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YIELD, QUALITY AND WATER CONSUMPTION OF CONILON COFFEE UNDER IRRIGATED AND DRYLAND MANAGEMENTS

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ABSTRACT: In this study the goal was to make an assessment and comparison of the yield, quality and water consumed by the Conilon coffee plants under irrigated and dryland types of cultivation, from seedlings raised in different containers and under varying shading levels. The experiment which extended from December 2007 to April 2012 was performed at the IFES, Alegre-ES Campus and involved the study of a total of four crops. The findings showed that the irrigated plants had 162% higher yield on average than did the rainfed plants. For the irrigated plants, the yield indices achieved were 4.5 kg of coffee of the planted / benefited area; 1.9 kg of coconut / beneficiated coffee and 5.6 balms of 80 L sc-^{1}; whereas, for the rainfed plants, the values recorded were 8.2 kg of coffee from the benefited field; 3.1 kg of coconut / beneficiated coffee and 12 balloons of 80 L sc-^{1}. The Conilon coffee grains harvested from the irrigated plants were superior in quality to those from the rainfed plants. For the irrigated plants, the water consumed on average was 7.9 m^3 per plant, while for the rainfall-dependent crop, it was 4.95 m^3. For the irrigated and rainfed plants the relations between the water consumption / kg of the beneficiated coffee was 8.8 m^3 and 30.3 m^3, respectively. The type of container and levels of shading exerted no influence on the Conilon coffee with respect to productivity, yield and quality.

Index terms: *Coffea canephora,* irrigation, productivity, growth.

RENDIMENTO, QUALIDADE E CONSUMO DE ÁGUA DO CAFEEIRO CONILON SOB MANEJO IRRIGADO E DE SEQUEIRO

RESUMO: Objetivou-se com este trabalho avaliar e comparar o rendimento, a qualidade e o consumo de água do cafeeiro conilon irrigado e de sequeiro, oriundo de mudas formadas em diferentes recipientes e níveis de sombreamento. O experimento foi conduzido no IFES, Campus de Alegre-ES, no período de dezembro de 2007 a abril de 2012, totalizando-se quatro colheitas. O valor médio de produtividade de plantas irrigadas foi 162% superior ao de plantas de sequeiro. Os índices de rendimento obtidos em plantas irrigadas foram de 4,5 kg de café da roça/beneficiado; 1,9 kg de café coco/beneficiado e 5,6 balaios de 80 L sc-^{1} e em plantas de sequeiro, de 8,2 kg de café da roça/beneficiado; 3,1 kg de café coco/beneficiado e 12 balaios de 80 L sc-^{1}. A qualidade dos grãos do cafeeiro conilon obtidos em plantas irrigadas foi superior à de plantas de sequeiro. O consumo médio de água em plantas irrigadas foi de 7,9 m^3 e em sequeiro de 4,95 m^3. A relação entre o consumo de água/kg de café beneficiado foi de 8,8 m^3 e 30,3 m^3, em plantas irrigadas e de sequeiro. Não houve influência do tipo de recipiente e níveis de sombreamento na produtividade, rendimento e qualidade do cafeeiro conilon.

Termos para indexação: *Coffea canephora,* irrigação, produtividade, crescimento.

1 INTRODUCTION

The genus *Coffea* comprises at least 124 species, of which *Coffea arabica* L. and *C. canephora* Pierre ex A.Froehner are economically important (DAVIS et al., 2011). In the 2016 crop, world coffee production exceeded 155.0 million bags, of this total, about 35% is conilon coffee, produced in countries considered emerging, such as Brazil. Of the total coffee produced in the world, about 30% is in Brazil (INTERNATIONAL COFFEE ORGANIZATION, 2017). The state of Espírito Santo is the largest Brazilian coffee conilon producer, in 2016, production was 5.0 million bags, corresponding to 63% of Brazilian conilon coffee (CONAB, 2017).

The irrigation management strategy utilized must be efficient in conserving water without affecting the crop yield (BONOMO et al., 2013). Therefore, further studies are required to accurately estimate the water consumption of the coffee plant under different phenological phases, with the objective of improving the irrigation management (SILVA et al., 2008; SILVA et al., 2011). In fact, two reproductive stages of coffee can be harmed by droughts: flowering and fruiting (DAMATTA et al., 2007).

Even in traditional areas of coffee cultivation, irrigation is justified by the fact that they suffer most of the time the effect of prolonged droughts in the critical periods of water demand by

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coffee (VICENTE et al., 2015). The growth rate of orthotropic and plagiotropic branches of Conilon coffee, in the Atlantic Region of the State of Bahia, Brazil, was higher under irrigation compared to non-irrigated plants, with the result that irrigation has been used with and to increase production by eliminating the risk of water deficiency at critical stages of cultivation (COVRE et al, 2016). Irrigation also influences the growth and distribution of the root system of coffee, in plants irrigated by drip irrigation, a greater amount of roots occurs in the area comprised by the humid irrigation bulb (COVRE et al, 2015).

However, very few studies linking irrigation with coffee quality are presently available. Irrigation provides a steady supply of water that ensures the correct formation, granulation and filling of the grains, while preventing the emergence of pimples and poor-quality grains; it modifies the microclimate, as well. However, it also stimulates the rise in diseases like rust, and supports pests such as the coffee borer. These exert a negative influence on the final quality for raw coffee grain grading. The fruit size is also greatly affected by the water supplied to the plant, as the fruit becomes larger when the humidity is suitable, which in turn improves the final grain quality (REZENDE et al., 2010).However, apart from assessing production and quality, the crop yield must be determined; this refers to the quantity of coffee required to fill a 60 kg bag of coffee (LIMA; CUSTÓDIO; GOMES, 2008). In the end, beverage quality will determine the final commercial value of the coffee.

It is known that the production of healthy and vigorous seedlings is of paramount importance for the success of a coffee crop. Tatagiba et al. (2010) verified that young plants of Conilon coffee kept under 88% of shade recorded the highest values for total dry matter accumulation, followed by the level of 22 and 50%, while the seedlings maintained in full sun registered the lowest values, factor that can influence the production of a good coffee is the container. Several researches have been carried out with the objective of combining quality with cost reduction. Silva et al. (2010) found that the pressed block, bag and tube (120 mL) were the most suitable containers for the production of Conilon coffee seedlings, in which higher vegetative growth and more vigorous seedlings were obtained. However, studies that associate productive characteristics of Conilon coffee plantations with seedlings from different levels of shade or different containers are still scarce.

In light of the facts mentioned, the aim in this work was to assess and compare the yield, quality and water consumption of Conilon coffee subjected to irrigation and dryland management techniques, from seedlings raised in different containers and under various levels of shade.

2 MATERIAL AL AND METHODS

The experiment was performed at the IFES (Federal Institute of Education, Science and Technology of Espírito Santo), Alegre-ES Campus, Farm Caixa D'Água, Rive district, located at latitude 20º 25' 51.61" S and longitude of 41º 27' 24.51" W and altitude of 136,82 m, with 1,250 mm annual average precipitation and annual average temperature of 26 ° C. The plant species in the study was *C. canephora* , Tropical Robusta variety (EMCAPER 8151), through seed propagation.

Adopting the randomized complete block design, the experiment was carried out over 2 x 2 x 4 sub-divided plots. The plots, managed on two levels (irrigated and dry), in the subplots the container used in the formation of the seedlings also in other stages, (and bag); shading in sub-subplots was performed by seedling formation at four levels (0%, 30%, 50% and 75%), involving three replicates. Each experimental plot comprised three plants.

The seedlings raised in tubes (120 mL) and bags (770 mL) filled with standard substrate and subjected to different levels of shading, were planted on December 13, 2007. Plant spacing of 3.0 x 1.1 m was maintained and sowing done was in sandy-clay textured Yellow Red Latosol (LVA) (EMBRAPA, 2006). Correctives and chemical fertilizers were applied, depending on the chemical analysis of the soil, based on the parameters suggested in the Manual of Recommendation of Liming and Fertilization for Espirito Santo: 5th Approach (PREZOTTI et al., 2007). Suitable cultural and phytosanitary treatments were performed to meet the crop needs, incorporating the guidelines for Conilon coffee (FERRÃO et al., 2007).

The conventional sprinkler type of irrigation system was employed in the irrigated plot, in two lateral lines, each provided with two sectoral sprinklers, 18 m apart, the Christiansen's Uniformity Coefficient (CUC) was 80.6%, with average depth length of 13.68 mm. The direct irrigation method via the soil was used. Using an electric oven set at 180º to 200ºC temperature, the soil moisture was assessed from the samples collected at the projection of the crown having 40g minimum weight and including six replications, with the aid of a soil withdrawal probe, at different depths according to the age of the coffee tree. The irrigation depth (Li) needed to increase the soil moisture content (Ua) to field capacity (23.8%) was calculated by the following equation 1:

$$Li = [(CC-Ua) / 10] \times Ds \times Z \qquad \text{(Eq. 1)}$$

in which:
Li = Irrigation depth (IRN), in mm
CC = moisture in field capacity, % by weight
Ua = current soil moisture, wt%
Ds = soil density in g cm-3 (0-20 cm = 1.73; 20-40 cm = 1.63; and 0-35 cm = 1.68 g cm-3)
Z = effective depth of the root system, in cm (Z = 20 cm in the first year, Z = 25 cm from 1.0 to 2.0 years, Z = 30 cm from 2.0 to 2.5 years, Z = 35 cm from 2.5 years).

Precipitation was determined using a rain gauge fixed in the experimental region, of the Ville de Paris brand, with daily values being recorded at 9 o'clock. Effective precipitation was assessed using the difference between the total soil water capacity (CTA) and precipitation recorded in the irrigation interval (equation 2). Water consumption was calculated by taking into account the irrigation depths and effective precipitation per month, corresponding to the evaluation time periods given: from planting to the 1st harvest - (12/2007 to 04/2009 - from 0 to 17 months); 2nd harvest (05/2009 to 04 / 2010- from 18 to 28 months); 3rd harvest (05/2010 to 05 / 2011- from 29 to 40 months); 4th harvest (06/2011 to 05 / 2012- from 41 to 52 months).

$$CTA= [(CC-Pm) / 10] \times Ds \times Z \qquad \text{(Eq. 2)}$$

in which:
DTA = total soil water capacity, in mm
Pm = permanent wilting point, % by weight
Ds = soil density in g cm^{-3} (0-20 cm = 1.73; 20-40 cm = 1.63; and 0-35 cm = 1.68 g cm^{-3})
Z = effective depth of the root system, in cm (Z = 20 cm in the first year, Z = 25 cm from 1.0 to 2.0 years, Z = 30 cm from 2.0 to 2.5 years, Z = 35 cm from 2.5 years).

Adopting the criterion of at least 50% of ripe fruits, harvesting was done using the nonselective method, with manual sifting through sieves, followed by post-harvest processing utilizing the dry process, avoiding washing the fruits and then dried. The yield was evaluated by weighing the fruits harvested from each plant, to obtain the quantity of coffee produced by the field (CR pl^{-1}). From the total collection taken from the experimental plot, a 2-kg sample was drawn, and subjected to drying (coconut coffee - CC). Next, the coconut coffee sample was harvested and weighed. The values achieved in kg of coffee benefited per plant (CB pl^{-1}) were transformed, and adjusted and filled in bags of 60 kg ha-1 capacity. Post treatment, the moisture content of the beans on average was ± 12.0%, determined using GEHAGA G 600, version 7.3.

The yield was determined using the relationships between the kg weight of the CR per kg of CB; liters of CR per kg of CB; kg of coffee in CC per kg of CB (yield of the pile) and breakage (number of balloons of 80L per sc^{-1} of 60 kg of CB). From 300g of the sample, based the sieve dimensions, the classification was determined as numbers 10, 11 and 12 by coffee sieve size for the Mocha Grains, and numbers 13, 15 and 17 for the Flat Grains, based on the percentage of grains retained in the respective sieves, foundations and Mocha grains. Classification based on type was achieved by adding the defective numbers present in 100 g of the sample, following the Official Classification Table of Brazil, and through sampling, based on the standards set up by the Technical Regulations of Identity and Quality for the Classification of Raw Benefited Coffee (BRASIL, 2003).

The experimental data was submitted to statistical analysis, in which the means were compared by the F (ANOVA) and Tukey tests, at the 5% level of probability, through the SAEG 9.1 (2007) computer program.

3 RESULTS AND DISCUSSION

Figure 1 reveals that the total of the monthly precipitation during the agricultural years (2008 to 2012) was more than 1,250 mm, which is the annual average of the climatological norm from 1976 to 2011 (INCAPER, 2012). The rainy season extends from October to April, while the dry period lasts from May to September. The lowest monthly average rainfall during the rainy season was observed between January and February, the time when acute droughts might result in losses in the coffee yield, as it corresponds to the granulation stage of the coffee bean (CAMARGO, CAMARGO, 2001).

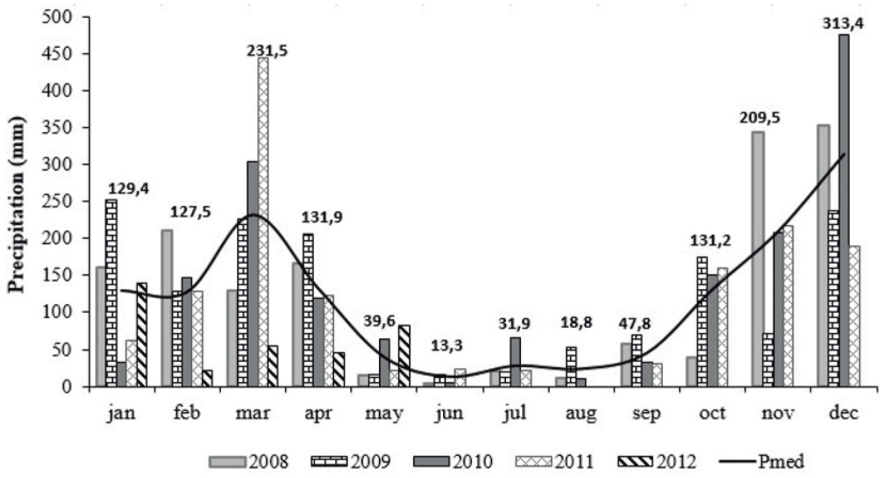


FIGURE 1 - Average monthly precipitation from 2008 to 2012; Alegre-ES.

According to Martins et al. (2015), water deficit and an air temperature are the meteorological elements that most influenced coffee productivity.

When the variations in the coffee production findings were submitted to the analysis of variance no interaction was noted among the factors and the study needed to be performed in isolation, as displayed in Table 1. However, during the harvest years (2010 and 2011), a noteworthy influence was seen for the management factor alone. Thus, it was concluded that neither the containers nor the levels of shading employed in seedling formation affected the benefited coffee production and, therefore, the harvest output during 2010/2011 / 2012 and yes the plant cultivation system. However, although the values recorded for the coefficients of variation of the variables in this work were notably high, between 33 and 47% magnitude, they fell within the acceptable range for experimentation with perennial crops. Ferrão et al. (2008) suggested that the higher coefficients of variation might be linked to sources like the long crop cycle, large size of the experiments, differentiated responses of the genotypes to high temperature and dry stress, and differentiated responses of the materials to the effects of pests and diseases, as well as winds and pruning.

Table 2 lists the productivity findings for the irrigated and rainfed Conilon coffee plants, assessed using the production of coffee benefited by the plant (Table 1). Only for the irrigated plants, the harvest was performed at 17 months, as the rainfed production was insignificant, analysis was not possible, and it was therefore disregarded for the evaluations of productivity, yield and quality. Karasawa et al. (2002), also reported that coffee plants lacking irrigation produced no grain during their first harvest.

The irrigated plants showed a higher yield than that of rainfed ones, less than 52 months in which no significant differences between the treatments were observed. For the irrigated plants, the yield obtained on average was 162% higher than that of the dryland plants, which translates to mean 23 bags more of the benefited coffee from 60 kg ha-¹. (Table 2). The results of several researchers indicated that irrigation promoted a profit of 20 to 30 bags ha^{-1} on average, notable among them being Gomes, Lima e Custódio (2007), Scalco et al. (2011) and Silva, Teodoro e Melo (2008). According to Leite Junior and Faria (2016) the lower the plant submission the water restriction, the greater the possibility of increases in coffee productivity. The national coffee industry typically experiences alternating high and low yields. This is largely due to the depletion of plant reserves during the high productivity season, causing the drop in the output during the following year. This phenomenon is clearly evident in the irrigated plants at 40 and 52 months. Faria and Siqueira (2005), as well as Silva, Teodoro and Melo (2008), similarly confirmed that irrigation did not minimize the biennial effect of productivity. They also showed that the irrigated *C. arabica* plants revealed lower productivity than the dryland variety, in the year just after a high harvest year. This ability of plants to recover after a period, often in abiotic stress conditions is due to their high resilience capacity, which is extremely important to ensure the acclimatization and sustainability of coffee production due to future scenarios of climate change (MARTINS et al., 2016; RODRIGUES et al., 2015).

TABLE 1 - Synthesis of analysis of variance and test of the means for the variable Conilon coffee production in kg of coffee benefited by plant, during the harvest years of 2010, 2011 and 2012.

Treatments	2010	2011	2012
Driving	486.07**	46.39**	3.7^{ns}
Irrigated	0.62 A	1.52 A	0.73 A
Dryland	0.07 B	0.19 B	0.84 A
Container	0.36^{ns}	2.23^{ns}	0.01^{ns}
Handling x Container	0.71^{ns}	0.11^{ns}	1.17^{ns}
Shading	0.86^{ns}	0.49^{ns}	0.59^{ns}
Shade x Handling	0.53^{ns}	1.70^{ns}	1.11^{ns}
Shade x Container	1.09^{ns}	0.41^{ns}	2.10^{ns}
Shade x Handling x Container	0.62^{ns}	0.38^{ns}	2.15^{ns}
CV (%)	39.45	33.85	47.08

** Significant at 1% probability by F test; ns - not significant by the F test; values followed by the same capital letter in the column do not differ from each other, at 5% probability, by Tukey test.

TABLE 2 - Conilon coffee productivity (sacks benefited from 60 kg ha^{-1}), from irrigated and rainfed plants, during four harvests (2009 to 2012).

Driving	Crops				
	2008/09 (17 months)	2009/10 (28 months)	2010/11 (40 months)	2011/12 (52 months)	Cumulative production
Irrigated	1.2	31.6 A	76.8 A	36.9 A	146.5
Dryland	0.0	3.6 B	9.6 B	42.7 A	55,9

Means followed by the same letter in the column do not differ from each other by the Tukey test, at the level of 5% probability.

Water is exclusively responsible for the higher yield obtained in the irrigated treatments, compared to that of the rainfed one. Thus, despite the annual rainfall of between 1,200 and 1,800 mm, which is within the optimal accepted range for the coffee plant, (Figure 1), typical summers in January / February were noted, particularly during the crop years of 2009/10 and 2010/11. This drought period tallied with the phenological stage characterized by the high water demand needed for the grain filling phase, which in turn resulted in the decline in yield of the rainfed plants cultivated in that period. This was verified because for the *C. arabica* and *C. canephora* plantations losses of productivity and lower grain quality were noted when the short dry seasons (*veranicos)* occurred during the critical phenological phases (SILVA et al., 2007; SILVA; TEODORO; MELO, 2008).

Productivity is as significant as grain yield, because poor yields will necessitate higher harvesting, drying and processing expenditures. When the crop yields at 28 and 40 months were analyzed, the irrigated plants showed values that exceeded those of the rainfed ones, and even at 52 months, although no statistical differences were observed between the yields (Table 2). For Conilon coffee, the weight ratio of the cherry fruits to the weight of coffee benefited is known to vary from 3.3 to 5.2: 1 and is contingent upon the genetic material; it increases at the time of harvesting the coffee plants, when they bear a greater percentage of green fruits (FERRÃO et al., 2007). For the irrigated plants, the average rates of coffee / coffee benefited and coffee / cocoa coffee ratio were recorded as being 4.5 and 1.9; and 5.6 balloons of 80 L sc-^{1} of 60 kg of coffee benefited. These results exhibited close similarity to those obtained in the *'Conilon Vitória'* variety (VITÓRIA INCAPER 8142), which recorded an average clone yield of 3.92 cherry / coffee beneficiated with a coconut/

benefited ratio of 1.8 (FERRÃO et al., 2007). For the rainfed plants, the mean indices noted were 8.2 for coffee / coffee ratio and 3.1 for coffee / coconut / coffee conversion; and 12 balloons of 80L sc-¹ of 60 kg of coffee benefited. According to Lima, Custódio e Gomes (2008), the non-irrigated *C. arabica* plants needed a more numbers of liters of coffee from the field to fill a 60 kg bag of coffee benefited (Table 3).

The dryland plants exhibited the lowest yield, which may be connected to the weight of 1000 grains, the average weight of which was 94 g over three harvests, while for the irrigated plants it was 108 g. Therefore, the insufficient rainfall distribution and paucity of water supplemented via irrigation might have contributed towards the pounding of the grains, as well as the greater percentage of poorly filled grains. This produces an intrinsic defect, leading to quality depreciation of the product and ultimately in low beneficiation yield.

Normally, for the irrigated seeds compared to the non-irrigated ones, a higher percentage of grains are retained in the sieves 13 and higher, which corresponds to higher coffee granulation in these treatments (Figure 2A). The irrigated plants showed values ranging from 65 to 93%, whereas in the rainfed ones, the range hovered from 40 to 88%. For the irrigated '*Conilon Vitória'* coffee variety Pereira (2015) reported 75.5% of the grains being retained in sieves 13 and higher and 40.5% for the non-irrigated plants. Rena and Maestri (2000) suggested that this occurred because the coffee bean size is determined between weeks 10 and 17, post flowering, when the fruit rapidly develops, with water being the factor solely responsible for the increase. Thus, the high percentages of the foundations recorded for the rainfed plants, referring to the grains that settle at the bottom after passing through the sieves, seem to contradict this affirmation (Figure 2B).

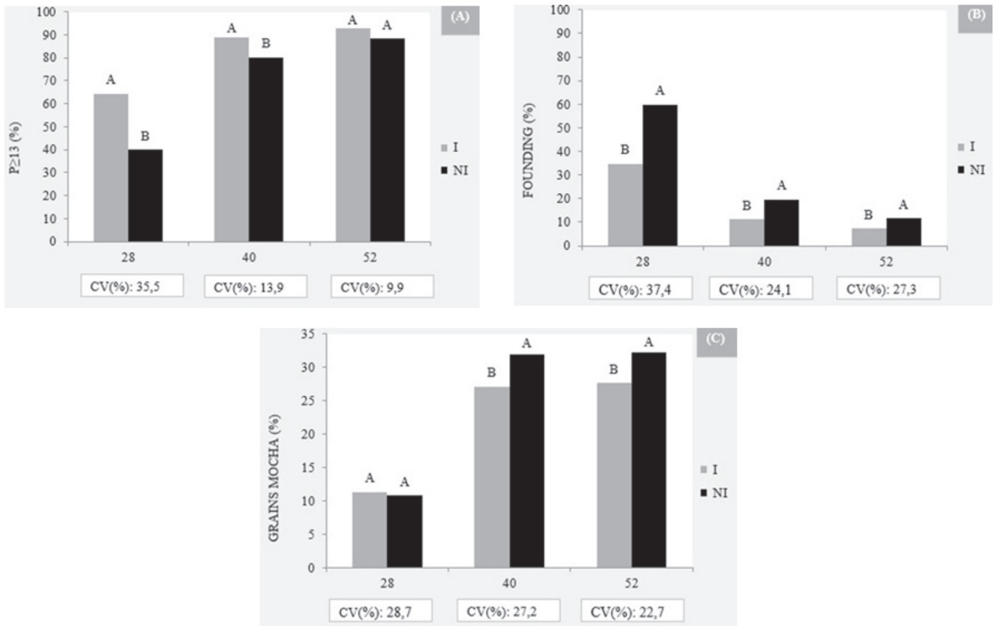
The Mocha type grain, promote lower yield when compared to those of the Flat type (flat-convex format). These grains are round in shape, having been formed from the development of a single seed, resulting from a gene abnormality (discoid endosperm) or environmental or physiological factors, including those of extended drought and nutrient deficiency (VACARELLI; MEDINA FILHO; FAZUOLI 2003). Figure 2C shows no statistical differences being recorded for the Mocha grains, between the treatments, at 28 months. However, at 40 and 52 months, the rainfed plants revealed values that surpassed those of the irrigated plants, as well as stayed higher than 21.4%, which was the mean value reported for the *'Conilon Vitória*' variety (FERRÃO et al., 2007).

Figures 3A and 3B indicate that at 28 and 40 months, the most number of defects were reported for the dryland management; they were classified as equivalence of types 7 and 6, based on the Brazilian Official Classification Table raw grain (BRASIL, 2003). The lesser equivalence in the number of defects for the irrigated management compared with the dryland coffee is due to the higher percentage of retention in sieves 13 and higher, which encouraged the beneficiation of the samples, producing lower quantities of broken grains, grains in shell *marinheiros* and bark, besides other aspects. At 52 months, no statistical differences were observed between the treatments, and the least numbers of defects were found and classified as type 5 and 4.

TABLE 3 - Yield, breakage and weight of 1,000 grains and percentage of low quality grains of Conilon coffee under irrigated and rainfed managements for three harvests (2010 to 2012).

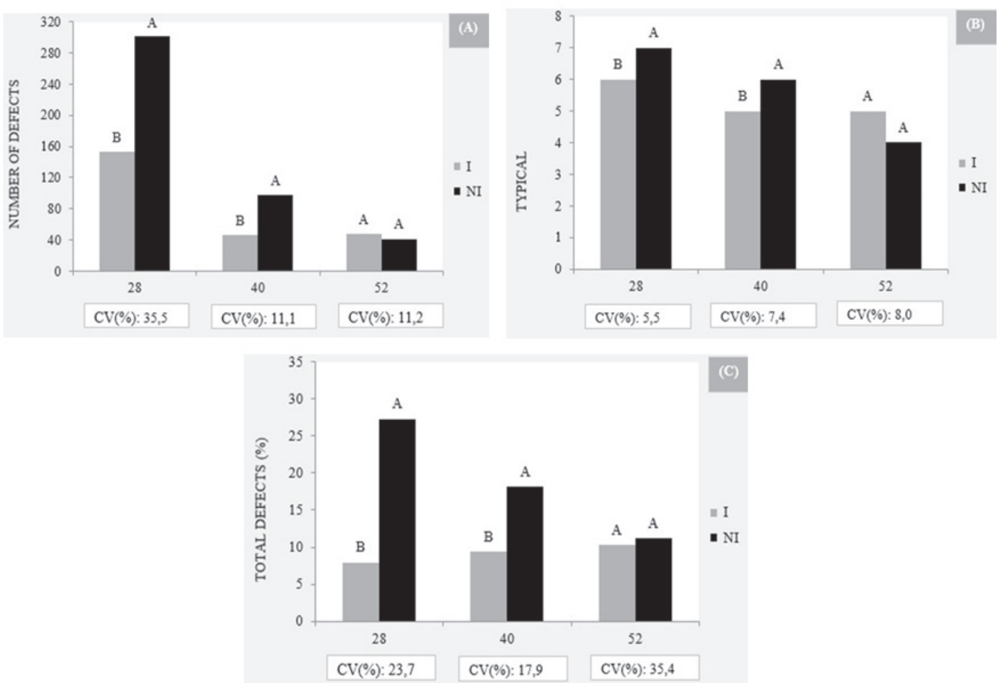
Indexes *	Relations	Harvests					
		28		40		52	
		I	NI	I	NI	I	NI
Yield	kgCR : kgCB	4,48	9,14	4,39	10,84	4,52	4,70
Stack yield	kgCC : kgCB	1,89	3,71	1,78	3,68	1,89	1,81
Breakage	NB 80L sc-¹	5,57	13,5	5,69	16,15	5,51	6,24
Weight of 1,000 grains	(g)	85,21	66,36	116,5	96,57	122,3	119,21
Percentage of low quality grains	(%)	1,5	9,7	3,0	9,0	1,54	5,02

* Yield: kgCR: kgCB- kg of coffee per kg of coffee benefited (CB); stack yield: kg Cc: kgCB - kg of coconut coffee per kg of CB; breakage: NB 80L sc^{-1} - number of balloons of 80L per sc of 60 kg of CB.



Means followed by the same letters do not differ from each other by the Tukey test, at the 5% probability level.

FIGURE 2 - Retention in sieve 13 and higher (P≥13), founding and percentage of coffee beans in Conilon under irrigated and rainfed management methods, during three harvests (2010 to 2012).



Means followed by the same letters do not differ from each other by the Tukey test, at the 5% probability level.

FIGURE 3- Classification by type, number of defects and total defects by weight for the Conilon coffee crop under irrigated and rainfed management methods, during three harvests (2010 to 2012).

Therefore, positive results for the number of defects may be linked (at least 50% of mature fruits), as well as to the care given during the fruit drying stages, which can be confirmed by the results total defects (Figure 3C). In this case the higher the percentage of total defects, the greater the problems in coffee quality. Thus, on average values of 9.2% and 18.9% were obtained respectively, for the irrigated and dry coffee plant harvests.

With respect to the irrigation depth (Li), the smallest leaf applied is consistent with the crop formation stage (17 months), largely because of the high incidence of precipitation. The next two productive periods (18 to 52 months), saw a decline in the values of the effective precipitation and the irrigation depth showed an increase as the crop developed (Figure 4). This concurs with the claims of Lena, Faria e Flumignan (2011), who stated that the water utilized by the coffee plants during the initial developmental phase was due to climatic variations, the greater exposure of the soil and small leaf area. However, the rise in the water consumption rates from the flowering stage to the commencement of grain filling resulted mostly from the large leaf area of the plants.

From Figures 5A and 5B, it is evident that the water consumed per plant and its relation with the production of coffee benefited, during the different growth phases and managements are higher for the irrigated plants than those for the rainfed plants, whose average values corresponded to 7.86 m³ and 4.95 m³, respectively. However, when this consumption was related to the production of coffee benefited (Figure 5B), the irrigated plants revealed an average value of 8.8 m3 of water kg-1 of coffee benefited, while the rainfed plants registered a value of 30.3 m3 of water kg-1 of coffee benefited. However, the low values seen for the rainfed plants at 52 months are noteworthy, and are due to their higher productivity in this cropping system. When the results of the research are adjusted according to the work of Bonomo et al. (2014), the water consumption on average of the Conilon coffee clones during the productive phase, while maintaining the soil water balance through irrigation, was 8.22 m³ which corresponded to 6.58 m³ of water per kg of coffee benefited. Thus, the water consumption observed for Conilon coffee is related to the phenological cycle, plant age, incident precipitation and production, besides irrigation management (Figure 5).

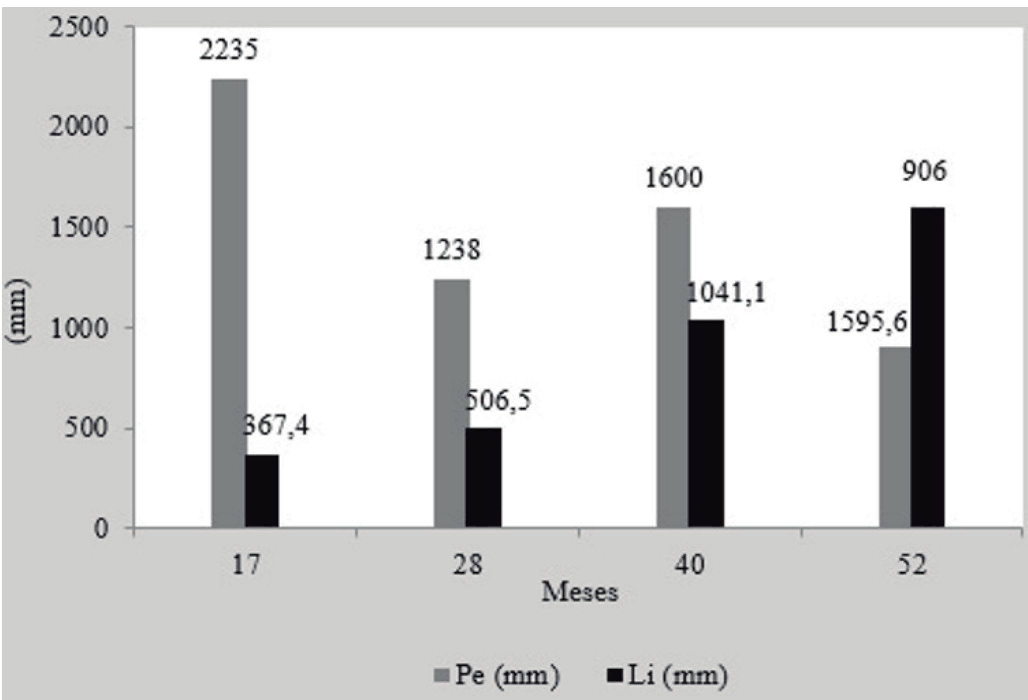
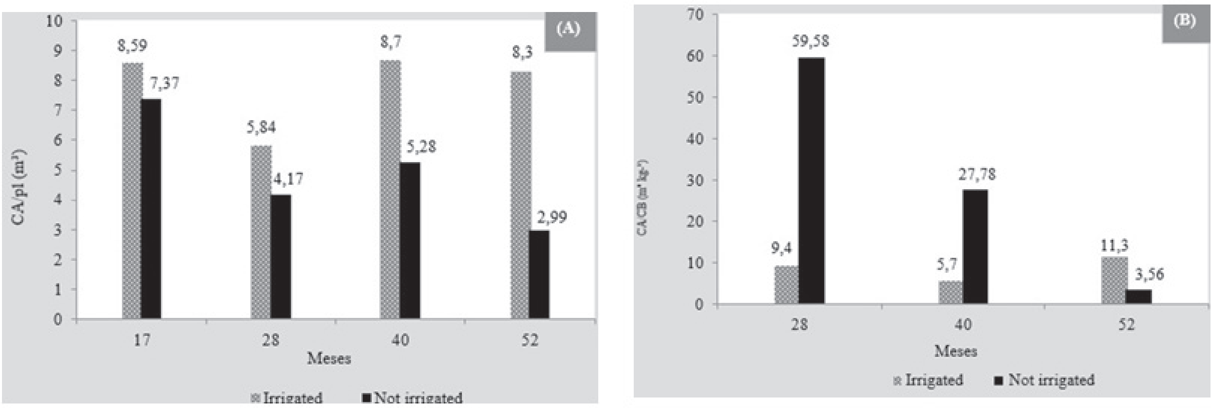


FIGURE 4 - Effective precipitation (Pe) and irrigation depth (Li) utilized for irrigation of the Conilon coffee plants during the different developmental phases.



* 17 months (Jan / 08 to April / 09); 28 months (May / 09 to April / 10); 40 months (May / 10 to April / 11); 52 months (May / 11 to April / 12).

