El Colegio de la Frontera Sur

Efecto de *Hemileia vastatrix* sobre la distribución del calcio y el potasio en las hojas de cafetos *Coffea arabica*

TESIS
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César Miguel Santiago Salazar

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A mi madre, Araceli

A mi esposa, Cris

A mi hija, Paulina

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Contenido

I. INTRODUCCIÓN

La roya del café, *Hemileia vastatrix* Berk. & Br. (Uredinales: Chaconiaceae), es la enfermedad más importante del café arábica (*Coffea arabica* L.) a nivel mundial, ya que provoca la caída prematura de las hojas, una severa reducción en la producción de fruto y el debilitamiento y muerte de las plantas enfermas (APS, 2011). Debido a que el cultivo del café es considerado como el producto agrícola más importante en el comercio internacional, una reducción en el rendimiento del fruto causado por *H. vastatrix* puede tener un gran impacto en los países productores cuya economía depende de forma importante de las exportaciones de café (APS, 2011).

Numerosos estudios dedicados a esta enfermedad han investigado la relación huésped-patógeno, y los factores bióticos y abióticos que favorecen el establecimiento y el éxito de la infección por *H. vastatrix*. Sin embargo, aún se desconocen los mecanismos por los que las enfermedades afectan la distribución de nutrientes, y su relación con la inducción de la respuesta de defensa en las plantas (Silva et al., 2006).

Los nutrientes pueden influir en la evolución de una enfermedad, al afectar la fisiología vegetal, a los patógenos, o a ambos (Dordas, 2008). La disponibilidad de nutrientes en los tejidos influye en el desarrollo de las plantas, lo que puede afectar las condiciones que facilitan el establecimiento de la infección y la esporulación del patógeno (Marschner, 1995). Además, los nutrientes pueden modificar la integridad de las paredes celulares, la fuga de metabolitos a través de las membranas y la composición química de los tejidos (Graham and Webb, 1991).

Los nutrientes como el potasio (K) y el calcio (Ca) están relacionados con la susceptibilidad de las plantas a los patógenos. El K interviene en la síntesis de

compuestos de alto peso molecular, por lo que su deficiencia en los tejidos vegetales conduce a una acumulación de compuestos precursores, de bajo peso molecular; además promueve el desarrollo de paredes celulares más gruesas en las células epidérmicas, evitando con ello la penetración de los agentes patógenos y la disponibilidad de nutrientes necesarios para su establecimiento (Dordas, 2008). El Ca participa en la composición de las membranas celulares, de ahí que niveles bajos de Ca provoca la pérdida de azúcares y aminoácidos, entre otros compuestos, hacia el exterior de las células, lo que facilita la infección por patógenos (Marschner, 1995); asimismo el Ca posee una acción inhibitoria sobre las enzimas destructoras de la pared celular empleadas por los patógenos para invadir a los tejidos vegetales (Collmer, 1986; Marschner, 1995) y se ha sugerido su función como un mensajero secundario (Maathuis, 2009; McAinsh and Pittman, 2009).

El empleo de técnicas como el microanálisis por energía dispersiva de rayos X, por ejemplo, ha permitido evaluar la relación de los nutrientes minerales con las interacciones huésped-patógeno en diferentes órganos vegetales (Leite and Andersen, 2009; Pozza et al., 2004) y bajo diferentes condiciones ambientales (Suginomoto et al., 2010). Williams et al. (2002) demostraron la acumulación de azufre en plantas de tomate (*Lycopersicon esculentum*) en respuesta a la infección por *Verticillium dahliae*; Ryan et al. (2003) cuantificaron la concentración de fósforo y otros elementos en diversas plantas, trébol subterráneo (*Trifolium subterraneum* L.) y blanco (*T. repens* L.), puerro (*Allium porrum* L.) y chícharos (*Pisum sativa* L.).

En patosistemas relacionados con *C. arabica* el microanálisis por rayos X ha sido empleado en diversas ocasiones. Rao y Tewari (1989) observaron la ocurrencia de

cristales de oxalato de magnesio en hojas de *C. arabica* que presentaban lesiones inducidas por *Mycena citricolor* Berk. & Curt. Así también Botelho et al. (2011) emplearon esta técnica para evaluar el efecto del silicio en la nutrición de *C. arabica* y la intensidad de la infección causada por el hongo *Cercospora coffeicola* Berk. & Cooke en este cultivo. Por su parte Belan et al. (2015) analizaron la composición de los nutrientes minerales en hojas y encontraron diferencias en la concentración de K y Ca entre zonas de tejido sintomático y asintomático de lesiones causadas por la bacteria *Pseudomonas syringae* pathovar *garcae* y los hongos *Colletotrichum gloeosporioides* Penz., *Cercospora coffeicola*, *Phoma tarda* (Stewart) Boerema & Bollen y *H. vastatrix.*

