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Changes in the chemical composition of lignin in the leaves of coffee plants supplied with silicon and infected with *Hemileia vastatrix*

Alterações na composição química da lignina nas folhas de plantas de café supridas com silício e infectadas por *Hemileia vastatrix*

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Abstract

Coffee leaf rust, caused by the fungus *Hemileia vastatrix*, is considered the main disease of coffee in Brazil. Aiming to find alternatives to control this disease, this study investigated, at the metabolic level, the possible effect of silicon (Si) on coffee resistance to coffee leaf rust. The analytical pyrolysis was used to evaluate possible changes in the chemical composition of lignin on the leaves of coffee plants supplied with Si and inoculated with *H. vastatrix*. There was no significant increase in the foliar Si concentration for plants supplied with this element, consequently, there was no reduction on coffee leaf rust symptoms. Eight compounds derived from lignin from the *p*-hidroxifenila precursor (H) were identified, seven from the precursor guaiacyl (G) and one from the precursor syringyl (S) on leaves of plants supplied or non-supplied with Si and non-inoculated or inoculated with *H. vastatrix*. For the inoculated plants supplied with Si, there was a tendency of occur higher concentrations of the compounds vanillin and caffeine compared to plants non-supplied with Si and non-inoculated. Plants supplied with Si, regardless of inoculation with *H. vastatrix*, showed higher values for S/G ratio when compared to non-supplied plants. Even though there was an increase in the concentrations of vanillin and caffeine and higher S/G ration on the leaves of plants supplied with Si and infected with *H. vastatrix*, this fact did not result in reduction in the symptoms of coffee leaf rust.

Additional keywords: *Coffea arabica*; lignin; phenolic compounds; phenylpropanoid pathway; rust.

Resumo

A ferrugem, causada pelo fungo *Hemileia vastatrix*, é considerada a principal doença do cafeeiro no Brasil. Com o objetivo de encontrar alternativas para o controle dessa doença, o presente trabalho investigou, a nível metabólico, o possível efeito do silício (Si) na resistência do cafeeiro à ferrugem. A pirólise analítica foi utilizada para avaliar possíveis alterações na composição química da lignina em folhas de plantas de café supridas com Si e inoculadas com *H. vastatrix*. Não houve aumento significativo na concentração foliar de Si para as plantas supridas com esse elemento, o que acabou por não reduzir os sintomas da ferrugem. Foram identificados oito compostos derivados da lignina de origem do precursor *p*-hidroxifenila (H), sete do precursor guaiacila (G) e um do precursor siringila (S) nas folhas das plantas supridas ou não supridas com Si e não inoculadas ou inoculadas com *H. vastatrix*. Para as plantas inoculadas e supridas com Si, houve tendência de ocorrer maiores concentrações dos compostos vanilina e cafeína em comparação às plantas não supridas com Si e não inoculadas. Plantas supridas com Si, independentemente da inoculação com *H. vastatrix*, apresentaram maiores valores para a relação S/G quando comparadas às plantas não supridas. Embora tenha ocorrido aumento nas concentrações de vanilina e cafeína, e maior relação S/G nas folhas das plantas de café supridas com Si e infectadas por *H. vastatrix*, tal fato não resultou em redução dos sintomas da ferrugem.

Palavras-chave adicionais: *Coffea arabica*; compostos fenólicos; ferrugem; lignina; rota dos fenilpropanoides.

Introduction

The coffee plant *(Coffea arabica* L.) is one of the most important commodities in international agricultural trade (Batista et al., 2012). Diseases in plants play an significant role in reducing coffee production in Brazil, especially rust, caused by fungus *Hemileia vastatrix* Berkley & Broome, which can cause losses of between 35 and 50% (Zambolim et al., 2005; Várzea & Marques, 2005; Carré-Missio et al., 2009). Among the main problems caused by rust, the defoliation of leaves, which can result in the death of branches and reduce coffee production in the following harvest (Zambolim et al., 2005).

Rust control is mainly carried out by applying fungicides and using resistant cultivars (Zambolim et al., 2005). However, it is currently common to find *H. vastatrix* populations that are unresponsive to fungicides and able to suppress the resistance of rust resistant cultivars (Várzea & Marques, 2005). Faced with these concerns, it is necessary to find alternative methods of rust control. Silicon (Si), despite not being considered an essential nutrient for plants, stands out because it reduces the intensities of diseases in various crops (Datnoff et al., 2007; Carré-Missio et al., 2014).