FIGURE 5 - Water consumption per plant (CA / pl) and the relation between the water consumed and coffee benefited (CA / CB) of the irrigated and rainfed Conilon coffee plants at different developmental stages.

4 CONCLUSIONS

The irrigated Conilon coffee plants revealed higher productivity and yield, as well as better grain quality than those from the rainfed ones.

Irrigation management induced higher water consumption per plant and lower consumption per kilogram of coffee benefited.

The container and levels of shading employed in the raising the seedlings did not affect the Conilon coffee in terms of productivity, yield and quality.

5 REFERENCES

BONOMO, D. Z. et al. Alternativas de manejo de água de irrigação em cultivos de conilon. **Coffee Science**, Lavras, v. 9, n. 4, p. 537-545, Oct/Dec. 2014.

BONOMO, D. Z. et al. Desenvolvimento vegetativo do cafeeiro conilon submetido a diferentes lâminas de irrigação. **Revista Brasileira de Agricultura Irrigada**, Fortaleza, v. 7, n. 2, p. 157-169, Mar/Apr. 2013.

_______. Ministério da Agricultura, Pecuária e Abastecimento. **Instrução Normativa n. 8**, de 11 de junho de 2003. Regulamento técnico de identidade e de qualidade para a classificação do café beneficiado grão cru. Brasília, 2003.

CAMARGO, A. P. de; CAMARGO, M. B. P. de. Definição e esquematização das fases fenológicas do cafeeiro arábica nas condições tropicais do Brasil. **Bragantia**, Campinas, V. 60, n. 1, p. 65-68, Jan/Apr. 2001.

CONAB. Companhia Nacional de Abastecimento**. Acompanhamento da Safra Brasileira Café Safra 2016, segunda estimativa, maio/2017**. Available on: <http://www.conab.gov.br>. Access in: jul. 2017.

COVRE, A. M. et al. Vegetative growth of Conilon coffee plants under two water conditions in the Atlantic region of Bahia State, Brazil. **Acta Scientiarum**, Maringá, v. 38, n. 4, p. 535-545, Oct/Dec. 2016.

COVRE, A. M. et al. Distribuição do sistema radicular de cafeeiro conilon irrigado e não irrigado. **Pesquisa Agropecuária Brasileira**, Brasília, v. 50, n. 11, p. 1006-1016, Nov. 2015.

DAMATTA, F. M. et al. Ecophysiology of coffee growth and production. **Brazilian Journal of Plant Physiology**, Campos dos Goytacazes, v. 19, n. 4, p. 485-510, Oct/Nov. 2007.

DAVIS, A. P. et al. Growing coffee: *Psilanthus* (Rubiaceae) subsumed on the basis of molecular and morphological data implications for the size, morphology, distribution and evolutionary history of *Coffea*. **Botanical Journal of the Linnean Society**, Londres, v. 167, n. 4, p. 357-377, Dec. 2011.

EMBRAPA. Centro Nacional de Pesquisa de Solos. Sistema Brasileiro de Classificação de Solos. Brasília: Embrapa, p. 354, 2006.

FARIA, R. T. de; SIQUEIRA, R. Produtividade do cafeeiro e cultivos intercalares sob diferentes regimes hídricos. **Bragantia**, Campinas, v. 64, p. 583-590, Oct/Dec. 2005.

FERRÃO, R. G. et al. Parâmetros genéticos em café Conilon**. Pesquisa Agropecuária Brasileira**, Brasília, v. 43, n. 1, p. 61-69, Jan. 2008.

FERRÃO, R. G. et al. **Café conilon**. Vitória, ES: Incaper, 2007. 702p.

GOMES, N. M.; LIMA, L. A.; CUSTÓDIO, A. de P. Crescimento vegetativo e produtividade do cafeeiro irrigado no Sul do Estado de Minas Gerais. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v. 11, n. 6, p. 564-570, Nov/Dec. 2007.

INCAPER. Instituto Capixaba de Pesquisa e Extensão Rural. **Meteorologia e recursos hídricos: dados médios da série histórica da estação meteorológica localizada no município de Alegre**. Available on: <http://www.hidrometeorologia.incaper.es.gov.br>. Access in: 30 July. 2017.

INTERNATIONAL COFFEE ORGANIZATION. **Total production by all exporting countries.** Available on: <http://www.ico.org/prices/po-production.pdf>. Access in: 30 Nov. 2017.

KARASAWA, S.; FARIA, M. A. de; GUIMARÃES, R. J. Resposta do cafeeiro cv. Topázio MG-1190 submetido a diferentes épocas de irrigação. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v. 6, n. 1, p. 28-34, Jan/Apr. 2002.

LEITE JUNIOR, M. C. R.; FARIA, M. A. Utilização da irrigação no controle do potencial matricial de água no solo e sua influência na produtividade do cafeeiro. **Revista da Universidade Vale do Rio Verde**, Três Corações, v. 14, n. 1, p. 533-544, Jan/June. 2016.

LENA, B. P.; FLUMIGNAN, D. L.; FARIA, R. T. de. Evapotranspiração e coeficiente de cultivo de cafeeiros adultos. **Pesquisa Agropecuária Brasileira**, Brasília, v. 46, n. 8, p. 905-911, Aug. 2011.

LIMA, L. A.; CUSTÓDIO, A. A. de P.; GOMES, N. M. Produtividade e rendimento do cafeeiro nas cinco primeiras safras irrigado por pivô central em Lavras, MG. **Ciência Agrotecnologia**, Lavras, v. 32, n. 6, p. 1832-1842, Nov/Dec. 2008.

MARTINS, E. et al. Influência das condições climáticas na produtividade e qualidade do cafeeiro produzido na região do sul de minas gerais. **Coffee Science**, Lavras, v. 10, n. 4, p. 499-506, Oct/Dec. 2015.

MARTINS, M. Q. et al. Protective Response Mechanisms to Heat Stress in Interaction with High [CO_2] Conditions in Coffea spp. Frontiers in Plant Science, v. 7, p. 1-18, June. 2016.

PEREIRA, L. R. **Crescimento, produção e rendimento dos clones da variedade 'Conilon Vitória' em condições de déficit hídrico e irrigado**. 2015. 54 f. Dissertação (Mestrado em Produção Vegetal) Universidade Federal do Espírito Santo, Alegre-ES, 2015.

PREZOTTI, L. C. et al. (ed.). **Manual de recomendações de calagem e adubação para o Estado do Espírito Santo – 5ª Aproximação**. Vitória, ES. SEEA/INCAPER/CEDAGRO. 305p, 2007.

RENA, A. B.; MAESTRI. Relações hídricas no cafeeiro. **ITEM: Irrigação & Tecnologia Moderna**, Brasília, n. 48, p. 34-41, 2000.

REZENDE, F. C. et al. Cafeeiro recepado e irrigado em diferentes épocas: produtividade e qualidade. **Coffee Science,** Lavras, v. 5, n. 3, p. 229-237, Sept/Dec. 2010.

RODRIGUES, W. P. et al. Long-term elevated air [CO2] strengthens photosynthetic functioning and mitigates the impact of supra-optimal temperatures in tropical *Coffea arabica* and *C. canefora* species. **Global Change Biology**, Illinois, v. 22, n. 1, p. 415-431, Jan. 2015.

SAEG. **SAEG**: sistema para análises estatísticas, versão 9.1. Viçosa: UFV, 2007.

SCALCO, M. S. et al. Cultivo irrigado e não irrigado do cafeeiro (*Coffea arabica* L.) em plantio superadensado. **Coffee Science**, Lavras, v. 6, n. 3, p. 193-202, Sept/Dec. 2011.

SILVA, A. C. et al. Características produtivas do cafeeiro arábica irrigado por pivô central na região de lavras/MG. **Coffee Science**, Lavras, v. 6, n. 2, p. 128-136, May/Aug. 2011.

SILVA, J. I. et al. Desenvolvimento de mudas de *Coffea canephora* PIERRE ex A. FROEHNER em diferentes combinações de substrato e recipiente. **Coffee Science**, Lavras, v. 5, n. 1, p. 38-48, Jan/Apr. 2010.

SILVA, C. A. da; TEODORO, R. E. F.; MELO, B. de. Produtividade e rendimento do cafeeiro submetido a lâminas de irrigação. **Pesquisa Agropecuária Brasileira**, Brasília, v. 43, n. 3, p. 387-394, Sept/Dec. 2008.

TATAGIBA, S. D. et al. Mudas de C*offea canefora* cultivadas sombreadas e a pleno sol. **Engenharia na Agricultura**, Viçosa, v. 18, n. 3, p. 219-226, Mar. 2010.

VACARELLI, V. N.; MEDINA FILHO, H. P.; FAZUOLI, L. C. Avaliação de frutos chochos e de sementes do tipo moca no rendimento de híbridos arabustas tetraploides (*Coffea arabica x Coffea canephora)*. **Boiscience Journal**, Uberlândia, v. 19, n. 3, p. 155-165, Sept/Dec. 2003.

VICENTE, M. R. et al. Efeito de diferentes lâminas de irrigação nas variáveis de desenvolvimento e produção do cafeeiro irrigado por pivô central. **Irriga**, Botucatu, SP, v. 20, n. 3, p. 528-543, July/Sept. 2015.

SAPROBIC FUNGI AS BIOCONTROL AGENTS OF HALO BLIGHT (*Pseudomonas syringae pv. garcae*) IN COFFEE CLONES

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ABSTRACT: Halo blight caused by *Pseudomonas syringae* pv. *garcae* is a limiting disease in coffee production. There are few efficient commercial products on the market to control this disease, and therefore, the prospection of different biocontrol agents is a promising alternative. The objectives in this study were (i) to select saprobic fungi with the potential to control halo blight in coffee clones, and (ii) to evaluate the contributions of induced resistance as control mechanisms. Plants were sprayed with *Gonytrichum chlamydosporium*, *Phialomyces macrosporus,* and *Moorella speciosa* 7 d before inoculation with *Pseudomonas. syringae* pv. *garcae*. The area under the halo blight progress curve (AUDPC) and plant growth parameters were evaluated. *M. speciose* and *G. clamydosporium* did not reduce the AUDPC and even reduced plant growth in none of the trails compared to the water control. *P. macrosporus* consistently reduced AUDPC by 42 - 72% and increased plant height by 40%. Thereafter, the contributions of induced resistance was evaluated for the *P. macrosporus*, selected as the most promising biocontrol agent.. In order to determine induced resistance, phenylalanine ammonia lyase (PAL), peroxidase (POX), and ascorbate peroxidase (APX) activity of plant leaves were measured at two time points after stress challenge. Enzyme activity evaluation demonstrated high activity of POX and PAL at seven days after treatment with the saprobe, and high APX activity after 14 days. The results of this study indicate that *P. macrosporus* has the potential to be used in the management of coffee halo blight in seedling production, and one mechanism likely involved is induced resistance.

Index terms: foliar disease, Induced systemic resistance, *Coffea arabica,* saprobe fungus, biological control.

FUNGOS SAPRÓBIOS COMO AGENTES DE BIOCONTROLE DA MANCHA AUREOLADA DO CAFEEIRO

RESUMO: A mancha aureolada causada por *Pseudomonas syringae* pv. *garcae* é uma doença limitante na produção de café. Há poucos produtos comerciais eficientes no mercado para controle desta doença e, portanto, a prospecção de diferentes agentes de biocontrole é uma alternativa promissora. Os objetivos deste estudo foram: (i) selecionar fungos sapróbios com o potencial de controle da mancha aureolada em clones de café, e (ii) avaliar a contribuição da indução de resistência como mecanismo de ação. Plantas foram pulverizadas com *Gonytrichum chlamydosporium*, *Phialomyces macrosporus* e *Moorella speciosa* 7 dias antes da inoculação com *P. syringae* pv. *garcae*. A área abaixo da curva de progresso da mancha aureolada (AACPMA) e variáveis relativas ao crescimento foram avaliadas. *M. speciose* e *G. clamydosporium* não reduziram a AACPMA em nenhum dos ensaios quando comparado à testemunha. *P. macrosporus* reduziu a AACPMA em 42 - 72% e aumentou a altura de plantas em 40%. Em seguida foi avaliada a indução de resistência como possível mecanismo de ação exercido por *P. macrosporus*, selecionado como o agente de biocontrole da mancha aureolada mais promissor. Para determinar a indução de resistência, as atividades das enzimas fenilalanina ammonia liase (PAL), peroxidase (POX) e ascorbato peroxidase (APX) de folhas tratadas e inoculadas em amostragens aos 7 e 14 dias após o tratamento. A avaliação da atividade de enzimas demonstrou atividade de POX e PAL aos sete dias, e atividade de APX aos 14 dias após tratamento com o sapróbio. Os resultados deste estudo indicam que *P. macrosporus* tem potencial de ser usado no manejo da mancha aureolada do cafeeiro na produção de mudas, e o mecanismo provavelmente envolvido nesta proteção é a indução de resistência.

Termos para indexação: Doença foliar, Indução da resistência sistêmica, *Coffea arabica,* Fungos sapróbios.

1 INTRODUCTION

Brazilian coffee has an important contribution to global trade in commodities (AGRICULTURE, 2016). However, several factors could contribute to the reduction of grain yield. One of them is losses due to diseases such as coffee rust, cercospora leaf spot, anthracnose, and halo blight caused by *Pseudomonas syringae* pv. *garcae* (BELAN et al., 2016). Recently, severe epidemics of halo blight have been reported as a limiting factor in coffee cultivation in cooler and

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more windswept regions, in forming or newly pruned crops, and in nurseries (PEIXOTO et al., 1999; SERA et al., 2002, 2004; ZOCCOLI et al., 2011).

Regarding the control of halo blight, there are few control strategies with proven management effectiveness (RODRIGUES et al., 2012). While no resistant cultivar is still available, the plant immune system can be boosted up through induced resistance, namely pathogen-associated molecular pattern-triggered immunity (RODRÍGUEZ et al., 2016). This mechanism is employed for the management of plant bacterial diseases (LLORENS, 2015) and result in the disease control or at least the delay of disease onset (ATHAYDE SOBRINHO et al., 2005; NOJOSA et al., 2005). Induced resistance (IR) in plants can be obtained through plant spray of biotic (plant extracts, microorganisms or their metabolites) or abiotic (chemical substances) compounds (CAVALCANTI et al., 2005). Several studies have proved the effectiveness of both chemical and biological products (MARTINS et al, 2013). One of the most used resistance abiotic inducer is Acibenzolar-S-methyl (ASM) which was also the first resistance inducer approved for commercial use (LYON; NEWTON, 1997 ; ZHANG, 2016). Since then, many products have been developed with a similar mode of action, some of them based on the use of living microorganisms or their derivatives (RESENDE et al., 2006). This control strategy is a promising tool to enhance the defensive capacity of plants providing long-term systemic resistance to a broad spectrum of pests and can be used in the integrated management of plant diseases (WALLING, 2001).

Microorganisms used to induce resistance can mobilize various strategies, including the production of elicitor molecules and their own structural components (BASHAN et al., 2016; SPADARO; DROBY, 2016). In addition, the use of microorganisms in disease management can tackle the disease through a hallmark of mechanisms: induced resistance, antibiosis, parasitism and competition, which culminates in disease control and in greater vigor and plant yield (MOYA-ELIZONDO et al., 2016; WANG et al., 2015). Another important advantage is the use in both organic and conventional agriculture, ensuring the commercial success of products generated from these fungi when used alone or in combination (KÖHL, 2015; OKON LEVY, 2015; ROMANAZZI, 2016). In the past few years, saprobic fungi have received increasing attention, not only for improving and preserving the quality of the coffee beverage as the commercial product called Cladosporin® (active ingredient *Cladosporium cladosporioides (Fresen.) G.A. de Vries*), but also as a potential bioagent for controlling coffee diseases (ANGELICO et al., 2017). As a consequence, the adoption of saprobic fungi for disease management in coffee is highly increasing, especially, due to the efficient disease control under adverse conditions (KÖHL et al., 1995, 2011).

Saprobe fungi are able to overwinter in the crop stubble and therefore withstand sudden variations in temperature and humidity (KÖHL et al., 1995), environmental impounds to microbioal establishment commonly encountered in the phylloplane, also a niche for *P. syringae* pv. *garcae* Amaral, Teixeira & Pinheiro. One such fungi, *Phialomyces macrosporus* Misra & Talbot, has previously been reported as fast-growing fungus that displaces *Colletotrichum gloeosporioides* from coffee leaves (RODRIGUEZ et al., 2016) and a possible endophyte of coffee plants (AHMAD et al. 2003).

A collection of saprophytic fungi isolated from leaf litter of an arid region of Brazil has proven to be efficient to control plant disease. Some of these isolates is a potential agent of biocontrol in coffee (RODRÍGUEZ et al., 2016), white mold in soybean (Barros et al., 2015) and brown eye spot (Laborde et al., 2018). However, the saprobe fungi potential for bacterial blight control has not been attempted for many species. The objectives of this study were (i) to select saprobic fungi with potential to control halo blight in coffee clones, and (ii) to evaluate induced resistance as the possible control mechanisms of the most promising biocontrol agent.

2 MATERIALS AND METHODS

Seedling production

Coffee clone seeds (Mundo Novo IAC 376-4 and Red Catuaí cultivars) were obtained from Agricultural Research Company of Minas Gerais (EPAMIG) and sown in trays containing sand. After the cotyledonary leaves stage, seedlings were transplanted into 2 L-bags. Soil and sand were used for the substrate in a 3:1 ratio, fertilized using super phosphate and NPK 04-14-08 added to the mixture. The seedlings were kept in the greenhouse at 25–28 during the entire experiment.

Saprobic fungi isolates

Three saprobe fungal strains were used in this study: *P. macrosporus*, *Gonytrichum chlamydosporium G.L.* Barron & G.C Bhatt and *Moorella speciosa* Rao & Rao obtained from the Culture Collection of the Microorganisms from Bahia, Feira de Santana State University (Bahia, Brazil).

They were grown in a medium of cornmeal carrot agar (CCA) for 7 d at 27°C. One disc (7 mm) containing conidia and mycelium was suspended in 100 mL of CCA liquid medium, grown in an orbital shaker at 120 rpm and 27°C. The suspension was shredded, homogenized in a blender and adjusted to $3.4{\times}10^7$ CFU x mL^{-1}. Antagonistic fungi were inoculated by spraying the leaves until run off.

Pseudomonas syringae pv. garcae

The isolate of *P. syringae* pv. *garcae* was obtained from the Microorganism Collection of the Laboratory of Plant Bacteriology, Universidade Federal de Lavras (UFLA). For all experiments, the pathogen was cultivated in Kado 523 liquid medium (MB1) (KADO; HESKETT, 1970) for 48 hours in an orbital shaker at 24°C and 90 rpm. Bacterial concentration used, was approximately 0.5 ($OD_{600\ nm}$) corresponded to $3.4{\times}10^7$ CFU x mL^{-1}. Bacterial pathogenicity was ensured before the installation of each essay, through inoculation in coffee plants followed re-isolation of the pathogen.

Disease prevention control screening

Each saprophytic fungus was sprayed until runoff onto 7-month-old coffee seedlings 7 d before the pathogen's inoculation. The inoculation of *P. syringae* pv. *garcae* on leaves was done by spraying the entire leaf until run-off. Plastic bags were placed 2 d before and after the inoculation, to simulate a moist micro-chamber. The chemical compound Acibenzolar-S-methyl (0.05 g L^{-1}) was included as a positive control for induced resistance and distilled water as a negative control in the initial screening step and evaluation of induced resistance potential, since such product compound is a putative resistance inducer against plant disease in coffee (RODRIGUEZ et al., 2016).

Plants were assessed for disease severity and plant growth at 7, 14, 21, 28 and 35 d after *P. syringae* pv. *garcae* inoculation. The disease rating was carried out using the diagrammatic scale adapted from SIDHU AND WEBSTER scale (1977). In all experiments, plants were distributed in a randomized block design with three replicates and two seedlings per plot. The experiment was performed twice. *P. macrosporus* was the fungus with the highest potential for disease reduction and its mode of action was further studied.

Defense-related enzyme activity

The fifth pair of leaves of 7-month-old coffee seedlings was used in these assays (FERNANDES et al., 2014). Seven days before pathogen inoculation, suspensions of *P. macrosporus*, ASM and water were sprayed on the seedlings. The leaves were stored in aluminum foil, quickly frozen in liquid nitrogen (N_2) and then stored in an ultrafreezer at -80°C for subsequent analysis. The plants were distributed in a randomized-block design with three replicates and two seedlings per plot. Treatments were: i) Water + *P. syringae* pv. *garcae*; ii) ASM + *P. syringae* pv. *garcae*; iii) *P. macrosporus* + *P. syringae* pv. *garcae*. Leaves samples of each plant were collected at 7 and 14 d after treatments to determine the activities of defense enzymes.

Ascorbate peroxidase - APX

To obtain the extracts used for determining the activity of APX and guaiacol peroxidase (POX), 0.2-g leaf tissue samples were added to liquid N_2 and polyvinylpyrrolidone (PVP) (1% w/v) and ground with mortar and pestle to a fine powder. The powder was homogenized with 1300 µL of extraction buffer (400 mM potassium phosphate, pH 7.8; 10 mM EDTA; 200 mM ascorbic acid; water). The homogenate was centrifuged at 14,000 × *g* for 25 min at 4°C, and the supernatant was used for enzymatic determination. The supernatant (20 µL) from each sample was added to three wells of an ELISA plate (UV plate). Then, incubation buffer (200 mM potassium phosphate, pH 7.0; 10 mM ascorbic acid; 2 mM hydrogen peroxide and water) was added to the wells. Absorbance was measured at 290 nm in the ELISA plate for 3 min after the addition of the extract to the mixture, with readings taken every 15 s. The temperature at the reading was 25°C. A molar extinction coefficient of 1.4 mM^{-1} cm^{-1} was used to calculate APX activity, which was expressed in µm produced per min mg^{-1} of protein.

Guaiacol peroxidase - POX

A 40-µL aliquot of supernatant from each sample was added to three wells of the

ELISA plate. Then, reaction buffer (100 mM potassium phosphate, pH 7.0; 50 mM guaiacol and 125 mM hydrogen peroxide) was added to the wells. Absorbance was measured at 420 nm in the ELISA plate for 2 min (reading every 15 s) after addition of the extract to the mixture. The temperature at the reading was 30°C. The molar extinction coefficient of 2.47 mM $^{-1}$ cm $^{-1}$ was divided by two (1.235) and used to calculate POX activity (CHANCE; MAEHLEY, 1955), which was expressed in µm produced per min mg $^{-1}$ of protein.

Phenylalanine ammonia-lyase - PAL

Phenylalanine ammonia lyase (PAL) activity was measured according to MORI et al. (2001). For each gram of macerated sample, 3 mL of extraction buffer (50 mM sodium phosphate, pH 6.5; 1 mM PMSF; 1% PVP) was added. A reaction mixture was prepared by combining Tris-HCl, buffer, pH 8.8, 40 mM phenylalanine and 5 µl of enzyme extract. The reaction mixture was incubated for 20 min (reading every two minutes) at 37°C, and readings were taken at 280 nm. The values are expressed in µm produced per min mg $^{-1}$ of protein.

Total protein

Soluble protein concentration was measured using a standard bovine serum albumin (BSA) curve as used by BRADFORD (1976).

Experimental design and statistical ana lysis

In all experiments, we used a randomized complete block design, with three blocks and one pot per plot with two seedlings per plot. The data were submitted to an analysis of variance (ANOVA), and Tukey's test (P=0.05). At the end of the experiment (five weeks), the AUDPC was calculated using the formula proposed by SHANNER; FINNEY (1977):

$$\text{AUDPC} = \sum_{i=1}^{n-1} \frac{(Y_i + Y_{i+1})}{2} * (T_{i+1} - T_i)$$

where AUDPC = area under the disease progress curve, Yi = proportion of disease in the i-the observation, Ti = time in days in the i-th observation, and n = total number of observations.

3 RESULTS AND DISCUSSION

The saprophytic fungus *P. macrosporus* reduced the disease severity (*P*=0.0001) as compared to the control with the pathogen alone (FIGURE 1). In the first experiment, treatment with *P. macrosporus* reduced the AUDPC by 42% and even with the presence of the pathogen, increased the vegetative seedling growth in 40% (*P*=0.03) (FIGURE 2). However, *M. speciosa* and *G. chlamydosporium* favored the development of disease, increasing AUDPC, as compared to controls. In addition, these fungi also reduced the seedling height (*P* = 0.03) (FIGURE 1 and 2). ASM neither reduced the AUDPC nor increase the plant height. In the second experiment, *P. macrosporus* reduced the AUDPC in 72% as compared to the control *with P. syringae pv. garcae* (*P*=0.000).

In the biocontrol interactions, there are four most reported modes of actions: antibiosis, induced resistance, competition for nutrients or space and parasitism, all of which has been studied by Rodriguez et al. (2016). We selected *P. macroscopus* among 10 saprophytic fungi for its higher activity against *C. gloeosporioides* in coffee seedlings and determined induced resistance and competition for nutrients as possible modes of action. Although, competition is reported as a mode of action of bacterial diseases, fungi do not play such role, this is a plausible mode of action for bacterial antagonists (BENEDUZI et al., 2012) and to the best of our knowledge there is not report of fungal antagonists that displace a bacterial pathogen due to the faster-growing nature of the pathogen and the co-evolution with the plant host to fast invade the plant tissue.

The same authors mentioned above using the same saprobes obtained similar control of anthracnose (*C.gloeosporioides*) in coffee seedlings. The protection against both fungal and bacterial diseases, both with necrotrophic-based parasitism is an indication that induced resistance may play a role in the plant protection, since the induced resistance follows the jasmonate pathway (THALER et al., 2004) which is in turn triggered by biotic elicitors such as biocontrol fungi (LORITO et al., 2010).

The induced resistance involved many pathways that are either exclusively regulated by the jasmonate pathway or other secondary messengers. In regard to the phenylpropanoid pathway we have studied the activity of an upstream enzyme, the phenylalanine ammonia lyase (PAL). The effect of PAL activity was significant for both evaluation periods (*P*=0.003 and 0.016).

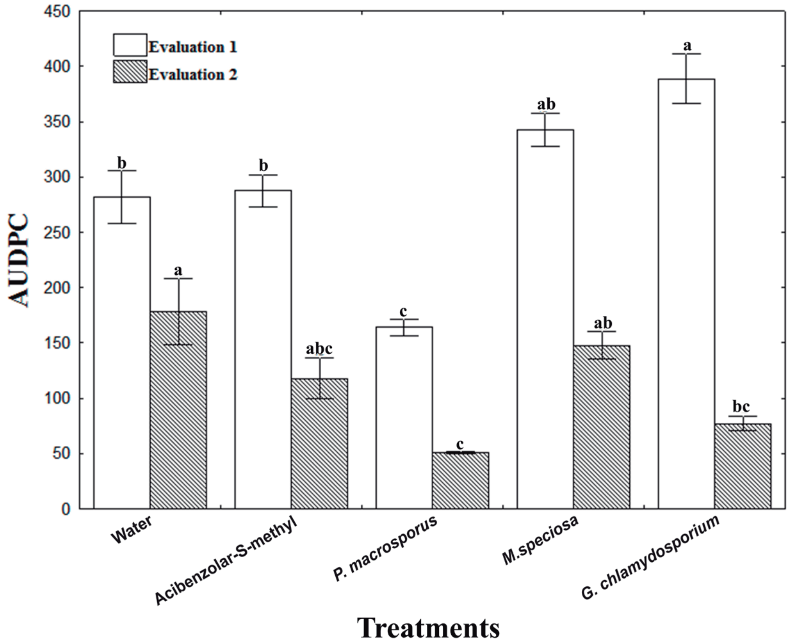

FIGURE 1 - Screening of saprophytic fungi against *P. syringae* pv. *garcae* on coffee seedlings. Seven-month-old coffee seedlings were sprayed with the antagonists and *P. syringae* pv. *garcae* was applied 7 d later and disease severity was assessed to calculate the area under the disease progress curve (AUDPC). The graph represents two equal experiments conducted in different periods. The bars with the same letter are similar at the 5% level according to the Tukey's test multiple range test. CV =11.40 % and 22.83 %, respectively. The line on each bar represents ±SE.

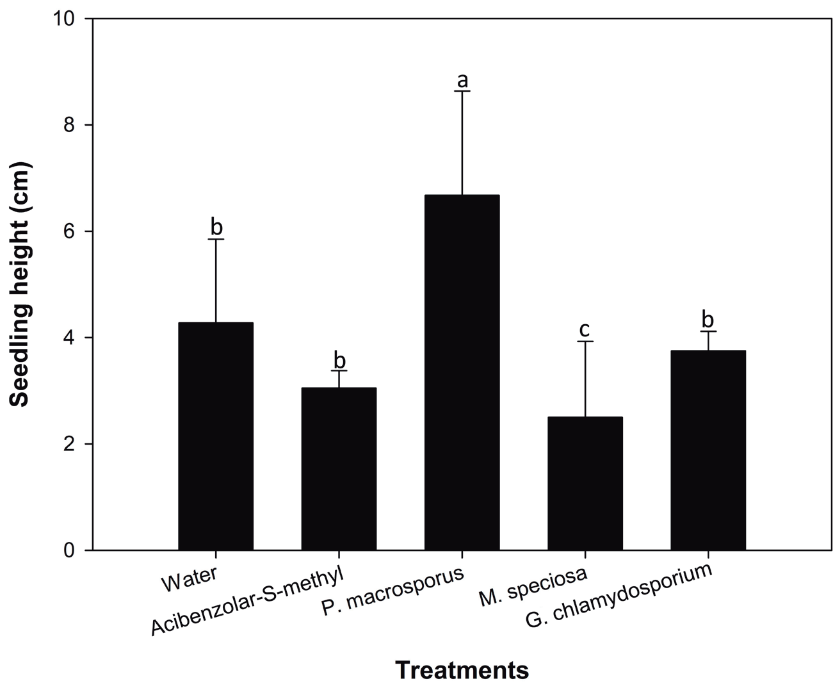

FIGURE 2 - Plant height of coffee seedlings after application of five treatments. The bars with the same letter are similar at the 5% level according to the Tukey' multiple range test. CV =32.87. The line on each bar represents ±SE.

At seven days after treatment, *P. macrosporus* increased PAL activity as compared to the water control, and this effect was similar to the chemical resistance inducer, ASM (FIGURE 3 A). While at 14 days, PAL activity declined in all treatments.

During the induced defense, the peroxidases play a role in modulating the production of reactive oxygen species (ROS). In this study, at seven days POX activity (FIGURE 3B) increased up to 20 mol mL^{-1} s^{-1} mg of protein⁻in plants treated with *P. macrosporus*, corresponding to 95%, and to 50% when compared to positive and negative controls, respectively (*P*=0.001). At 14 days, treatment with *P. macrosporus* resulted in the enzyme activity similar to water control and lower than ASM (*P*=0.021) (FIGURE 3 B). Regarding APX activity, at seven days, there was no difference among treatments (*P*=0.26). While at 14 days, *P. macrosporus* treatment resulted in higher activity than the other treatments (*P*=0.008), while ASM was similar to the water control (FIGURE 3 C).

In this study, at the time of bacterial inoculation, higher Phenylalanine ammonia-lyase (PAL) and Guaiacol peroxidase (POX) responses were observed. A later enhancement of the activity of APX was observed in plants treated with the antagonist. This finding is in agreement with the study of Rodríguez et al (2016), who showed that the application of *P. macrosporus* increased the permeability of the cuticle followed by increased activities of guaiacol and phenylalanine ammonia-lyase (PAL) reducing anthracnose severity by 32 to 41% . Thus, in our study treatments with *P. macrosporus* activated defense enzymes, balanced the content of O_2 − and H_2O_2 and induced the disease resistance. As *P. macrosporus* is reported as endophytic of coffee seeds, it suggests this characteristic may ensure longer-lasting plant protection (HARMAN 2000; AHMAD et al. 2003). Although, future studies are necessary to evaluate the necessity for the reapplication at 7-d intervals or a formulation to foster the antagonist survival and activity over the 7-d period.

One strategy that may be involved in the induction of plant defenses is the production of pectolytic enzymes. These enzymes can hydrolyze components of the middle lamella of the plant, releasing oligogalacturonides, which are recognized by plant and trigger defense response (L'HARIDON, 2011). In agreement with our hypothesis, DURAN-FLORES AND HEIL (2014) studied the response of common bean (*Phaseolus vulgaris* L.) to the application of leaf homogenate, as a source of DAMPs.

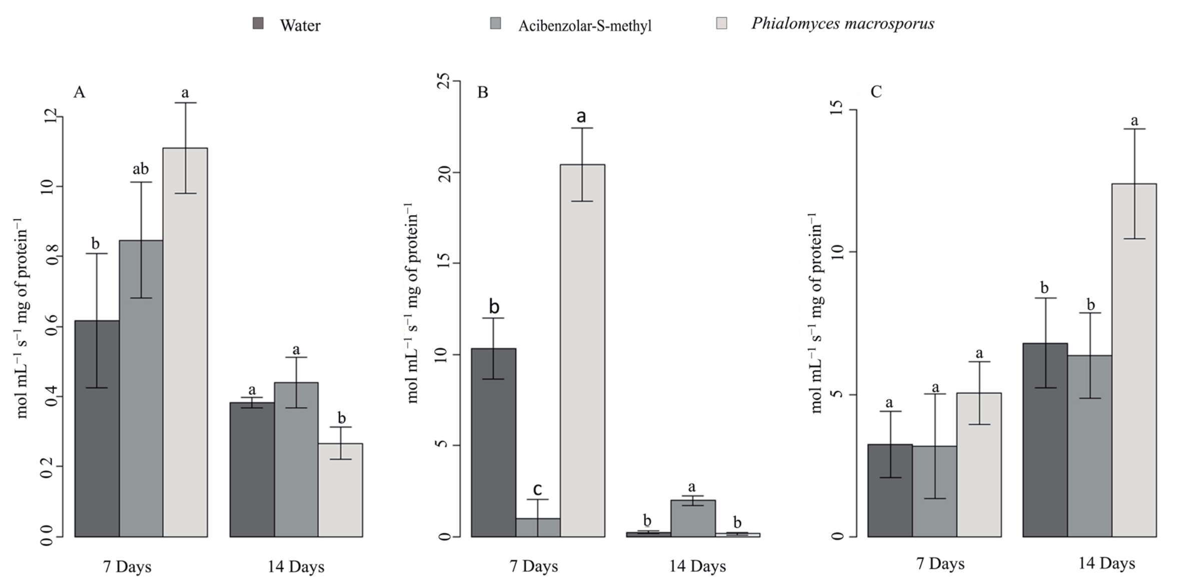

FIGURE 3 - Enzyme activities (mol mL^{-1} s^{-1} mg of $protein^{-1}$) after treatments applications. (A) Phenylalanine ammonia-lyase; (B) ascorbate peroxidase and (C) guaiacol peroxidase; Letters indicate differences between treatments detected by one way ANOVA in each sampling time (7 and 14 d). The bars with the same letter are similar at the 5% level according to the Tukey' multiple range test($P \leq 0.05$). CV = 21.73%, 13.98%; 10.91%, 71.99%, 36.83% and 19.75% for graphs A, B, and C, at 7 and 14 d, respectively.