No obstante, aún no se tienen estudios sobre la variación de la distribución de nutrientes entre variedades susceptibles y resistentes a la roya, ni sobre la distribución de los nutrientes en diferentes partes de las plantas atacadas por esta enfermedad. Conocer la forma en la que se distribuyen los nutrientes en los tejidos vegetales en un patosistema podría explicar algunos mecanismos de ataque-respuesta ante patógenos en plantas, y contribuir al desarrollo de estrategias de manejo (Belan et al., 2015). El propósito de este estudio fue examinar la distribución de K y Ca en plantas resistentes y alrededor de lesiones foliares causadas por *H. vastatrix* en plantas susceptibles de *C. arabica* y su relación con la posición de las hojas en la planta, por medio de microanálisis por rayos X.

Nuestra hipótesis de trabajo propuso que, si la distribución de K y Ca está relacionada con la reacción de defensa ante la infección por *H. vastatrix*, entonces su concentración en hojas infectadas será diferente a la que muestran hojas no infectadas; asimismo, se

parte del supuesto que la concentración de estos elementos minerales dependerá de la posición de las hojas en la planta.

II.

Efecto de *Hemileia vastatrix* en la distribución del calcio y el potasio en las hojas de cafetos *Coffea arabica*

Sometido a Australasian Plant Pathology

1 *H. vastatrix* and nutrients distribution in coffee leaves

2

3 Effect of *Hemileia vastatrix* on calcium and potassium distribution in *Coffea arabica* leaves

4

5 C. M. Santiago-Salazar[a]

6 A. González-Díaz[b]

7 J. Gómez-Ruiz[a]

8 J. C. Rojas[a]

9 J. F. Barrera[a] *

10

11 [a] Departamento Agricultura Sociedad y Ambiente, El Colegio de la Frontera Sur. Carretera
12 Antiguo Aeropuerto km 2.5. Tapachula, Chiapas. C.P. 30700, Mexico.

13 [b] División Académica de Ingeniería y Arquitectura, Universidad Juárez Autónoma de
14 Tabasco. Carretera Cunduacán-Jalpa de Méndez, Km. 1, Col. La Esmeralda, Cunduacán,
15 Tabasco. C.P. 86690. México.

16 * Corresponding author: Juan F. Barrera (jbarrera@ecosur.mx).

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18 **Keywords**: coffee leaf rust, plant-pathogen interactions, plant defense, mineral elements,
19 scanning electron microscopy/energy dispersive X-ray spectroscopy.

20

Abstract

Coffee leaf rust (*Hemileia vastatrix*) is one of the major diseases of *Coffea arabica* worldwide. There is still no clear knowledge of the mechanisms by which this disease affects nutrient distribution in the plant. Therefore, the objective of this study was to examine the distribution of potassium (K) and calcium (Ca) in leaves of resistant plants and in susceptible plants, with and without *H. vastatrix*, and the relationship of these elements with the position of the leaves on the plant. Samples of leaf fragments, with and without lesions, were taken from leaves from different parts of the plants in a field infected naturally by *H. vastatrix*. Using scanning electron microscopy/energy dispersive X-ray spectroscopy, we quantified K and Ca (% mass) around the lesions and in tissue from the asymptomatic area to the symptomatic area. The results suggest that *C. arabica* reacts to *H. vastatrix* attack, sending Ca to lesions in the leaves. The process was more intense in the lower and middle part of the plant where the disease begins its development. The content of K in infected leaves was not statistically different from that observed in healthy leaves in either susceptible or resistant plants.

Keywords: coffee leaf rust, plant-pathogen interactions, plant defense, mineral elements, scanning electron microscopy/energy dispersive X-ray spectroscopy.

Introduction

Coffee leaf rust, *Hemileia vastatrix* Berk. & Br. (Uredinales: Chaconiaceae), is one of the major diseases that attacks *Coffea arabica* L. It causes defoliation and reductions of production of coffee beans in severe cases (Avelino and Rivas 2014). Numerous studies on this disease have investigated the host-pathogen relationship and biotic and abiotic factors that favor establishment and successful infection by *H. vastatrix*. However, it is still not clear how the disease affects nutrient distribution in the plant or what relationship exists between nutrients and induction of plant defense response (Silva et al. 2006).