One of the hypotheses for the reduction in the intensities of disease by Si is partly due to the polymerization of monosilicic acid under the cuticle forming a double cuticle-silicon layer which acts as a physical barrier that impedes or delays fungus penetration (Datnoff et al., 2007). Another hypothesis accepted by researchers is that Si can enhance physiological and/or biochemical defense mechanisms in plants (Datnoff et al., 2007) such as increasing the concentration of phenolic compounds (Fortunato et al., 2014), lignin and its precursors (Schurt et al., 2013) and of phytoalexins (Rodrigues et al., 2004); greater activity of defense enzymes such as phenylalanine ammonia-lyases, peroxidases, chitinases and *β*-1,3-glucanases (Tatagiba et al., 2014) as well as fast and intensive defense genes transcription (Rodrigues et al., 2005).

Phenolic compounds, lignin and its precursors are toxic to fungal pathogens in plants (Weete, 1980). The lignification is a biochemical process that involves two phases: biosynthesis of monolignols mediated by intrinsic enzymes and polymerization of lignin in the cellular wall through the reaction of dehydrogenative oxidation of the available monolignols (Nicholson & Hammerschmidt, 1992; Monteiro et al., 2004). The monolignols-phenylpropanoids are the *p*-cumarilic *(p*-hydroxyphenyl lignin), coniferyl (guaiacol lignin) and sinapyl (siringyl lignin) alcohols that are considered to be majority precursors of lignin (Nicholson & Hammerschmidt, 1992; Monteiro et al., 2004).

In vegetables, lignin is present in the cellular wall and in the middle lamella of tissues in concentrations varying from 10 to 30 dag kg^{-1} (Hon & Shiraishi, 2001). However, concentrations of lignin in tissues depend on the plant species, tissue age, and also of the organ (Hon & Shiraishi, 2001). Moreover, different levels of lignin have been reported in plants infected with pathogens as a defense mechanism (Nicholson & Hammerschmidt, 1992) as well as plants with resistance to disease potentiated by Si (Schurt et al., 2013; Fortunato et al., 2014; Tatagiba et al., 2014). For example, banana plants supplied with Si produced more lignin, lignin precursors and phenolic compounds in the roots reducing, therefore, the infection by *Fusarium oxysporum* f. sp. *cubense* due to strengthening of the cellular wall as well as a reduction in the diffusion of non-host selective toxins and lytic enzymes produced by the pathogen (Fortunato et al., 2014).

This study aimed to evaluate possible alterations in the chemical composition of lignin in the leaves of coffee plants supplied with Si and inoculated with *H. vastatrix*. The hypothesis is that supply of Si or inoculation with *H. vastatrix* can increase the lignin levels in coffee leaves increasing, therefore, coffee resistance to rust.

Material and methods

Coffee seeds from the red Catauaí cultivar 44 were seeded in moistened sand and, after 60 days, the seedlings were transplanted into plastic pots containing 3.5 L of Clark (1975) nutrient solution with some modifications. The nutrient solution was composed, in mmol/L, of 5.7 $N\text{-}NO_3^-$; 1 $N\text{-}NH_4^+$; 0.1 $P\text{-}H_2PO_4^-$; 2.4 K; 1.2 Ca^{+2}; 0.6 Mg^{+2}; 0.7 $S\text{-}SO_4^{-2}$; and in µmol.L, 35 Fe; 0.8 Cu; 1.5 Zn; 5 Mn; 17 B; 0.1 Mo and 25 ethylenediaminetetraacetic disodium acid. The Si was supplied to the plants in the form of monosilicic acid, which was obtained from passage through a potassium silicate solution (PQ Corporation, São Paulo) using a cation exchange column (Amberlite IR-120B, H^+ form, Sigma-Aldrich, São Paulo) (Ma et al., 2002). The Si doses used in the nutrient solution were 0 (-Si) and 2 (+Si) mM. The aerated nutrient solution was changed every seven days and the pH monitored every six days and kept close to 5.5.

Urediniospores of *H. vastatrix* were obtained from leaves showing symptoms of rust in coffee plants (cv. 44 red Catuaí) kept in a greenhouse. Before inoculation, the viability of the urediniospores was tested based on their germination in agar-water (2%). Only urediniospores with germination above 35% were used in the experiments. The abaxial face of the 2nd pair of leaves, from the apex of seedlings grown in nutrient solution for 45 days, with or without the presence of Si, was inoculated with a suspension of urediniospores (1 mg/mL) of *H. vastatrix* using a Paasche (VL-SET model) atomizer (Carré-Missio et al., 2009; 2012; 2014; Honorato Júnior et al., 2015). After inoculation, plants were transferred to

a moist chamber (relative humidity > 95%, 24 ± 1 ºC) and remained in the dark for 48 h. Subsequently, plants were transferred to a growth chamber (22 ºC) until finalization of the experiment.