The authors showed that the use of this oligogalacturonides resulted in ROS signaling and reduction of bacterial pathogen infection, *P. syringae* pv. *garcae*.

Foliar application of *P. macrosporus* to coffee seedlings 7 d before the inoculation of *P. syringae* pv. *garcae* increased activities of PAL, POX and APX, consistent with the induced resistance phenomenon. Similar studies showed that the resistance inducer requires time before pathogen application to trigger defense responses (MEDEIROS et al. 2011). Ahmad et al. (2014) working with biocontrol of early blight in tomato (*Alternaria alternata (*Fr.) Keissler), observed that when the antagonist *Penicillium oxalicum* was applied ten days before pathogen inoculation, tomato innate antifungal resistance boosted up and remarkably controlled disease incidence.

4 CONCLUSION

P. macrosporus has the potential to be used in the management of coffee halo blight in seedling production, and its mechanism of action is linked to the activation of plant defense responses.

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6 REFERENCES

AGRICULTURE USDA. **Production, Supply and Distribution.** Available on : <http://www.fas.usda.gov/commodities/coffee>**.** Access in 16 Dec. 2017

AHMAD, R. B.; THARAPPAN, D. R.; BONGIRWAR. Impact of gamma irradiation on -the monsooning of coffee beans. **Journal of Stored Products**, Helsingin, v. 39, n. 2, p. 149-157. July. 2003.

AHMAD, A.; SHAFIQUE, S.; AKRAM, W. *Penicillium oxalicum* directed systemic resistance in tomato against *Alternaria alternata.* **Acta Physiologiae Plantarum**, Kraków, v. 36, n. 5, p. 1231-1240, May. 2014.

ANGELICO, C. L.; CHALFOUN, S.M.; REZENDE, M.L.V. Hiperparasitism on mycotoxigenic fungus *Aspergillus ochraceus* G. Wilh. by *Cladosporium cladosporioides* (Fresen) de Vries. **International journal of environmental & agriculture research**, v. 3, n.11, p. 14, Nov. 2017.

ATHAYDE SOBRINHO, C. P. T.; FERREIRA, L. S., CAVALCANTI, L. S. Indutores abióticos. In: ________. **Indução de resistência em plantas a patógenos e insetos**. 1 ed. Piracicaba: Fundação de Estudos Agrários “Luiz de Queiroz”, 2005. p. 51-80.

BARROS, D. C. M. et al. Biocontrol of *Sclerotinia sclerotiorum* and white mold of soybean using saprobic fungi from semi-arid areas of Northeastern Brazil. **Summa Phytopathologica**, Botucatu, v. 41, n. 4, p. 251–255, Dec. 2015.

BASHAN, Y.; DE BASHAN, L. E.; PRABHU, S. R. Superior polymeric formulations and emerging innovative products of bacterial inoculants for sustainable agriculture and the environment. In: ________. **Agriculturally Important Microorganisms.** Singapore: Springer Singapore, 2016. p. 15-46.

BELAN, L. L. et al. Occurrence of’ *Pseudomonas syringae* pv. *garcae*’in coffee seeds. **Australian Journal of Crop Science**, Melbourne, v. 10, n. 7, p. 1015, July. 2016.

BENEDUZI, A. et al. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. **Genetics and molecular biology**, São Paulo, vol. 35, n. 4, Jan. 2012.

BRADFORD, M. M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, Valhalla, v. 72, n. 1-2, p. 248-254, May. 1976.

CAVALCANTI, L. S. et al. **Indução de resistência em plantas contra patógenos e insetos.** 1 ed. Piracicaba : Fealq, 2005. 263pp.

CHANCE, B. ; MAEHLEY, A. C. Assay of catalases and peroxidases. **Methods in Enzymology**, Baltimore: Christine Ziegler, 1955. 764-775p.

DURAN-FLORES, D.; HEIL, M. Damaged-self recognition in common bean (*Phaseolus vulgaris*) shows taxonomic specificity and triggers signaling via reactive oxygen species (ROS). **Frontiers in plant science**, Rockville Pike, v. 5, p. 585, Oct. 2014

FERNANDES, L. H. M. et al. Inductors of resistance and their role in photosynthesis and antioxidant system activity of coffee seedlings. **American Journal of Plant Sciences**, Rockville Pike, v. 5, n. 25, p. 3710-3716, Dec, 2014

HARMAN, G. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. **Plant Disease**, Saint Paul, v. 84, n. 4, p. 377-393, Apr. 2000.

KADO, C. I.; HESKETT, M. G. Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas* and *Xanthomonas*. **Phytopathology**, Saint Paul, v. 60, n. 6, p. 969-976. Jan. 1970

KÖHL, J. et al. Effect of interrupted leaf wetness periods on suppression of sporulation of *Botrytis allii* and *B. cinerea* by antagonists on dead onion leaves. **European Journal of Plant Pathology**, Wageningen, v. 101, n. 6, p. 627-637, Nov. 1995.

KÖHL, J. et al. Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. **Biological Control**, Wageningen, v. 57, n. 1, p. 1-12, Apr. 2011.

KÖHL, J.; SCHEER, C.; HOLB, I. J. Toward an integrated use of biological control by *Cladosporium cladosporioides* H39 in apple scab (*Venturia inaequalis*) management. **Plant Disease**, Saint Paul, v. 99, n. 4, p. 535-543, Apr, 2015.

LABORDE, M. C. F. **Avaliação de fungos sapróbios na sobrevivência de *Cercospora coffeicola*.** 2014. 48 p. Dissertação (Thesis in Agronomy/ Plant disease) - Universidade Federal de Lavras, Lavras, 2014.

L'HARIDON, F. et al. Permeable cuticle is associated with the release of reactive oxygen species and induction of innate immunity. **PLoS Pathogens**, San Francisco, v. 7, n. 7, p. 1–17, July. 2011.

LLORENS, E. et al. Induced resistance in sweet orange against *Xanthomonas citri* subsp. *citri* by hexanoic acid. **Crop Protection**, Amsterdam, v. 74, p. 77-84, April. 2015.

LORITO, M. et al. Translational research on *Trichoderma*: from 'omics to the field. **Annual review of phytopathology**, Palo Alto, v. 48, p. 395-417, May. 2010.

LYON, G. D.; NEWTON, A. C. Do resistance elicitors offer new opportunities in integrated disease control strategies?. **Plant Pathology**, Edgmond, v. 46, n. 5, p. 636-641, Oct. 1997.

MARTINS, S. J. et al. Biological control of bacterial wilt of common bean by plant growth-promoting rhizobacteria. **Biological Control**, Wageningen, v. 66, n. 1, p. 65-71, July. 2013.

MEDEIROS, F. H. V. et al. Transcriptional profiling in cotton associated with *Bacillus subtilis* (UFLA 285) induced biotic-stress tolerance. **Plant and Soil**, Wageningen, v. 347, n. 1-11, p. 327-337, June. 2011.

MORI, T.; SAKURAI, M.; SAKUTA, M. Effects of conditioned medium on activities on PAL, CHS, DAHP synthase (DS-Co and DS-Mn) and anthocyanin production in suspension cultures of *Fragaria ananassa*. **Plant Science**, Chicago, v. 160, n. 2, p. 355-360, Dec. 2001

MOYA-ELIZONDO, E. A.; JACOBSEN, B. J. Integrated management of Fusarium crown rot of wheat using fungicide seed treatment, cultivar resistance, and induction of systemic acquired resistance (SAR). **Biological Control**, Wageningen, v. 92, p. 153-163, Jan. 2016.

NOJOSA, G. B. A.; RESENDE, M. L. V.; RESENDE, A. V. Uso de fosfitos e silicatos na indução de resistência. In: CAVALCANTI, L. S. **Indução de resistência em plantas a patógenos e insetos**. Piracicaba: Fundação de Estudos Agrários "Luiz de Queiroz", 2005. p. 139-153.

OKON LEVY, N. et al. Induced resistance to foliar diseases by soil solarization and *Trichoderma harzianum*. **Plant Pathology**, Edgmond, v. 64, n. 2, p. 365-374, June. 2015.

PEIXOTO, P. H. P. et al. Aluminum effects on lipid peroxidation andon the activities of enzymes of oxidative metabolism in sorghum. **Revista Brasileira de Fisiologia Vegetal**, Campinas, v. 11, n. 3, p. 137-143, Aug. 1999.

RESENDE, M. L. V. et al. Produtos comerciais à base de bioindutores de resistência em plantas. **Revisão Anual de Patologia de Plantas**, Passo Fundo, v. 14, p. 361-380, Oct. 2006.

RODRIGUES, L. M. R. et al. Agressiveness of *Pseudomonas syringae* pv. *garcae* strains in *Coffea arabica* cvs. Mundo Novo and Borboun Amarelo. **Abstracts, International Conference on Coffee Science**, San José: ASIC, p. 199, 2012.

RODRÍGUEZ, G. A. A. et al. *Phialomyces macrosporus* decreases anthracnose severity on coffee seedlings by competition for nutrients and induced resistance. **Biological Control**, Wageningen, v. 103, p. 119-128, Dec. 2016.

ROMANAZZI, G. et al. Integrated management of postharvest gray mold on fruit crops. **Postharvest Biology and Technology**, Potsdam, v. 113, p. 69-76. Mar. 2016.

SERA, G. H. et al. Associação de *Pseudomonas syringae* pv. *garcae* com algumas características agronômicas em cafeeiros F2 segregantes para o gene erecta. **Ciência e Agrotecnologia,** Lavras, v. 28, n. 5, p. 974-977, Oct. 2004.

SERA, T.; ALTEIA, M. Z.; PETEK, M. R. Melhoramento do cafeeiro: variedades melhoradas no Instituto Agronômico do Paraná (IAPAR). In: ZAMBOLIM, L.**O estado da arte de tecnologias na produção de café.** Viçosa: UFV, 2002, p. 217-251.

SHANNER, G.; FINNEY, R. F. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in knox wheat. **Phytopathology**, Saint Paul, v. 67, n. 8, p. 1051-1056, Feb. 1977.

SIDHU, G. S.; WEBSTER, J. M. The use of amino acid fungal auxotrophs to study the predisposition phenomena in the root-knot: wilt fungus disease complex. **Physiological Plant Pathology**, London, v. 11, n. 11, p. 117-127, Sept, 1977.

SPADARO, D.; DROBY, S. Development of biocontrol products for postharvest diseases of fruit: the importance of elucidating the mechanisms of action of yeast antagonists. **Trends in Food Science & Technology**, London, v. 47, p. 39-49, Jan. 2016.

THALER, J. S.; OWEN, B.; HIGGINS, V. J. The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. **Plant physiology**, Bronx, v. 135, n. 1, p. 530-538, May. 2004.

WALLING, L. L. Induced resistance: from the basic to the applied. **Trends in plant science**, Hampshire, v. 6, n. 10, p. 445-447, Oct. 2001.

WANG, X. et al. Biocontrol and plant growthpromoting activity of rhizobacteria from Chinese fields with contaminated soils. **Microbial biotechnology**, Barcelona, v. 8, n. 3, p. 404-418, Sept. 2015.

ZHANG, Q. et al. *Streptomyces rochei* A-1 induces resistance and defense-related responses against *Botryosphaeria dothidea* in apple fruit during storage. **Postharvest Biology and Technology**, Leuven, v. 115, p. 30-37, May. 2016.

ZOCCOLI, D. M.; TAKATSU, A.; UESUGI, C. H. Ocorrência de mancha aureolada em cafeeiros na região do Triângulo Mineiro e Alto Paranaíba. **Bragantia**, Campinas, v. 70, n. 4, p. 843-849, Apr. 2011.

PHYSIOLOGICAL SELECTIVITY OF INSECTICIDES TO EGGS AND LARVAE OF PREDATOR *Chrysoperla externa* (HAGEN) (NEUROPTERA: CHRYSOPIDAE)

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ABSTRACT: Given the importance of green lacewings as agents of biological pest control, the present study aimed to evaluate the toxicity of insecticides used on coffee crops on the eggs and larvae of *Chrysoperla externa*. The insecticides tested were (g or mL a.i./L) chlorpyrifos (2.25), cartap hydrochloride (1.66), pyriproxyfen (0.33), profenofos/lufenuron (1.33/0.13), fenpropathrin (0.40), triazophos/deltamethrin (0.70/0.02) and zetacypermethrin (0.05). The insecticides, when applied directly on the eggs, caused no adverse effects on the duration of the embryonic period. After the application of triazophos/deltamethrin, pyriproxyfen, profenofos/lufenuron and zetacypermethrin, a reduction in egg viability was induced. The insecticides triazophos/deltamethrin, chlorpyrifos, and profenofos/lufenuron reduced the survival of newly hatched first instar larvae from treated eggs. The first instar larvae that were treated directly were sensitive to the effects of the products used, with the effect of triazophos and chlorpyrifos/deltamethrin being high. The survival of the second instar larvae was reduced by zetacypermethrin, fenpropathrin, profenofos/lufenuron, and cartap hydrochloride. The products chlorpyrifos and triazophos/deltamethrin also did not allow second instar larvae survival. For treated third instar larvae, chlorpyrifos and triazophos/deltamethrin allowed survival of only 20.0 and 57.5%. Eggs and larvae of *C. externa* showed sensitiveness to insecticides chlorpyrifos and triazophos, being needed more studies in semi-field and field conditions for the confirmation or not of the toxicity aiming the conservation of this predator specie on the coffee agroecosystem.

Index terms: *Coffea arabica*, natural enemy, plant protection, side effects.

SELETIVIDADE FISIOLÓGICA DE INSETICIDAS PARA OVOS E LARVAS DO PREDADOR C*hrysoperla externa* (HAGEN) (NEUROPTERA: CHRYSOPIDAE)

RESUMO: Diante da importância dos crisopídeos como agentes de controle biológico de pragas, o presente estudo teve como objetivo avaliar a toxicidade de inseticidas utilizados na cultura cafeeira sobre ovos e larvas de *Chrysoperla externa*. Os inseticidas testados foram (g ou mL i.a./L) clorpirifós (2,25), cloridrato de cartape (1,66), piriproxifem (0,33), profenofós/lufenurom (1,33/0,13), fenpropatrina (0,40), triazofós/deltametrina (0,70/0,02) e zetacipermetrina (0,05). Os produtos fitossanitários aplicados diretamente sobre ovos não causaram efeitos negativos sobre a duração do período embrionário. Após a aplicação de triazofós/deltametrina, piriproxifem, profenofós/lufenurom e zetacipermetrina ocorreu redução na viabilidade dos ovos. Os produtos triazofós/deltametrina, clorpirifós e profenofós/lufenurom provocaram diminuição na sobrevivência de larvas de primeiro instar recém-eclodidas, oriundas de ovos tratados. As larvas de primeiro instar diretamente tratadas foram sensíveis aos efeitos dos produtos utilizados, sendo que clorpirifós e triazofós/deltametrina foram mais tóxicos. A sobrevivência das larvas de segundo instar tratadas foi reduzida por zetacipermetrina, fenpropatrina, profenofós/lufenurom e cloridrato de cartape. Os produtos clorpirifós e triazofós/deltametrina também não permitiram que larvas de segundo instar tratadas sobrevivessem. Para larvas de 3° instar tratadas, clorpirifós e triazofós/deltametrina permitiram a sobrevivência de apenas 20,0 e 57,5%. Ovos e larvas de *C. externa* mostraram-se sensíveis aos inseticidas clorpirifós e triazofós/deltametrina, necessitando de novos estudos em condições de semicampo e campo para confirmação ou não da toxicidade, visando à conservação dessa espécie de predador no agroecossistema cafeeiro.

Termos para indexação: *Coffea arabica,* inimigo natural, proteção de plantas, efeitos colaterais.

1 INTRODUCTION

Although the average productivity of coffee plantations is around 48.64 bags of coffee per hectare in some regions, the average Brazilian productivity is around 28.41 bags per hectare (COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB, 2018). Several factors contribute to this drastic difference, among which the most prominent are pests and diseases that attack the crop and cause productivity losses, reduced grain quality, and depletion of plants (POZZA et al., 2010; SILVA et al., 2010).

The coffee agroecosystem hosts a great diversity of arthropod pests such as the coffee leaf miner *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae), coffee berry borer *Hypothenemus hampei* (Ferrari, 1867) (Coleoptera: Scolytidae), cicada *Quesada gigas* (Olivier, 1790) (Hemiptera: Cicadidae), red coffee mite *Oligonychus ilicis* (McGregor, 1919) (Acari: Tetranychidae), broad mite *Polyphagotarsonemus latus* (Banks, 1904) (Acari: Tarsonemidae), false spider mite *Brevipalpus phoenicis* (Geijskes, 1939) (Acari: Tenuipalpidae), among other species (MESQUITA et al., 2016; SILVA et al., 2010; SOUZA; REIS; SILVA, 2007).

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To control these insect pests, pesticides with high toxicity and broad-spectrum action have been frequently used, which is a major cause of biological imbalances in the agroecosystems. Furthermore, this has caused phenomena such as upwelling and selection of resistant insect-pests populations. In this context, preservation of the natural enemies of these insect pests is one of the most important practices in integrated pest management of coffee crop. The association between chemical and biological methods of control is possible only for pesticides that present selectivity to natural enemies, either physiological or ecological (BUENO et al., 2017; PARRA; REIS, 2013; RIGITANO; CARVALHO, 2001).

Among the various existing species of predator insects, those belonging to the family Chrysopidae play an important role as regulators of the populations of agricultural pests. The green lacewings are predators with high reproductive capacity and great voracity and may feed on the eggs and small caterpillars of moths, aphids, mealybugs, whiteflies, psyllids, among others (AUAD et al., 2007; BARBOSA et al., 2008; GONÇALVES-GERVÁSIO; SANTA-CECÍLIA, 2001; PITWAK; MENEZES JR; VENTURA, 2016). There are reports that *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) is effective for the control of *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae), *L. coffeella* and phytophagous mites on trees, and can be used to regulate the population of these pests (BEZERRA et al., 2006; ECOLE et al., 2002; SILVA et al., 2006).

Given the importance of green lacewings as agents of biological pest control, the present study aimed to evaluate the toxicity of insecticides used in coffee crop on the eggs and larvae of the predator *C. externa*.

2 MATERIAL AND METHODS

The experiments were performed in the laboratory at temperature of 25 ± 2 °C, RH 70±10%, and photophase of 12 h.

The products used are shown in Table 1. The control treatment consisted only of water. The experimental study was a completely randomized design with 8 treatments and 10 replicates, with each plot consisting of 4 eggs or larvae.

Effect of insecticides on eggs of *C. externa*

Eggs of about 24 h old and obtained from the maintenance building were placed in 15 cm diameter Petri dishes for treatment with the products. The compounds were sprayed directly on the eggs using a Potter tower with an application volume of 1.5 ± 0.5 mg/cm^2 and pressure of 15 lb/in^2. Then, the eggs were placed on individual glass tubes of 2.5 cm diameter x 8.5 cm height, and the tubes were sealed using laminated polyvinyl chloride (PVC) film. The surviving larvae that originated from the treated eggs were fed with eggs of *Anagasta kuehniella* (Zeller) (Lepidoptera: Pyralidae) every day ad libitum.

The parameters evaluated were egg viability (%), duration of embryonic period (days), survival (%) and duration of larval instars (days) and duration of pupae (days).

TABLE 1 - Trade name, active ingredient, chemical group, doses of insecticides and concentrations of the active ingredients of compounds registered for control of *Leucoptera coffeella* in coffee crop (Sistema de agrotóxicos fitossanitários - AGROFIT, 2009), tested in eggs and larvae of *Chrysoperla externa*, under laboratory conditions.

Trade name	Active ingredient	Chemical group	Doses [1]	Concentrations[2]
Astro	Chlorpyrifos	Organophosphate	1.5	2.25
Cartap BR 500	Cartap hydrochloride	Thiocarbamate	1.0	1.66
Cordial 100	Pyriproxyfen	Ether piridiloxipropílico	1.0	0.33
Curyom 550 CE	Profenofos/ Lufenuron	Organophosphate/ Benzoylureas	0.8	1.33/ 0.13
	Fenpropathrin	Pyrethroid	0.4	0.40
Danimen 300 CE	Triazophos/ Deltamethrin	Organophosphate/ Pyrethroid	0.6	0.70/ 0.02
Deltaphos CE	Zetacypermethrin	Pyrethroid	0.04	0.05

[1] Doses (L or kg c.p.ha^{-1}); [2]Concentrations (g or mL a.i.L^{-1} water).

Effect of insecticides on the larvae of *C. externa*

Approximately 24h after hatching or instar change, larvae of the first or second or third instars for each treatment were placed in 15 cm diameter Petri dishes. The products were sprayed directly on the larvae by using the same methodology applied earlier in the egg stage of the predator. Survivors of the treated larvae were fed ad libitum every other day with eggs of *A. kuehniella*.

We evaluated the duration (days) and survival of larval instars (through the presence of exuviae in Petri dishes) and pupae.

Statistical analysis

Data were submitted to Shapiro-Wilk and Bartlett tests in order to verify normality of distribution and homoscedasticity. On confirmation of these assumptions, data were submit ted to analysis of variance (ANOVA), and means were compared using the Scott-Knott test (SCOTT; KNOTT, 1974). In all tests, the level of statistical significance was set at 5%.

3 RESULTS AND DISCUSSION

Side effects of insecticides on eggs of the *C. externa*

The predator eggs treated using pyriproxyfen showed an embryonic period shorter than that for other insecticide treatments (Table 2). Despite the fact that this reduction was statistically significant, the difference while using pyriproxyfen when compared with the control was only 9.6 h, which does not prevent the use of the product in integrated pest management programs, because a short embryonic period can be advantageous. This is because green lacewing eggs are easy prey for other insects. For other compounds, there were no differences in the duration of the embryonic period, which was similar to the results shown by Carvalho et al. (2002) and Vilela et al. (2010a), who treated the eggs of *C. externa* using fenpropathrin at concentrations of 0.09, 0.15, and 0.3 g a.i./L of water, and Ferreira et al. (2005), who assessed the effect of the etofenprox (pyrethroid) and organophosphate chlorpyrifos, both at doses of 150 g c.p./100 L of water.

The viability of eggs was reduced with the usage of triazophos/deltamethrin, pyriproxyfen, profenofos/lufenuron and zetacypermethrin. Treatments using the other insecticides showed a high survival rate (Table 2).

Although the use of some insecticides reduced the egg viability, the morphology of the chorion of green lacewing eggs can hinder the penetration of the chemical molecules, thereby not affect neither the embryonic period nor embryos' survival.

This result is similar to that in the study performed by Grafton-Cardwell and Hoy (1985), which showed that the egg and pupal stages of green lacewings are more tolerant to pesticides. Further, the decrease in viability after the use of juvenoid ether piridiloxypropyl (pyriproxyfen) might be because the embryogenesis was affected, besides suppressing metamorphosis and prolonging the larval period (BUENO et al., 2017; FARIA, 2009).

The reduction in viability of *C. externa* eggs caused by triazophos/deltamethrin, pyriproxyfen, profenofos/lufenuron and zetacypermethrin can be related to the octanol-water partition coefficient (log K_{ow}) of these insecticides (3.34/6.20, 5.5, 4.82/5.12 and 6.6, respectively). High log K_{ow} values implies in higher lipophilicity, facilitating the penetration of a larger amount of the product through the chorion and its translocation to action sites (FERNANDES et al., 2010).

Studies by some authors corroborate the results presented in this work, such as those obtained by Carvalho et al. (2002), which showed a viability of 73.3% when they applied fenpropathrin (0.09 g a.i./L of water) on the eggs of *C. externa*. Vilela et al. (2010a) observed 85.0 and 70.0% viability, respectively, when they tested fenpropathrin at concentrations of 0.15 and 0.30 g a.i./L of water. Godoy et al. (2004) observed a viability of 76.6% for predator eggs treated using deltamethrin (0.0125 g a.i./L of water) and Rimoldi et al. (2008) verified an average of 96.7% viability using cypermethrin (0.025 g a.i./L of water). Preetha et al. (2009) found 81.5% viability of *Chrysoperla carnea* Stephens (Neuroptera: Chrysopidae) eggs treated using the organophosphorus monocrotophos (2 mL c.p./L of water); Gandhi et al. (2005) showed that the use of imidacloprid (350 g c.p./L of water) and endosulfan (350 g c.p./L of water) exhibited viabilities of 43.5 and 55.0 %. The toxic effects of fifteen insecticides, at their highest recommended concentrations for wheat crop, were evaluated by Pasini et al. (2018), who concluded that only etofenprox and imidacloprid + beta-cyflutrin reduced the viability of *C. externa* eggs in 40 and 70%.

TABLE 2 - Duration of the embryonic period (days) and viability (%) (±SE) of treated eggs of *Chrysoperla externa*. Temperature of 25 ± 2°C, RH 70 ± 10% and photophase of 12 hours.

Treatment	Embryonic period (days)	Viability (%)
Chlorpyrifos	5.3 ± 0.14 a	87.5 ± 3.95 a
Cartap hydrochloride	5.4 ± 0.16 a	90.0 ± 3.88 a
Pyriproxyfen	4.8 ± 0.19 b	80.0 ± 5.92 b
Profenofos/Lufenuron	5.3 ± 0.16 a	80.0 ± 5.92 b
Fenpropathrin	5.2 ± 0.12 a	95.0 ± 4.74 a
Triazophos/Deltamethrin	5.1 ± 0.17 a	70.0 ± 6.89 b
Zetacypermethrin	5.3 ± 0.15 a	82.5 ± 5.02 b
Control	5.2 ± 0.14 a	92.5 ± 3.62 a
CV (%)	3.64	9.95

Means followed by the same letter in the column are not statistically different according to the Scott-Knott test (P < 0.05).

Although the use of insecticides allowed a large number of eggs to hatch, only 22.5, 27.5, and 12.5% of larvae survived from the eggs treated using chlorpyrifos, profenofos/lufenuron and triazophos/deltamethrin, respectively (Table 3). The larval mortality was probably because of the presence of waste products in the chorion, which may have been the point-of-contact of the *C. externa* larvae at the time of hatching. Normally, organophosphates are highly toxic to insects, because they inhibit the action of the enzyme acetylcholinesterase through its structural conformation of molecules that allow them to occupy the docking site on the enzyme via the phosphate group. The hydrolysis of the phosphorylated enzyme occurs very slowly, resulting in the accumulation of molecules of acetylcholine in the synapse, causing the insect to die by hyperexcitation of the nervous system (OMOTO, 2000). Rugno, Zanardi and Yamamoto (2015) applied insecticides on *Ceraeochrysa cubana* (Hagen, 1861) (Neuroptera: Chrysopidae) eggs and found that newly hatched larvae exposed to chlorpyrifos-contaminated chorion presented less than 60% survival.

High mortality of first instar larvae of *C. externa* from the treated eggs was observed by Bueno and Freitas (2001, 2004) and Rimoldi et al. (2008), when they applied cypermethrin (0.025 g a.i./L of water), imidacloprid (3.5, 7.0, 10.5, 14.0, 17.5 and 21.0 g a.i./100 L water), and lufenuron (2.50, 3.75, 5.0, 6.25, 7.5 and 10.0 g a.i./100 L water) and found no surviving larvae. Godoy et al. (2004) assessed the toxicity of deltamethrin (0.0125 g a.i./L of water) for this predator and showed that only 38.3% of the newly hatched larvae survived.

Apart from the first instar larvae, the other larval instars were not affected by the products tested (Table 3), thus confirming the results obtained by Godoy et al. (2004) regarding the effects of the insecticides thiacloprid, deltamethrin, lufenuron, tebufenozide, fenbutatin oxide and abamectin (0.0360, 0.0125, 0.0375, 0.1200, 0.4000 and 0.0054 g a.i./L of water, respectively) on *C. externa* eggs that none of the compounds reduced the survival of the second and third instar larvae. Similar results were observed by Vilela et al. (2010a) for the products (g a.i./L of water) spirodiclofen (0.12), fenpropathrin (0.15 and 0.3), sulfur (4.0 and 8.0) and abamectin (0.0067 and 0.0225). The survival of the larvae may have been because the molecules of the products used were degraded by the insects through various metabolic processes, by which the products were converted into nontoxic forms or even quickly eliminated from the body of the insect. Further, various enzymes and enzyme systems may be involved, such as esterases, oxidases and transferases (HEMINGWAY, 2000).

The application of insecticides had no detrimental effect on the duration of the larval instars. Additionally, the compounds showed no negative effect on the pupal stage (Table 3).

Effect of products on the first instar larvae of the predator

The products chlorpyrifos and triazophos/deltamethrin were highly toxic to the treated first instar larvae. Moreover, the use of other products decreased the larval survival rates for profenofos/lufenuron, fenpropathrin, zetacypermethrin, cartap hydrochloride and pyriproxyfen, thus indicating that the first larval stage of green lacewings, in general, is more sensitive to chemical compounds (Figure 1).

TABLE 3 - Duration (days) and survival (%) (± SE) of three larval instars and pupal stage of *Chrysoperla externa*, from eggs treated with the insecticides (n=40). Temperature of 25 ± 2°C, RH 70 ± 10% and photophase of 12 hours.

Treatment	First instar		Second instar	
	Duration**	Survival*	Duration**	Survival**
Chlorpyrifos	4.3 ± 0.21	22.5 ± 7.39 b	3.5 ± 0.19	95.0 ± 4.74
Cartap hydrochloride	4.1 ± 0.15	70.0 ± 7.07 a	3.5 ± 0.17	100.0 ± 0.00
Pyriproxyfen	4.3 ± 0.19	62.5 ± 12.9 a	3.6 ± 0.16	97.5 ± 2.37
Profenofos/Lufenuron	4.5 ± 0.23	27.5 ± 8.20 b	3.5 ± 0.24	95.0 ± 4.74
Fenpropathrin	4.1 ± 0.15	67.5 ± 4.15 a	3.7 ± 0.16	100.0 ± 0.00
Triazophos/Deltamethrin	4.6 ± 0.17	12.5 ± 4.15 b	3.0 ± 0.00	100.0 ± 0.00
Zetacypermethrin	4.6 ± 0.18	62.5 ± 4.15 a	3.4 ± 0.18	100.0 ± 0.00
Control	4.4 ± 0.17	92.5 ±4.15 a	3.6± 0.20	100.0 ± 0.00
CV (%)	4.96	24.55	5.22	4.76

Treatment	Third instar		Pupae	
	Duration**	Survival**	Duration**	Survival**
Chlorpyrifos	4.1 ± 0.13	100.0 ± 0.00	10.1 ± 0.20	97.5 ± 2.37
Cartap hydrochloride	4.2 ± 0.24	100.0 ± 0.00	10.1± 0.11	92.5 ± 3.62
Pyriproxyfen	4.6 ± 0.27	100.0 ± 0.00	10.0 ± 0.12	95.0 ± 4.74
Profenofos/Lufenuron	4.9 ± 0.19	95.0 ± 4.74	10.1 ± 0.19	97.5 ± 2.37
Fenpropathrin	4.6 ± 0.21	100.0 ± 0.00	10.0 ± 0.13	100.0 ± 0.00
Triazophos/Deltamethrin	4.6 ± 0.21	100.0 ± 0.00	10.0 ± 0.27	100.0 ± 0.00
Zetacypermethrin	4.1 ± 0.17	100.0 ± 0.00	10.0 ± 0.17	97.5 ± 1.67
Control	4.2 ± 0.22	100.0 ± 0.00	10.2 ± 0.26	97.5 ± 2.37
CV (%)	6.14	3.25	2.55	4.40

*Means followed by the same letter in the column are not statistically different according to the Scott-Knott test ($P < 0.05$).

** Not statistically difference ($P > 0.05$).

In this case, the product chlorpyrifos acted by contact and prevented the degradation of acetylcholine by acetylcholinesterase inhibition, causing neurological disorders. For pyrethroids, such as deltamethrin, fenpropathrin and zetacypermethrin, it may have been that the insecticide molecules were positioned in some units of the binding site of the sodium ion channels, which thereby stayed open for long periods, increasing the influx after an action potential and triggering death by nervous hyperexcitation (OMOTO, 2000; RIGITANO; CARVALHO, 2001).

According to Fernandes et al. (2010), penetration rate of insecticides through the integument of the insect depends on its affinity with insecticides, physiological factors and chemical composition of products. The lipophilicity of insecticides is inversely proportional to their solubility in water, therefore more lipophilic compounds may have different penetration rates. In the case of first-instar larvae, another factor that may have influenced the penetration of chemical compounds is the thickness of the cuticle (CHAPMAN, 2013). The newly hatched larvae (about 24 hours) evaluated in this study were very small and their cuticle have low thickness.

Several studies have shown the toxic effects of compounds used directly on first instar larvae. Maroufpoor et al. (2010) evaluated the toxicity of spinosad on *C. carnea* first instar larvae and observed a larval mortality rate of about 70.0% at the highest tested concentration (2500 ppm).

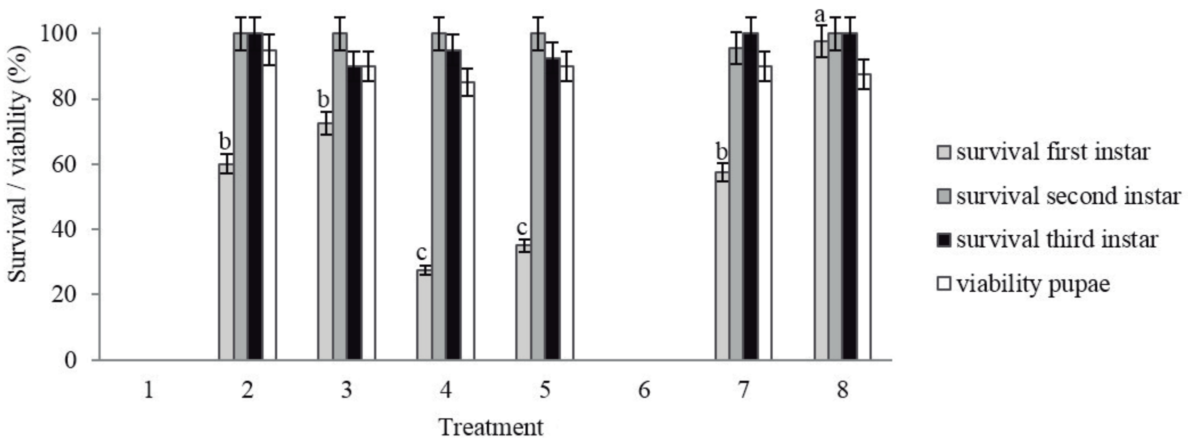

FIGURE 1 - Survival of three larval instars and viability pupal (%) (± SE) stage of *Chrysoperla externa*, from first instar larvae treated with the insecticides. Treatments: 1: chlorpyrifos; 2: cartap hydrochloride; 3: pyriproxyfen, 4: profenofos/lufenuron; 5: fenpropathrin; 6: deltamethrin/triazophos; 7: zetacypermethrin and 8: control.