Plant susceptibility to pathogens is related to, among other factors, deficiencies of nutrients such as potassium (K) and calcium (Ca). K activates a large variety of specific enzymes, it intervenes in protein synthesis (Maathuis 2009), it strengthens resistance through regulation of secondary metabolism, and it facilitates the transport of a large amount of resources to prevent infection and repair damage (Wang et al. 2013). Ca is related to resistance to infections because of its structural feature. Invasion of tissues by pathogenic fungi is strongly inhibited by Ca (Huber et al. 2012).

Using techniques, such as X-ray energy dispersive microanalysis, for example, has enabled discerning the relationship between mineral nutrients and host-pathogen interactions in different plant organs (Pozza et al. 2004) and under different environmental conditions (Suginomoto et al. 2010). In coffee (*C. arabica*), Belan et al. (2015) analyzed mineral nutrient composition in leaves and found that concentrations of K and Ca in symptomatic zones with lesions caused by *H. vastatrix* was different from those in asymptomatic zones. However, there are no studies on variation of nutrient distribution between coffee leaf rust-susceptible

and coffee leaf rust-resistant varieties, nor on the distribution of nutrients in different parts of plants attacked by the disease. Knowledge of how nutrients are distributed in plant tissue in a pathosystem might explain some of the mechanisms of response to attack by pathogens in plants and contribute to development of management strategies (Belan et al. 2015). The purpose of this study was to examine the distribution of K and Ca in resistant *C. arabica* plants and in the tissue surrounding leaf lesions caused by *H. vastatrix* in susceptible plants and to study its relationship with the position of the leaves on the plant.

Materials and methods

Site of biological material collection

Samples of *C. arabica* leaves were collected in July 2015 from cultivated plants in a 50 x 50 m lot located on the Finca La Concepción (15°00'49" N, 92°11'20" W) in Cacahoatán, Chiapas, Mexico at an altitude of 480 masl where mean annual temperature is 25.4 °C (INEGI 2016). All the lesions on the leaves were caused by natural infection. The leaf samples were taken from plagiotropic growth branches of the coffee plants; both healthy and infected leaves were collected from a susceptible variety (*C. arabica* var. bourbon). Leaves (healthy) from a resistant variety (*C. arabica*, Catimor) were collected too. Susceptible and resistant plants were found interspersed in the same lot separated by distances that varied from 0.70 to 1.5 m.

Sample collection in the field

87

88 Leaf samples with 1-5 mm diameter lesions and a severity of 2-5 lesions per leaf were
89 selected from susceptible coffee plants to *H. vastatrix*. As the control, samples of healthy
90 leaves were collected from the same branch node from which each of the infected leaves were
91 obtained. This procedure was followed also with healthy leaves of resistant plants.

92 Each plant was divided into thirds of its height (lower, middle and upper parts). One
93 plagiotropic branch was selected at random from each of these thirds and divided in two parts
94 with respect to distance from the stem (proximal and distal). From each of these branch parts,
95 one pair of opposite leaves was selected at random. In this way, 12 leaves were obtained from
96 each of 10 plants for analysis. The samples were taken to the laboratory for processing.

97

98 *Sample preparation and X-ray microanalysis*

99

100 In the laboratory, *H. vastatrix* infected leaves with at least one 5 mm diameter lesion
101 were selected. From each of the selected leaves, a 2 x 5 mm fragment of tissue was obtained;
102 this fragment included areas with asymptomatic tissue, transition areas (with or without
103 yellow halo) and areas with symptomatic tissue (Belan et al. 2015). The procedure was
104 repeated to obtain five fragments (five replications). This method was also applied with
105 healthy leaves, but the leaf fragments were obtained from asymptomatic tissue.

106 The leaf fragments were distributed over a glass slide covered with absorbent paper.
107 Nine fragments were placed on each slide and properly identified. The slides were
108 immediately placed in a drier with silica gel (SiO_2) (Hycel, Mexico) for 48 h to dehydrate the

plant material. After this time, the samples were observed under a Scanning Electron Microscopy coupled with Energy Dispersive X-ray Spectroscopy (SEM/EDX) (JEOL JSM-6010LA; Tokyo, Japan) located at Universidad Juárez Autónoma de Tabasco, Mexico.

On each leaf fragment, scanning areas were delimited on a straight line containing zones with asymptomatic to symptomatic tissue (susceptible variety with leaf rust) (Belan et al. 2015) or healthy tissue only (susceptible variety without leaf rust and resistant variety). With the SEM/EDX, distribution of K and Ca was quantified at 20 reading points along this line. The images were generated at 20 kV with a distance of 9 mm and range of 3 to 5 Kcps. Quantification of K and Ca was expressed in percentage of mass per reading point.