The severity of rust was evaluated at 10, 15, 20, 25, and 30 days after inoculation (DAI) with the help of a scale developed by Kushalappa (1978). The severity data were used to calculate the area under the rust progress curve (AURPC) according to Shaner & Finney (1977).

After 20 DAI, leaves from ten coffee plants were collected from each treatment. During sampling, leaves were frozen in liquid nitrogen and stored in an ultra-freezer at -80°C. The leaf samples were lyophilized, ground in a Thomas-Wiley type grinder and the fine power obtained was passed through a 40 mesh sieve. The powder obtained from each leaf sample (approximately 100 µg) was placed in platinum mortar and pyrolyzed at temperatures of 300, 400, 500, 550 and 600 ºC for 10 s. Pyrolysis analysis is a quick technique for analyzing lignified materials using only thermal energy (Schurt et al., 2013). Thus, for pyrolysis of the coffee plant samples, a vertical micro-oven (PYR-4A Shimadzu) attached to a gas chromatograph and Pi-CG-EM (PQ5050A, Shimadzu) mass spectrometer was used. This gas chromatograph helped in separating the phenolic compounds, while the mass spectrometer detected these substances (Schurt et al., 2013). The products from the pyrolysis were automatically injected into the chromatograph using helium as a carrier gas at a flow rate of 1 mL/min and split rate of 1/10 in a Veri-Flow 500 (Agilet) model electronic flow meter. To separate the compounds obtained, a DB-5 fused-silica capillary column (30 m × 0.25 mm in diameter × 0.25 µm of film) was used. The initial temperature of the column was 45 °C for 4 min, increasing from 45 to 240 °C at a rate of 4 °C min^{-1}, remaining for 10 min thereafter. The detector temperature was 250 °C and 290 °C in the CG-EM interface. The mass detector operated by ionization through the impact of electrons (70 eV) and scanning masses at an interval of m/z 40 to 400 Da. The compounds were identified via comparison of the mass spectra of the samples. The quantification of the carbohydrate and lignin derivatives were based on the peak areas obtained, where the sum corresponded to 100%. The percentage of the area of the lignin derivatives such as *p*-hydroxyphenyl (H), guaiacol (G) and siringyl (S) corresponded to the sum of the areas of the units of the three derivatives. The area of the total lignin derivatives was the sum of the areas of the lignin derivatives: H, G, and S. The S/G ratio was calculated as the ratio of the sums of the areas relative to the signs corresponding to the S and G structures (Schurt et al., 2013).

At 35 DAI, five leaves from plants from replication of each treatment were collected and dried in oven at 65°C for four days until reaching constant mass. After this procedure, the leaves were ground in Wiley type grinders to determine the foliar Si concentration according to Korndörfer et al. (2004).

Two experiments in 2 x 2 factorial design, composed of plants non-inoculated and inoculated with *H. vastatrix* and non-supplied or supplied with Si, were installed in an entirely randomized design with 15 replications for each sampling time. Each replication corresponded to a plastic pot containing two coffee plants. For the pyrolyzed samples, from the 15 replications of each treatment, three pooled samples were obtained for pyrolyzation. Data from foliar Si concentration and AURPC were combined after calculating the variance homogeneity according to Cochran´s test (Gomez & Gomez, 1994). Data for these two variables were submitted to ANOVA and the averages for the treatments were compared using Tukey´s test ($P \leq 0.05$) using the SAS 9.0 program (SAS Institute Inc., Cary, NC). Data from pyrolysis analysis were quantitatively interpreted using only the data from one experiment.

Results and discussions

There was no significant difference for the foliar Si concentration and AURPC between plants non-supplied or supplied with Si (Table 1). The foliar Si concentration was considered low (values lower than 0.5 dag/kg) when compared to plants that accumulate this element, for example rice (values higher than 5 dag/kg) (Datnoff et al., 2007; Carré-Missio et al., 2009, 2012). In general, coffee plants have a low efficiency in translocating Si from the roots to the shoots when grown in nutrient solution, which may influence coffee resistance to diseases (Carré-Missio et al., 2009; 2012; 2014; Rodrigues et al., 2011). In the present study, the lower foliar Si concentration may have contributed for not having reduction in the levels of rust in plants supplied with this element.

Table 1 - Foliar silicon concentration (SiC; dag/kg) and area under the rust progress curve (AURPC) in leaves of coffee plants supplied (+Si) or non-supplied (-Si) with silicon and inoculated with *Hemileia vastatrix*.