The juvenile hormone analogue (pyriproxyfen) were highly harmful to *C. cubana* first instar larvae, causing 100% mortality before they reach the pupal stage (ONO et al., 2017). Silva et al. (2005) observed 100% mortality of first instar larvae of *C. externa* when using chlorpyrifos (1.2 g a.i./L of water) and beta-cyfluthrin (150 g a.i./100 L of water). Additionally, Ferreira et al. (2006) showed 100% mortality in predators treated using chlorpyrifos (0.72 g a.i./L of water). Vilela et al. (2010b) verified that treatments using fenpropathrin (0.15 and 0.3 g a.i./L of water) did not allow the survival of any *C. externa* larvae, and similar results were obtained by Moura et al. (2012) when *C. externa* larvae were treated (g a.i./L) using trichlorfon (1.5), carbaryl (1.73), fenitrothion (0.75) and methidathion (4.0).

The first instar larval duration was not reduced by treatment using all the products, and no differences were observed between treatments for the survivors of the second and third instar larvae (Figure 2). The averages were similar to those obtained for larvae of *C. externa* that fed on different prey, which were not contaminated by chemicals (AUAD et al., 2007; COSTA et al., 2002).

Both duration and survival of pupae from the treated first instar larvae were not adversely affected by the usage of the different insecticides (Figure 2).

Effect of the insecticides on the second instar predator larvae

Similarly as occurred to first instar larvae, chlorpyrifos and triazophos/deltamethrin caused 100% mortality of treated second instar larvae (Figure 3), confirming the results obtained by Silva et al. (2005) when using chlorpyrifos (1.2 g a.i./L of water) on the second instar larvae of *C. externa*. The second instar larval survival was low when they were treated using zetacypermethrin, fenpropathrin, profenofos/lufenuron and cartap hydrochloride (Figure 3), thus showing that in addition to sensitivity toward organophosphates and pyrethroids, the second instar larvae of *C. externa* also showed sensitivity to another group of chemical neurotoxic insecticides, thiocarbamates, which are acetylcholine antagonists and prevent the transmission of nerve impulses in the synapse, causing rapid paralysis of the insect and death (OMOTO, 2000).

The higher lipophilicity presented by chlorpyrifos and triazophos/deltamethrin probably favored their the penetration through the cuticle of the larvae, favoring their translocation to the site of action of the insecticides (FERNANDES et al., 2010).

Carvalho et al. (2003) observed the median survival of the second instar larvae of *C. externa* at around 43.3% and 10.0%, respectively, 96 h after the application of fenpropathrin (0.125 g a.i./100 mL of water) and trichlorfon (0.020 g a.i./100 mL of water). In their study, treatment by using chlorpyrifos ensured that no larvae survived.

Although pyriproxyfen acts as an agonist of juvenile hormone, and its action is more pronounced in the last instar of insect development, it had no effect on the treated larvae (FARIA, 2009; FERREIRA, 1999). Velloso et al. (1999) showed that pyriproxyfen (0.1 g a.i./L of water) was toxic to *C. externa*, thereby ensuring that none of the treated second instar larvae survived.

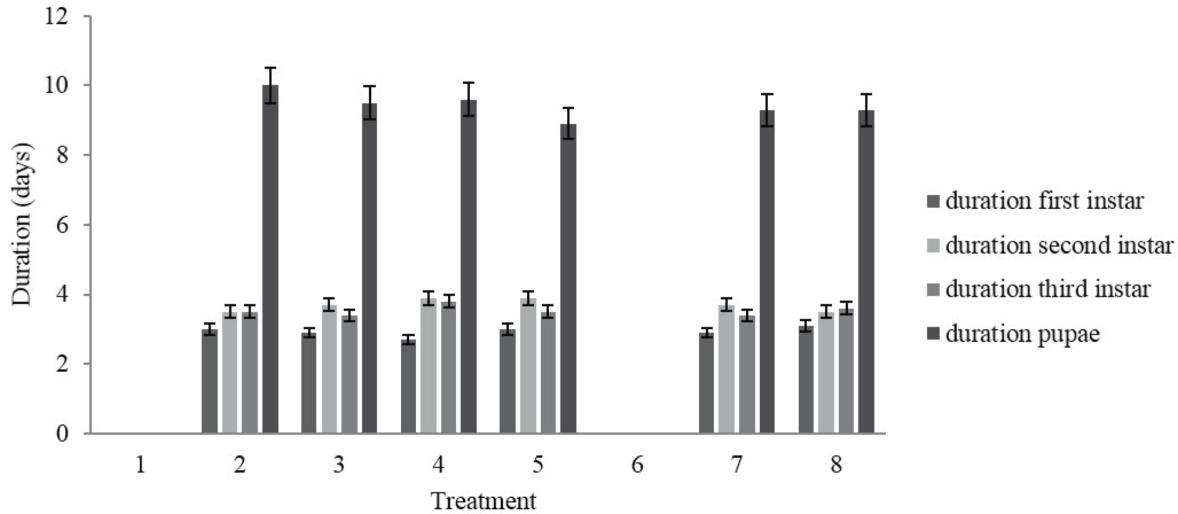

FIGURE 2 - Duration (days) of three larval instars and pupal stage of *Chrysoperla externa*, from first instar larvae treated with the pesticides. Treatments: 1: chlorpyrifos; 2: cartaphydrochloride; 3: pyriproxyfen, 4: profenofos/lufenuron; 5: fenpropathrin; 6: deltamethrin/triazophos; 7: zetacypermethrin and 8: control.

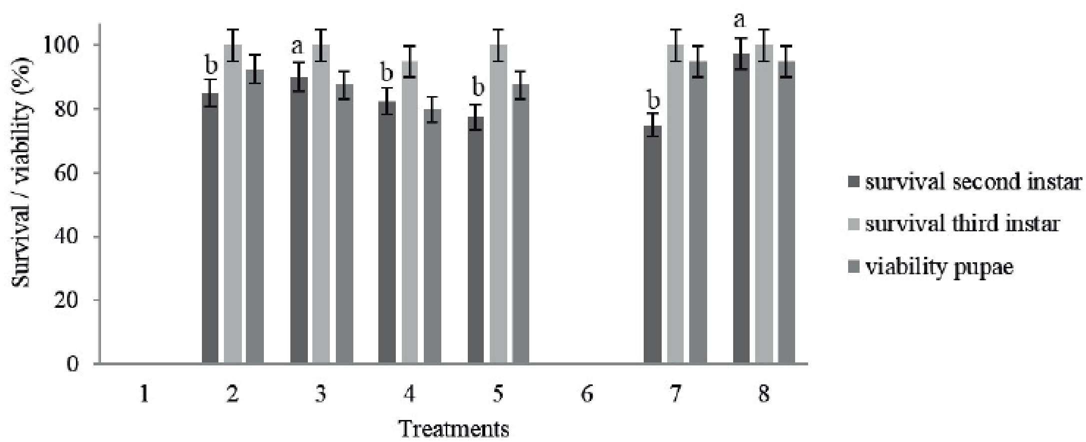

FIGURE 3 - Survival (%) (± SE) of immature stages of *Chrysoperla externa* from second instar larvae treated with the pesticides. Treatments: 1: chlorpyrifos; 2: cartap hydrochloride; 3: pyriproxyfen, 4: profenofos/lufenuron; 5: fenpropathrin; 6: triazophos/deltamethrin; 7: zetacypermethrin and 8: control.

This discrepancy can be explained by the methodology applied, since these authors sprayed compounds on the larvae that were maintained in treated Petri dishes; hence, increasing the exposure of the insects to the insecticide residues in addition to the dosage used, which was higher than that used in the present study.

Amarasekare, Shearer and Mills (2016) applied insecticides (mg a.i. L^{-1}) directly to *C. carnea* second instar larvae and found that cyantraniliprole (160.2), chlorantraniliprole (117.9), spinetoram (131.1), novaluron (388.5) and lambda -cyhalothrin (49.9) were lethal to the predator. Castilhos et al. (2017) observed, under semi-field conditions, that insecticides (% a.i.) lufenuron (0.005) proved to be harmless (class 1); deltamethrin (0.001) and malathion (0.200) were slightly harmful (class 2), and fenthion (0.05) and phosmet (0.100) moderately harmful (class 3) to second instar larvae of *C. externa*.

The surviving second instar larvae showed no significant differences in the duration of further larval instar stages. Further, the duration and survival of pupae from the treated second instar larvae were not affected by the insecticides used (Figure 4).

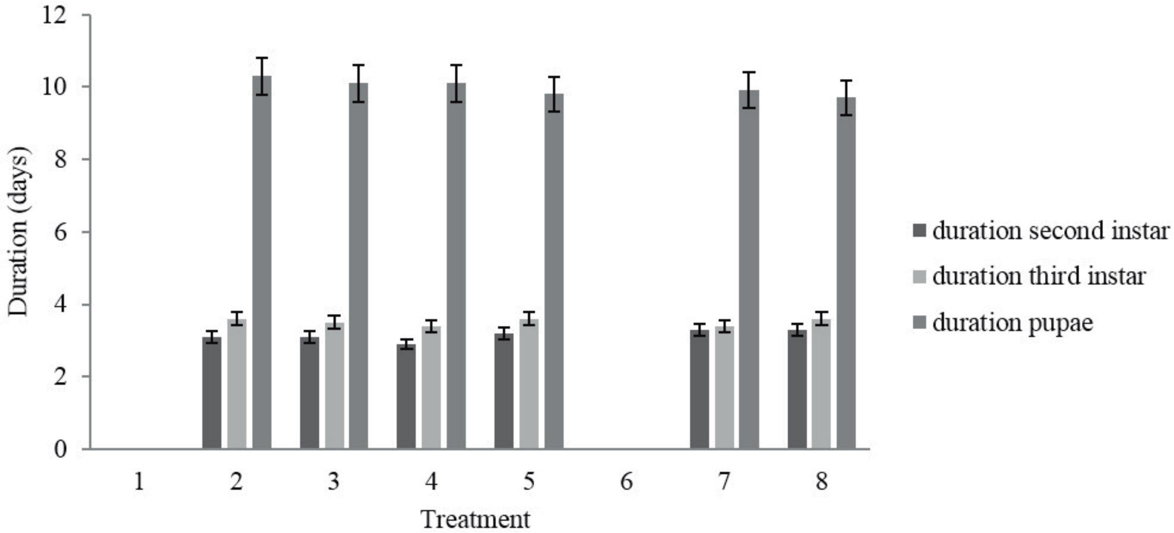

FIGURE 4 - Duration (days) of immature stages of *Chrysoperla externa* from second instar larvae treated with the insecticides. Treatments: 1: chlorpyrifos; 2: cartap hydrochloride; 3: pyriproxyfen, 4: profenofos/lufenuron; 5: fenpropathrin; 6: triazophos/deltamethrin; 7: zetacypermethrin and 8: control.

Effect of the insecticides on the third instar predator larvae

The treated third instar larvae were more tolerant to the tested insecticides, due to the survival of the insects was reduced only by treatments using triazophos and chlorpyrifos products/deltamethrin (Table 4). The same product used at the same concentration can cause different toxicity, depending on the development stage of exposed natural enemy (SOUZA et al., 2014; BUENO et al., 2017). Another factor that must be considered is that insects in advanced development stages can better support the effect of some groups of insecticides. It occurs due to increase of reserves and degradation capacity of insecticides by several metabolic processes, in which insecticide molecules are converted into non-toxic forms or even quickly eliminated from insects' body (CROFT, 1990; HEMINGWAY, 2000).

Nonetheless, Moura et al. (2011) showed 100% mortality of the third instar predator larvae to the organophosphates (g a.i./L of water), fenitrothion (0.75) and methidathion (0.4). Several factors may be associated with the discrepancies in the results such as the differences in the dosages of the insecticides used and origin of the populations of the treated green lacewings.

Regarding insect growth regulators, Bortolotti, Sbrenna and Sbrenna (2005) found that the same dose of the insecticide fenoxycarb, applied in third instar larvae of *C. carnea*, at different intervals after ecdysis (24, 48, 60 and 72 hours) had different effects. The effect on larvae survival was more severe for those treated after 60 hours of ecdysis, indicating the beginning of metamorphosis, where insects have less capacity to metabolize the insecticidal molecules. This may explain why *C. externa* larvae treated with pyriproxyfen and profenofos/lufenurom were not affected by insecticides in the present study, since they were treated with products via spraying 24 hours after ecdysis.

In general, an increased tolerance of the predator to several chemical compounds was observed during the third instar larval stage (CARVALHO et al., 2003; FERREIRA et al., 2006; GODOY et al., 2004; SILVA et al., 2005).

The duration of the third instar was not affected by treatments using any of the insecticides. Similar results were observed by Silva et al. (2005), who presented that the duration of the third instar larvae of *C. externa* after treatment using beta-cyfluthrin and pyrethroids was 3.3 days. For the duration of the pupal stage, there were no differences between treatments. Furthermore, no decrease in the rates of emergence of *C. externa* predators from the treated larvae was observed (Table 4).

TABLE 4 - Duration (days) and survival (%) (± SE) of immature stages of *Chrysoperla externa*, from third instar larvae treated with the insecticides (n=40). Temperature of 25 ± 2°C, RH 70 ± 10% and photophase of 12 hours.

Treatment	Third instar		Pupae	
	Duration**	Survival*	Duration**	Survival**
Chlorpyrifos	3.5 ± 0.13	20.0 ± 7.07 b	9.0 ± 0.12	90.0 ± 5.00
Cartap hydrochloride	4.0 ± 0.20	77.5 ± 2.16 a	9.1 ± 0.15	92.5 ± 6.50
Pyriproxyfen	4.6 ± 086	87.5 ± 4.15 a	8.8 ± 0.15	95.0 ± 4.33
Profenofos/Lufenuron	3.7 ± 0.11	82.5 ± 2.16 a	9.1 ± 0.15	97.5 ± 2.16
Fenpropathrin	3.7 ± 0.13	92.5 ± 4.15 a	8.7 ± 0.14	95.0 ± 4.33
Triazophos/Deltamethrin	4.2 ± 0.14	57.5 ± 8.93 b	9.0 ± 0.17	87.5 ± 8.20
Zetacypermethrin	4.2 ± 0.15	87.5 ± 4.15 a	8.8 ± 0.12	95.0 ± 4.33
Control	3.9 ± 0.13	95.0 ± 4.33 a	9.0 ± 0.18	97.5 ± 2.16
CV (%)	12.94	6.87	4.23	3.14

* Means followed by the same letter in the column are not statistically different according to the Scott-Knott test ($P < 0.05$).

** Not statistically difference ($P > 0.05$)

4 CONCLUSIONS

None of the tested insecticides exerted a negative effect on the duration of the embryonic period and viability of the treated eggs.

The first larval instar was more sensitive to the effects of the insecticides than those by the second and third larval instars.

The products chlorpyrifos and triazophos/ deltamethrin were toxic to larvae of the first, second and third instars.

Products considered toxic to the predator should be evaluated in semi-field and field conditions to confirm their toxicity. This information is important to the conservation of *C. externa* in the coffee agroecosystem and its compatibility in IPM programs.

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6 REFERENCES

AMARASEKARE, K. G.; SHEARER, P. W.; MILLS, N. J. Testing the selectivity of pesticides effects on natural enemies in laboaratory biossays. **Biological Control**, Montreal, v. 102, p. 7-16, Nov. 2016.

AUAD, A. M. et al. Potencial de *Chrysoperla externa* (Hagen) no controle de *Bemisia tabaci* (Gennadius) biótipo B em tomateiro. **Acta Scientiarum Agronomy**, Maringá, v. 29, n. 1, p. 29-32, 2007.

BARBOSA, L. R. et al. Eficiência de *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) no controle de M*yzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae) em pimentão (C*apsicum annum* l.). **Ciência e Agrotecnologia**, Lavras, v. 32, n. 4, p. 1113-1119, July/Aug. 2008.

BEZERRA, G. C. D. et al. Aspectos biológicos da fase adulta de *Chrysoperla externa* (Hagen,1861) (Neuroptera: Chrysopidae) oriunda de larvas alimentadas com *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae). **Ciência e Agrotecnologia**, Lavras, v. 30, n. 4, p. 603-610, July/ Aug. 2006.

BORTOLOTTI, L.; SBRENNA, A. M.; SBRENNA, G. Action of fenoxycarb on metamorphosis and cocoon spinning in *Chrysoperla carnea* (Neuroptera: Chrysopidae): identification of the JHA-sensitive period. **European Journal Entomology**, Ceske Budejovice, v. 102, n. 1, p. 27-32, Jan/Mar. 2005.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Agrofit**: Sistema de Agrotóxicos

Fitossanitários. Brasília, DF. Available on: <http://extranet.agricultura.gov.br/agrofit_cons/principal_agrofit_cons>. Access in: 20 jun. 2009.

BUENO, A. F. et al. Pesticide selectivity to natural enemies: challenges and constraints for research and field recommendation. **Ciência Rural**, Santa Maria, v. 47, n. 1, p. 1-10, Jan. 2017.

BUENO, A. F.; FREITAS, S. Efeito do hexythiazox e imidacloprid sobre ovos, larvas e adultos de *Chrysoperla externa* (Hagen) (Neuroptera: Chrisopidae). **Revista Ecossistema**, Espírito Santo do Pinhal, v. 26, n. 1, p. 74-76, 2001.

BUENO, A. F.; FREITAS, S. Effect of the insecticides abamectin and lufenuron on eggs and larvae of *Chrysoperla externa* under laboratory conditions. **BioControl**, Dordrecht, v. 49, n. 3, p. 277-283, May/June. 2004.

CARVALHO, G. A. et al. Seletividade de inseticidas a *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae). **Neotropical Entomology**, Londrina, v. 31, n. 4, p. 615-621, Oct/Dez. 2002.

CARVALHO, G. A. et al. Efeitos de inseticidas usados na cultura do algodoeiro sobre *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae). **Neotropical Entomology**, Londrina, v. 32, n. 4, p. 699-706, Oct/Dez. 2003.

CASTILHOS, R. V. et al. Selectivity of insecticides used in peach farming to larvae of *Chrysoperla externa* (Neuroptera: Chrysopidae) in semi-field conditions. **Revista Caatinga**, Mossoró, v. 30, n. 1, p. 109-115, Jan/Mar. 2017.

CHAPMAN, R. F. The integument, gas exchange and homeostasis. In:_____.**The insects: structure and function.** 15. ed. Cambridge: Cambridge University Press, 2013, chap. 16, p. 463-498.

COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira de café - safra 2018 (primeiro levantamento)**. Brasília, DF, 2018. Available on: <http://www.conab.gov.br/index.php/info-agro/safras/cafe>. Access in: 13 abr. 2018.

COSTA, R. I. F. et al. Duração e viabilidades das fases pré-imaginais de *Chrysoperla externa* (Hagen) alimentadas com *Aphis gossypii* Glover e *Sitotroga cerealella* (Olivier). **Acta Scientiarum Agronomy**, Maringá, v. 24, n. 2, p. 353-357, 2002.

CROFT, B. A. Management of pesticide resistance in arthropod pests. In: GREEN, M. B.; MOBERG, W.K.; LEBARON, H. (eds.). **Managing resistance to agrochemicals**: fundamental and practical approaches to combating resistance. Washington: American Chemical Society, 1990, p. 149-168.

ECOLE, C. C. et al. Predação de ovos, larvas e pupas do bicho-mineiro-do-cafeeiro, *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) por *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae). **Ciência e Agrotecnologia**, Lavras, v. 26, n. 2, p. 318-324, Mar/Apr. 2002.

FARIA, A. B. C. Revisão sobre alguns grupos de inseticidas utilizados no manejo integrado de pragas florestais. **Ambiência**, Guarapuava, v. 5, n. 2, p. 345-358, May/Aug. 2009.

FERNANDES, F. L. et al. Impact and selectivity of insecticides to predators and parasitoids. **Entomobrasilis**, Vassouras, v. 3, n. 1, p. 1-10, Jan/Apr. 2010.

FERREIRA, A. J. et al. Seletividade de inseticidas usados na cultura da macieira a ovos de *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae). **Ciência Rural**, Santa Maria, v. 35, n. 4, p. 756-762, July/Aug. 2005.

FERREIRA, A. J. et al. Seletividade de inseticidas usados na cultura da macieira a duas populações de *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae). **Ciência Rural**, Santa Maria, v. 36, n. 2, p. 378-384, Mar/Apr. 2006.

FERREIRA, W. L. B. Inseticidas de uso domiciliar e controle de vetores de doenças. In: MARICONI, F.A.M. (Ed.). **Insetos e outros invasores de residências**. Piracicaba: FEALQ, 1999, v. 6, p. 440-452.

GANDHI, P. I. et al. Laboratory evaluation of relative toxicities of some insecticides against *Trichogramma chltonis* (Hymenoptera: Trichogrammatidae) and *Chrysoperla carnea* (Neuroptera: Chrysopidae). **Journal Asia-Pacific Entomology**, Seoul, v. 8, n. 4, p. 381-386, Dec. 2005.

GODOY, M. S. et al. Seletividade de inseticidas utilizados na cultura dos citros para ovos e larvas de *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae). **Neotropical Entomology**, Londrina, v. 33, n. 5, p. 639-646, Sept/Oct. 2004.

GONÇALVES-GERVÁSIO, R. C. G.; SANTA-CECÍLIA, L. V. C. Consumo alimentar de *Chrysoperla externa* sobre as diferentes fases de desenvolvimento de *Dysmicoccus brevipes*, em laboratório. **Pesquisa Agropecuária Brasileira**, Brasília, v. 36, n. 2, p. 387-391, Feb. 2001.

GRAFTON-CARDWELL, E. E.; HOY, M. A. Short-term effects of permethrin and fenvalerate on oviposition by *Chrysoperla carnea* (Neuroptera: Chrysopidae). **Journal of Economic Entomology**, Annapolis, v. 78, n. 4, p. 955-959, 1985.

HEMINGWAY, J. The molecular basis of two contrasting metabolic mechanisms of insecticide resistance. **Insect Biochemistry and Molecular Biology**, Oxford, v. 30, n. 11, p. 1009-1015, Nov. 2000.

MAROUFPOOR, M. et al. Lethal effects of spinosad on *Chrysoperla carnea* larvae (Neuroptera: Chrysopidae) under laboratory conditions. **Journal of Plant Protection Research**, Poznań, v. 50, n. 2, p. 179-183, Apr/June. 2010.

MESQUITA, C. M. et al. **Manual do café:** distúrbios fisiológicos, pragas e doenças do cafeeiro (*Coffea arabica* L.). Belo Horizonte: EMATER-MG, 2016, p. 22- 42.

MOURA, A. P. et al. Toxicological and ultrastructural analysis of the impact of pesticides used in temperate fruit crops on two populations of *Chrysoperla externa* (Neuroptera, Chrysopidae). **Revista Brasileira de Entomologia**, Curitiba, v. 55, n. 3, p. 411-418, July/Sept. 2011.

MOURA, A. P.; CARVALHO, G. A.; BOTTON, M. Residual effect of pesticides used in integrated apple production on *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) larval. **Chilean Journal of Agricultural Research**, Chillán, v. 72, n. 2, p. 217-223, Apr/June. 2012.

OMOTO, C. Modo de ação de inseticidas e resistência de insetos a inseticidas. In: GUEDES, J. C.; COSTA, I. D.; CASTIGLIONI, E. (Org.) **Bases e técnicas do manejo de insetos**. Santa Maria: Pallotti, 2000. p. 31-49.

ONO, E.K. et al. Susceptibility of *Ceraeochrysa cubana* larvae and adults to six insect growth-regulator insecticides. **Chemosphere**, Oxford, v. 168, n. 1, p. 49-57, Feb. 2017.

PARRA, J. R. P.; REIS, P. R. Manejo integrado para as principais pragas da cafeicultura, no Brasil. **Visão Agrícola**, Piracicaba, n. 12, p. 47-50, Jan/June. 2013.

PASINI, R. A. et al. Side effects of insecticides used in wheat crop on eggs and pupae of *Chrysoperla externa* and *Eriopis connexa.* **Phytoparasitica,** v. 46, n. 1, p. 115-125, Jan/Feb. 2018.

PITWAK, J.; MENEZES JR, A.O.; VENTURA, M.U. Development and reproductive performance of *Chrysoperla externa* (Neuroptera: Chrysopidae) using preys from wheat crop. **Revista Colombiana de Entomologia**, Bogotá, v. 42, n. 2, p. 118-123, July/Dec. 2016.

POZZA, E. A.; CARVALHO, V. L.; CHALFOUN, S. M. Sintomas de injúrias causadas por doenças em cafeeiro. In: GUIMARÃES, R. J.; MENDES; A. N. G.; BALIZA, D. P. **Semiologia do cafeeiro**: sintomas de desordens nutricionais, fitossanitárias e fisiológicas. 1. ed. Lavras: Editora UFLA, 2010. cap. 2, p. 69-101.

PREETHA, G. et al. Toxicity of imidacloprid and diafenthiuron to *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) in the laboratory conditions. **Journal of Plant Protection Research**, Poznań, v. 49, n. 3, p. 290-296, July/Sept. 2009.

RIGITANO, R. L. O.; CARVALHO, G. A. **Toxicologia e seletividade de inseticidas**. Lavras: UFLA/FAEPE, 2001. 72p.

RIMOLDI, F.; SCHNEIDER, M. I.; RONCO, A. E. Susceptibility of *Chrysoperla externa* eggs (Neuroptera: Chrisopidae) to conventional and biorational insecticides. **Environmental Entomology**, Annapolis, v. 37, n. 5, p. 1252-1257, Sept/Oct. 2008.

RUGNO, G. R.; ZANARDI, O. Z.; YAMAMOTO, P. T. Are the pupae and eggs of the lacewing *Ceraeochrysa cubana* (Neuroptera: Chrysopidae) tolerant to insecticides? **Journal of Economic Entomology**, Annapolis, v. 108, n. 6, p. 2630-2639, Nov/Dec. 2015.

SCOTT, A. J.; KNOTT, M. A. A cluster analysis method for grouping means in the analysis of variance. **Biometrics**, Washington, v. 30, p. 507-512, 1974.

SILVA, R. A. et al. Toxicidade de produtos fitossanitários utilizados na cultura do cafeeiro a larvas de *Chrysoperla externa* (Hagen) (Neuroptera: Chrysopidae) e efeitos sobre as fases subsequentes do desenvolvimento do predador. **Neotropical Entomology**, Londrina, v. 34, n. 6, p. 951-959, Nov/Dec. 2005.

SILVA, R. A. et al. Predatory capacity of *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae) on *Brevipalpus phoenicis* (Geijskes, 1939) (Acari: Tenuipalpidae). **Coffee Science**, Lavras, v. 1, n. 1, p. 50-54, Jan/June. 2006.

SILVA, R. A. et al. Sintomas de injúrias causadas pelo ataque de pragas em cafeeiro. In: GUIMARÃES, R. J.; MENDES; A. N. G.; BALIZA, D. P. **Semiologia do cafeeiro**: sintomas de desordens nutricionais, fitossanitárias e fisiológicas. 1. ed. Lavras: Editora UFLA, 2010. cap. 3, p. 107-142.

SOUZA, J. C.; REIS, P. R.; SILVA, R. A. **Cigarras-do-cafeeiro em Minas Gerais**: histórico, reconhecimento, biologia, prejuízos e controle. Belo Horizonte: Epamig, 2007. 47p. (Epamig. Boletim Técnico, 80).

SOUZA, J. R. et al. Toxicity of some insecticides used in maize crop on *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) immature stages. **Chilean Journal of Agriccultural Research**, v. 74, n. 2, p. 234-239, Apr/June. 2014.

VELLOSO, A. H. P. P. et al. Efeitos de compostos reguladores de crescimento de insetos sobre larvas e adultos de *Chrysoperla externa* (Hagen, 1861) (Neuroptera: Chrysopidae). **Ciência e Agrotecnologia**, Lavras, v. 23, n. 1, p. 96-101, Jan/Mar. 1999.

VILELA, M. et al. Ação de acaricidas utilizados em cafeeiro sobre ovos e fases subsequentes do desenvolvimento de crisopídeos. **Coffee Science**, Lavras, v. 5, n. 1, p. 10-16, Jan/Apr. 2010.

VILELA, M. et al. Seletividade de acaricidas utilizados em cafeeiro para larvas de crisopídeos. **Revista Ceres**, Viçosa, v. 57, n. 5, p. 621-628, Sept/Oct. 2010.

SELECTION OF *Coffea arabica* L. HYBRIDS USING MIXED MODELS WITH DIFFERENT STRUCTURES OF VARIANCE-COVARIANCE MATRICES

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ABSTRACT: This study aimed to evaluate different structures of variance-covariance matrices in modeling of productive performance of coffee genotypes over the years, and select hybrids of *Coffea arabica* using mixed models. A mixed linear model was used to estimate variance components, heritability coefficients, and prediction of genetic values of hybrids and cultivars. Three commercial cultivars and eight hybrids of *C. arabica* L. were evaluated. The field production after acclimatization of seedlings was conducted in March 2006. The yield averages from 2009, 2010, 2011, 2013, and 2014 agricultural years were evaluated. The selection criteria of models were used to test 10 structures of variance-covariance matrices, and later a model was chosen to estimate the components of variance, heritability coefficients, and prediction of genetic values. According to Bayesian information criterion (BIC), the best structure was ARMA (Autoregressive Moving Average); however, considering the Akaike Information Criterion (AIC) and corrected Akaike Information Criterion (AICC), the CSH (Heterogeneous Composite Symmetric) was indicated. The Spearman correlation between the genotypic values obtained in the models with ARMA and CSH type R matrix was 0.84. The high and positive correlation indicates that the best model could involve the R matrix with ARMA or CSH structure. The heritability of individual genotypes differed from heritability in broad sense, which considers the independence among agricultural years. Hybrids with higher performance were identified by ordering the genotypic effects, among them, H 2.2, H 4.2, and H 6.1 hybrids were highlighted.

Index terms: Plant breeding, repeated measures, yield.

SELEÇÃO DE HÍBRIDOS DE *Coffea arabica* L. POR MEIO DE MODELOS MISTOS COM DIFERENTES ESTRUTURAS DE MATRIZES DE VARIÂNCIA E COVARIÂNCIA

RESUMO: Neste trabalho buscou-se avaliar diferentes estruturas de matrizes de variâncias e covariâncias na modelagem do comportamento produtivo de genótipos de cafeeiro ao longo dos anos, e selecionar híbridos de *Coffea arabica* utilizando um modelo linear misto Foram avaliadas três cultivares comerciais e oito híbridos de *C. arabica* L., utilizando mudas clonais obtidas por meio do enraizamento de estacas caulinares de ramos ortotrópicos. O plantio em campo, após aclimatação das mudas, foi realizado em março de 2006. Foram avaliadas as produções médias dos anos agrícolas 2009, 2010, 2011, 2013 e 2014. Critérios de seleção de modelos (RLL, AIC e BIC) foram utilizados para testar 10 estruturas da matriz R de variâncias e covariâncias, e posteriormente selecionou-se um modelo para estimar os componentes de variância, os coeficientes de herdabilidades e predição dos valores genéticos. Segundo o critério de informação Bayesiano (BIC) a melhor estrutura foi a ARMA, porém considerando o critério de Akaike e Akaike corrigido, a CSH foi indicada. A correlação de Spearman entre os valores genotípicos obtidos nos modelos com matriz R do tipo ARMA e CSH foi 0,84, alta e positiva, indicando que nesse conjunto de dados o melhor modelo pode envolver a matriz R com estrutura ARMA ou CSH. A herdabilidade individual dos genótipos foi diferente da herdabilidade no sentido amplo, que considera a independência entre os anos agrícolas. Pelo ordenamento dos efeitos genotípicos foram identificados os híbridos de desempenho superior, dentre os quais destacaram os híbridos H 2.2, H 4.2 e H 6.1.

Termos para indexação: Melhoramento genético, medidas repetidas, produtividade.

1 INTRODUCTION

The importance of coffee agribusiness can be evaluated not only by production and profits, but also by its role in the job market as a generator of jobs and as a factor of fixation of labor in the rural environment (SANTOS et al., 2009). Given this outlook and the importance of coffee production chain, coffee breeding programs have an important role in growing coffee in Brazil and become more economically competitive. The breeding of arabic coffee is more directed to development of pure-lines cultivars (MEDINA-FILHO et al., 2008), however, there is a great potential for use of hybrids with high heterosis (BERTRAND et al., 2011; MOHHAMED, 2011) and with multiple resistance to important diseases (ANDREAZI et al., 2015).

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Perennial plant species such as coffee trees have peculiar aspects, including a long reproductive cycle, marked annual oscillation of production resulting in a biennial cycle, overlap of generations, expression of characters over several years, and differences in precocity and productive longevity (BRUCKNER, 2008). Due to these agronomic peculiarities, coffee breeding is difficult (OLIVEIRA et al., 2011). Therefore, the use of special methods to estimate genetic parameters and predict genetic values is recommended (DE FAVERI et al., 2016).

When researchers deal with segregating coffee populations, the selection on a single plant level is relevant and is not only based on the average progeny. Thus, special methods to estimate the genetic parameters and prediction of genotypic values are necessary (PEREIRA et al., 2013; RESENDE, 2007). The application of mixed models in plant breeding has emphasized the estimation of variance components and appropriate identification of experimental error to test the hypotheses of fixed effects (HENDERSON, 1975). A general proposal has rarely been used when considering the modeling of variance-covariance genetic structures and random effects predictions (PIEPHO et al., 2008). Some studies have detected the small changes in the parameter estimation as a function of the variance-covariance structures used (APIOLAZA; GARRICK, 2001), including a change in genotype ordering when applying the selection to different models with various structures (ANDRADE et al., 2016).

The objective of this work was to evaluate different matrix structures of variance-covariance in the modeling of the yield performance of coffee genotypes over the years. A mixed linear model was used for the estimation of variance components, heritability coefficients, and genetic values prediction of hybrids and existing cultivars.

2 MATERIAL AND METHODS

The experiments were conducted at the Department of Agriculture, Coffee Sector at the Federal University of Lavras (UFLA). Three cultivars and eight hybrids were evaluated, as described in Table 1.