Data analysis

The data of the distribution of K and Ca (% mass) in the leaf fragments were analyzed for each mineral element with the mixed experimental model (Littell et al. 2006). Reading points (n=20) on the tissue represented the random factor, while the location of the leaf fragments from different parts of the plant (lower, middle and upper thirds of its height; proximal and distal branch parts), leaf condition (healthy or infected by *H. vastatrix*) and type or variety of coffee (susceptible or resistant to *H. vastatrix*) were considered fixed effects. Three analyses of variance (ANOVA) were conducted for each mineral element: healthy vs infected tissue from susceptible plants; healthy tissue from susceptible plants vs (healthy) tissue from resistant plants; and infected tissue from susceptible plants vs (healthy) tissue from resistant plants. Data were analyzed with R software (R Core Team 2014).

Results

Potassium (K)

Healthy vs infected tissue from susceptible plants

The ANOVA showed highly significant differences (F= 21.48; df= 1, 1139; P<0.0001) in the distribution of K along the 20 reading points obtained with SEM/EDX in leaf fragments. Graphing these points revealed that K content tended to decrease linearly from the asymptomatic zone toward the symptomatic zone affected by coffee leaf rust (Fig. 1a). However, the average (±standard error) of K concentration in susceptible plants to *H. vastatrix* (0.558±0.017%) was not significantly different (F= 0.13; df= 1, 48; P= 0.71) from that of healthy plants (0.519±0.021%) (Table 1). Nor were there significant differences in the distribution of K (F = 0.05; df= 1, 48; P = 0.83) with respect to the position of the leaves on the branch, either in healthy leaves (proximal: 0.457± 0.029%, distal: 0.580±0.031%) or in leaves infected by *H. vastatrix* (proximal: 0.596± 0.023%, distal: 0.519±0.025%). Neither were there differences in the distribution of this element among the branches positioned in the different thirds of the plant height (F = 2.03; df= 2, 48; P = 0.14), either in healthy tissue (lower: 0.629±0.039%, middle: 0.471±0.039%, upper: 0.455±0.031) or in infected tissue (lower: 0.747±0.035%, middle: 0.448±0.026%, upper: 0.478±0.021%) (Table 1).

153 *Healthy tissue from susceptible plants vs (healthy) tissue from resistant plants*

154

155 Distribution of K along the 20 reading points obtained with SEM/EDX in leaf
156 fragments was highly significant (F= 17.04; df= 1, 1139; P<0.0001). Analysis of the
157 distribution of K found no significant difference (F= 0.30; df= 1, 48; P= 0.58) between
158 healthy leaves from susceptible plants (0.519±0.021%) and those from resistant plants to *H.*
159 *vastatrix* (0.453±0.019%) (Table 1). No significant differences (F = 0.05; df= 1, 48; P = 0.82)
160 between healthy leaves from different parts of branches of susceptible plants (proximal:
161 0.457± 0.029%, distal: 0.580±0.031%) and those of resistant plants (proximal: 0.487±
162 0.028%, distal: 0.418±0.025%). Nor were there statistical differences (F = 0.49; df= 2, 48; P
163 = 0.62) between the healthy leaves of susceptible plants at different thirds (lower:
164 0.629±0.039%, middle: 0.471±0.039%, upper: 0.455±0.031%) of the plant and those of *H.*
165 *vastatrix* resistant plants (lower: 0.507±0.036%, middle: 0.397±0.032%, upper:
166 0.454±0.028%) (Table 1).

167

168 *Infected tissue from susceptible plants vs (healthy) tissue from resistant plants*

169

170 As in the two previous cases, distribution of K at the 20 reading points obtained with
171 SEM/EDX in the leaf fragments was highly significant (F= 29.79; df= 1, 1139; P<0.0001).
172 However, the ANOVA did not detect statistical differences (F= 1.33; df= 1, 48; P= 0.58) in
173 the distribution of K between leaves of infected plants of the susceptible variety
174 (0.558±0.017%) and those of *H. vastatrix* resistant variety (0.453±0.019%) (Table 1). There

175 were no differences (F = 0.64; df= 1, 48; P = 0.43) in K distribution between the parts of
176 branches with infected leaves from susceptible plants (proximal: 0.596± 0.023%, distal:
177 0.519±0.025%) and those from resistant plants (proximal: 0.487± 0.028%, distal:
178 0.418±0.025%). Nor were there differences (F = 1.87; df= 2, 48; P = 0.16) found between
179 infected leaves of susceptible plants among the thirds in which plant height was divided
180 (lower: 0.642±0.053%, middle: 0.507±0.041%, upper: 0.408±0.030%) and those of resistant
181 plants (lower: 0.507±0.036%, middle: 0.397±0.032%, upper: 0.454±0.028%) (Table 1).