Variables	+Si	-Si
SiC	0.41	0.35 n.s.
AURPC	2827	2654 n.s.

n.s. = not statistically significant.

Nineteen phenolic compounds were identified after pyrolyzation of the leaves of coffee plants non-supplied or supplied with Si and non-inoculated or inoculated with *H. vastatrix* (Figure 1 and Table 2). Analysis of these compounds allowed them to be grouped into three compounds deriving from the degradation of carbohydrates and 16 lignin derivatives. Among the 16 compounds derived from lignin, eight originated from the H precursor (monophenol, 2.5-dimythylephenol, 2-ethylphenol, 3-ethylphenol, 2.5-dimythyl-4-methoxyphenol, coumaric acid, 4-ethyl-2-

methoxyphenol and 4-heptyl-phenol), seven from the G precursor (2-methylphenol, 4-methylphenol, guaiacol, pyrocatechol, metoxyeugenol, vanillin, and isoeugenol) and one from the S (syringol) precursor (Figure 1 and Table 2). Some compounds identified in the coffee leaves did not have their origins determined due to the low resolution of their mass spectra (hydroxyacetone, pyruvic acid, toluene, phenylacetone, 2-methylindole, 3-methylindole, dodecanol, dihydrophitol, neophytadiene, and caffeine) (Figure 1 and Table 2). The pyrolysis connected to the gaseous phase chromatography and to the mass spectrometry has shown itself to be a quick and highly sensitive method for characterizing lignin chemical structures (Barbosa et al., 2008; Schurt et al., 2013). Lignin, after pyrolyzed, consists of a relatively simple mixture of phenols, which result from the separation of ether and certain carbon-carbon connections (Barbosa et al., 2008; Schurt et al., 2013). Although various authors have reported on the importance of lignin as an important defense mechanism in plants (Rodrigues et al., 2004, 2005; Fortunato et al., 2014; Tatagiba et al., 2014), few studies have studied lignin properties in host-pathogen interactions (Schurt et al., 2013).

For plants non-supplied or supplied with Si and non-inoculated or inoculated with *H. vastatrix* showed values for lignin derivatives ranging from 4.8 to 6.3% of the area for the H type; from 2.8 to 3.7% for G and from 0.2 to 1.0% for S (Table 2). The sum of the areas of these lignin derivatives was approximately 11% (Table 2). Lignin is classified according to the relative quantity of H, G and S monomers in its structure (Barbosa et al., 2009). In pteridophyte plants, gymnosperms and angiosperms, lignins are predominated in the forms of cumarilic (formed by units of H), cumarilic-guaiacollic (formed by units of H and G) and guaiacollic-siringylic (formed by units of H and G), respectively (Monteiro et al., 2004). However, in many angiosperms the formation of lignin involves polymerization of the three types of monomer units (Barbosa et al., 2008). In the sheaths of rice plants non-supplied or supplied with Si and infected with *Rhizoctonia solani*, 23 compounds derived from lignin were identified, with eight originating from the H type, 11 from the G type and four from the S type (Schurt et al., 2013). In the present study, the lignification mechanism in coffee leaves appears to depend on polymerization of the three majority precursors of lignin (H-G-S), both in plants non-supplied or supplied with Si and non-inoculated or inoculated with *H. vastatrix.*

The 4-heptyloxy-phenol and dodecanol compounds were found only in the leaves of non-inoculated plants (Figure 1 and Table 2). In contrast, the vanillin compound was identified only in the leaves of inoculated plants (Figure 1 and Table 2). All of the phenolic compounds detected in the leaves of plants supplied with Si were also found in the leaves of plants non-supplied with this element (Figure 1 and Table 2). In the leaves of plants supplied with Si and inoculated with *H. vastatrix*, there was a tendency of occur higher peak values and relative area of the caffeine compound in comparison to leaves from plants non-supplied with Si (Figure 1 and Table 2). Moreover, among the compounds identified through pyrolysis analysis, the caffeine was the compound detected in greater concentrations in the leaves of coffee plants regardless of Si supply and inoculation with *H. vastatrix* (Figure 1 and Table 2). The biosynthesis of lignin, lignin precursors and phenolic compounds in the plants are carried out by the phenylpropanoid pathway, being considered one of the basal or induced defense mechanisms against pathogens (Nicholson & Hammerschmidt, 1992). The antimicrobial effect of vanillin and caffeine against pathogens has been reported as a defense mechanism in plants against pathogen infection (Nicholson & Hammerschmidt, 1992). Maize kernels from a resistant cultivar to infection by *F. graminearum* had high concentrations of vanillin in contrast to kernels from a plants of susceptible cultivar (Atanasova-Penichon et al., 2012). Moreover, it was also proven that kernels of maize plants inoculated with *F. graminearum* exhibited higher concentrations of vanillin in comparison to those non-inoculated regardless of the cultivar used (Atanasova-Penichon et al., 2012). In another study, mango plants with high concentrations of phenolic compounds, such as caffeine (7-metilxantine), exhibited greater resistance to Ceratocystis wilt compared with plants that had low concentrations of the compounds (Araujo et al., 2015). In contrast, in coffee plants supplied with Si and inoculated with *H. vastatrix* the increase in the concentration of chlorogenic acid and caffeoyl-quinic acid in leaves was not sufficient to reduce the severity of rust (Rodrigues et al., 2011). In the present study, despite higher concentrations of vanillin and caffeine having occurred in the leaves of plants supplied with Si and inoculated with *H. vastatrix*, these compounds did not contribute to greater resistance of the coffee plants to rust, in agreement with the results obtained by Rodrigues et al. (2011).

