Results showed that phenolic levels were higher during rainy than the dry seasons but no interaction with e[CO_2] occurred. Additionally, diversity and abundance of mites in coffee leaves were not affected by e[CO_2], but the diversity of mites

Table III. Earson correlation coefficient (R) between variables evaluated in coffee leaves of Catuaí and Obatã cultivars collected in two seasons (dry/rainy) and cultivated in two levels of [CO_2] (elevated/ambient).

Variables		General Catuaí	Cultivar		Season		[CO_2]	
			Obatã	Dry	Rainy	Ambient	Elevated	
Chlorogenic acid (5-CQA)	CAF	0.24**	0.44**	0.23*	-0.15ns	0.30**	0.18ns	0.28**
	TSP	0.67**	0.63**	0.69**	0.69**	0.55**	0.66**	0.68**
	*sp*Mites	-0.49**	-0.66**	-0.43**	-0.53**	-0.36**	-0.48**	-0.48**
	*n*Mites	-0.22*	-0.31*	-0.18ns	-0.11ns	-0.18ns	-0.23ns	-0.32*
Caffeic acid (CAF)	TSP	0.19**	0.23*	0.19ns	-0.31**	0.47**	0.24*	0.14ns
	*sp*Mites	-0.11ns	-0.29*	-0.01ns	0.09ns	-0.50**	0.03ns	-0.21ns
	*n*Mites	-0.01ns	0.06ns	-0.05ns	0.15ns	-0.44**	0.11ns	-0.29*
Total soluble phenols (TSP)	*sp*Mites	-0.52**	-0.60**	-0.47**	-0.47**	-0.43**	-0.46**	-0.60**
	*n*Mites	-0.24*	-0.18ns	-0.35*	-0.11ns	-0.45**	-0.18ns	-0.48**
***sp*Mites**	*n*Mites	0.10ns	0.12ns	0.68**	0.09ns	0.79**	0.45**	0.10ns

ns = no significant ; * = significant at 5%; ** = significant at 1%.

were strongly related to the seasonal variability of coffee leaf phenolics. In general, high levels of phenolics were negatively correlated to abundance of mites, while the diversity was negatively correlated with 5-CQA and TSP levels. Considering that 5-CQA is known to be responsible for many aspects of coffee beverage quality and to have important participation on ecological interactions, like suggested by our results with mite population analysis, reductions of 5-CQA levels in coffee leaves is an undesirable effect of e[CO_2], especially during dry seasons, when high incidence of mites and other pests are observed in many crop systems. Further investigations may highlight the contribution of different secondary metabolites in the mite-coffee leaf interactions. Finally, the relationship between [CO_2] atmospheric and phenolic compounds in coffee plants was described in this work for the first time and draws attention to the need to consider the natural variability of plant defenses for the phytosanitary management of coffee plantations.

Acknowledgments

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