182 Only in this case, significant interactions were found (P<0.01). These interactions
183 were reading points (n=20, random factor) and the fixed effects of plant height (F = 19.22;
184 df= 2, 1128; P < 0.0001); reading points, branch part and coffee variety (F = 7.79; df= 1,
185 1128; P = 0.005); reading points, plant height and coffee variety (F = 11.00; df= 2, 1128; P <
186 0.0001); and reading points, branch part, plant height and coffee variety (F = 4.13; df= 2,
187 1128; P = 0.02).

188

189 *Calcium (Ca)*

190

191 *Healthy vs infected tissue from susceptible plants*

192

193 The analysis revealed highly significant differences (F= 64.60; df= 1, 1139; P<0.0001)
194 in the distribution of Ca along the 20 reading points on leaf fragments obtained with
195 SEM/EDX. It was observed that Ca content tended to increase almost linearly from the
196 asymptomatic zone toward the symptomatic zone affected by *H. vastatrix* (Fig. 1b).

197 Moreover, the average (±standard error) of Ca concentration in susceptible plants with healthy
198 leaves (0.091±0.004%) was lower (F= 11.15; df= 1, 48; P= 0.002) than in leaves infected by
199 *H. vastatrix* (0.174±0.007%) (Table 2). However, no differences were found in Ca
200 distribution (F = 0.46; df= 1, 48; P = 0.50) between the position of the analyzed tissue on the
201 branch, regardless of whether leaves were healthy (proximal: 0.074± 0.005%, distal:
202 0.108±0.005%) or infected (proximal: 0.174± 0.009%, distal: 0.173±0.011%). Nor were there
203 differences in the distribution of this element in the thirds in which plant height was divided
204 (F = 2.49; df= 2, 48; P = 0.09), either in healthy tissue (lower: 0.096±0.006%, middle:
205 0.095±0.008%, upper: 0.081±0.005) or infected (lower: 0.245±0.015%, middle:
206 0.120±0.009%, upper: 0.115±0.010%) (Table 2).

207

208 *Healthy tissue from susceptible plants vs healthy tissue from resistant plants*

209

210 The SEM/EDX indicated that Ca had a highly significant distribution (F = 13.63; df=
211 1, 1139; P=0.0002) along the 20 reading points on the leaf fragments. Ca concentration in
212 leaves of healthy susceptible plants (0.091±0.004%) and *H. vastatrix* resistant plants
213 (0.089±0.004%) was not different (F= 0.01; df= 1, 48; P= 0.92) (Table 2). No differences
214 were found between healthy leaves from different parts of the branches of susceptible plants
215 (proximal: 0.074±0.005%, distal: 0.108±0.005%) and healthy leaves from resistant plants
216 (proximal: 0.091± 0.006%, distal: 0.089±0.005%). Nor were there differences between
217 healthy leaves from the different thirds (lower: 0.096±0.006%, middle: 0.095±0.008%,
218 up0p0er: 0.081±0.005%) of susceptible plants (F = 0.20; df= 2, 48; P = 0.82) and those from

219 the different parts of resistant plants (lower: 0.097±0.006%, middle: 0.067±0.006%, upper:
220 0.103±0.007%) (Table 2).

221

222 *Infected tissue from susceptible plants vs (healthy) tissue from resistant plants*

223

224 In this case, Ca had a highly significant distribution (F= 71.15; df= 1, 1139;
225 P<0.0001) at the 20 reading points obtained with SEM/EDX. The ANOVA also detected
226 differences (F= 11.95; df= 1, 55; P= 0.001) in the distribution of Ca between infected leaves
227 from susceptible plants to *H. vastatrix* (0.174±0.007%) and those from resistant plants to *H.*
228 *vastatrix* (0.089±0.004%) (Table 2). Another difference was found among the infected leaves
229 from different thirds of susceptible plants (lower: 0.045±0.015%, middle: 0.120±0.009%,
230 upper: 0.115±0.010%) and leaves from resistant plants (lower: 0.097±0.006%, middle:
231 0.067±0.006%, upper: 0.103±0.007%). However, there was no difference (F= 0.010; gl= 1,
232 55; P= 0.92) between parts of the branch in infected leaves of susceptible plants (proximal:
233 0.174± 0.009%, distal: 0.173±0.011%) and those of resistant plants (proximal: 0.091±
234 0.006%, distal: 0.087±0.005%) (Table 2).