For leaves of plants supplied with Si, regardless of inoculation with *H. vastatrix*, there was a tendency for the occurrence of higher values for the S/G ratio in comparison to plants non-supplied with Si (Table 3). Generally, some vegetables that are little lignified contain mainly G in their structure with few unit of S, while the ones that are highly lignified contain proportional units of G-S in their structure (Brebu & Vasile, 2010). It is known that Si can potentiate some biochemical mechanisms related to host defense against pathogens (Rodrigues et al., 2004, 2005; Datnoff et al., 2007; Fortunato et al., 2014). For example, rice plants supplied with Si had high foliar concentrations of total phenolic compounds and lignin derivatives of the thioglycolic acid which contributed to reduce the severity of leaf scald (Tatagiba et al., 2014). In another study, an increase in the S/G ratio in rice plants supplied with Si was

associated with greater resistance to sheath blight (Schurt et al., 2013). The lignin and its precursors are important for plant resistance since they are toxic to pathogens and help to reinforce the cell walls (Weete, 1980; Nicholson & Hammerschmidt, 1992). However, in the present study, despite plants supplied with Si exhibited a high S/G ratio, it was converted into greater resistance (lignification) on the leaves since there was no reduction on rust symptoms in comparison to plants non-supplied with Si. One probable explanation for this is due to the biotrophy of *H. vastatrix* (Zambolim et al., 2005) not giving it the ability to produce lytic enzymes and non-host selective toxins in abundance in comparison to *F. oxysporum* f. sp. *cubense* considered to be necrotrophic pathogen (Fortunato et al., 2014). Lignin also has the ability to restrict the diffusion of lytic enzymes and non-host selective toxins produced by fungi in the cellular tissues increasing, therefore, the resistance of plants to pathogens (Fortunato et al., 2014). This would explain, at least partly, the lower effect of lignin in coffee resistance to rust.