The seedlings of hybrids were obtained by rooting stem cuttings of orthotropic branches harvested from matrices plants. Segments 3–5 cm in length were treated with phytohormone indole-3-butyric acid (IBA) in inert talc and placed into commercial substrate to rooting. Rooting was conducted in greenhouse that had humidity and temperature control and was equipped with automatic irrigation system. The substrate used for the rooting was a mixture of washed sand and vermiculite [1:1]. After rooting, cuttings were transplanted to conventional polyethylene sacks (10 × 20 cm) for half-year seedlings. The sacks contained Plantmax®, a commercial substrate and standard substrate in a 1:1 ratio. Seedlings were then transferred to a nursery under sunlight with 50% of shading, where they remained until they reached the recommended seedling size for field planting.

After acclimatization, seedlings were planted in March 2006, following recommendations of planting (typical cultural practices) for the region. The average grain yield of years 2009, 2010, 2011, 2013, and 2014 was evaluated using mixed model analyses. The following matrix model was considered assuming normal multivariate distribution:

$$Y = X_r + Z_g + W_a + ZW_{ga} + \varepsilon$$

$$Where \begin{cases} y|b, V \sim N\,(Xb, V) \\ g \sim N\,(0, I\sigma_g^2) \\ a \sim N(0, I\sigma_a^2) \\ ga \sim N(0, I\sigma_{ga}^2) \\ \varepsilon \sim N\,(0, R) \end{cases}$$

In this model, Y is the data vector, *r* is the vector of blocks effects (assumed to be fixed) added to the general mean, *g* is the vector of individual genotypic effects (assumed to be random), *a* is the vector of effects of agricultural years (random), *ga* is the vector of the interaction effects of genotypes and agricultural years. In the previous description, the random error vector associated with the experimental unit is distributed as N (O,R), that is, normal with mean 0 and a positive and defined dimension covariance matrix *R*. In general, crop data are analyzed together by assuming a composite symmetry (CS) of matrix *R*, with equal variances and null correlations, or experimental design considering model as split-plots in time. However, this kind of assumption may not be accurate. To verify the validity of this assumption, sphericity hypothesis of residual covariance matrix was applied by Mauckhly sphericity test (MAUCKHLY, 1940) using SAS® University version (PROC GLM).

TABLE 1 - Identification and description of the materials (C: cultivars; H: hybrids) evaluated in the trial.

Identification	Description
C 1	Icatu IAC-2942
C 2	Catuaí IAC-62
C 3	Catuaí IAC-99
H 1.2	(Icatu IAC-2942 x Catuaí IAC-62); Plant 2
H 1.3	(Icatu IAC-2942x Catuaí IAC-62); Plant 3
H 2.1	(Icatu IAC-2942x Icatu IAC-5002); Plant 1
H 2.2	(Icatu IAC-2942x Icatu IAC-5002); Plant 2
H 4.1	(Icatu IAC-4040-179 x Catuaí IAC-17); Plant 1
H 4.2	(Icatu IAC-4040-179 x Catuaí IAC-17); Plant 2
H 6.1	(Icatu IAC-4040-179 x Catuaí IAC-99); Plant 1
H 6.2	(Icatu IAC-4040-179 x Catuaí IAC-99); Plant 2

After sphericity test, 10 alternative structures of *R* matrix were tested in order to deal with present reality, to better explore data, and thus, obtain accurate estimates. The structures tested were: Antidependence (ANTE), Autoregressive (AR), Autoregressive with heterogeneous variances (ARH), Autoregressive moving averages (ARMA), Composite symmetry with heterogeneous variances (CSH), Analytical Factor (FA), Huynh-Feldt (HF), Toeplitz (TOEP), Toeplitz with Heterogeneous Variance (TOEPH), and Unstructured (UN).

The parameters related to variances of each evaluation are generally located within the main diagonal of matrices. Outside of diagonal are covariance parameters for each year pair considered. The number of parameters estimated by each covariance structure is a function of global dimension (t) of covariance matrix. For matrix *R* modeling, the (t) variable corresponds to the number of harvests (Table 2).

Three criteria were considered when selecting models under different covariance matrix structures: 1) Maximum likelihood ratio (ML) test, 2) Akaike model selection criteria (AIC), and 3) Schwarz criterion (BIC) (KONISH and KITAGAWA, 2008; GURKA, 2006). These criteria indicate which model is the most likely among analyzed ones and referring to data in question and do not guarantee the choice of the true model.

The likelihood ratio testing evaluates whether the additional parameters significantly improve the model. This test considered $L1 = -2\log(L)$ as the model with the lowest number of parameters and $L2 = -2\log(L)$ as the model with the highest number of parameters. The tested hypothesis was whether the two models were equivalent (i.e., the extra parameters do not differ from zero). Under normality, the difference between L1 and L2 is asymptotically distributed as L1 - L2 ~ $\chi 2$ [r] (chi-square with r degrees of freedom).

As an alternative to maximum likelihood ratio test, it is possible to calculate measures based on information such as AIC and BIC criteria. The information is calculated as a penalty term applied to the likelihood function. The AIC criterion is based on decision theory and in order to avoid excessive parameterization, penalizes models with large number of parameters. It is defined by expression:

$$AIC = -2\log(L) + 2p$$

Where log (L) is the logarithm of the maximum likelihood function of the model, and *p* is the number of parameters of the variance-covariance matrix. According to this criterion, the variance-covariance matrix model to be chosen is the one with the lowest AIC value. The Schwarz criterion or Bayesian information criterion (BIC) was derived from the Bayes' theorem to problem of model identification. The BIC value is minimized asymptotically in order of the model with the highest probability later. It is defined by expression:

$$BIC = -2\log(L) + \log(N) * p$$

TABLE 2 - Description of the tested variance-covariance structures, and the number of parameters tested in each structure.

Structures	Description	Parameters*
ANTE(1)	Ante-Dependence	2t-1
AR(1)	Autoregressive	2
ARH(1)	Heterogeneous Autoregressive	t+1
ARMA(1,1)	Autoregressive Moving Average	3
CSH	Composite Symmetric with Heterogeneous variance	t+1
FA(1)	Factor Analytic	q/2(2t-q+1)+t
HF	Huynh-Feldt	t+1
TOEP	Toeplitz	t
TOEPH	Heterogeneous Toeplitz	2t-1
UN	Unstructured	t(t+1)/2

*t is number of harvests

Where log (L) is the logarithm of the maximum likelihood function of the model, *N* is the total number of observations and *p* is the number of parameters of the variance-covariance matrix. While ML, AIC, and BIC are conceptually different, they use the same statistical criterion: the maximum of the likelihood which functions as a measure of adjustment. However, these criteria define different critical values (LITTELL et al., 2006).

After selection of the best structure of variance-covariance matrix, it was possible to obtain variance estimates by restricted maximum likelihood (REML) method and to predict the genetic values by Empirical Best Linear Unbiased Prediction (E-BLUPs) using SAS University Version (PROC MIXED). The E-BLUP has good predictive accuracy compared to other procedures (PIEPHO et al., 2008); a common estimator used was based on E-BLUP expression, that is: $E\text{-}BLUP = h^2(\bar{Y}_{i..} -$ (BERNARDO, 2010). Therefore, it was used to calculate the heritability of each genotype by expression:

$$h_i^2 = \frac{E\text{-}BLUP}{(\bar{Y}_{i..} - \bar{Y}_{...})}$$

Where $\bar{Y}_{i..}$ is the genotype *i* mean in all harvests, and $\bar{Y}_{...}$ is the overall mean of all genotypes in all harvests under consideration. And broad sense heritability considering the composite symmetry according to Ramalho et al. (2012):

$$h^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ga}^2}{a} + \frac{\sigma_r^2}{ga}}$$

Where σ_g^2 is the variance of the genotypes; σ_{ga}^2 is the variance of the interaction genotypes and agricultural years; σ_r^2 is the residual variance; a is the number of agricultural years and g is the number of genotypes.

A coincidence index (CI) of the top three selected genotypes was obtained according to Hamblin and Zimmerman (1986):

$$CI = \frac{A - C}{B - C}$$

Where, A is number of coincident progenies among the top three genotypes selected; B is the number of selected progenies. C is the expected amount of coincident progenies.

3 RESULTS AND DISCUSSION

Before comparing the quality of adjustment associated with the different models, the correlations between the years were estimated according to the means of all genotypes in all replicates (Table 3).

The highest positive correlation was identified between the years 2011 and 2014 (r = 0.41, $p < 0.05$). Negative correlations were identified in subsequent years (2010 and 2011; 2013 and 2014).

TABLE 3 - Pearson's correlations among the evaluation years regarding genotype yield (hybrids and cultivars).

Years	2009	2010	2011	2013
2010	0.304			
2011	0.220	-0.16		
2013	0.131	0.030	-0.012	
2014	0.0192	0.22	0.412	-0.400

Therefore, it was demonstrated that the assumption of independence among years cannot be accepted as a rule to support a model for traditional variance analysis (FREITAS et al., 2008). This fact is confirmed by Mauchly's sphericity test (Table 4).

The hypothesis of sphericity was rejected by the Mauchly's test, which indicated that the composite symmetry structure (CS) for the variance-covariance matrix might not be the most adequate for the data in question. According to Resende (2007), the sphericity rejection in perennial plants has been very common. With the sphericity rejection, 10 alternative structures were examined for the covariance matrices associated with matrix R.

The answers of the different criteria of information and selection of models diversify according to some characteristics—such as the probability distribution of the data—and, primarily, the covariance pattern present in such characteristics. The AIC and BIC criteria are RLL adjustments and they are the most used in the literature (GURKA, 2006). A lower value of these statistics indicates a better structure. At the 5% probability level, the structure $I\sigma^2$ (used in PROC GLM) is not the most appropriate compared to the structures tested. Thus, according to BIC, the best structure was ARMA. This brings together characteristics of first order autoregressive structure with the moving average technique (COOPER and THOMPSON, 1977). However, when considering AIC and AICC, the CSH structure is the best option (Table 5). In general, the two criteria produce concordant results (FLORIANO, 2006). However, in this way, the choice must account for number of parameters.

The use of these techniques is fundamental to decision theory in mixed models; besides the quality of adjustment, such techniques consider the principle of parsimony, which penalizes models with a greater number of parameters (BURNHAM and ANDERSON, 2004).

For perennial and semi-perennial crops, trials of which are usually evaluated in different years, there is a case of repeated measures in time (PIEPHO and ECKL, 2014). The correlation between years may decrease with the temporal distance between harvests (SONG, 2007). One reason is that genes expressed in the first agricultural year may not be expressed in subsequent years (PASTINA, 2012).

The best structure regarding the ability of the model to fit the observed data varies between trials, revealing the impossibility of previously indicating a structure for the analysis of similar experiments (SILVA, DUARTE, REIS, 2015). For example, a similar study examining *C. arabica*, Andrade et al. (2016) identified that the Toeplitz (TOEP) structure for the variance-covariance matrix is the most appropriate because it considers specific correlations for each interval between harvests. In addition, Burgueño et al. (2011) demonstrated that the predictive power of a model increased up to 6% when the variance- covariance structure was adequately modeled.

Table 6 presents the genotypic values of genotypes under evaluation considering the two primary variance and covariance structures.

The cultivars presented low genotypic values when compared to hybrids. The order of classification of genotypic values for ARMA and CSH were coincident just in first two positions, with hybrids H 2.2 and H 4.2 and in last position with C3. However, by Spearman's correlation, there was a high (0.82) and significant (P-value = 0.002) correlation between the E-BLUPS of two structures in question. In practical terms, in selection of three best genotypes, the coincidence index was 66%. However, when the four best genotypes were selected, the coincidence index was 75%. These values are high due to low population sampled. However, in larger populations, it is possible that the coincidence percentage decreases, requiring even more apropriate choice of the best structure of variances-covariance matrix.

To calculate the heritability of the progenies, the *R* matrix of the ARMA type was considered. As a result, a low heritability of the genotype (12.3%) was found in comparison to the heritability in the broad sense (73.5%). This fact is an alert for the mild selection of genotypes. The genetic variance was lower than the environmental variance, due to the field experimentation conditions that tended to increase environmental variance.

TABLE 4 - P-values of the Mauchly's sphericity test according to coffee yield evaluated in the years 2009, 2010, 2011, 2013, and 2014.

Degrees of freedom	Mauchly's criterion(W)	X^2	p-value
9	0.2136	26.8851	0.0015

TABLE 5 - Results of the tests for the best *R* matrix choice for the model involving coffee yield evaluated in years 2009, 2010, 2011, 2013, and 2014.

Structures	F Qui	p-value	ML[1]	AIC[2]	AICc[3]	BIC[4]
ANTE(1)	4 10.14	0.0381	1476.4	1486.4	1486.8	1496.5
AR(1)	1 3.87	0.0491	1482.7	1486.7	1486.8	1490.7
ARH(1)	3 10.14	0.0174	1476.4	1484.4	1484.7	1492.5
ARMA(1,1)	1 6.85	0.0089	1479.7	1483.7	1483.8	1487.7
CSH	3 13.28	0.0041	1473.3	1481.3	1481.5	1489.3
FA(1)	5 13.60	0.0183	1473.0	1485.0	1485.5	1497.0
HF	3 11.62	0.0088	1474.9	1482.9	1483.2	1491.0
TOEP	2 6.99	0.0304	1479.6	1485.6	1485.7	1491.6
TOEPH	4 13.60	0.0087	1473.0	1483.0	1483.4	1493.0
UN	5 13.60	0.0183	1473.0	1483.0	1483.4	1493.0

1: Maximum Likelihood Test

2: Akaike Information Criterion

3: Akaike Information Criterion Corrected

4:BayesianInformationCriterion(BIC)

TABLE 6 - Genotypic values of the coffee genotypes (C: cultivars, H: hybrids) in terms of yield considering the years 2009, 2010, 2011, 2013 and 2014.

	Structures of *R* matrix	
Identification of coffee genotypes*	ARMA(1,1) Autoregressive Moving Average	CSH Heterogeneous Composite Symmetric
C 1	0.20	0.01
C 2	-0.66	-0.02
C 3	-4.26	-0.14
H 1.2	-2.57	0.13
H 1.3	1.38	0.50
H 2.1	-1.82	-0.07
H 2.2	9.39	9.46
H 4.1	3.68	0.12
H 4.2	5.81	7.68
H 6.1	1.60	2.51
H 6.2	1.54	2.42

* Description in Table 1

4 CONCLUSIONS

The best variance-covariance structures for the data analysis of the experiment in question were ARMA and CSH. The genotypic values obtained by these two structures are correlated, however, when simulating genotype selection, a minimum of four genotypes is expected so that at least three genotypes are selected based on the analyzes with the two structures. Therefore the three best hybrids were H 2.2 [(Ic 2942 x 5002); Plant 2], H 4.2 [(Ic 4040-179 x Ct 17); Plant 2] and H 6.1 [(Ic 4040-179 x Ct 99); Plant 1].

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6 REFERENCES

ANDRADE, V. T. et al. Statistical modeling implications for coffee progenies selection. **Euphytica,** Wageningen, v. 207, n. 1, p. 177-189, Jan. 2016.

ANDREAZI, E. et al. Desempenho de híbridos F_1 de café arábica com resistência simultânea a ferrugem, mancha aureolada e bicho mineiro. **Coffee Science**, Lavras, v. 10, n. 3, p. 375-382, July/Sept. 2015.

APIOLAZA, L. A.; GARRICK, D. J. Analysis of longitudinal data from progeny tests: some multivariate approaches. **Forest Science**, Bethesda, v. 47, n. 2, p. 129-140, May. 2001.

BERNARDO, R. N. **Breeding for quantitative traits in plants**. 2. ed. Woodbury: Stemma Press, 2010, 390p.

BERTRAND, B. et al. Performance of *Coffea arabica* F1 hybrids in agroforestry and full-sun cropping systems in comparison with American pure line cultivars. **Euphytica**, Wageningen, v. 181, n. 2, p 147-158, Sept. 2011.

BRUCKNER, C. H. **Fundamentos do melhoramento de fruteiras.** 1. ed. Viçosa: Editora UFV, 2008, 202 p.

BURGUEÑO, J. et al. Genomic prediction of breeding values when modeling genotype×environment interaction using pedigree and dense molecular markers. **Crop Science**, Madison, v. 52, n. 2, p. 707-719, Mar/Apr. 2012.

BURNHAM, K. P.; ANDERSON, D. R. Multimodel Inference - Understanding AIC and BIC in model selection. **Sociological Methods and Research**, Cambridge, v. 33, p. 261-304, 2004.

CARVALHO, A. Principles and practice of coffee plant breeding for productivity and quality factors. In: CLARKE, R. J.; MACRAE, R. (Eds.). **Coffee: Agronomy**. London: Elsevier Applied Science, 1988. p. 129–165.

COOPER, D. M.; THOMPSON, R. A note on the estimation of the parameters of the autoregressive-moving average process. **Biometrika**, London, v. 64, n. 3, p. 625-628, Sept/Dec. 1977.

DE FAVERI J. et al. Residual variance–covariance modeling in analysis of multivariate data from variety selection trials. **Journal of Agricultural, Biological, and Environmental Statistics**, v. 22, n. 1, p. 1–22, Jan/Mar. 2016.

FLORIANO, E. P. et al. Ajuste e seleção de modelos tradicionais para série temporal de dados de altura de árvores. **Ciência Florestal**, Santa Maria, v. 16, n. 2, p. 177-199, Apr/June. 2006.

FREITAS, E. G. de. et al. Modelo univariado aplicado a dados longitudinais de cana-de-açúcar. **Revista Brasileira de Biometria**, Lavras, v. 26, p. 93-16, 2008.

GURKA, M. J. Selecting the best linear mixed model under REML. **The American Statistician**, Washington, v. 60, n. 1, p. 19-26, Jan/Mar. 2006.

HENDERSON, C. R. Best linear unbiased estimation and prediction under a selection model. **Biometrics**, Bethesda, v. 31, p. 423-449, 1975.

KONISHI, S.; KITAGAWA, G. **Information criteria and statistical modeling.** 1. ed. New York: Springer, ISBN 0387718869. 2008. 321p.

LITTELL, R. C. et al. **SAS system for mixed models**. 2. ed. Cary: Statistical Analysis System Institute, 2006. 828 p.

MAUCHLY, J. W. Significance test for sphericity of a normal n-variate distribution. **Annals of Mathematical Statistics**, Ann Arbor, v. 11, n. 2, p. 204- 209, Apr/June. 1940.

MEDINA FILHO, H. P.; BORDIGNON, R.; CARVALHO, C. H. S. de. Desenvolvimento de novas cultivares de café arábica. In: CARVALHO, C. H. S. de, (Ed.). **Cultivares de café: origem características e recomendações**. Brasília: Embrapa Café, 2008. p. 79-101.

MOHAMMED, W. Heterosis and combining ability analysis for coffee quality in diallel crosses of diverse coffee (*Coffea arabica* L.) parents in origin. **East African Journal of Sciences**, Haramaya, v. 5, n. 1, p. 12-21, Jan/June. 2011.

OLIVEIRA, A. C. B. et al. Prediction of genetic gains from selection in Arabica coffee progenies. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 2, p. 106-113, 2011.

PASTINA, M. M. et al. A mixed model QTL analysis for sugarcane multiple-harvest-location trial data. **Theoretical and Applied Genetics**, New York, v. 124, n. 5, p. 835-849, Mar. 2012.

PEREIRA, T. B. et al. Eficiência da seleção de progênies de café F4 pela metodologia de modelos mistos (REML/BLUP). **Bragantia**, Campinas, v. 72, n. 3, p. 230-236, July/Sept. 2013.

PIEPHO, H. P.; ECKL, T. Analysis of series of variety trials with perennial crops. **Grass and Forage Science**, Victoria, v. 69, n. 3, p. 431–440, July/Sept. 2014.

PIEPHO, H. P. et al. BLUP for phenotypic selection in plant breeding and variety testing. **Euphytica**, Wageningen, v. 161, n. 1-2, p. 209-228, May. 2008.

RAMALHO, M. A. P. et al. **Aplicações da genética quantitativa no melhoramento de plantas autógamas**. 1. ed. Lavras: Editora UFLA, 2012. 522 p.

RESENDE, M. D. V. **Matemática e estatística na análise de experimentos e no melhoramento genético.** 1. ed. Colombo: Embrapa Florestas, 2007. 435 p.

SANTOS, V. E. dos. et al. Análise do setor de produção e processamento de café em Minas Gerais: uma abordagem matriz insumo-produto. **Revista de Economia e Sociologia Rural**, Brasília, v. 47, n. 2, p. 363–388, jun. Apr/June. 2009.

SILVA, E. N.; DUARTE, J. B.; REIS, A. J. dos S. Seleção da matriz de variância-covariância residual na análise de ensaios varietais com medidas repetidas em cana-de-açúcar. **Ciência Rural**, Santa Maria, v. 45, n. 6, p. 993-999, June. 2015.

SONG, P. X. -K. **Correlated data analysis: modeling, analytics, and applications**. New York: Springer. 2007. 352 p.

DEVELOPMENT OF A METHODOLOGY TO DETERMINE THE BEST GRID SAMPLING IN PRECISION COFFEE GROWING

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ABSTRACT: Precision agriculture is based on a set of techniques that explore the spatial variability of properties related to a determined area. The aim of this study was to develop and test a methodology to evaluate the quality of grid sampling. The experiment was performed in three areas of 112, 50 and 26 ha, in coffee plantations (*Coffea arabica*) with cultivar Catuai 144, in the Três Pontas Farm, located in Presidente Olegário, MG, Brazil, in 2014 and 2015. A total of 224, 100, and 52 georeferenced points (2.0 points/ha) were plotted in the areas regarding the soil chemical properties, respectively: phosphorus, potassium, calcium and magnesium. For the application methodology the standardized accuracy index (SAI), the standardized precision index (SPI) and the standardized optimal grid indicator (SOGI) were developed and tested. From grid 1 (2 points/ha), another three sampling grids (1.0, 0.7 and 0.5 point/ha) were adopted. The indexes were important to analyze the grid quality, whereas the SOGI allowed selecting the grid that best represented the properties.

Index terms: Precision agriculture, geostatistics, grids, soil fertility.

DESENVOLVIMENTO DE UMA METODOLOGIA PARA DETERMINAÇÃO DA MELHOR MALHA AMOSTRAL EM CAFEICULTURA DE PRECISÃO

RESUMO: A agricultura de precisão baseia num conjunto de técnicas que explora a variabilidade espacial dos atributos de uma área. O objetivo deste trabalho foi desenvolver e testar uma metodologia para avaliar a qualidade de malhas amostrais. O experimento foi desenvolvido nos anos de 2014 e 2015 na fazenda Três Pontas, Presidente Olegário/MG, em três áreas de 112, 50 e 26 ha, todas de lavoura de cafeeiro (*Coffea arabica*) cultivar Catuai 144. Demarcaram-se nas áreas 224, 100 e 52 pontos georreferenciados respectivamente (2,0 pontos/ha), os atributos químicos do solo testados: fósforo, potássio, cálcio e magnésio. Para aplicação da metodologia foi desenvolvido e testado o índice de exatidão padronizado (IEP), índice de precisão paradronizado (IPP) e o indicador de malha ótima padronizado (IMOP). A partir da malha 1 (2 pontos/ha) foram adotadas mais 3 malhas amostrais (1,0; 0,7 e 0,5 ponto/ha). Os índices mostraram importantes para analisar a qualidade das malhas e o IMOP permitiu a escolha da malha que melhor representou os atributos.

Termos para indexação: Agricultura de precisão, geoestatistica, *grids,* fertilidade do solo.

1 INTRODUCTION

Coffee is one of the main agricultural products of Brazilian agribusiness. Because it is an activity of great importance for the Brazilian trade balance, there is a need to seek the deep knowledge of all the productive processes involved from planting to harvesting (FERRAZ et al., 2012b).

The coffee growers are in a continuing search for technologies that aid in the management and improve the production of their crop, adapting to the market demands.

Precision agriculture comes as a tool that contributes to cost savings. For Rodrigues Junior et al. (2011), precision agriculture might bring countless benefits to the coffee growing, since it has a high income per area and the main parameter is bean quality. According to Ferraz (2012), precision agriculture in coffee growing has been referred as "precision coffee growing".

Ferraz et al. (2012) define it as a set of techniques and technologies capable of assisting the coffee farmer to manage the crop, based on the spatial variability of soil and plant properties, in order to maximize profitability, increase efficiency of fertilization, spraying and harvesting, thus increasing productivity and the product's final quality.

The understanding on spatial variability in the crop requires a greater amount of information, which can be obtained from sampling operations (SOUZA; MARQUES JÚNIOR; PEREIRA, 2004). These samplings, mainly referring to grid sampling, still generate discussions among scientists, technicians and producers, which do not yet have well-established standards for coffee growing.

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According to Ferraz et al. (2017), the use of unsatisfactory grid sizes can generate maps that do not reflect the field and thus generate wrong technical recommendations, which could result in losses to its users.

According to Nanni et al. (2011), the grid sampling used in the most diverse Brazilian cultures are around one point every two to three hectares and, in some cultures, up to one point is used every four hectares. In relation to the coffee crop, few studies refer to the adequate size of grid sampling, thus, there is a great need for research with this purpose.

The aim in this study was to develop and test a methodology to evaluate and compare the quality of different grid samples in different size areas and define the one that best characterizes the spatial variability for each tested property.

2 MATERIAL AND METHODS

The experiment was performed in three different areas (112 ha, 50 ha and 26 ha) at Três Pontas Farm, Presidente Olegário, MG, Brazil, in 2014 and 2015, all based on wet process (*Coffea arabica* L.) with cultivar Catuaí IAC 144, planted in December 2005 (11-year crop), December 2011 (5 years) and December 2004 (12 years), respectively, spacing of 4.0 m between rows and 0.5 m between plants, totaling 5,000 plants.ha^{-1}.

They were demarcated in the study area, using a GPS signal receiver equipment, 224, 100 and 52 georeferenced sample points (2.0 points/ha), respectively. From grid 1, another three grid samples were adopted (1.0, 0.7 and 0.5 point/ha).

Using a GPS signal receiver, the demarcated points were found and then the samples collection was made. The grid 1 has the greater number of georeferenced sample points, with 2.0 points/ha, the grid 2 has 1.0 point/ha, the grid 3 refers to 0.7 point/ha and the grid 4 is composed by 0.5 point/ha.

Soil fertility data were collected at every georeferenced point in the area. Each sampling point corresponded to four plants, being that the collection was performed in July 2014 and July 2015 using a quadricycle, by removing subsamples in the projection of coffee canopy at depth from 0 to 20 cm. The samples were sent to the Brazilian Laboratory of Agricultural Analysis (LABRAS), in Monte Carmelo, MG. The chemical attributes of the soil analyzed were Phosphorus, Potassium, Calcium and Magnesium.

In order to reduce costs with the collection of soil samples in 2015, in the area of 112 ha, the sampling was done from grid 2, referring to one point per hectare.

Semivariograms are used in order to analyze the spatial dependence of properties under study. Semivariance is classically estimated by equation 1:

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} \left[Z(x_i) - Z(x_i + h) \right]^2 \quad (1)$$

where, N (h) is the number of experimental pairs of observations $Z(x_i)$ and $Z(x_i + h)$ separated by a distance h. The semivariogram is represented by the graph $\hat{\gamma}(h)$ versus h. From the fit of a mathematical model to the calculated values of $\hat{\gamma}(h)$, the coefficients of the theoretical model were estimated for the semivariogram called nugget effect (C_0); sill (C_0+C_1); and range (a), as described by Bachmaier and Backers (2011).

The weighted least squares method and the spherical, exponential and Gaussian models were used according to the best fit for each property and for tested grids. With the semivariograms ready, ordinary kriging and kriging validation procedures were performed to assess the interpolation quality.

The software used for geostatistical analysis and map generation was ArcGIS 10. The geostatistical index proposed by Seidel and Oliveira (2014) was used to measure the spatial dependence. Where, the spatial dependence is weak whether ratio is lower than 12.5%; moderate, between 12.5% and 25%, and strong spatial dependence when the ratio is greater than 25%. This index can be used for the spherical, exponential and Gaussian semivariogram models, which is expressed by equation 2:

$$IDE_{model(\%)} = MI.\left(\frac{C_1}{C_0 + C_1}\right).\left(\frac{a}{q.MD}\right).100 \quad (2)$$

where MI is the model index; C_0, the nugget effect; C_1, the contribution; a, the practical range; and q.MD, the value corresponding to the fraction (q) reached at maximum distance (MD) between sampled points. Whether the ratio (*a/q.MD)* results in a value greater than 1, the ratio is then set to 1, so that it assumes only values between zero and 1. Moreover, the greater the MI value, the greater the spatial dependence of model.

One of the ways to evaluate the quality of estimation and fitting of semivariograms, besides other research characteristics, such as the sample mesh, is through validation. It makes possible to extract some useful values for the observation of errors presented by each grid, such as the mean of standard error (M_{SE}) (Equation 3), which should have the value closer to zero, and the standard deviation of standard error (SD_{SE}) (Equation 4), which should be as lower as possible.

$$M_{(SE)} = \frac{1}{n}\sum_{i=1}^{n} \frac{[\hat{z}(x_i) - z(x_i)]}{\sigma^2{}_{k(xi)}} \tag{3}$$

$$SD_{(SE)} = \sqrt{\frac{1}{n}\sum_{i=1}^{n} \frac{[\hat{z}(x_i) - z(x_i)]^2}{\sigma^2{}_{k(xi)}}} \tag{4}$$

Where n is the number of data; $\hat{Z}(x_i)$ is a value predicted or estimated by ordinary kriging at point xi, without considering the observation Z(xi); Z(xi) is the value observed at point xi; and $\sigma_{k(xi)}$ is the standard deviation.

Based on these values, a methodology was developed and tested covering as main characteristic the use of standard errors, which can be applied in several circumstances, such as in the comparison between grids from different cultures, representing a great differential in relation to other methodologies developed previously.

Based on the MSE and SDSE values obtained it was developed and tested the Standardized Accuracy Index (SAI) and the Standardized Precision Index (SPI). These indexes make possible to identify the best grid for areas up to 100 ha. It was used attributes of the soil to test the indexes, being evaluated Phosphorus (P), Potassium (K), Calcium (Ca) and Magnesium (Mg); which were sampled in the years 2014 and 2015.

The value of the MSE, obtained by validation, reflects the accuracy of grid sampling. The SAI concept (Equation 5) was proposed and developed to determine an accuracy component that would allow comparing among grids.

$$SAI = \frac{M_{(SE)}}{mM_{(SE)}} \tag{5}$$

where $M_{(SE)}$ is the value of the mean of standard error, in module, of the grid to be compared and $mM_{(SE)}$ is the greatest value of the mean of standard error, in module, among all analyzed grids.

The value of the SD_{SE}, obtained by validation, reflects the grid accuracy. Moreover, the SPI was developed and proposed to compare the accuracy component of the grid among the different studied grids (Equation 6).

$$SPI = 1 - \frac{SD_{(SE)}}{mSD_{(SE)}} \tag{6}$$

where SD_{SE} is the value of the standard deviation of standard error of the studied grid and mSD_{SE} is the greatest value of the standard deviation of standard error presented by the group of analyzed grids. The SAI and SPI values range from zero to one, and the closer to one, the more accurate/precise is the grid sample, while the closer to zero, the more inaccurate/imprecise is the grid sample.

The SOGI was developed and tested in order to choose the best grid among those under study, taking into account the weighting between the standardized accuracy and prediction indices (Equation 7).

$$SOGI = (0.5 \times SAI) + (0.5 \times SPI) \tag{7}$$

In the equation it is calculated a proportion between SAI and SPI indexes, since both accuracy and precision are important for the grid quality index. As the two indicators (accuracy and precision) were considered equally important, it was given equal weight to both, that is, 50% for each one.

The SOGI ranges from zero to one and the closer to one (or 100%), the better the grid (more accurate and more precise), while the closer to zero (0%), the worse (the more inaccurate and imprecise) is the grid.

3 RESULTS AND DISCUSSION

Cherubin et al. (2015) studied the phosphorus (P) property and observed that the increase in the number of collected samples (n), provided by smaller grid samples, makes it possible to identify sites in the area with extreme P levels that, if not corrected, might result in crop restriction zones.

Carvalho (2016) studied the optimal sampling density for precise coffee growing in an area of 22 ha and found that the grid with 2.0 points/ha were the most suitable for productivity and soil fertility of coffee.

By evaluating grid samples for an area of 22 ha, Ferraz et al.(2017) applied another methodology and found the 3.0 points per hectare as the most suitable for the soil fertility variables Phosphorus (P), remaining Phosphorus (P-rem), Potassium (K), and changeability of calcium at pH 7.0 (T). The methodology proposed by Ferraz et al. (2017) encompasses the standard error of the data and was studied for smaller areas, up to 22 ha, while the methodology of this work is based on standardized errors and it was studied in larger areas, up to 100 ha. Moreover, the great difference between the two methodologies is that the one used in this work can be applied in study of different cultures, differing from the one applied by Ferraz which can only be used for coffee cultivation.

Comparative studies were also performed to evaluate grid samples for different variables. Cherubin et al. (2014), Nanni et al. (2011) and Ragagnin, Sena Júnior and Silveira Neto (2010) recommend the use of a sampling with more points per hectare.

Table 1 presents the parameters estimated by the semivariogram fitted by the weighted least squares method for the properties Phosphorus (P) and Potassium (K) in the years 2014 and 2015 in an area of 112 ha. For the variable P, in 2014, the geostatistical index proposed by Seidel and Oliveira (2014) showed values considered as weak, since they had a percentage lower than 12.5% in all grid samples. The SOGI relates the SAI and SPI indices, ranging from 0 to 100%, indicating that the best grid for Phosphorus was the grid 3 in the year 2014. For the parameters estimated by the semivariogram fitted by the weighted least squares method and by the exponential model of P in 2015, the spatial dependence index (SDI) showed weak spatial dependence for all grids, and the best grid sample in the year 2015 for P was the grid 4, according to SOGI.

For the parameters estimated by the semivariogram fitted by the weighted least squares method and by the spherical and exponential model for the property Potassium (K) in 2014, it is observed that the geostatistical index is presented as weak for all grid samples, i.e., a spatial dependence lower than 12.5%. In contrast, grids 1 and 3 showed values close to 40% for SOGI. The parameters estimated by the semivariogram fitted by the weighted least squares method and by the spherical model for the Potassium variable, for the year 2015. The IDE was weak for grid samples 2 and 3, and moderate for grid 4. Analyzing all the grid samples, the grid 4 showed the most indicated value for the SOGI, with 44%.

Table 2 presents the parameters estimated by the semivariogram fitted by the weighted least squares method for the properties calcium (Ca) and Magnesium (Mg) in the years 2014 and 2015 in an area of 112 ha.

For the property Calcium (Ca), the geostatistical index was weak for most of the grids fitted by the weighted least squares method and by the spherical model for 2014 and 2015, and only the grid 3 of the year 2015 was moderate, according to the index. The SOGI showed values of 45.14% in grid 1 and 43.11% in grid 3 for 2014 and 44.96% in grid 2 in the year 2015, being the best values among the grid samples. The parameters estimated by the weighted least squares method and the spherical model for the variable Magnesium (Mg) in the years 2014 and 2015, presented the geostatistical index as weak and moderate for grid samples in both years, respectively. The SOGI presented values of 43.33% for grid 1 and 40.7% for grid 4 in 2014. For the year 2015 the values presented for grids 1 and 4 were of 42.44% and 40.56% respectivelywhich are the closest to 100% for both years.