235

236 **Discussion**

237

238 Recently the distribution of mineral nutrients has been studied using this technique in
239 *C. arabica* leaf tissue around lesions caused by the bacterium *Pseudomonas syringae* pathovar
240 *garcae* and the fungi *Colletotrichum gloeosporioides* Penz., *Cercospora coffeicola*, *Phoma*

241 *tarda* (Stewart) Boerema & Bollen and *H. vastatrix* (Belan et al. 2015), and variations were
242 found in the content of K and Ca around the lesions. Specifically, Belan et al. (2015) found
243 that the content of K was higher in the asymptomatic tissues that surrounded the lesions
244 caused by *P. syringae* pathovar *garcae* and the fungi *C. coffeicola* and *P. tarda*; toward the
245 transition zone it decreased and in symptomatic tissues its content was minimal. These
246 authors found that Ca content had the opposite trend with the same pathogens. That is, it
247 increased toward the transition zone and reached a maximum content in symptomatic tissue.
248 In the case of *C. gloeosporioides* and *H. vastatrix*, K content was similar to that observed in
249 the pathogens mentioned above, but Ca remained relatively constant in both the infected zone
250 and healthy tissue. Because of these results, our study proposed to determine whether the
251 variations in K and Ca contents around the lesions caused by *H. vastatrix* might be affected by
252 factors such as susceptibility or resistance to the pathogen and position of the infected leaves
253 on the plant.

254 When we analyzed the variations of these mineral elements in leaf tissue infected by
255 *H. vastatrix* and healthy leaves from susceptible coffee plants, we found that our results
256 coincided with the findings reported by Belan et al. (2015); that is, the K content in infected
257 leaves decreased from the symptomatic area toward the symptomatic area. However, in our
258 study, this decrease was not statistically different from that observed in healthy leaves from
259 either susceptible or resistant plants. Therefore, based on the information obtained by our
260 research, this response of content of K, in the case of *H. vastatrix*, cannot be linked to effect
261 of the pathogen.

262 In contrast to the results obtained by Belan et al. (2015) for coffee leaf rust, our results
263 indicate that the content of Ca increased significantly in the symptomatic zone—as occurred

with *P. syringae* pathovar *garcae* and the fungi *C. coffeicola* and *P. tarda* in the study of the authors mentioned above. However, healthy leaf tissue from the coffee variety susceptible to *H. vastatrix* remained without change, and thus this response could be attributed to coffee leaf rust. We observed the same response when we compared the Ca content in infected tissue from plants susceptible to *H. vastatrix* with that in healthy tissue from resistant coffee plants. While Ca content in healthy leaf tissue from susceptible plants was statistically similar to that in tissue from the resistant variety, the content of this element in infected tissue was significantly higher than that obtained in both the susceptible variety without coffee leaf rust and in the resistant variety.

It is known that during the infection process in plant tissues, pathogenic fungi release enzymes that promote degradation or modification of the cell wall, altering its permeability to facilitate access to nutrients (Mäkelä et al. 2014). This process induces a response of hypersensitivity in the plant as a defense mechanism, producing a flow of anions and water from the infected tissue toward areas of healthy tissue. This facilitates cell contraction and mechanisms of cell death to restrict pathogen growth at the infection site (Hückelhoven 2007; Shabala and Pottosin 2014).

The increase in Ca in symptomatic leaf tissues infected by *H. vastatrix*, could be related to the plant's defense response to pathogens and to intracellular homeostasis of this element. It has been found that elevation of Ca moderates a diversity of responses to biotic and abiotic pressure (Ranf et al. 2008), which regulate different ion transporters, among which is that of K (Gaymard et al. 1998). Accumulation of Ca in infected tissue is likely related to the damage caused by enzymes released by the pathogen that degrade the cell wall and decrease its mechanical resistance and its ability to limit expansion of cell turgidity

287 (Hématy et al. 2009). The mechanical stress in the plasmatic membrane could activate the Ca
288 channels and modify its intracellular concentration and initiate signalling events, as
289 Nakagawa et al. (2007) suggested. Furthermore, the inhibiting function that Ca exerts on the
290 degrading enzymes (Huber et al. 2012) could explain the increase in the quantity of Ca
291 observed in infected tissues. Ca could also, according to Hückelhoven (2007), contribute to
292 containing the pathogen at the infection site since it intervenes in non-covalent intercrossing
293 of the cell wall, favoring its increased rigidity.