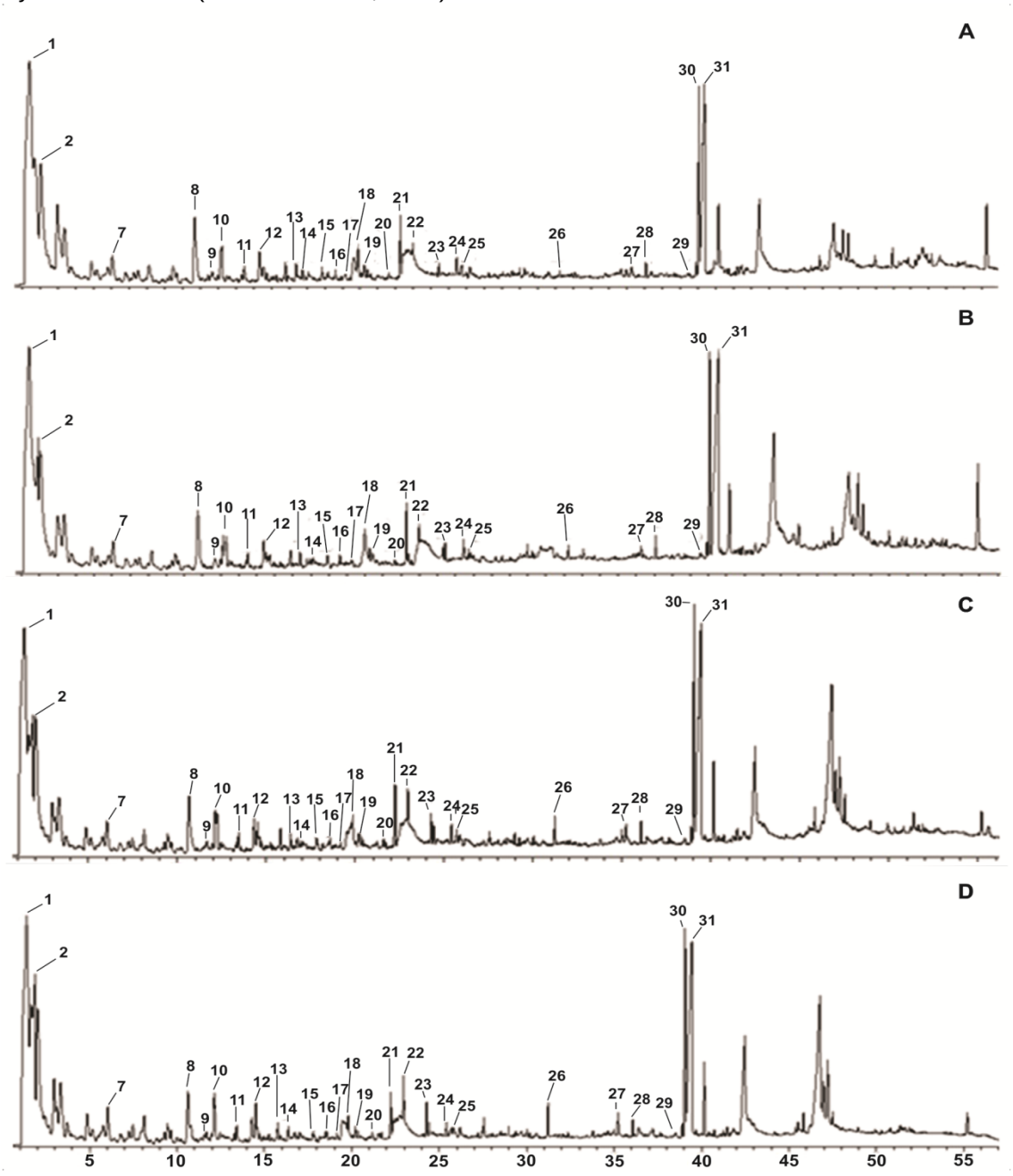

Figure 1 - Pyrograms from leaves of coffee plants supplied (A, C) or non-supplied (B, D) with silicon and non-inoculated (A, B) or inoculated (C, D) with *Hemileia vastatrix*. Samples of coffee leaves were collected at 20 days after inoculation with *H. vastatrix*. The numbers of the peaks in each pyrogram refer to the compounds presented in Table 2. Data from the pyrolytic analysis were interpreted qualitatively using only data from one experiment.

Table 2 - Phenolic compounds identified using the pyrolysis technique in leaves of coffee plants supplied (+Si) or non-supplied (-Si) with silicon and non-inoculated (NI) or inoculated (I) with *Hemileia vastatrix*.

Peaks[1]	RT[2]	NC[3]	Relative areas (%)				Origin[4]
			NI		I		
			+Si	-Si	+Si	-Si	
1	1.39	U.I.	27.76	32.14	27.12	23.15	nd
2	1.68	Acetic acid	8.60	7.67	12.48	13.56	C
3	2.03	Hydroxyacetone	9.15	5.80	8.23	8.00	nd
4	2.96	Pyruvic acid	3.47	7.55	0.98	6.86	nd
5	3.15	U.I.	1.16	1.16	2.21	1.45	nd
6	3.34	Toluene	3.00	2.22	0.95	2.29	nd
7	6.05	Phenylacetone	1.04	0.98	0.00	0.96	nd
8	10.70	Monophenol	3.50	2.72	2.26	1.93	LH
9	12.14	2-Cyclopenten-1-one	1.79	2.03	1.94	1.96	C
10	12.26	2-Methylphenol	0.37	0.30	0.34	0.26	LG
11	13.49	4-Methylphenol	1.04	0.92	0.85	0.65	LG
12	14.35	Guaiacol	0.49	0.23	0.44	0.81	LG
13	16.40	2-Cyclopenten-1-one	0.41	0.30	0.24	0.29	C
14	16.77	2,5-Dimethyl-phenol	0.32	0.26	0.46	0.12	LH
15	17.88	2-Ethyl-phenol	0.26	0.15	0.58	0.62	LH
16	19.76	3-Ethyl-phenol	0.26	0.13	0.24	0.50	LH
17	19.87	2,5-D-M-4	0.18	0.11	0.35	0.29	LH
18	19.92	Pyrocatechol	1.50	0.75	0.24	0.62	LG
19	20.23	Coumaric acid	1.30	0.86	0.66	0.99	LH
20	20.41	Syringol	0.97	0.18	0.58	0.50	LS
21	22.28	2-Methylindole	1.36	1.51	1.23	1.26	nd
22	22.98	4-ethyl-2-methoxy-phenol	0.11	0.35	0.60	0.52	LH
23	24.28	Methoxyeugenol	0.10	0.16	0.35	0.40	LG
24	25.44	3-Methylindole	0.42	0.42	0.27	0.20	nd
25	25.74	4-Heptyloxy-phenol	0.37	0.26	0.00	0.00	LH
26	31.19	Vanillin	0.00	0.00	0.60	0.52	LG
27	35.27	Isoeugenol	0.24	0.38	0.36	0.27	LG
28	36.08	Dodecanol	0.15	0.29	0.00	0.00	nd
29	38.91	Dihydrophytol	0.29	0.29	0.27	0.20	nd
30	39.07	Neophytodiene	4.70	4.88	5.29	9.07	nd
31	39.37	Caffeine	13.00	15.66	15.75	9.70	nd
Area of the carbohydrate derivatives (%)			10.80	10.01	14.66	15.81	
p-Hydroxyphenyl type lignin (%)			6.30	4.84	5.14	4.96	
Guaiacyl type lignin (%)			3.73	2.75	3.19	3.53	
Syringyl type lignin (%)			0.97	0.18	0.58	0.50	
Area of the total lignin derivatives (%)			11.00	7.77	8.91	8.99	