Table 3 presents the parameters estimated by the semivariogram fitted by the weighted least squares method for the properties Phosphorus (P) and Potassium (K) in the years 2014 and 2015, in an area of 50 ha.

For the variable P, in 2014, the geostatistical index presented values considered as moderate for grid 3 and weak for grids 1, 2 and 4, which had percentage lower than 12.5%. Through the SOGI, it was observed that grid 2 was the best grid for Phosphorus in 2014. For the parameters estimated by the semivariogram fitted by the weighted least squares method and by the exponential model of the P in 2015, the SDI showed weak spatial dependence for grids 1 and 2 and moderate for grids 3 and 4. According to SOGI, the grid 4 was the best grid sample for Phosphorus in the year 2015, with 49.48%.

For the property Potassium (K), in the year 2014, the SDI was weak for all grid samples and, according to the SOGI classification, the best grid sample was grid 1, which showed an index value of 36.51%. For the year 2015, still for the variable K, the SDI was weak for all grids, and the greatest SOGI was 51.67% for the grid 1.

Table 4 presents the parameters estimated by the semivariogram fitted by the weighted least squares method for the properties calcium (Ca) and Magnesium (Mg) in the years 2014 and 2015 in an area of 50 ha.

TABLE 1 - Parameters estimated by the semivariogram fitted by the weighted least squares method and by the spherical (*) and exponential (**) model for the properties Phosphorus (P) and Potassium (K), in the area of 112 ha in the years 2014 and 2015.

Grid	NPG	C_0	C_1	$C_0 + C_1$	A	SDI_Seidel		CV(%)	$M_{(SE)}$	$SD_{(SE)}$	SAI	SPI	SOGI
						Phosphorus 2014							
1*	224	2100.03	103.02	2203.05	166.31	0.50	Weak	33.45	0.0119	0.9697	0.5704	0.0260	29.82
2*	112	1395.83	833.87	2229.70	174.12	4.18	Weak	33.03	0.0139	0.9817	0.4982	0.0140	25.61
3*	79	1941.97	0.00	1941.97	1093.17	0.00	Weak	31.22	0.0073	0.9580	0.7365	0.0377	38.71
4*	57	2008.40	0.00	2008.40	434.93	0.00	Weak	31.54	0.0277	0.9956	0.0000	0.0000	0.00
						Phosphorus 2015							
2**	112	536.18	615.51	1151.69	228.23	6.62	Weak	25.87	0.0084	1.0118	0.5307	0.0180	27.43
3**	79	527.63	490.51	1018.14	176.39	4.62	Weak	25.75	0.0179	1.0303	0.0000	0.0000	0.00
4**	57	805.02	193.85	998.87	246.24	2.61	Weak	26.07	0.0015	1.0086	0.9162	0.0211	46.86
						Potassium 2014							
1*	224	1516.27	343.21	1859.48	167.95	1.99	Weak	33.29	0.0061	0.9796	0.8365	0.0000	41.82
2*	112	1476.09	808.29	2284.38	522.04	11.87	Weak	33.48	0.0122	0.9562	0.6729	0.0239	34.84
3*	79	640.22	1508.17	2148.39	161.53	7.31	Weak	34.63	0.0096	0.9125	0.7426	0.0685	40.56
4**	57	1897.91	0.00	1897.91	258.26	0.00	Weak	37.02	0.0373	0.9555	0.0000	0.0246	1.23
						Potassium 2015							
2*	112	1035.23	854.72	1889.95	281.92	8.18	Weak	65.58	0.0077	0.9748	0.6435	0.0000	32.18
3*	79	1677.76	242.76	1920.52	289.83	2.35	Weak	68.60	0.0216	0.9429	0.0000	0.0327	1.64
4*	57	0.00	1351.08	1351.08	264.97	17.09	Mod	59.26	0.0025	0.9637	0.8843	0.0114	44.78

NPG - Number of points of the grid sampling; C_0 - Nugget effect; C_1 - Contribution; C_0+C_1 - Sill; a - range; SDI - Spatial dependence index; Str - Strong; Mod - Moderate; Weak - Weak; CV (%) - Coefficient of variation; $M_{(SE)}$ - Mean of standard error; SD_{SE} - Standard deviation of standard error; SAI - Standardized accuracy index; SPI - Standardized precision index; SOGI - Standard optimal grid indicator.

TABLE 2 - Parameters estimated by the semivariogram fitted by the weighted least squares method by the spherical model (*) for the properties Calcium (Ca) and Magnesium (Mg) in the area of 112 ha in the years 2014 and 2015.

Grid	NPG	C_0	C_1	$C_0 + C_1$	a	SDI_Seidel		CV(%)	$M_{(SE)}$	$SD_{(SE)}$	SAI	SPI	SOGI
Calcium 2014													
1*	224	1.08	0.06	1.14	296.00	1.08	Weak	64.66	0.0031	0.9690	0.8495	0.0532	45.14
2*	112	0.89	0.65	1.54	208.14	5.62	Weak	69.22	0.0056	0.9720	0.7282	0.0503	38.92
3*	79	0.60	1.40	2.00	214.02	9.65	Weak	74.91	0.0039	0.9707	0.8107	0.0516	43.11
4*	57	1.69	0.00	1.69	390.83	0.00	Weak	71.57	0.0206	1.0235	0.0000	0.0000	0.00
Calcium 2015													
2*	112	1.51	0.28	1.79	1583.29	15.92	Weak	60.06	0.0036	1.0235	0.8992	0.0000	44.96
3*	79	1.41	0.38	1.79	1571.7	13.32	Mod	59.20	0.0357	1.0197	0.0000	0.0037	0.19
4*	57	0.74	0.68	1.42	246.24	7.60	Weak	63.34	0.0098	1.0005	0.7255	0.0225	37.40
Magnesium 2014													
1*	224	0.50	0.15	0.65	313.12	4.65	Weak	65.89	0.0031	0.9775	0.8622	0.0044	43.33
2*	112	0.46	0.30	0.76	266.78	6.80	Weak	66.86	0.0225	0.9708	0.0000	0.0112	0.56
3*	79	0.52	0.43	0.95	267.17	7.71	Weak	71.72	0.0196	0.9443	0.1289	0.0382	8.35
4*	57	0.37	0.35	0.72	258.26	8.15	Weak	64.34	0.0042	0.9818	0.8133	0.0000	40.67
Magnesium 2015													
2*	112	0.35	0.12	0.47	1583.29	12.99	Mod	77.98	0.0036	1.0475	0.8487	0.0000	42.44
3*	79	0.34	0.03	0.37	176.40	0.92	Weak	79.19	0.0238	1.0180	0.0000	0.0282	1.41
4*	57	0.26	0.06	0.32	530.25	6.41	Weak	81.34	0.0055	1.0032	0.7689	0.0423	40.56

NPG - Number of points of the grid sampling; C0- Nugget effect; C1- Contribution; C0+C1- Sill; a - range; SDI - Spatial dependence index; Str - Strong; Mod - Moderate; Weak - Weak; CV (%) - Coefficient of variation; M(SE)- Mean of standard error; SDSE - Standard deviation of standard error; SAI - Standardized accuracy index; SPI - Standardized precision index; SOGI - Standard optimal grid indicator.

TABLE 3 - Parameters estimated by the semivariogram fitted by the weighted least squares method and by the spherical (*) and exponential (**) model for the properties Phosphorus (P) and Potassium (K) in the area of 50 ha in the years 2014 and 2015.

Grid	NPG	C_0	C_1	$C_0 + C_1$	A	SDI_Seidel		CV(%)	$M_{(SE)}$	$SD_{(SE)}$	SAI	SPI	SOGI
Phosphorus 2014													
1*	100	900.11	553.15	1453.26	120.48	3.50	Weak	49.68	0.0193	1.0116	0.5896	0.0455	31.76
2*	50	881.78	885.71	1616.41	658.95	12.19	Weak	44.28	0.0150	1.0318	0.6809	0.0264	35.36
3**	33	782.41	1166.08	1856.10	770.09	16.39	Mod	42.69	0.0333	1.0598	0.2915	0.0000	14.57
4*	25	1287.53	0.00	1287.53	1058.60	0.00	Weak	41.58	0.0470	1.0313	0.0000	0.0269	1.34
Phosphorus 2015													
1**	100	247.47	203.37	450.84	167.56	5.77	Weak	43.09	0.0115	0.9871	0.6338	0.1016	36.77
2*	50	365.64	150.74	516.38	910.77	10.62	Weak	42.99	0.0119	1.0785	0.6210	0.0184	31.97
3*	33	381.34	264.56	645.90	993.74	16.31	Mod	45.47	0.0314	1.0987	0.0000	0.0000	0.00
4*	25	295.88	327.65	623.53	705.73	14.81	Mod	46.39	0.0009	1.0787	0.9713	0.0182	49.48
Potassium 2014													
1*	100	1185.50	729.43	1914.93	386.94	11.25	Weak	32.09	0.0103	1.0379	0.6971	0.0332	36.51
2*	50	1440.04	500.16	1940.20	1092.93	11.25	Weak	28.99	0.0289	1.0665	0.1500	0.0065	7.83
3*	33	1523.92	191.04	1714.96	287.65	2.57	Weak	31.20	0.0340	1.0735	0.0000	0.0000	0.00
4*	25	1692.79	0.00	1692.79	705.73	0.00	Weak	30.73	0.0214	1.0328	0.3706	0.0379	20.42
Potassium 2015													
1*	100	159.29	439.32	598.61	134.91	7.55	Weak	46.04	0.0092	0.9539	0.8999	0.1334	51.67
2*	50	482.20	86.29	568.49	176.40	2.14	Weak	46.93	0.0306	0.9918	0.6670	0.0990	38.30
3*	33	626.58	76.05	702.63	203.50	1.76	Weak	51.33	0.0079	1.0264	0.9140	0.0676	49.08
4*	25	122.17	203.09	325.26	213.44	10.64	Weak	42.02	0.0919	1.1008	0.0000	0.0000	0.00

NPG - Number of points of the grid sampling; C_0 - Nugget effect; C_1 - Contribution; C_0+C_1 - Sill; a - range; SDI - Spatial dependence index; Str - Strong; Mod - Moderate; Weak - Weak; CV (%) - Coefficient of variation; $M_{(SE)}$ - Mean of standard error; SD_{SE} - Standard deviation of standard error; SAI - Standardized accuracy index; SPI - Standardized precision index; SOGI - Standard optimal grid indicator.

TABLE 4 - Parameters estimated by the semivariogram fitted by the weighted least squares method by the spherical (*) and Gaussian model (***) for the properties Calcium (Ca) and Magnesium (Mg) in the area of 50 ha in the years 2014 and 2015.

Grid	NPG	C_0	C_1	$C_0 + C_1$	A	SDI_Seidel		CV(%)	$M_{(SE)}$	$SD_{(SE)}$	SAI	SPI	SOGI
Calcium 2014													
1*	100	1.53	0.17	1.70	1137.16	4.40	Weak	42.00	0.0068	0.9932	0.5310	0.0259	27.85
2*	50	1.66	0.18	1.83	1092.93	4.18	Weak	38.95	0.0057	1.0196	0.6069	0.0000	30.34
3*	33	1.78	0.34	2.13	993.74	6.40	Weak	39.22	0.0066	0.9820	0.5448	0.0369	29.09
4*	25	1.82	0.17	2.00	1058.60	3.66	Weak	40.92	0.0145	0.9882	0.0000	0.0308	1.54
Calcium 2015													
1*	100	0.96	1.55	2.51	154.07	7.26	Weak	47.80	0.0009	0.9715	0.9833	0.0506	51.70
2*	50	0.83	1.44	2.27	197.23	9.99	Weak	46.02	0.0182	1.0074	0.6630	0.0155	33.93
3*	33	2.01	0.01	2.02	229.56	0.09	Weak	43.39	0.0540	0.9265	0.0000	0.0946	4.73
4*	25	1.80	0.00	1.80	372.15	0.00	Weak	46.07	0.0134	1.0233	0.7519	0.0000	37.59
Magnesium 2014													
1*	100	0.31	0.06	0.37	159.02	2.00	Weak	73.09	0.0158	0.9952	0.5798	0.1017	34.08
2*	50	0.32	0.07	0.39	235.97	3.17	Weak	71.90	0.0047	1.0246	0.8750	0.0752	47.51
3*	33	0.13	0.30	0.44	224.47	12.49	Weak	71.57	0.0376	1.0024	0.0000	0.0952	4.76
4***	25	0.05	0.49	0.54	223.86	21.79	Mod	76.12	0.0131	1.1079	0.6516	0.0000	32.58
Magnesium 2015													
1*	100	0.25	0.18	0.43	161.41	5.16	Weak	67.64	0.0122	0.9656	0.8082	0.0724	44.03
2*	50	0.25	0.16	0.41	185.00	5.77	Weak	68.77	0.0222	1.0410	0.6509	0.0000	32.55
3*	33	0.33	0.00	0.33	418.25	0.00	Weak	65.16	0.0636	1.0264	0.0000	0.0140	0.70
4*	25	0.38	0.00	0.38	1058.60	0.00	Weak	69.72	0.0103	1.0276	0.8381	0.0129	42.55

NPG - Number of points of the grid sampling; C_0 - Nugget effect; C_1 - Contribution; C_0+C_1 - Sill; a - range; SDI - Spatial dependence index; Str - Strong; Mod - Moderate; Weak - Weak; CV (%) - Coefficient of variation; $M_{(SE)}$ - Mean of standard error; SD_{SE} - Standard deviation of standard error; SAI - Standardized accuracy index; SPI - Standardized precision index; SOGI - Standard optimal grid indicator.

For the property Calcium (Ca), the geostatistical index was weak for all the grids fitted by the weighted least squares method and by the spherical model for 2014 and 2015. The SOGI showed greater values in grids 2 (2014) and 1 (2015), being 30.34% and 51.70%, respectively.

For the variable Magnesium (Mg), the geostatistical index was weak for all the grids of the years 2014 and 2015, except for the grid 4 of the year 2014, where the index was moderate. The SOGI presented values of 47.51% for grid 4 in 2014, and 44.03% and 42.55% for grids 1 and 4, respectively, in the year 2015, which are the closest to 100%.

Table 5 presents the parameters estimated by the semivariogram fitted by the weighted least squares method for the properties Phosphorus (P) and Potassium (K) in the years 2014 and 2015 in an area of 26 ha.

For the property Phosphorus (P), the geostatistical index was moderate for the grid 1 and weak for the other grids in the year 2014. For the year 2015, the SDI was moderate for grids 1 and 2 and weak for grids 3 and 4. The SOGI showed values of 38.44% in grid 2 and 37.33% in grid 3 for 2014 and 32.79% in grid 3 in the year 2015, being the best values among the grid samples. For the variable Potassium (K), the geostatistical index was moderate for grid 1 and weak for grid 2, 3, and 4, in the year 2014. For the year 2015, the SDI for K was weak for grids 1 and 3, moderate for grid 3, and strong for grid 4. The SOGI presented values of 35.90% for grid 1 in the year 2014 and 55.04% for grid 1 in the year 2015.

Table 6 presents the parameters estimated by the semivariogram fitted by the weighted least squares method for the Calcium (Ca) and Magnesium (Mg) in the years 2014 and 2015 in an area of 26 ha.

For the property Magnesium (Mg), the geostatistical index was moderate for grid 3 and weak for grid 1, 2 and 4 in the year 2014. For the year 2015, the SDI was weak for all grids. The SOGI presented values of 46.71% for grid 1 in the year 2014 and 38.07% for grid 4 in the year 2015.

Based on Table 7, the grids considered as more adequate for the area of 112 ha were grids 1 and 2, not statistically different from each other, by the Scott Knott Test, at 5% probability. Grid 1 is more recommended for areas of 50 ha and 26 ha, followed by grid 2, 4, and 3. Therefore, for precision coffee growing, based on this study conditions, it is recommended to use a grid from 2.0 to 1.0 points/ha, for areas above 100.0 ha and a grid with 2.0 points/ha for areas equal or smaller than 50.0 ha.

TABLE 5 - Parameters estimated by the semivariogram fitted by the weighted least squares method by the spherical (*) and Gaussian model (***) for the properties Phosphorus (P) and Potassium (K) in the area of 26 ha in the years 2014 and 2015.

Grid	NPG	C_0	C_1	$C_0 + C_1$	A	SDI_EnioSeidel		CV(%)	$M_{(SE)}$	$SD_{(SE)}$	SAI	SPI	SOGI
						Phosphorus 2014							
1***	52	64.92	1912.09	1977.01	112.03	16.50	Mod	39.58	0.0088	0.9526	0.4500	0.0811	26.56
2*	26	1600.37	801.01	2401.38	525.38	9.93	Weak	42.64	0.0037	1.0367	0.7688	0.0000	38.44
3*	17	1699.97	67.69	1767.66	317.38	1.38	Weak	36.57	0.0055	0.943	0.6563	0.0904	37.33
4*	13	2084.43	804.59	2889.02	684.51	11.59	Weak	38.94	0.0160	1.0191	0.0000	0.0170	0.85
						Phosphorus 2015							
1*	224	0.00	1415.65	1415.65	130.66	14.80	Mod	42.81	0.0173	0.9120	0.4627	0.1095	28.61
2*	112	424.68	810.82	1235.50	185.00	13.75	Mod	37.64	0.0239	1.0242	0.2578	0.0000	12.89
3*	79	1159.76	0.00	1159.76	813.13	0.00	Weak	34.57	0.0138	0.9378	0.5714	0.0844	32.79
4*	57	1118.67	0.00	1118.67	821.42	0.00	Weak	36.43	0.0322	0.9670	0.0000	0.0558	2.79
						Potassium 2014							
1*	52	0.00	779.92	779.92	110.60	12.53	Mod	28.63	0.0115	1.0145	0.6875	0.0305	35.90
2*	26	673.89	178.26	852.16	293.52	6.96	Weak	28.79	0.0140	1.0465	0.6195	0.0000	30.98
3*	17	816.07	69.38	885.46	813.13	3.61	Weak	27.77	0.0368	1.0224	0.0000	0.0230	1.15
4*	13	878.64	0.00	878.64	547.61	0.00	Weak	27.24	0.0165	0.9665	0.5516	0.0764	31.40
						Potassium 2015							
1*	52	95.11	62.41	157.52	153.23	6.88	Weak	24.95	0.0004	0.9657	0.9934	0.1074	55.04
2***	26	39.89	119.72	159.61	183.96	15.63	Mod	23.34	0.0325	0.9925	0.4646	0.0826	27.36
3*	17	75.71	29.14	104.85	470.71	7.41	Weak	19.03	0.0080	1.0819	0.8682	0.0000	43.41
4***	13	22.00	84.64	106.64	284.38	27.44	Str	19.17	0.0607	1.0781	0.0000	0.0035	0.18

NPG - Number of points of the grid sampling; C_0 - Nugget effect; C_1 - Contribution; C_0+C_1 - Sill; a - range; SDI - Spatial dependence index; Str - Strong; Mod - Moderate; Weak - Weak; CV (%) - Coefficient of variation; $M_{(SE)}$ - Mean of standard error; SD_{SE} - Standard deviation of standard error; SAI - Standardized accuracy index; SPI - Standardized precision index; SOGI - Standard optimal grid indicator.

TABLE 6 - Parameters estimated by the semivariogram fitted by the weighted least squares method by the spherical model (*) for the properties Calcium (Ca) and Magnesium (Mg) in the area of 26 ha in the years 2014 and 2015.

Grid	NPG	C_0	C_1	$C_0 + C_1$	a	SDI_Seidel		CV(%)	$M_{(SE)}$	$SD_{(SE)}$	SAI	SPI	SOGI
Calcium 2014													
1*	52	1.26	1.08	2.34	259.44	13.60	Mod	46.09	0.0064	1.0234	0.8867	0.0000	44.34
2*	26	0.79	1.18	1.98	213.01	14.45	Mod	40.75	0.0565	0.9463	0.0000	0.0753	3.77
3*	17	1.18	0.59	1.77	284.09	10.75	Weak	38.32	0.0306	0.9467	0.4584	0.0749	26.67
4*	13	1.50	0.00	1.50	547.61	0.00	Weak	34.57	0.0237	0.9550	0.5805	0.0668	32.37
Calcium 2015													
1*	100	1.66	0.89	2.55	114.87	5.07	Weak	54.04	0.0148	1.0275	0.5502	0.0000	27.51
2*	50	2.30	0.00	2.30	359.06	0.00	Weak	47.77	0.0223	0.9772	0.3222	0.0490	18.56
3*	33	2.39	0.00	2.39	505.14	0.00	Weak	49.43	0.0329	0.9982	0.0000	0.0285	1.43
4*	25	1.34	0.00	1.34	547.61	0.00	Weak	39.46	0.0187	1.0136	0.4316	0.0135	22.26
Magnesium 2014													
1*	52	0.30	0.09	0.38	162.48	4.16	Weak	51.04	0.0042	1.0024	0.9302	0.0039	46.71
2*	26	0.19	0.13	0.32	244.26	11.26	Weak	45.77	0.0525	1.0063	0.1279	0.0000	6.40
3*	17	0.11	0.24	0.35	255.30	19.73	Mod	46.10	0.0602	0.9400	0.0000	0.0659	3.29
4*	13	0.26	0.08	0.34	821.42	12.04	Weak	43.69	0.0196	0.9477	0.6744	0.0582	36.63
Magnesium 2015													
1*	52	0.34	0.12	0.46	120.48	3.56	Weak	61.50	0.0100	1.0399	0.5215	0.0143	26.79
2*	26	0.50	0.04	0.54	813.38	3.41	Weak	55.67	0.0065	1.0131	0.6890	0.0397	36.44
3*	17	0.59	0.02	0.61	813.13	1.51	Weak	57.19	0.0209	1.0550	0.0000	0.0000	0.00
4*	13	0.36	0.00	0.36	821.42	0.00	Weak	46.17	0.0056	1.0240	0.7321	0.0294	38.07

NPG - Number of points of the grid sampling; C_0 - Nugget effect; C_1 - Contribution; C_0+C_1 - Sill; a - range; SDI - Spatial dependence index; Str - Strong; Mod - Moderate; Weak - Weak; CV (%) - Coefficient of variation; $M_{(SE)}$ - Mean of standard error; SD_{SE} - Standard deviation of standard error; SAI - Standardized accuracy index; SPI - Standardized precision index; SOGI - Standard optimal grid indicator.

TABLE 7 - Ranking of grids as a function of the mean SOGI (%), considering soil fertility and collection seasons.

Area:	Ranking	Grid	Number of points	Mean SOGI (%)
112ha	1st	1	224	34.28 a
	1st	2	112	27.43 a
	2nd	4	57	20.08 b
	2nd	3	79	17.32 b
50ha	1st	1	100	38.59 a
	2nd	2	50	28.78 b
	2nd	4	25	27.81 b
	3rd	3	33	6.30 c
26ha	1st	1	52	32.58 a
	2nd	2	26	22.92 b
	2nd	4	13	19.68 b
	2nd	3	17	16.67 b

4 CONCLUSIONS

By applying the methodology, through the SAI, SPI and SOGI, it was possible to identify the most recommended grid for the tested properties.

Based on this study conditions, it is recommended the georeferenced sampling, obeying the use of grids with 2.0 points per hectare, for precision coffee growing.

5 REFERENCES

BACHMAIER, M.; BACKERS, M. Variogram or semivariogram? variance or semivariance? Allan variance or introducing a new term? **Mathematical Geosciences**, Berlin, v. 43, n. 6, p. 735-740, Aug. 2011.

CARVALHO, L. C. C. **Determinação da densidade amostral ótima para a geração de mapas temáticos na Cafeicultura de Precisão**. 2016. 230 p. Tese (Doutorado em Engenharia Agrícola) - Universidade Federal de Lavras, Lavras, 2016.

CHERUBIN, M. R. et al. Eficiência de malhas amostrais utilizadas nacaracterização da variabilidade espacial de fósforo e potássio. **Ciência Rural**, Santa Maria, v. 44, n. 3, p. 425-432, Mar. 2014.

CHERUBIN, M. R. et al. Dimensão da malha amostral para caracterização da variabilidade espacial de fósforo e potássio em Latossolo Vermelho. **Pesquisa Agropecuária Brasileira**, Brasília, v. 50, n. 2, p. 168-177, Feb. 2015.

FERRAZ, G. A. S. **Cafeicultura de precisão:** malhas amostrais para o mapeamento de atributos do solo, da planta e recomendações. 2012. 134 p. Tese (Doutorado em Engenharia Agrícola) - Universidade Federal de Lavras, Lavras, 2012.

FERRAZ, G. A. S. et al. Geostatistical analysis of fruit yield and detachment force in coffee. **Precision Agriculture**, Dordrecht, v. 13, n. 1, p. 76-89, Jan. 2012.

FERRAZ, G. A. S. et al. Methodology to determine the soil sampling grid for precision agriculture in a coffee field. **DYNA,** Medellín, v. 84, p. 316-325, 2017.

NANNI, M. R. et al. Optimum size in grid soil sampling for variable rate application in site-specific management. **Scientia Agricola**, Piracicaba, v. 68, n. 3, p. 386-392, May/June. 2011.

RAGAGNIN, V. A.; SENA JÙNIOR, D. G.; SILVEIRA NETO, A. N. Recomendação de calagem a taxa variada sob diferentes intensidades de amostragem. Revista Brasileira de Engenharia Agrícola e Ambiental, Campina Grande, v. 14, n. 6, June. 2010.

RODRIGUUES JUNIOR, F. A. et al. Geração de zonas de manejo para a cafeicultura empregando sensor SPAD e análise foliar. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v. 15, n. 8, Aug. 2011.

SEIDEL, E. J.; OLIVEIRA, M. S. Novo índice geoestatístico para a mensuração da dependência espacial. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 38, n. 3, p. 699-705, May/June. 2014.

SOUZA, Z. M.; MARQUES JÚNIOR, J.; PEREIRA, G. T. Variabilidade espacial da estabilidade de agregados e matéria orgânica em solos de relevos diferentes. **Pesquisa Agropecuária Brasileira**, Brasília, v. 39, n. 5, p. 491-499, Feb. 2004.

EFFECT OF BIOSTIMULANT AND MICRONUTRIENT ON EMERGENCE, GROWTH AND QUALITY OF ARABICA COFFEE SEEDLINGS

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ABSTRACT: The micronutrients and biostimulant use can promote root, shoot and seedling growth. The aim of this work was evaluated the micronutrients and biostimulant application effects on arabica coffee seedling development. The experimental design was a randomized block in factorial 5 x 3, with four replicates. Five cultivars of arabica coffee (Topázio, Catuaí Amarelo, Catuaí Vermelho 99, Catuaí Vermelho 144 and Catiguá) cultivated in green house, combined with biostimulant (Stimulate®), of micronutrients (Mo+Co) and control. 150 days after sowing the plants was evaluated. The higher seed germination velocity (0.10) was obtained with micronutrients and biostimulant. The higher height of plant, 8.88 cm, was attained with biostimulant on Catuaí Amarelo. Catuaí Amarelo and Vermelho obtained higher number of leaves (4.5) with biostimulant use and the Catuaí Amarelo (4.38) with micronutrients use. In Catuaí Amarelo was attained higher shoot dry mass and leaf area with biostimulant use, reach 1.69 g and 19.55 cm^2, respectively. The higher root dry mass values (0.76 g) and Dickson quality index (0.47 and 0.48) was attained with micronutrients use to the Catuaí Amarelo e vermelho cultivars. Seedling development of Catuaí Amarelo was benefited with Stimulate® application and the cultivar Topázio did not get positive development with Stimulate® and micronutrients application.

Index terms: *Coffea arabica* ., Stimulate®, molybdenum + cobalt, Dickson quality index.

EFEITO DE BIOESTIMULANTE E MICRONUTRIENTE NA EMERGÊNCIA, CRESCIMENTO E QUALIDADE DE MUDAS DE CAFÉ ARÁBICA

RESUMO: O uso de bioestimulante e micronutriente pode promover o crescimento de raízes e parte aérea de mudas de plantas. Assim, o objetivo do trabalho foi avaliar o efeito da aplicação de bioestimulante e micronutriente no desenvolvimento de mudas de café arábica. O delineamento experimental foi em blocos ao acaso, em esquema fatorial 5 x 3, com quatro repetições. Foi utilizado cinco cultivares de café arábica (Topázio, Catuaí Amarelo, Catuaí Vermelho 99, Catuaí Vermelho 144 e Catiguá) cultivadas em casa de vegetação, associado a aplicação do bioestimulante (Stimulate®), de micronutriente (Mo+Co) e controle. As plantas foram avaliadas 150 dias após a semeadura. O maior índice de velocidade de germinação de sementes (0,10) foi obtido com bioestimulante e com micronutriente. A maior altura de planta, 8,88 cm, foi atingida com o bioestimulante em Catuaí Amarelo. O Catuaí Amarelo e o Catuaí Vermelho obtiveram maior número de pares de folhas (4,5) com uso de bioestimulante e o Catuaí Amarelo (4,38) com uso de micronutriente. Em Catuaí Amarelo foram atingidos maiores massa seca da parte aérea e área foliar com uso do bioestimulante, chegando a 1,69 g e 19,55 cm^2, respectivamente. Os maiores valores de massa seca de raiz (0,76 g) e índice de qualidade de Dickson (0,47 e 0,48) foram obtidos com o uso de micronutrientes para as cultivares de Catuaí Amarelo e vermelho. O desenvolvimento de mudas de Catuaí Amarelo foi beneficiado com a aplicação de Stimulate® e a cultivar Topázio não obteve desenvolvimento positivo quando aplicado o Stimulate® e o micronutriente.

Termos de indexação: *Coffea arabica* , Stimulate®, molibdênio + cobalto, índice de qualidade de Dickson.

1 INTRODUCTION

Brazil has 2.21 million hectares planted with coffee, in which 81% corresponds to crops planted with arabica coffee (*Coffea arabica* L.), despite the decrease in area planted with coffee in recent years, the country stands out as the leader in exports of this species. Nevertheless, studies show that there was a gain in productivity due to the use of new technologies in the production of vigorous seedlings, new varieties, adequate fertilization, irrigation, among other types of cultural treatments (Companhia Nacional de Abastecimento - CONAB, 2017).

A good performance in the development of coffee seedlings can be achieved by the application of biostimulants and micronutrients, which can enhance nutrient uptake by plants (BINSFELD et al., 2014), in which the biostimulant is characterized as being a natural substance or synthetic, produced from plant regulators such as

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cytokinins, gibberellins and auxins, and can be mixed with other nutritional elements, thus acting on the cellular metabolism due to its natural or synthetic composition from regulators (SILVA et al., 2012; SILVA; TEODORO; MELO, 2008).

Micronutrients, as in this case, molybdenum and cobalt are of extreme physiological importance for plants, especially for the biological nitrogen fixation process in legumes (WEISANY; RAEI; ALLAHVERDIPOOR, 2013). Molybdenum, in turn, acts as a cofactor in the enzymatic complex of nitrate reductase for nitrogenase and sulfide oxidase, donating electrons, being one of the responsible for the nitrate assimilation in vegetables, this in turn, for being an anion has its availability in synergism with increasing pH, and its deficiency in acid pH, in coffee cultivation, molybdenum is required in a few quantities, about 3 mg per bag of coffee (TAIZ et al., 2017; SFREDO; OLIVEIRA, 2010; MALAVOLTA, 2006). However, for cobalt, there are few reports of its importance in coffee cultivation, but it is known that this essential element has great importance for nitrogen-fixing plants, in which it acts in the synthesis processes of vitamin B_{12}, cobamide and leghemoglobin in nitrogen-fixing nodules (SFREDO; OLIVEIRA, 2010). Some authors describe that micronutrient seed treatment has the potential to meet cultural needs in relation to mineral nutrition and to improve plant emergence, yield and grain enrichment. In addition, Mo application in seed is more effective compared to application in the soil for many cultures (FAROOQ; WAHID; SIDDIQUE, 2012).

Nevertheless, in the Brazilian academic field, different studies have been carried out with the aim of promoting better coffee development and productivity, connected studies to identify molecular markers related to the coffee resistance (*C. arabica*) to rust (*Hemileia vastatrix*) (ALVARENGA et al., 2011), to the productive and economic performance of the arabica coffee consortium, under rainfed and drip irrigated conditions (PERDONÁ; SORATTO; ESPERANCINI, 2015; BONOMO et al., 2008) are examples of the broad approach of Brazilian coffee research, although there is much information on the cultivation of arabica coffee, few studies have described the influence of the addition of biostimulant and micronutrients on the seedlings production in nursery. Thus, it is necessary to use new technologies that promote good development of vigorous seedlings, which provide good survival, reduced replanting and fast initial plant growth (ALVES; GUIMARÃES, 2010).

The use of biostimulant in the production of seedlings of different species has been tested with diverse results. The application of biostimulant in seeds of *Genipa americana* L. increased the seed emergence velocity and root length (PRADO NETO et al., 2007), also increased seedling emergence in *Dimorphandra mollis* Benth. (CANESIN et al., 2012) and mass of dry matter and leaf area of *Campomanesia adamantium* (Cambess.) O.Berg, (SCALON et al., 2009). On the other hand, it did not result in the growth in height and diameter of *Jacaranda decurrens* Cham. (KISSMANN et al., 2011) and in high doses, inhibited the emergence and development of *Hymenaea courbaril* L. seedlings (PIERAZAN; SCALON; PEREIRA, 2012).

Due to the great economic potential provided by this crop in the country, this work had as objective to evaluate the effect of the application of biostimulants and micronutrients (molybdenum and cobalt) in the production of Arabica coffee seedlings.

2 MATERIAL AND METHODS

The work was carried out in a greenhouse, belonging to the Forest Engineering Department, from experimental area of the Federal University of *Mato Grosso do Sul, Chapadão do Sul* campus - *Chapadão do Sul - MS*. The climate is classified by the Köppen method as tropical humid the annual temperature is from 13 to 29 °C, the average rainfall is 1.850 mm, with rainfall concentration in summer and winter dryness (CUNHA; MAGALHÃES; CASTRO, 2013).

The experimental design was a randomized complete block, in a 5 x 3 factorial scheme, with 4 replicates. The treatments resulted in the use of five arabica coffee varieties (Topázio, Catuaí Amarelo, Catuaí Vermelho 99, Catuaí Vermelho 144 and Catiguá) combined with the application of biostimulant (25 mL L^{-1} of water), micronutrient (25 mL L^{-1} of water) and in the absence of biostimulant and micronutrient (control), the seeds were immersed in solution for two hours. The plots were composed of polyethylene trays of 96 tubes with conical shape, capacity of 120 mL.