294 Regarding the effect of the position of infected leaves on the plant on variation of K
295 and Ca contents, our results did not provide any indication that would suggest that *H.*
296 *vastatrix* affected the distribution of K in the plant. However, in the case of Ca, the results
297 indicate that the content of this mineral element in the infected tissue located in leaves of the
298 lower and middle thirds of susceptible plants was significantly higher than that found in the
299 same thirds of resistant plants. This result coincides with Avelino and Rivas (2014), who state
300 that *H. vastatrix* begins its development in old leaves located in the lower part of the coffee
301 plant and progressively infects younger leaves in the upper part of the plant.

302 The results obtained in our study suggest that the *C. arabica* coffee plant reacts
303 defensively against the attack of *H. vastatrix* sending Ca to the lesions on the leaves, a process
304 that is more intense in the lower and middle parts of the plant where the disease begins its
305 development.

306

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308

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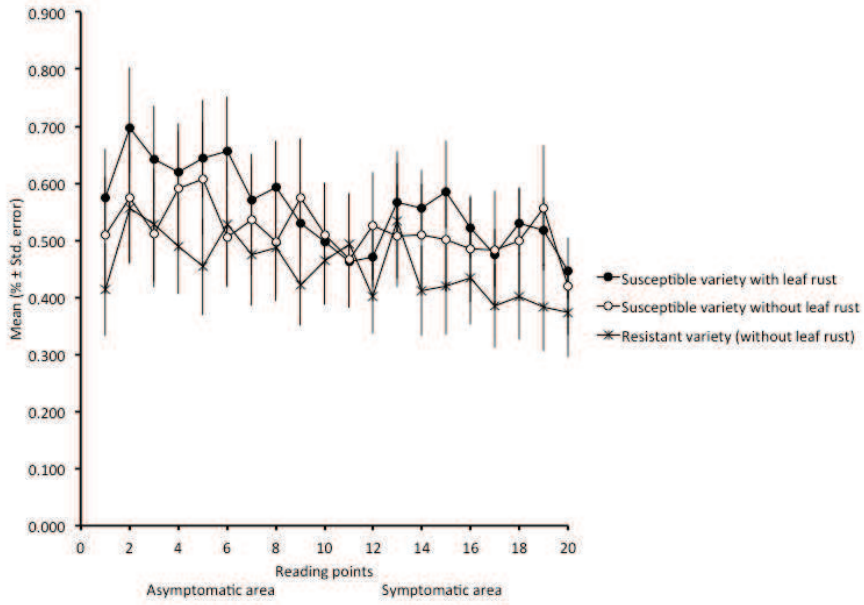
372 Table 1. Distribution of potassium (% mass) in leaf fragments according to coffee variety,
373 infection of coffee leaf rust (*H. vastatrix*), parts of branch and plant height (*C. arabica*).
374 Scanning electron microscopy/energy dispersive X-ray spectroscopy generated the data.

Coffee variety, infection of rust			Parts of branch			Plant height (L=lower, M= middle, U=upper thirds)			
Mean	Std. error	n	Mean	Std. error	n		Mean	Std. error	n
Susceptible variety, without rust									
			Proximal						
0.519	0.021	600	0.457	0.029	300	L=	0.531	0.057	100
						M=	0.403	0.055	100
						U=	0.437	0.038	100
			Distal						
			0.580	0.031	300	L=	0.727	0.052	100
						M=	0.540	0.055	100
						U=	0.473	0.050	100
						Mean			
						L=	0.629	0.039	200
						M=	0.471	0.039	200
						U=	0.455	0.031	200
Susceptible variety, with rust									
			Proximal						
0.558	0.017	600	0.596	0.023	300	L=	0.852	0.043	100
						M=	0.390	0.032	100
						U=	0.547	0.029	100
			Distal						
			0.519	0.025	300	L=	0.642	0.053	100
						M=	0.507	0.041	100
						U=	0.408	0.030	100
						Mean			
						L=	0.747	0.035	200
						M=	0.448	0.026	200
						U=	0.478	0.021	200
Resistant variety, without rust									
			Proximal						
0.453	0.019	600	0.487	0.028	300	L=	0.523	0.052	100
						M=	0.362	0.041	100
						U=	0.576	0.049	100
			Distal						
			0.418	0.025	300	L=	0.492	0.050	100
						M=	0.432	0.050	100
						U=	0.362	0.041	100
						Mean			
						L=	0.507	0.036	200
						M=	0.397	0.032	200
						U=	0.454	0.028	200

375 Table 2. Distribution of calcium (% mass) in leaf fragments according to coffee variety,
376 infection of coffee leaf rust (*H. vastatrix*), parts of branch and plant height (*C. arabica*).
377 Scanning electron microscopy/energy dispersive X-ray spectroscopy generated the data.