[1]The peak number refers to the signs designated in Figure 1. [2]Retention time in minutes of the compound in the column. [3]Name of the compounds: U.I. = unidentified compound; 2,5-D-M-4 = 2,5-dimethyl-4-methoxyphenol. [4]Compound origin: C = carbohydrate; LH = p-hydroxyphenyl lignin; LG = guaiacyl lignin; LS = syringyl lignin, and nd = non-determined compound. Samples of coffee leaves were collected at 20 days after inoculation with *H. vastatrix*. Data from the pyrolytic analysis were interpreted qualitatively using only data from one experiment.

Table 3 - Syringyl/guaiacyl ratios in leaves of coffee plants supplied (+Si) or non-supplied (-Si) with silicon and non-inoculated (NI) or inoculated (I) with *Hemileia vastatrix*.

Treatments	+Si	-Si
NI	0.26	0.06
I	0.18	0.14

Data from the pyrolytic analysis were interpreted qualitatively using only data from one experiment.

Conclusions

Coffee plants supplied with Si and infected with *H. vastatrix* exhibited high concentrations of vanillin and caffeine as well as a high S/G ratio in the leaves.

Coffee plants supplied with Si did not show an increase on their resistance to rust in comparison to non-supplied plants.

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References

Araujo L, Bispo WMS, Rios VS, Fernandes SA, Rodrigues FA (2015). Induction of the phenylpropanoid pathway by acibenzolar-S-methyl and potassium phosphite increases mango resistance to *Ceratocystis fimbriata* infection. Plant Disease 99(4):447-459.

Atanasova-Penichon V, Pons S, Pinson-Gadais L, Picot A, Marchegay G, Bonnin-Verdal MN, Ducos C, Barreau C, Roucolle J, Sehabiague P, Carolo P, Richard-Forget F (2012). Chlorogenic acid and maize ear rot resistance: a dynamic study investigating Fusarium graminearum development, deoxynivalenol production, and phenolic acid accumulation. *Molecular Plant-Microbe Interactions* 25(12):1605-1616.

Barbosa LCA, Maltha CRA, Silva VL (2008). Determinação da relação siringila/guaiacila da lignina em madeiras de eucalipto por pirólise acoplada à cromatografia gasosa e espectrometria de massas (PI-CG/EM). Química Nova 31(8):2035-2041.

Batista KD, Araújo WL, Antunes WC, Cavatte PC, Moraes GABK, Martins SCV, DaMatta FM (2012). Photosynthetic limitations in coffee plants are chiefly governed by diffusive factors. Trees 26(2):459-468.

Brebu M, Vasile C (2010). Thermal degradation of lignin - a review. Cellulose Chemistry and Technology 44(9):353-363.

Clark RB (1975). Characterization of phosphatase of intact maize roots. Journal of Agricultural and Food Chemistry 23(3):458-460.

Carré-Missio V, Rodrigues FA, Schurt DA, Pereira SC, Oliveira MG, Zambolim L (2009) Ineficiência do silício no controle da ferrugem do cafeeiro em solução nutritiva. Tropical Plant Pathology 34(6):416-421.