The description of the biostimulant and the micronutrient was carried out according to the manufacturer's information. The biostimulant used was Stimulate®, a liquid product composed of three plant regulators containing 90 mg L^{-1} (0.009%) kinetin, 50 mg L^{-1} (0.005%) gibberellic acid, 50 mg L^{-1} (0.005% %) of indolebutyric acid and 99.981% of inert ingredients. The Power Seed® is a liquid fertilizer, foliar application, composed of two micronutrients, with 12.50% Molybdenum and 1.25% Cobalt.

Coffee cultivars sowing was carried out in 96-cell trays, filled with a standard substrate, composed of 70% sifted soil and 30% commercial Plantmax® substrate. Three coffee seeds were placed in each cell at a depth of 2 cm. After seedlings establishment that occurred 7 to 10 days after germination, thinning was performed leaving only one plant per cell. Germination occurred from 56 to 102 days after sowing. The evaluations were performed 150 days after sowing, in which the seedlings were removed from the trays, washed with tap water to remove the adherent substrate.

The evaluated parameters were: germination speed seed obtained by the formula: GSI = G1/N1 + G2/N2 + ... Gn/Nn (MAGUIRE, 1962); seedling height (cm) measured with millimeter ruler, from the collar to the apical bud; collar diameter, expressed in mm, measured using a pachymeter with an accuracy 0.01 mm; number of leaf pairs; leaf area (cm^2), estimated with leaf area meter LI-COR model LI-3000; shoot/root dry mass (grams) determined in a forced circulation oven at 75 ºC until constant weight; Dickson quality index obtained by the formula DQI = [total dry mass/(HDR + SRDMR)] (DICKSON; LEAF; HOSNER, 1960); shoot height/collar diameter ratio (HDR); height/shoot dry mass ratio (HSDMR), shoot/root dry mass ratio (SRDMR); total dry mass (grams), obtained by the sum of dry mass from leaves, stem and root.

The obtained data were submitted to variance analysis ($p< 0.05$), Tukey test was used, at a 5% probability level, according to the Sisvar computational program (FERREIRA, 2008).

3 RESULTS AND DISCUSSION

There was a significant effect ($p <0.05$), by the F test in all evaluated parameters. For the germination speed index (GSI), the seeds from different arabica coffee cultivars submitted to biostimulant and micronutrient application obtained different responses when compared to the control (Table 1).

The comparison among the treatments by the Tukey test (5%) indicates that the biostimulant presented better germination efficiency for the cultivars Catiguá, Catuaí Amarelo and Catuaí Vermelho 99 (Table 1). The highest total averages reached by the GSI were obtained for the Catuaí Amarelo cultivar, in the treatments with biostimulant and micronutrient, for these, there was no significant difference between them, indicating that when the biostimulant and the micronutrient were used the seedlings had better GSI. The results obtained for the germination speed index show different responses as to the type of cultivar within the treatments. The obtained results were similar to those obtained by Medina et al. (2016), in which there was greater stimulation of seed germination when the biostimulant FitoMas-E (obtained from a biochemical compound with high content of amino acids, nitrogenous bases, saccharides and polysaccharides potentially active, is used to increase and accelerate biochemical reactions, such as seed germination, root stimulation (ESQUIROL et al., 2016)) was applied in arabica coffee seeds.

The Catuaí Amarelo exhibited higher average values for plant height and number of leaf pairs per plant when compared to other cultivars. For plant height the highest average was obtained with the Catuaí Amarelo cultivar, and the Catuaí Vermelho 99 cultivar presented lower average for plant height and collar diameter, the biostimulant application provided higher plant height and number of leaf pairs for the Catuaí Amarelo variety (Table 2).

For the Topázio and Catuaí Vermelho 144 cultivars, the biostimulant use resulted in a lower plant height compared to the micronutrient and the control. The micronutrient application did not result in gains in height for coffee seedlings, but also did not affect the growth in height of the cultivars, unlike what was observed with the biostimulant in the Topázio and Catuai vermelho 99 cultivars (Table 2). According to Pierazan, Scalon and Pereira (2012), the combination of plant regulators, present in the biostimulant, can increase plant growth and development, stimulating cell division, differentiation and cell elongation.

More specifically, the gibberellic acid present in the biostimulants can influence in the seedlings elongation, acting in the stem and the leaves of the plants growth regulating the height (LAVAGNINI et al., 2014). Thus, we can infer that Catuaí Vermelho 99, Catiguá and Catuaí Amarelo cultivars were influenced by the Stimulate® composition, due to the gibberellin and cytokinin presence. The cytokinins act to promote the shoot growth, due to the increase of the cellular proliferation in the apical meristem of the stem (TAIZ et al., 2017); in addition, it can interact with other hormones, including gibberellin.

TABLE 1 - Germination speed index from the first emerged seedling of arabica coffee cultivars submitted to biostimulant and micronutrient application, *Chapadão do Sul - MS, 2015.*

	Cultivars				
	Topázio	Catuaí Vermelho 99	Catiguá	Catuaí Amarelo	Catuaí Vermelho 144
TRE	Germination speed index				
CONT	0.07 aB	0.07 aB	0.07 cB	0.08 bA	0.06 bB
BIO	0.06 bC	0.07 aB	0.10 aA	0.10 aA	0.04 cD
Co + Mo	0.04 cC	0.03 bC	0.08 bB	0.10 aA	0.10 aA
CV (%) = 4.79		DMS TRE = 0.007		DMS Cultivars = 0.006	

Averages followed by the same lowercase in the column and upper case in the line, do not indicate a significant difference between them by the Tukey test (5%). TRE = treatment; CONT = control; BIO = biostimulant; Co + Mo = cobalt + molybdenum; CV (%) = coefficient of variation; DMS = significant minimal difference.

TABLE 2 - Evaluation of plant height, collar diameter, number of leaf pairs at 196 days after sowing for different cultivars of arabica coffee submitted to biostimulant and micronutrient application, *Chapadão do Sul - MS*, 2015.

	Cultivars				
	Topázio	Catuaí Vermelho 99	Catiguá	Catuaí Amarelo	Catuaí Vermelho 144
TRE	Evaluation of plant height (cm)				
CONT	6.69 aC	5.66 aD	7.04 aBC	7.78 bA	7.20 aB
BIO	5.90 bD	5.68 aD	7.15 aB	8.88 aA	6.73 bC
Co + Mo	6.35 aC	5.70 aD	7.05 aB	7.75 bA	7.40 aAB
CV (%) = 2.94		DMS TRE = 0.41		DMS Cultivars = 0.35	
	Collar diameter (mm)				
CONT	2.50 aAB	2.13 aC	2.46 aB	2.59 aA	2.55 aAB
BIO	2.38 bA	2.14 aB	2.35 bA	2.23 bB	2.40 bA
Co + Mo	2.49 aB	2.11 aD	2.35 bC	2.63 aA	2.58 aAB
CV (%) = 2.10		DMS TRE = 0.10		DMS Cultivars = 0.08	
	Number of leaf pairs				
CONT	4.13 aB	4.25 aA	3.75 aB	4.13 bB	4.24 aB
BIO	3.63 bC	4.13 bA	3.88 aB	4.25 abA	4.25 aA
Co + Mo	3.63 bD	3.63 cD	3.88 aC	4.38 aA	4.13 aB
CV (%) = 1.82		DMS TRE = 0.15		DMS Cultivars = 0.13	

Averages followed by the same upper case in the line and lowercase in the column, do not differ each other by Tukey test (5%). TRE = treatment; CONT = control; BIO = biostimulant; Co + Mo = cobalt + molybdenum; CV (%) = coefficient of variation; DMS = significant minimal difference.

The Catuaí Vermelho 144 cultivar had the highest collar diameter average, and the Catuaí Vermelho 99 variety was the one that presented the lowest average value within this parameter (Table 2). The different cultivars obtained very different responses to the stem increase both in the presence or absence of the products. It was observed that with the biostimulant application for Topázio, Catuaí Amarelo and Catuaí Vermelho 144 cultivars presented positive results, differing from the other cultivars. With the micronutrient application, the lowest stem diameter growth result was for the Catuaí Vermelho 99 cultivar, differing from the other cultivars. The micronutrient use did not statistically differ when compared to the control within the cultivars, except for the Catiguá cultivar. The cultivars that exhibited the best growth of stem diameter in the three treatments were Topázio, Catuaí Amarelo and Catuaí Vermelho 144.

The biostimulant application damaged the leaves production of Topázio and Catuaí Vermelho 99 cultivars while the micronutrients use was harmful only to the Topázio cultivar (Table 2). The control statistically differed from the other treatments for Topázio cultivar, do not differ from the treatment with micronutrients within the Catuaí Vermelho 99 cultivar. For the number of leaf pairs it is observed that only Catuaí Amarelo cultivar responded positively to the biostimulant treatment, do not differ from the Mo+Co application. Corroborating with Medina et al. (2016), in which the FitoMas-E application also at dose 3.0 ml $^{-1}$ in the seeds imbibition and a second foliar application after 150 days of sowing promoted a greater number of coffee leaf pairs. Tecchio et al. (2015), evaluating the effect of Stimulate® on the growth of Kunquat 'Nagami' seedlings applied at different concentrations (0, 50,100, 150 and 200 mL L of solution), describe in their results that the dose of 200 mL L of Stimulate® promoted increase in plant height, number of leaves, root length and cup diameter of Kunquat 'Nagami' seedlings. For the treatment with biostimulant and micronutrient, the lowest average number of leaf pairs was observed in the Topázio cultivar, with a statistical difference among the others. The cultivars presented differentiated leaf pairs formation as a function of the products application.

Shoot, root and leaf area dry mass of arabica coffee cultivars obtained positive responses to the biostimulant and micronutrient application (Table 3).

The biostimulant application damaged the shoot dry mass production of Topázio and Catuaí Vermelho 144 cultivars, while the micronutrient was harmful to the Catuaí Vermelho 99 cultivar. The Catuaí Amarelo cultivar obtained the highest shoot dry mass, when compared to the other cultivars, both for the absence of products, and in the biostimulant and micronutrient presence (Table 3).

Regarding root dry mass, Catuaí Amarelo cultivar had a higher total averages value for root dry mass production, and the lowest root dry mass production occurred within the Catuaí Vermelho 99 cultivar. The biostimulant application provided the highest root growth for Catuaí Vermelho 99 cultivar, differing from the treatment with micronutrient and the control. The biostimulant application damaged the root growth for Catuaí Amarelo cultivar, differing from the other treatments (Table 3). It can be inferred that the presence of the cytokinin hormone had harmed the root development, because it acts on the cellular differentiation in the apical meristem, a contrary effect to that cytokinin and auxin, in which it promotes the cellular division of the apical meristem (TAIZ et al., 2017).

The micronutrient application in the Catiguá, Catuaí Amarelo and Catuaí Vermelho 144 cultivars presented a better root mass development, differing from the control for the Catiguá cultivation, and differing from the other treatments of Catuaí Amarelo and Catuaí Vermelho 144 cultivars. When comparing the cultivars within the treatments, the micronutrients application had the highest production of averages in relation to the biostimulant application and the control (Table 3).

The leaf area statistical evaluation showed that the biostimulant application resulted in higher averages for the Catuaí Amarelo cultivar, which differed from the other cultivars, had obtained an average higher than its control (Table 3). In results obtained by Medina et al. (2016) the use of Fitomas-E biostimulant applied at planting and after 150 days of planting in arabica coffee promoted increase to leaf area, when compared to the control. Scalon et al. (2009) also observed increased leaf area of *C. adamantium* seedlings with the use of biostimulant, however, Canesin et al (2012) warn that the use of high doses of biostimulant can inhibit metabolic processes. Thus, the general action of the plant regulators presents on the biostimulant, acting on the cellular division (PIERAZAN; SCALON; PEREIRA, 2012) and gibberellic acid acting on the leaves (LAVAGNINI et al., 2014) may have contributed to the increase of leaf area.

Comparing the treatments within the cultivars, the highest leaf area average was produced by the control, in relation to the other treatments, and the biostimulant presented the lowest average value. The Catuaí Amarelo cultivar presented the best performance of leaf area production, differing from the other cultivars in all treatments. Catuaí Vermelho 99 cultivar showed the lowest averages among the treatments differing from the other cultivars, except for Topázio cultivar within the biostimulant treatment (Table 3).

Catuaí Amarelo cultivar presented greater leaf area, whereas the Catuaí Vermelho 99 cultivar was the one that presented smaller leaf area.

TABLE 3 - Evaluation of shoot dry mass, root dry mass, and leaf area for arabica coffee cultivars submitted to biostimulant and micronutrient application, *Chapadão do Sul - MS*, 2015.

	Cultivars				
	Topázio	Catuaí Vermelho 99	Catiguá	Catuaí Amarelo	Catuaí Vermelho 144
TRE	Evaluation of shoot dry mass (g)				
CONT	1.29 aC	0.84 bE	1.17 cD	1.59 bA	1.44 bB
BIO	0.89 cE	0.97 aD	1.29 bB	1.69 aA	1.22 cC
Co + Mo	1.05 bC	0.59 cD	1.37 aB	1.62 bA	1.62 aA
CV (%) = 2.78		DMS TRE = 0.07		DMS Cultivars = 0.06	
	Root dry mass (g)				
CONT	0.54 aB	0.38 bC	0.53 bB	0.69 bA	0.63 bA
BIO	0.49 abC	0.45 aC	0.58 abB	0.64 cAB	0.64 bA
Co + Mo	0.48 bC	0.30 cD	0.59 aB	0.76 aA	0.76 aA
CV (%) = 5.30		DMS TRE = 0.06		DMS Cultivars = 0.05	
	Leaf area (cm²)				
CONT	17.10 aC	15.39 aD	16.95 aC	18.58 cA	17.94 aB
BIO	15.29 cC	15.41 aC	17.16 aB	19.55 aA	17.09 bB
Co + Mo	16.02 bD	14.52 bE	17.14 aC	18.88 bA	18.22 aB
CV (%) = 0.28		DMS TRE = 0.33		DMS Cultivars = 0.97	

Averages followed by the same upper case in the line and the same lowercase in the column, do not differ each other by Tukey test (5%). TRE = treatment; CONT = control; BIO = biostimulant; Co + Mo = cobalt + molybdenum; CV (%) = coefficient of variation; DMS = significant minimal difference.

There was no statistically difference of the treatments in the Catiguá cultivar, for Topázio and Catuaí Vermelho 99 cultivars the control did not differ from the biostimulant treatment, differing only from the micronutrient application. The biostimulant application for Catuaí Amarelo cultivar was the one that presented higher average value, differing from the control and the treatment of micronutrient. The micronutrient treatment was the best for Catuaí Vermelho 144 cultivar do not differ from the control (Table 3).

The height/diameter ratio (HDR), height/shoot dry mass ratio (HSDMR), shoot/root dry mass ratio (SRDMR), Dickson Quality Index (DQI) showed significant responses to the different cultivars as a function of the biostimulant and micronutrient application (Table 4).

When the biostimulant was applied to height/diameter ratio (HDR), the cultivar that obtained the highest average was Catuaí Amarelo, differing from the other cultivars and being larger than the average of the control (Table 4).

Using micronutrients, the Catiguá cultivar obtained the highest average, but there was no statistical differentiation of Catuaí Amarelo and Catuaí Vermelho 144 cultivars. The Catiguá and Catuaí Vermelho 99 cultivars obtained higher averages than those of their control. The Topázio cultivar had the lowest average, but it was not statistically different from Catuaí Vermelho 99 cultivar (Table 4). The values from height/diameter ratio (HDR) varied from 2.48 to 3.98, different from the recommendation by Marana et al. (2008), wherein reasonable values for arabica coffee are from 3.5 to 4.0. These authors argue that values greater than 4.0 indicate excessive seedlings development at height, and smaller values indicate less development.

For the height/shoot dry mass ratio (HSDMR), with the biostimulant application, it was observed that Topázio cultivar obtained the highest average, differing from the other cultivars and being superior to control. The Catuaí Amarelo cultivar presented the lowest average, but there was no difference with the Catiguá and Catuaí Vermelho 144 cultivars.

TABLE 4 - Average values for shoot height/collar diameter ratio, height/shoot dry mass ratio, shoot/root dry mass ratio and Dickson quality index for cultivars of arabica coffee submitted to biostimulant and micronutrient application, *Chapadão do Sul*, MS, 2015.

	Cultivars				
	Topázio	Catuaí Vermelho 99	Catiguá	Catuaí Amarelo	Catuaí Vermelho 144
TRE	Height/collar diameter ratio				
CONT	2.69 aBC	2.66 aC	2.86 bAB	3.00 bA	2.82 aABC
BIO	2.48 bD	2.66 aCD	3.04 aB	3.98 aA	2.80 aC
Co + Mo	2.55 abC	2.70 aBC	3.00 abA	2.94 bA	2.87 aAB
CV (%) = 3.25		DMS TRE = 0.19		DMS Cultivars = 0.16	
	Height/shoot dry mass ratio				
CONT	5.19 cC	6.75 bA	6.00 aB	4.89 abC	5.00 bC
BIO	6.63 aA	5.85 cB	5.55 bBC	5.25 aC	5.54 aBC
Co + Mo	6.05 bB	9.68 aA	5.16 bC	4.79 bCD	4.58 bD
CV (%) = 4.22		DMS TRE = 0.49		DMS Cultivars = 0.42	
	Shoot/root dry mass ratio				
CONT	2.41 aA	2.22 aA	2.23 aA	2.30 bA	2.28 aA
BIO	1.84 bC	2.18 aB	2.25 aB	2.67 aA	1.90 bC
Co + Mo	2.22 aA	1.95 bB	2.32 aA	2.13 bAB	2.13 aAB
CV (%) = 5.89		DMS TRE = 0.26		DMS Cultivars = 0.22	
	Dickson quality index				
CONT	0.36 aB	0.25 bC	0.33 bB	0.43 bA	0.41 bA
BIO	0.32 bC	0.29 aC	0.35 abB	0.35 cB	0.39 bA
Co + Mo	0.32 bC	0.19 cD	0.37 aB	0.47 aA	0.48 aA
CV (%) = 4.30		DMS TRE = 0.03		DMS Cultivars = 0.03	

Averages followed by the same upper case in the line and the same lowercase in the column, do not differ from each other by the Tukey test (5%). TRE = treatment; CONT = control; BIO = biostimulant; Co + Mo = cobalt and molybdenum; CV (%) = coefficient of variation; DMS = significant minimal difference.

The Catuaí Vermelho 99 cultivar presented the highest average in relation to the others with the micronutrients application. Catuaí Vermelho 144 showed the lowest average, but did not differ statistically from Catuaí Amarelo cultivar (Table 4). According to Gomes and Paiva (2011) as lower this index, more lignified will be the seedling and greater its survival capacity in the field. The biostimulant application obtained higher performance for the Topázio and Catuaí Vermelho 144 cultivars differing from the other treatments, whereas for the Catuaí Amarelo cultivar it also presented higher performance, but did not differ from the control. The best cultivar for the control was Catuaí Vermelho 99, differing from the other cultivars. In the treatment of Co + Mo the cultivar with the best response to treatment was Catuaí Vermelho 99, differing statistically from the other cultivars.

The biostimulant application in shoot/root dry mass ratio (SRDMR) the Catuaí Amarelo cultivar obtained the highest average, differing from all other cultivars. Topázio and Catuaí Vermelho 144 cultivars presented the smallest average, do not differ from each other. The Catiguá cultivar obtained the highest average with the micronutrients application, but there was no difference with Topázio, Catuaí Amarelo and Catuaí Vermelho 144 cultivars. From the cultivars cited, only Catiguá obtained higher average than control. Catuaí Vermelho 99 cultivar obtained the lowest average in relation to the others (Table 4).

For shoot/root dry mass ratio (SRDMR), Marana et al. (2008) reached the value 4.7 as the best ratio. The same authors affirm that values smaller than 4.7 suggest that the seedling did not have a good shoot increment, and values over 7.0 the development of the root system apparently was insufficient. According to Lima et al. (2008), this imbalance can damage the seedlings adaptation after planting in a definitive location, due to the fact that the root system is small, making it difficult to absorb water and support seedling in the soil.

Regarding to the Dickson Quality Index (DQI), Marana et al. (2008) report that quality seedlings are those with a minimum value 0.21. When the biostimulant was used, Catuaí Vermelho 144 cultivar had the highest DQI, but this index was lower than the control, proving that Trazzy, Caldeira e Colombi (2010) concluded in their work that Dickson Quality Index is variable according to species, management of seedlings in the nursery, substrate proportion type, container volume and age at which seedling was evaluated. Topázio and Catuaí Vermelho 99 obtained the lowest DQI and did not differ statistically.

When applied the micronutrient, Catuaí Vermelho 144 cultivar was that obtained the highest quality index that did not differ statistically from Catuaí Amarelo. Both had higher quality index than their control (Table 4). Gomes and Paiva (2011) affirm that when higher the index value, better the seedlings standard quality.

4 CONCLUSIONS

The application of biostimulant and micronutrient did not present uniformity among the evaluated parameters for the different cultivars, and the cultivar Catuaí Amarelo presented better performance in the parameters evaluated with the application of biostimulant, indicating that the application of Stimulate® is an alternative with potential for the use of seedlings of this cultivar. However, there was a negative effect on the application of biostimulant and micronutrient to Topázio cultivar.

5 REFERENCES

ALVARENGA, S. M. et al. Marcadores moleculares derivados de sequências expressas do genoma café potencialmente envolvidas na resistência à ferrugem. **Pesquisa Agropecuária Brasileira,** Brasília, v. 46, n. 8, p. 890-898, Aug. 2011.

ALVES, J. D.; GUIMARÃES, R. J. Sintomas de desordens fisiológicas em cafeeiro. In: GUIMARÃES, R. J.; MENDES, A. N. G.; BALIZA, D. P. (Ed.). **Semiologia do cafeeiro: sintomas de desordens nutricionais, fitossanitárias e fisiológicas.** Lavras: UFLA, 2010. p. 169-215.

BINSFELD, J. A. et al. Uso de bioativador, bioestimulante e complexo de nutrientes em sementes de soja. **Pesquisa Agropecuária Tropical,** Goiás, v. 44, n. 1, p. 88-94, Jan/Mar. 2014.

BONOMO, R. et al. Produtividade de cafeeiros arábica irrigados no Cerrado Goiano. **Pesquisa Agropecuária Tropical**, Goiás, v. 38, n. 4, p. 233-240, Oct/Dec. 2008.

CANESIN, Â. et al. Bioestimulante no vigor de sementes e plântulas de faveiro (*Dimorphandra mollis* Benth.). **Cerne**, Lavras, v. 18, n. 2, p. 309-315, Apr/June. 2012.

CONAB, Companhia Nacional de Abastecimento. **Acompanhamento de safra brasileira de café,** Safra 2017. Conab, v. 4, n. 2, p. 1-104, May. 2017.

CUNHA, F. F.; MAGALHÃES, F. F.; CASTRO, M. A. Métodos para estimativa da evapotranspiração de referência para Chapadão do Sul – MS. **Engenharia na agricultura**, Viçosa, v. 21, n. 2, p. 159-172, Mar. 2013.

DICKSON, A.; LEAF, A. L.; HOSNER, J. F. Quality appraisal of white spruce and white pine seedling stock in nurseries. **Forest Chronicle**, Ontário, v. 36, n. 1, p. 10-13, 1960.

ESQUIROL, C. F. R. et al. FitoMas-E: una alternativa para el enraizamiento in vitro de cultivares de caña de azúcar. **Biotecnología Vegetal**, Cuba, v. 16, n. 4, p. 239-244, Oct/Dec. 2016.

FAROOQ, M.; WAHID, A. Y.; SIDDIQUE, K. H. M. Micronutrient application through seed treatments. **Journal of Soil Scienceand Plant Nutrition**, Temuco, v. 12, n. 1, p. 125-142, Jan/Mar. 2012.

FERREIRA, D. F. SISVAR: um programa para análises e ensino de estatística. **Revista Symposium**, Lavras, v. 6, n. 2, p. 36-41, July. 2008.

GOMES, J. M.; PAIVA, H. N. DE. **Viveiros Florestais: propagação sexuada**. 1. ed. Viçosa: UFV, 2011. 116 p.

KISSMANN, C. et al. Biorregulador e pré-condicionamento osmótico na germinação de sementes e no crescimento inicial da muda de carobinha (*Jacaranda decurrens* subsp. *symmetrifoliolata* Farias & Proença) -Bignoniaceae. **Revista Brasileira de Plantas Medicinais**, Botucatu, v. 13, n. 1, p. 58-67, Jan/Mar. 2011.

LAVAGNINI, C. G. et al. Fisiologia vegetal-hormônio giberelina. **Revista Científica Eletrônica de Agronomia,** Garças, v. 25, n. 1, p. 48-52, July. 2014.

LIMA, J. D. et al. Efeitos da luminosidade no crescimento de mudas de *Caesalpinia ferrea* Mart. ex Tul. (*Leguminosae, Caesalpinoideae*). **Acta Amazonica**, Manaus, v. 38, n. 1, p. 5-10, Jan/Mar. 2008.

MAGUIRE, J. D. Speed of germination aid in selection and evaluation for seedling emergence and vigor. **Crop Science,** Madison, v. 2, n. 2, p. 176-77, Mar. 1962.

MALAVOLTA, E. **Manual de nutrição mineral de plantas**. 2. ed. São Paulo: Agronômica Ceres, 2006. 638 p.

MARANA, J. P. et al. Índices de qualidade e crescimento de mudas de café produzidas em tubetes. **Ciência Rural**, Santa Maria, v. 38, n. 1, p. 39-45, Jan/Fev. 2008.

MEDINA, A. D. et al. Influencia del bioestimulante FitoMas-E sobre la producción de posturas de cafeto (*Coffea arabica* L.). **Centro Agrícola,** Santa Clara, v. 43, n. 4, p. 29-35, Oct/Dec. 2016.

PERDONÁ, M. J.; SORATTO, R. P.; ESPERANCINI, M. S. T. Desempenho produtivo e econômico do consórcio de cafeeiro arábica e nogueira-macadâmia. **Pesquisa Agropecuária Brasileira**, Brasília, v. 50, n. 1, p. 12-23, Jan. 2015.

PIERAZAN, L.; SCALON, S. P. Q.; PEREIRA, Z. V. Emergência de plântulas e crescimento de mudas de jatobá com uso de bioestimulante e sombreamento. **Cerne**, Lavras, v. 18, n. 1, p. 127-133, Jan/Mar. 2012.

PRADO NETO, M. et al. Germinação de sementes de jenipapeiro submetidas à pré-embebição em regulador e estimulante vegetal. **Ciência e Agrotecnologia,** Lavras, v. 31, n. 3, p. 693-698, May/June. 2007.

SCALON, S. P. Q. et al. Germinação de sementes e crescimento inicial de mudas de *Campomanesia adamantium* Camb.: Efeito da lavagem, temperatura e de bioestimulantes. **Revista Brasileira de Sementes**, Londrina, v. 31, n. 2, p. 96-103, Apr/June. 2009.

SFREDO, G. J.; OLIVEIRA, M. C. N. DE. **Soja: molibdênio e cobalto**. 21. Ed. Paraná: Documentos Embrapa Soja, 2010. 36 p.

SILVA, L. C. F. et al. Agricultura bioestimulada. **Cultivar Grandes Culturas**, São Carlos, v. 14, n. 162, p. 34-35, Nov. 2012.

SILVA, C. A.; TEODORO, R. E. F.; MELO, B. Produtividade e rendimento do cafeeiro submetido a lâminas de irrigação. **Pesquisa Agropecuária Brasileira**, Brasília, v. 43, n. 3, p. 387-394, Mar. 2008.

TAIZ, L. et al. (eds.). **Fisiologia e desenvolvimento vegetal**. 6 ed. Porto Alegre: Artmed, 2017. 858 p.

TECCHIO, M. A. et al. Stimulate no desenvolvimento de mudas de Kunquat ‘nagami’. **Irriga,** Botucatu, v. 1, n. 1, p. 97-106, Jan/Apr. 2015.

TRAZZY, P. A.; CALDEIRA, M. V. W.; COLOMBI, R. Avaliação de mudas de *Tecoma stans* utilizando biossólido e resíduo orgânico. **Revista de Agricultura**, Piracicaba, v. 85, n. 1, p. 218-226, 2010.

WEISANY, W.; RAEI, Y.; ALLAHVERDIPOOR, K. H. Role of some of mineral nutriens in biological nitrogen fixation. **Bulletin of Environment, Pharmacology and life sciences**, India, v. 2, n. 4, p. 77–84, Mar. 2013.

CONTRIBUTION OF AGRONOMIC TRAITS TO THE COFFEE YIELD OF *Coffea canephora* Pierre ex A. Froehner IN THE WESTERN AMAZON REGION

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ABSTRACT: The evaluation of morphological characters related to the hulled coffee yield subsidizes the selection of *Coffea canephora* plants that combine a set of favorable traits. The aim of this study was to evaluate the direct and indirect effects of agronomic traits on the production of hulled coffee to subside the plant selection. To this, nine morphological descriptors were evaluated of 130 clones of the botanical varieties Conilon and Robusta over two crop years in the experimental field of Embrapa, in the municipality of Ouro Preto do Oeste, state of Rondônia (RO). To quantify the genetic variability the path analysis and the Scott Knott cluster test were used. The effect of genotype x year interaction was significant for eight of the nine characteristics analyzed. The genotypes were clustered in three to five classes, subsidizing the establishment of a scale to evaluate the variability of this genetic resource. Pathway analysis indicated that the number of plagiotropic branches and the number of rosettes per productive branch were the traits that exhibited the greatest direct effect on hulled coffee yield. These results show that it is possible to select plants with complementarity traits which favor a higher production of hulled coffee.

Index terms: Conilon coffee, Robusta coffee, path analysis, genetic variability.

CONTRIBUIÇÃO DE CARACTERES AGRONÔMICOS PARA A PRODUTIVIDADE DE CAFÉ BENEFICIADO DE *Coffea canephora* Pierre ex A. Froehner NA REGIÃO AMAZONICA OCIDENTAL

RESUMO: A utilização simultânea de caracteres morfológicos associados à produtividade de café beneficiado subsidia a seleção de plantas de *Coffea canephora* que apresentem um conjunto de características favoráveis. O objetivo desse estudo foi avaliar os efeitos diretos e indiretos de características agronômicas sobre a produção de café beneficiado de *C. canephora* subsidiando a seleção de plantas. Para isso foram considerados nove descritores morfológicos avaliados em 130 clones das variedades botânicas Robusta e Conilon ao longo de dois anos agrícolas no campo experimental da Embrapa, no município de Ouro Preto do Oeste, estado de Rondônia (RO). Para quantificar a variabilidade genética, utilizou-se a análise de trilha e o teste do agrupamento Scott Knott. O efeito da interação genótipo x ano foi significativo para oito das nove características analisadas. Os genótipos foram agrupados em três a cinco classes, delimitando uma escala que pode ser utilizada para avaliar a variabilidade deste recurso genético. A análise de trilha indicou que o número de ramos plagiotrópicos e o número de rosetas por ramo produtivo foram as características que apresentaram o maior efeito direto sobre o rendimento do café beneficiado.

Termos para indexação: Café Conilon, café Robusta, análise de trilha, variabilidade genética.

1 INTRODUCTION

The *Coffea canephora* Pierre ex A. Froehner represents approximately 35% of world coffee production and it is characterized by high plant vigor and high yield (Ico, 2016). In the Western Amazon, the state of Rondônia stands out as a traditional coffee producer with approximate production of 1.93 million bags of hulled coffee in 2017 (Conab, 2017).

This species presents two botanical varieties with distinct characteristics that are commercially cultivated (Davis et al., 2006). The botanical variety Robusta is characterized by erect growth, larger leaf size, higher average sieve, late maturation, less tolerance to water deficit, and greater resistance to diseases and pests. The botanical variety Conilon is characterized by shrub growth, early maturation, elongated leaves, greater tolerance to drought, and greater susceptibility to pests and diseases (Montagnon et al., 2012; Oliveira et al., 2018).

In modern agriculture, two main strategies are considered to increase yield per area: planting genetically superior materials and improving growing conditions (Ramalho et al., 2012; Ferrão et al., 2017). In various crops, an additive relationship between these factors has been observed, the result of the expression of superior genetic potential with improvement of growing conditions (Dubberstein et al., 2017; Rodrigues et al., 2016). In classical breeding of *C. canephora*, the aim is to select plants with superior yield potential associated with greater uniformity of ripening, greater sieve size, and smaller plant architecture (Teixeira et al., 2017).

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In addition to the long reproductive cycle, the overlapping of generations, and the vigor that is manifested in plants from divergent crosses; association among the coffee bean yield components has considerable implications for breeding of this species (Souza et al., 2017; Rocha et al., 2015). According to Cruz et al. (2014), genetic gain from direct selection is defined as changes in the population mean resulting from selection practiced on the trait itself. However, it is known that selection in one main trait may result changes that can favor or hinder the selection of plants that combine a series of favorable characteristics.

Pathway analysis, originally proposed by Wright (1923), allows the correlation coefficients to be broken down into their direct and indirect effects on a main trait. Appropriate interpretation of pathway analysis depends on the creation of a cause and effect diagram, which represents the relationship of secondary traits on a main trait when complex relationships are involved (Cruz et al., 2014).

Bikila and Sakiyama (2017) observed significant estimates of the simple correlation coefficient between yield, number of plagiotropic branches and plant height. Cilas et al. (2006) observed significant phenotypic correlations among the distance between rosettes, the number of rosettes and hulled coffee yield. Ferrão et al. (2008a) observed higher magnitude of the genotypic correlations compared to the environmental correlations, selecting plants of higher yield potential. Studies were not found in the literature that estimated the direct and indirect effects of other agronomic traits on *C. canephora* hulled coffee yield.

In this context, the aim of this study was to quantify the direct and indirect effects of morphological and yield traits on the hulled coffee bean yield of *C. canephora,* for the purpose of subsidizing the characterization and use of the genetic variability of this species.

2 MATERIALS AND METHODS

Field experiment

Over the 2013-2014 and 2014-2015 crop years, nine morphological and yield descriptors were evaluated from 130 genotypes with characteristics of the botanical varieties Robusta (37), Conilon (75), and interspecific hybrids (18): plant height (PHt), number of plagiotropic branches (NPLAG), number of rosettes per branch (NROS), length of plagiotropic branches (LPLAG), number of fruits per rosette (FROS), distance between rosettes (DROS), number of days to fruit ripening (NDAYS), hulled coffee bean yield (YLD), and mean sieve size (SIEV). The field experiment was managed according to Marcolan et al. (2009).