Coffee variety, infection of rust			Parts of branch			Plant height (L=lower, M= middle, U=upper thirds)			
Mean	Std. error	n	Mean	Std. error	n		Mean	Std. error	n
Susceptible variety, without rust									
			Proximal						
0.091	0.004	600	0.074	0.005	300	L=	0.079	0.009	100
						M=	0.069	0.009	100
						U=	0.073	0.007	100
			Distal						
			0.108	0.005	300	L=	0.114	0.008	100
						M=	0.122	0.013	100
						U=	0.089	0.007	100
						Mean			
						L=	0.096	0.006	200
						M=	0.095	0.008	200
						U=	0.081	0.005	200
Susceptible variety, with rust									
			Proximal						
0.174	0.007	600	0.174	0.009	300	L=	0.234	0.017	100
						M=	0.122	0.013	100
						U=	0.166	0.013	100
			Distal						
			0.173	0.011	300	L=	0.257	0.024	100
						M=	0.119	0.013	100
						U=	0.144	0.015	100
						Mean			
						L=	0.245	0.015	200
						M=	0.120	0.009	200
						U=	0.155	0.010	200
Resistant variety, without rust									
			Proximal						
0.089	0.004	600	0.091	0.006	300	L=	0.102	0.010	100
						M=	0.041	0.004	100
						U=	0.129	0.011	100
			Distal						
			0.087	0.005	300	L=	0.091	0.007	100
						M=	0.093	0.011	100
						U=	0.077	0.006	100
						Mean			
						L=	0.097	0.006	200
						M=	0.067	0.006	200
						U=	0.103	0.007	200

(a)



(b)

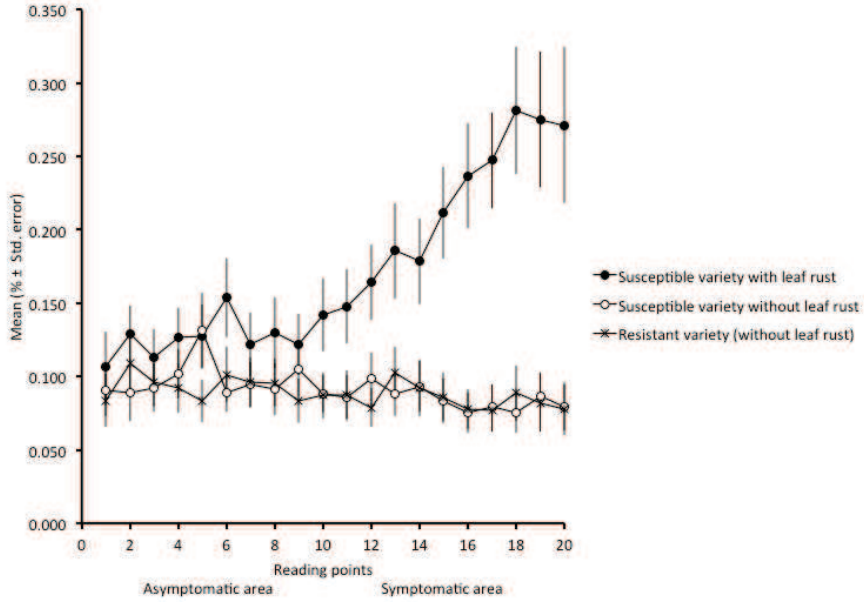


Fig. 1. Potassium (a) and calcium (b) content (% mass), in leaf fragments of resistant and susceptible (with and without *H. vastatrix*) varieties of coffee. Scanning electron microscopy/energy dispersive X-ray spectroscopy generated the data. In the case of susceptible variety with leaf rust, the analyzed fragments included areas from asymptomatic to symptomatic tissues.

III. CONCLUSIONES

Los resultados obtenidos en este trabajo sugieren que el aumento significativo en el contenido de Ca en la zona sintomática de las lesiones provocadas por *H. vastatrix* en hojas de *C. arabica*, podría tener relación con la respuesta de defensa de la planta ante el patógeno. Sin embargo, no hubo evidencia que sugiera que la disminución del contenido de K de la zona asintomática hacia la zona sintomática de las lesiones causadas por *H. vastatrix* en hojas de *C. arabica*, esté relacionada al efecto del patógeno.

Por otro lado, los resultados de este estudio no proporcionaron indicación alguna que permita sugerir que *H. vastatrix* afectó la distribución del K en la planta. Por el contrario, la evidencia sugiere que la planta de café *C. arabica* reacciona defensivamente ante el ataque de *H. vastatrix* enviando Ca hacia las lesiones en las hojas, proceso que fue más intenso en la parte baja y media de la planta donde la enfermedad inicia su desarrollo.

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