Carré-Missio V, Rodrigues FA, Schurt DA, Moreira WR, Rezende DC, Korndörfer GH, Zambolim L (2012) Proteção local, não sistêmica, do silicato de potássio reduz os sintomas da ferrugem do cafeeiro. Tropical Plant Pathology 37(4):275-280.

Carré-Missio V, Rodrigues FA, Schurt DA, Resende RS, Souza NFA, Rezende DC, Moreira WR, Zambolim L (2014). Effect of foliar-applied potassium silicate on coffee leaf infection by *Hemileia vastatrix.* Annals of Applied Biology 164(3):396-403.

Datnoff LE, Elmer WH, Huber DM (2007). Mineral Nutrition and Plant Disease. The American Phytopathological Society Press:St. Paul, MN, 278 p.

Fortunato AA, da Silva WL, Rodrigues FA (2014). Phenylpropanoid pathway is potentiated by silicon in the roots of banana plants during the infection process of *Fusarium oxysporum* f. sp. *cubense*. Phytopathology 104(6):597-603.

Gomez KA, Gomez AA (1994). Statistical Procedures for Agricultural Research, 2nd Ed. New York: John Wiley, 704 p.

Hon DNS, Shiraishi N (2001). Wood and cellulosic chemistry. Marcel Dekker:New York, 802 p.

Honorato Júnior J, Zambolim L, Aucique-Perez CE, Resende RS, RodriguesFA (2015) Photosynthetic and antioxidative alterations in coffee leaves caused by epoxiconazole and pyraclostrobin sprays and *Hemileia vastatrix* infection, Pesticide Biochemistry and Physiology 123(2):31-39.

Korndörfer GH, Pereira HS, Nolla A (2004). Análise de silício: solo, planta e fertilizante (Boletim Técnico, No. 2). Ed. Universidade Federal de Uberlândia,Uberlândia, MG, 34 p.

Kushalappa AC, Chaves GM (1978) Escala para avaliar a percentagem de área foliar com ferrugem do cafeeiro. Fitopatologia Brasileira (3):119.

Ma JF (2004). Role of silicon in enhancing the resistance of plants to biotic and abiotic stresses. Soil Science and Plant Nutrition 50(1):11-18.

Monteiro MBO, Pereira RPW, Abreu HS (2004). Bioquímica da lignificação de células xilemáticas. Floresta e Ambiente 11(2):48-57.

Nicholson RL, Hammerschmidt R (1992). Phenolic compounds and their role in disease resistance. Annual Review of Phytopathology 30(1):369-389.

Rodrigues FA, McNally DJ, Datnoff LE, Jones JB, Labbé C, Benhamou N, Menzies J G, Bélanger RR (2004). Silicon enhances the accumulation of diterpenoid phytoalexins in rice: a potential mechanism for blast resistance. Phytopathology 94(2):177-183.

Rodrigues FA, Jurick WM, Datnoff LE, Jones JB, Rollins JA (2005). Silicon influences cytological and molecular events in compatible and incompatible rice-*Magnaporthe grisea* interactions. Physiological and Molecular Plant Pathology 66(4):144-159.

Rodrigues FA, Carré-Missio V, Jham GN, Berhow M, Schurt DA (2011). Chlorogenic acid levels in leaves of coffee plants supplied with silicon and infected by *Hemileia vastatrix*. Tropical Plant Pathology 36(6):404-408.

Shaner G, Finney RE (1977). The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. Phytopathology 67(8):1051-1056.

Schurt DA, Rodrigues FA, Carré-Missio V, Soares NFF (2013). Silício alterando compostos derivados da pirólise de bainhas foliares de plantas de arroz infectadas por *Rhizoctonia solani*. Bragantia 72(1):52-60.

Tatagiba SD, Rodrigues FA, Filippi MCC, Silva GB, Silva LC (2014). Physiological responses of rice plants supplied with silicon to *Monographella albescens* infection. Journal of Phytopathology 162(9):596-606.

Várzea VMP, Marques DV (2005). Population variability of *Hemileia vastatrix* vs. coffee durable resistance. In: Zambolim L (ed) Durable resistance to coffee leaf rust. Viçosa MG: Suprema Gráfica e Editora. p.53-74.

Weete ER (1980). Lipid Biochemistry of Fungi and Other Organisms. Plenum Press, New York, 388p.

Zambolim L, Vale FXR, Zambolim EM (2005) Doenças do cafeeiro. In: Kimati H, Amorim L, Rezende JAM, Bergamin Filho A, Camargo LEA (ed.) Manual de Fitopatologia: Doenças das Plantas Cultivadas. São Paulo SP: Agronômica Ceres. p.165-180.