The assay installed in November 2011 was carried out in the experimental field of Embrapa, in the municipality of Ouro Preto do Oeste, state of Rondônia (RO). The Climate in the region, according to Köppen, is tropical rainy, with mean annual rainfall of 1939 mm/year and mean annual temperatures from 21.2ºC to 30.3ºC. The region is located at 10º37'03''S and 62º51'50''W, with relative humidity throughout the year around 81%. Soil in the experimental area is eutrophic red-yellow oxisol with clayey texture and flat topography, it is a deep and well-drained soil. The experimental design was randomized blocks with four replications of four plants per plot at spacing of 3.5 x 1.5 meters, between plants and between rows, respectively.

Multicollinearity diagnosis and pathway analysis

Data were subjected to analysis of variance and analysis of homoscedasticity. With the aim of clustering the clones in divergent and mutually exclusive groups, the Scott Knott mean clustering test was used at 5% probability (Cruz, 2013).

Pathway analysis was conducted by estimating the phenotypic correlations (r_f) between the traits and their significance was tested at 1% and 5% probability by the t test at $(n-2)$ degrees of freedom (Cruz et al., 2014). Multicollinearity was diagnosed according to the condition number (CN) of the singular matrix X'X, defining the ratio between the largest and smallest eigen value of the correlation matrix. According to Montgomery & Peck, (1981), if CN<100, multicollinearity is weak and does not constitute a problem for analysis; if 100<CN<1000 multicollinearity is moderate to strong; and if CN>1000, it is severe.

To obtain estimates of the direct and indirect effects, a causal diagram was established that considers the logical, additive relationship among the primary and secondary yield components (Figure 1). The statistical analyses were done using the statistical software Genes, version 1990.2018.18 (Cruz, 2013).

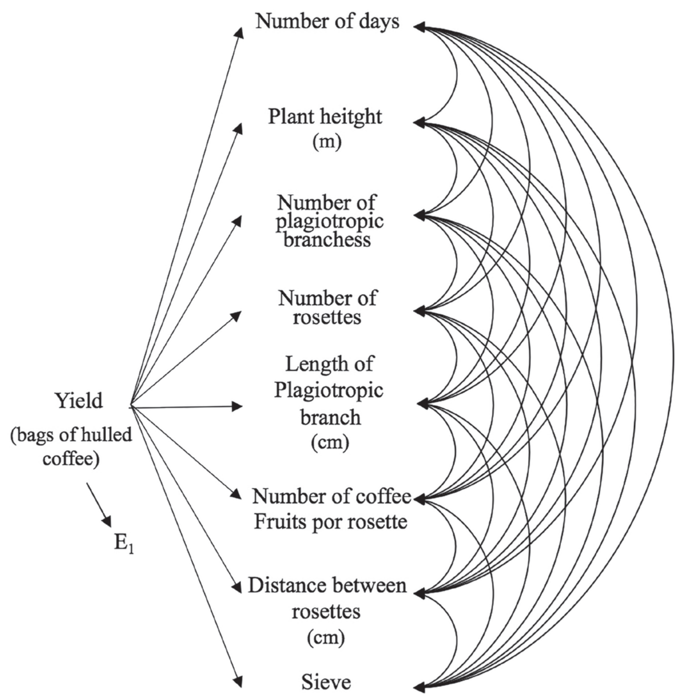

FIGURE 1 - Illustrative diagram representing in the single-headed arrows, the direct effects of eight explanatory variables on the main variable, yield. The bidirectional arrows represent the indirect effects quantified by the phenotypic correlation coefficients.

3 RESULTS AND DISCUSSION

The effect of the clone × year interaction wasn't significant for only one of the nine traits analyzed (Table 1). The significant effect of the clones x year interaction difficult the improvement of perennial species, as it results in a decrease in the genetic progress obtained with plant selection resulting from changes in the order of clones selected from one year to the next. Expressive clone × year interaction were also observed by Souza et al. (2013) and Montagnon et al. (2012) in genotypes evaluated in the state of Espírito Santo and in Ivory Coast.

Characterization of *C. canephora* genotypes should consider both superior performance and maintenance of superiority over time (Silva et al., 2017). Although the effect of the genotype × year interaction was significant, except for SIEV, the repeatability estimates of the traits evaluated indicate the predominance of simple interaction (Resende; Duarte, 2007) (Table1). Simple interaction is characterized by smaller changes in ordering of the genotypes over time, unlike complex interaction, which is characterized by the absence of correlation in ordering of genotypes over time (Cruz et al., 2014).

The Scott Knott cluster test at 5% probability was used with the aim of grouping the genotypes in mutually exclusive classes. This test allows the genotypes to be grouped in sets of minimum variation within groups and maximum variation among groups, making it easier to interpret results, due to the absence of ambiguity (Bhering et al., 2008). The clones evaluated were clustered in three to five mutually exclusive groups, depending on the trait (Table 2).

The grouping based in the number of days to fruit ripening (NDAYS) indicated the existence of three different times of fruit ripening, with approximately 20 days difference between them (Table 2). This grouping corroborates the variability observed in the field in which clones that mature in Abril, May, and June are called early, intermediate, and late clones, respectively. Partelli et al. (2014) and Morais et al. (2012) observed genotypes with fruit ripening in March and July called super early and super late, respectively.

For hulled coffee yield (YLD), four different yield classes were observed, with amplitude from 8.8 to 72.9 $bags.ha^{-1}$ in the first harvest and from 11.3 to 123.7 $bags.ha^{-1}$ in the second harvest (Table 2).

TABLE 1 - Summary of the F estimates of the joint analyses variance for the traits: number of days to fruit ripening (NDAYS), plant height (PHt), number of plagiotropic branches (NPLAG), number of rosettes per branch (NROS), length of plagiotropic branches (LPLAG), number of fruits per rosette (FROS), distance between rosettes (DROS), yield (YLD), and mean sieve size (SIEV) of *C. canephora* coffee, evaluated in the 2013-2014 and 2014-2015 crop years.

S.V.	NDAYS	PHt	NPLAG	NROS	LPLAG	FROS	DROS	SIEV	YLD
Blocks									
Clones	6.78^{**}	5.52^{**}	5.92^{**}	1.79^{**}	4.94^{**}	1.51^{*}	2.72^{**}	4.57^{**}	3.64^{**}
Years	2.10^{**}	1387.98^{**}	169.47^{**}	68.43^{**}	14.96^{**}	26.33^{**}	24.67^{**}	0.51^{NS}	148.38^{**}
Clones xYears	2.94^{**}	4.43^{**}	2.29^{**}	3.11^{**}	2.70^{**}	8.12^{**}	7.84^{**}	2.96^{NS}	3.90^{**}
Residue									
Mean	302.0	1.58	81.34	10.33	0.79	17.01	4.91	15.09	43.31
$Mean_{1st\ measure}$	302.7	1.32	67.95	9.44	0.77	18.09	5.06	15.12	33.70
$Mean_{2nd\ measure}$	301.2	1.84	94.72	11.22	0.80	15.92	4.76	15.06	52.91
Repeatability	85.25	81.89	83.13	44.38	79.83	33.68	63.23	78.14	72.51
CV	3.25	6.65	26.91	19.09	9.43	14.03	7.25	5.43	29.72

*and ** = significant at 1% and 5% probability by the F test, respectively. r = repeatability; CV = coefficient of variation.

TABLE 2 - Grouping by interval of classes of the *C. canephora* coffee yield traits. Embrapa, RO, Brazil.

NDAYS 2013-2014		NDAYS 2014-2015		PHt 2013-2014		PHt 2014-2015		NPLAG 2013-2014		NPLAG 2014-2015	
Classes	f	Classes	f	Classes	f	Classes	f	Classes	f	Classes	f
271|--|288	34	262 |--| 286	28	0.9|--| 1.3	59	1.4|--|1.6	32	13|--|71	79	13|--|73	31
295|--|314	71	288 |--| 314	78	1.3|--| 1.5	52	1.6|--|1.8	27	75|--|100	32	74|--|102	44
317|--|332	25	317 |--| 349	24	1.5|--| 1.6	14	1.8|--|1.9	44	104|--|121	16	103|--|128	34
-	-	-	-	1.6|--| 1.9	5	2.0|--|2.4	27	136|--|143	3	129|--|206	21
LPLAG 2013-2014		**LPLAG 2014-2015**		**NROS 2013-2014**		**NROS 2014-2015**		**FROS 2013-2014**		**FROS 2014-2015**	
Classes	**f**	**Classes**	**f**	**Classes**	**f**	**Classes**	**f**	**Classes**	**f**	**Classes**	**f**
0.50|--|0.77	70	0.50|--|0.78	60	5.7|--|7.6	21	5.7|--|11.4	74	9|--|17	52	10|--|15	75
0.78|--|0.86	38	0.79|--|0.86	41	7.7|--|9.5	48	11.5|--|13.3	34	18|--|22	66	16|--|19	35
0.87|--|0.94	16	0.87|--|0.94	17	9.6|--|11.2	40	13.5|--|14.4	14	23|--|27	10	20|--|23	12
0.96|--|1.00	6	0.95|--|1.12	12	11.3|--|14.5	21	14.7|--|22.0	8	30|--|35	2	23|--|30	8
DROS 2013-2014		**DROS 2014-2015**		**YLD 2013-2014**		**YLD 2014-2015**		**SIEV 2013-2014**		**SIEV 2014-2015**	
Classes	**f**	**Classes**	**f**	**Classes**	**f**	**Classes**	**f**	**Classes**	**f**	**Classes**	**f**
3.6|--|4.3	18	3.4|--|4.3	34	8.8|--|20.8	36	11|--|46	54	19|--|18	10	19|--|18	11
4.4|--|5.4	81	4.4|--|5.4	50	21.8|--|46.8	66	47|--|70	45	17|--|16	33	17|--|16	33
5.5|--|5.9	20	5.1|--|5.9	10	47.5|-- | 56.5	20	72|--|81	21	15|--|14	65	15|--|14	67
6.0|--|7.2	11	6.0|--|7.5	36	58.4 |--| 72.9	8	85|--|123	10	13|--|12	18	13|--|12	17
-	-	-	-	-	-	-	-	11|--|10	4	11|--|10	2

f = simple absolute frequency; NDAYS = number of days to fruit ripening; PHt = plant height; NPLAG = number of plagiotropic branches; LPLAG = length of plagiotropic branches; NROS = number of rosettes; DROS = distance between rosettes; FROS = number of fruits per rosette; YLD = hulled coffee yield; SIEV = mean sieve size.

Hulled coffee yield is a quantitative trait of continuous distribution expression, influenced both by the genotype and by the environment (Cruz et al., 2014). The establishment of mutually exclusive groups ordered according to the variability of the trait, allowed establishment of scales that differentiate plants with low, medium, high, and very high yield (Table 2).

Plant height (PHt) was grouped in four different classes, with amplitude from 0.92 to 1.87 m in the first harvest and from 1.36 to 2.39 min the second; a mean increase of 0.90 m between the first and second year of evaluation was observed. Bergo et al. (2008) observed genotypes of *C. canephora* that after four years of growth in the field exhibited mean height of 3.55 m in comparison with the cultivars Icatu and Catuaí of *C. arabica*, which exhibited 3.33 m and 2.71 m height, respectively. According to Ferrão et al. (2008b), this trait is predominantly under genetic control, with heritability estimates higher than 0.60.

The NPLAG and LPLAG traits were grouped in four different classes (Table 2). For NPLAG, an increase in the mean value from 26 to 47 plagiotropic branches was observed between the first and second year; and for LPLAG, a mean increase of 120 cm was observed between the first and the second year (Table 2). This classification allows to identify plants of greater yield potential, which stand out through a higher number and greater length of productive branches.

The NROS, FROS, and DROS traits were also grouped in four different classes. The NROS exhibited an increase in the mean number of rosettes over time, from 8.8 to 16.3, unlike FROS, which exhibited a similar mean performance over time of 18.09 in the first year and 15.92 in the second year of evaluation (Table 2). For DROS were identified groups with minimal, small, intermediate, and large distance between rosettes (Table 2).

In relation to mean sieve size (SIEV), distribution of clones was observed in five classes, with amplitude from 10 to 19, with a small variation over time of 14.82 in the first year and 14.75 in the second year. Ramalho et al. (2016) observed greater amplitude in genotypes of the Conilon botanical variety, from 13.6 to 17 and a mean sieve size of 15.4.

The study of the direct and indirect effects of the plant traits on coffee yield allows to identify the degree of importance of the explanatory variables to the main variable (Sureshkumar et al., 2013). The pathway diagram shows the relationship between the primary components and hulled coffee yield (Figure 1). To estimate the direct and indirect effects of pathway analysis, it is necessary that the X'X matrix not have high levels of multicollinearity, since in the presence of multicollinearity, the variances associated with the estimators of the pathway coefficients can reach excessively high values (Cruz et al., 2014). According to Montgomery & Peck (1981), the estimates of phenotypic correlations showed weak multicollinearity, and it was not necessary to discard any of the traits analyzed (Table 3).

The coefficient of determination of the pathway analysis (R^2), which measures the fraction of total variation explained by the variables under study, was estimated at 68% in the first harvest and 55% in the second harvest (Table 3). In the second year the residual effect exceed the coefficient of determination. According to Cruz et al. (2014), the values observed allow to interpret the cause and effect relations since a representative fraction of the variability is explained by the traits of the diagram.

In evaluation of the direct effects of the plant traits on the main variable (YLD), the highest magnitude was found for NPLAG that was the most important for hulled coffee yield, followed in smaller proportion by NROS in the first and second year of evaluation (Table 3). Positive associations between development of plagiotropic branching and hulled coffee yield were also observed by Dalcomo et al. (2017) in *C. canephora*.

Different associations can facilitate or hinder the selection process. The effects of DROS were negative in relation to the FROS trait and positive in relation to SIEV, since a lower distance between rosettes was associated with a lower number of fruits per rosette and with a lower mean sieve size. Cilas et al. (2006) observed that a higher number of fruits per rosette were also associated with a greater distance between them.

The small direct effect of the PHt trait on yield indicates the possibility of selecting plants of smaller size and of superior yield (Table 3). On the other hand, Bikila and Sakiyama (2017) observed a positive association between plant height and yield. This association was not observed in the germplasm evaluated, which has genotypes of the Conilon botanical variety that stand out for lower height and good yield potential. Rocha et al. (2014) also observed a positive correlation between plant height and distance between rosettes.

TABLE 3 - Estimates of the direct and indirect effects of the secondary traits of number of days to fruit ripening (NDAYS), number of plagiotropic branches (NPLAG), number of rosettes (NROS), length of plagiotropic branches (LPLAG), number of fruits per rosette (FROS), distance between rosettes (DROS), and mean sieve size (SIEV) on the primary trait of hulled coffee yield (YLD). Bold print highlights the associations of greatest relevance.

1st measurement - 2013-2014										
Trait	**Direct Effect**	**...Indirect effect...**								**Total effects**
		PHt	**NPLAG**	**NROS**	**LPLAG**	**DROS**	**FROS**	**NDAYS**	**SIEV**	
...Hulled coffee yield...										
PHt	-0.009		0.131	0.003	-0.052	-0.062	0.001	-0.001	0.004	0.015
NPLAG	**0.614**	-0.002		0.002	0.023	0.068	0.010	-0.007	-0.018	**0.691**
NROS	**0.252**	-0.002	0.133		-0.028	-0.003	-0.001	-0.006	-0.009	**0.336**
LPLAG	-0.165	-0.003	-0.087	0.002		-0.086	-0.007	0.005	0.104	-0.237
DROS	-0.011	-0.002	-0.105	0.001	-0.057		**-0.208**	0.003	**0.250**	-0.107
FROS	0.031	-0.001	0.066	-0.001	0.352	**-0.202**		-0.003	-0.029	**0.213**
NDAYS	0.039	0.002	-0.106	-0.001	-0.023	-0.019	-0.003		0.041	-0.072
SIEV	0.103	-0.001	-0.109	-0.001	-0.016	-0.036	-0.009	0.015		-0.053
R^2	0.68									
Residual effect	0.49									
2nd measurement - 2014-2015										
Trait	**Direct effect**	**...Indirect effect...**								**Total effects**
		PHt	**NPLAG**	**NROS**	**LPLAG**	**DROS**	**FROS**	**NDAYS**	**SIEV**	
...Hulled coffee yield...										
PHt	0.107		0.013	0.044	0.008	-0.046	-0.035	0.001	0.023	0.115
NPLAG	**0.424**	0.003		0.133	-0.004	0.041	0.007	-0.003	-0.021	**0.581**
NROS	**0.293**	0.016	0.193		0.003	0.028	-0.006	-0.001	-0.004	**0.521**
LPLAG	0.018	0.047	-0.092	0.056		-0.052	-0.028	0.004	0.018	-0.029
DROS	-0.125	0.039	-0.139	-0.065	0.007		-0.037	0.003	**0.265**	-0.005
FROS	-0.111	0.034	-0.025	0.169	0.005	**-0.342**		0.004	0.021	**-0.245**
NDAYS	0.017	0.001	-0.070	-0.024	0.004	-0.020	-0.028		0.026	-0.093
SIEV	0.098	0.025	-0.093	-0.012	0.003	-0.021	-0.024	0.005		-0.019
R^2	0.55									
Residual effect	0.58									

R^2 : Coefficient of determination

Yield potential is related to the indirect effects of NROS and LPLAG on hulled coffee yield (Table 3). Although mean sieve size (SIEV) is important for better beverage quality, the small direct and indirect effects of this trait on yield indicate that hulled coffee yield was more related to the capacity of the plant to produce fruits than to the variability of coffee bean size.

These results reveal that it is possible to select plants that aggregate a set of favorable traits associated with higher hulled coffee yield, such as higher number of productive branches, higher number of rosettes per branch, and lower distance between rosettes. The complementarity of the Conilon and Robusta traits favors selection of hybrid genotypes that express the best traits of each breeding population.

4 CONCLUSIONS

Although the effect of the genotype × year interaction was not significant for only one trait, the repeatability estimates indicate predominance of simple interaction over the years. The establishment of mutually exclusive groups, ordered according to the variability of the traits, subsidizes the establishment of a scale to evaluate the variability of this genetic resource. Pathway analysis indicated that the number of plagiotropic branches and the number of rosettes per productive branch were the traits that exhibited the greatest direct effect on hulled coffee yield.

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6 REFERENCES

BERGO, C. L.; PEREIRA, R. C. A.; SALES, F. Avaliação de genótipos de cafeeiros Arabica e Robusta no Estado do Acre. **Ciência e Agrotecnologia**, Lavras, v. 32, n. 1, p. 11-16, Jan/Feb. 2008.

BHERING, L. L. et al. Alternative methodology for Scott-Knott test. **Crop Breeding And Applied Technology**, Viçosa, v. 8, n. 1, p. 9-16, Jan/Mar. 2008.

BIKILA, B. A.; SAKIYAMA, N. S. Estimation of Genetic Parameters in *Coffea canephora* Var. Robusta. **Advances in Crop Science and Technology**, v. 5, n. 5, p. 310, Sept/Oct. 2017.

CILAS, C. et al. Definition of architectural ideotypes for good yield capacity in *Coffea canephora*. **Annals of Botany**, v. 97, n. 3, p. 405–411, Mar. 2006.

CONAB (2017). **Companhia Nacional de Abastecimento**. Café: Safra 2016: Primeira Estimativa. Available on: <http://www.conab.gov.br> Access in: 05 April 2016.

CRUZ, C. D. Genes: a software package for analysis in experimental statistics and quantitative genetics. **Acta Scientiarum**, Maringá, v. 35, n. 3, p. 271-276, July/Sept. 2013.

CRUZ, C. D.; REGAZZI, A. J.; CARNEIRO, P. C. S. **Modelos biométricos aplicados ao melhoramento genético**. 3ed, Viçosa: Editora UFV, 2014, 560p.

DALCOMO, J. M. et al. Growth comparison of 22 genotypes of conilon coffee after regular pruning cycle. **African Journal of Agricultural Research**, v. 12, n. 1, p. 63-70, Jan. 2017.

DAVIS, A. P. et al. An annotated taxonomic conspectus of the genus *Coffea* (Rubiaceae). **Botanical Journal of the Linnean Society**, London, v. 152, n. 4, p. 465-512, Dec. 2006.

DUBBERSTEIN, D. et al. Matéria seca em frutos, folhas e ramos plagiotrópicos de cafeeiros cultivados na Amazônia Ocidental. **Coffee Science**, Lavras, v. 12, n. 1, p. 74-81, Jan/Mar. 2017.

FERRÃO, R. G. et al. Parâmetros genéticos em café Conilon. **Pesquisa Agropecuária Brasileira**, Brasília, v. 43, n. 1, p. 61-69, Jan. 2008b.

FERRÃO, R. G. et al. Inter-trait relations for direct and indirect selection in *Coffee*. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 8, n. 4, p. 271-278, Oct/Dec. 2008a.

FERRÃO, R. G. et al. **Café Conilon**. 2 ed, Vitória, ES: Incaper, 2017, p. 784.

ICO - International Coffee Organization Robusta cupping protocols (2010) PSCB 123/10. Available on: <http://dev.ico.org/documents/pscb-123-e-robusta.pdf>. Access in: 09 Sept. 2016.

MARCOLAN, A. L. et al. **Cultivo dos cafeeiros Conilon e Robusta para Rondônia**. 3. ed. rev. atual. Porto Velho: Embrapa Rondônia, (Embrapa Rondônia. Sistema de produção, 33), 2009, 67p.

MONTAGNON, C.; CUBRY, P.; LEROY, T. Amélioration génétique du caféier *Coffea canephora* Pierre: connaissnacesacquises, stratégieset perspectives. **Cahiers Agricultures**, Montipellier, v. 21, n. 2-3, p. 143-153, Mar/June. 2012.

MONTGOMERY, D. C.; PECK, E. A. **Introduction to linear regression analysis**. New York: J. Wiley, 1981, p. 504.

MORAIS, L. E. et al. The effects of pruning at different times on the growth, photosynthesis and yield of conilon coffee (*Coffea canephora*) clones with varying patterns of fruit maturation in southeastern Brazil. **Experimental Agriculture**, Baltimore, v. 48, n. 2, p. 210-221, Apr/June. 2012.

OLIVEIRA, L. N. L. D. et al. Selection of *Coffea canephora* parents from the botanical varieties Conilon and Robusta for the production of intervarietal hybrids. **Ciência Rural**, Santa Maria, v. 48, n. 4, Apr. 2018.

PARTELLI, F. L. et al. Dry matter and macronutrient accumulation in fruits of conilon coffee with different ripening cycles. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 38, n. 1, p. 214-222, Jan/Feb. 2014.

RAMALHO, A. R. et al. Progresso genético da produtividade de café beneficiado com a seleção de clones de cafeeiro 'Conilon'. **Revista Ciência Agronômica**, Ceará, v. 47, n. 3, p. 516-523, July/Sept. 2016.

RAMALHO, M. A. P.; DIAS, L. A. D. S.; CARVALHO, B. L. Contributions of plant breeding in Brazil: progress and perspectives. **Crop Breeding and Applied Biotechnology**, v. 12, n. edição especial, p. 111-120, Nov. 2012.

RESENDE, M. D. V.; DUARTE, J. B. Precisão e controle de qualidade em experimentos de avaliação de cultivares. **Pesquisa Agropecuária Tropical**, Goiânia, v. 37, n. 3, p. 182-194, July/Sept. 2007.

ROCHA, R. B. et al. Adaptabilidade e estabilidade da produção de café beneficiado em *Coffea canephora*. **Ciência Rural**, Santa Maria, v. 45, n. 9, p. 1531-1537, Sept. 2015.

ROCHA, R. B. et al. Caracterização e uso da variabilidade genética de banco ativo de germoplasma de *Coffea canephora* Pierre ex Froehner. **Coffee Science**, Lavras, v. 8, n. 4, p. 478-485, Oct/Dec. 2013.

RODRIGUES, W. P. et al. Assessment of genetic divergence among coffee genotypes by Ward-MLM procedure in association with mixed models. **Genetics and Molecular Research**, Ribeirão Preto, v. 15, n. 2, p. 1-7, Apr/June. 2016.

SILVA, V. A. et al. Adaptability, stability, and genetic divergence of conilon coffee in Alto Suaçuí, Minas Gerais, Brazil. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 17, n. 1, p. 25-31, Jan/Mar. 2017.

SOUZA, C. A. et al. Componentes genéticos do desenvolvimento e maturação de frutos de *Coffea canephora* Pierre ex A. Froehner. **Coffee Science**, Lavras, v. 12, n. 3, p. 355-364, July/Sept. 2017.

SOUZA, F. D. F. et al. Molecular diversity in *Coffea canephora* germplasm conserved and cultivated in Brazil. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 13, n. 4, p.221-227, Oct/Dec. 2013.

SURESHKUMAR, V. B. et al. Interrelationship and association of characters in Robusta Coffee (*Coffea Canephora* Var. Robusta). **Agriculture, Forestry and Fisheries**, New York, v. 2, n. 2, p. 98-104, Mar/Apr. 2013.

TEIXEIRA, A. L. et al. Performance of intraspecific hybrids (Kouillou x Robusta) of *Coffea canephora* Pierre. **African Journal of Agricultural Research**, Lagos, v. 12, n. 35, p. 2675-2680, Aug. 2017.

WRIGHT, S. Theory of path coefficients. **Genetics**, Madison, v. 8, n. 3, p. 239-285, May/June. 1923.

SPATIAL VARIABILITY OF SOIL PENETRATION RESISTANCE IN COFFEE GROWING

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ABSTRACT: The intensive use of machines in agriculture tends to cause soil compaction, which can hamper the expansion of root system and the absorption of water and nutrients, thus affecting the crop development. In view of the above, the present study aimed to identify critical zones of soil compaction, through the spatial distribution of soil penetration resistance (SPR), having positions within the coffee rows and soil depth ranges as variables. The study was performed in a coffee plantation of 7.32 ha, belonging to the Bom Jardim Farm, located in the municipality of Bom Sucesso, MG, Brazil. The SPR was measured using a penetrometer in the depth range from 0 to 0.40 m, with discretization in four layers of 0.10 m. The data were interpreted based on geostatistics, in order to identify if there is spatial dependence of the SPR and generate thematic maps demonstrating the variable's spatial behavior. It is concluded that there is spatial dependence of soil penetration resistance, being possible to use geostatistical tools to generate thematic maps based on classes of soil penetration resistance. The values of SPR in the tractor trail, for layers from 0.10 to 0.20 and from 0.20 to 0.30 m, were classified in the high SPR class and could cause damage to the crop.

Index Terms: Precision agriculture, penetrometer, soil compaction, *Coffea arabica*.

VARIABILIDADE ESPACIAL DA RESISTÊNCIA DO SOLO À PENETRAÇÃO NA CAFEICULTURA

RESUMO: O uso intensivo de máquinas na agricultura tende a causar a compactação do solo, processo esse que pode prejudicar a expansão do sistema radicular e a absorção de água e nutrientes, afetando, assim, o desenvolvimento da cultura. Diante do exposto, o presente trabalho tem o objetivo de identificar zonas críticas de compactação do solo, através da distribuição espacial da resistência do solo à penetração (RSP), tendo como variáveis posições dentro da linha do cafeeiro e faixas de profundidade do solo. O trabalho foi desenvolvido em uma gleba de lavoura cafeeira de 7,32 ha, pertencente à fazenda Bom Jardim, localizada no município de Bom Sucesso-MG. A medição da RSP foi feita com o uso de um penetrômetro na faixa de profundidade de 0 a 0,40 m, com discretização em 4 camadas de 0,10 m. Os dados foram interpretados com base na geoestatística, de modo a identificar se há dependência espacial da RSP e gerar mapas temáticos demonstrando o comportamento espacial da variável. Conclui-se que existe a dependência espacial da RSP, sendo possível utilizar das ferramentas da geoestatística para geração dos mapas temáticos, baseando-se nas classes de RSP, presentes na literatura. Os valores de RSP no rastro do rodado do trator, para as camadas de 0,10 a 0,20 m e 0,20 a 0,30 m, foram enquadradas na classe de alta RSP, podendo causar danos à cultura.

Termos de indexação: Agricultura de precisão, penetrômetro, compactação do solo, *Coffea arabica*.

1 INTRODUCTION

Agriculture plays an important role in the social and economic development of Brazil (SOUZA et al., 2014). In this context, coffee stands out as one of the main commodities and revenue generator for the country. Due to globalization and market competitiveness, maintaining sustainably the high productivity is the main challenge for agricultural production (ST-MARTIN; BOMMARCO, 2016).

In this scenario, mechanization in agriculture has been intensified, since this process allows increasing the operating capacity and reducing production costs. Currently, in fully mechanized areas, all operations during the crop cycle are performed mechanically by different machines and implements (CUNHA; SILVA; DIAS, 2016).

However, the environmental impacts caused by mechanization negatively influence this process. Among the impacts, the soil compaction caused by the traffic of agricultural equipment in the crop is highlighted, which strongly influences the coffee cultivation, affecting the crop development and reducing productivity (CARVALHO et al., 2013; MARTINS et al., 2012).

Compaction process affects soil structure and physical properties (CARMO et al., 2011), reducing macroporosity and permeability and increasing resistance by the action of excessive loads on the soil. According to Nawaz, Bourrié and Trolard (2013), since soil compaction directly affects soil physical properties, such as density, penetration resistance and porosity, these parameters can be used to quantify soil compaction. The authors also state that the consequences caused by soil compaction are still underestimated.

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According to Fernandes, Santinato and Santinato (2012), the presence of the compacted layer in the soil diminishes its useful depth, hindering the coffee growing due to the difficulty of water infiltration in the soil and precluding the root growth of plants, thus resulting in low yield. In a study to evaluate the effect of subsoiling on coffee yield, the authors verified the superiority of all subsoiling treatments in relation to the control, increasing from 26 to 65% in the average of three analyzed harvests, indicating that the superiority of the subsoil treatments is resulting from the reduced soil compaction with the practice of subsoiling, justifying the use of subsoiler for this purpose.

However, subsoiling is a costly process (SALVADOR; BENEZ; MION, 2009), requiring high use of labor force and equipment. In this way, it can be noted the importance of the compaction study in the cultivated areas, seeking to identify and quantify critical compaction locations. Thus, in possession of this information, the management of subsoiling can become more efficient, showing better results and reducing costs.

Measurement of soil penetration resistance is one of the identification methods of soil compaction, where high resistance values indicate compacted areas. This indicator is widely used due to its practicality, providing a good estimation of the soil compaction at each sampling point. The knowledge on the spatial variability of soil penetration resistance may allow identifying areas that need specific management. In this context, the use of precision agriculture or "precision coffee growing" techniques, become an interesting alternative for the management of compacted zones and as a decision tool.

According to Ferraz et al. (2012), precision coffee growing can be defined as a set of techniques and technologies capable of assisting the coffee grower in the crop management, based on the spatial variability of soil and plant properties, in order to increase process efficiency and maximize profitability, thus increasing productivity and the product's final quality.

Geostatistics is highlighted among the tools used in precision agriculture, which differs from classical statistics because it considers that the data sampled are spatially dependent. Moreover, it makes possible to identify whether or not there is spatial dependence for the analyzed factors and to characterize the magnitude of spatial variability, allowing generating thematic maps that aid in the decision making in the crop (CARVALHO et al., 2013).

In this context, the aim in this study was to identify the critical soil compaction zones, through soil penetration resistance data, using geostatistical tools, comparing positions within the coffee row and in soil depth ranges.

2 MATERIAL AND METHODS

The study was developed in a coffee plantation of 7.32 ha, belonging to the Bom Jardim Farm, located in the municipality of Bom Sucesso, MG, Brazil, at coordinates 21°01'20" S and 44°55'9" W, and 990 m altitude. The coffee variety in the plot is Catuaí IAC 62 with spacing of 4.0 x 1.0 m. The soil was characterized as dystrophic red latosol (Brazilian Agricultural Research Agency - EMBRAPA, 2013).

The studied area has been managed by mechanized operations, except harvesting, for 20 years. In total, 16 mechanized operations are carried out per year. However, there is no record of soil subsoiling in the area since the installation of the coffee, in 1973.

For the accomplishment of the study values of soil penetration resistance (SPR) were collected in December 2016, using a regular grid with 29 points (four points per hectare), as observed in Figure 1 (a). At each point, RSP values were collected in three different positions: under coffee canopy (UCC), in the tractor trail (TT) and in the midway between coffee rows (MCR), according to Figure 1 (b). The positions were determined using the coffee row as reference. The distances from the coffee row were 0.3 m, 1.3 m and 2 m, for the positions UCC, TT and MCR, respectively. For each plot, two replicates were performed and data were collected at depths from 0 to 0.10; 0.10 to 0.20; 0.20 to 0.30, and 0.30 to 0.40 m.

For the measurement of SPR, it was used an electronic penetrometer of the brand Falker (PenetroLOG), model PLG1020. The apparatus was programmed to operate up to 40 cm depth, with data then transferred to the computer and tabulated, finding an average value for each layer of 10 cm of soil. The data collection points were generated through the Farm Works™ software, and for localization in the plot it was used the CR Campeiro, a free distribution software developed by the Department of Rural Engineering of the Federal University of Santa Maria.

The spatial dependence of SPR was analyzed through semivariogram fitting by the classical estimator, as follows (Equation 1):

$$\hat{\gamma}(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(x_i) - Z(x_i + h)]^2 \qquad (1)$$

where, N (h) is the number of experimental pairs of observations Z(xi) and Z (xi + h) separated by a distance h. The semivariogram is represented by the graph $\hat{\gamma}(h)$ versus h. From the fit of a mathematical model to the calculated values of $\hat{\gamma}(h)$ the coefficients of the theoretical model were estimated for the semivariogram; these coefficients are: nugget effect (C_0), sill $(C_0 + C)$ and range (a).

In order to analyze the spatial dependence index (SDI) of properties under study, the classification of Cambardella et al. (1994) was used, calculated by Equation 2, in which are considered as spatial dependence:

- Strong: semivariograms with nugget effect lower than or equal to 25% of the sill (SDI $\leq$ 0.25);
- Moderate: semivariograms between 25 and 75% of the sill (0.25 $\leq$ SDI $\leq$ 0.75);
- Weak: nugget effect greater than or equal to 75% of the sill (SDI $\geq$ 0.75).

$$SDI = \frac{C_0}{C_0 + C} \quad (2)$$

The fitting of semivariograms models was chosen according to the ordinary least squares (OLS), using the spherical model. Such model is the most used in geostatistical studies related to the soil and to the coffee growing (SILVA et al., 2007).

After fitting the semivariograms, the data were interpolated by ordinary kriging. Thereby, it is possible to show spatial distribution patterns of variables in the crop, in the form of thematic maps. The maps were created based on SPR classes, according to Table 1, classifying the SPR in pressure ranges that when related to crops may or may not influence the plant development.

For the geostatistical analysis, the R statistical software, free distribution, was used through the geoR package (RIBEIRO JUNIOR; DIGGLE, 2001), while the software Farm Works™ was used to generate the thematic maps. The maps were generated in the Universal Transverse Mercator (UTM) coordinate system in zone 23K, where is located the experimental area.

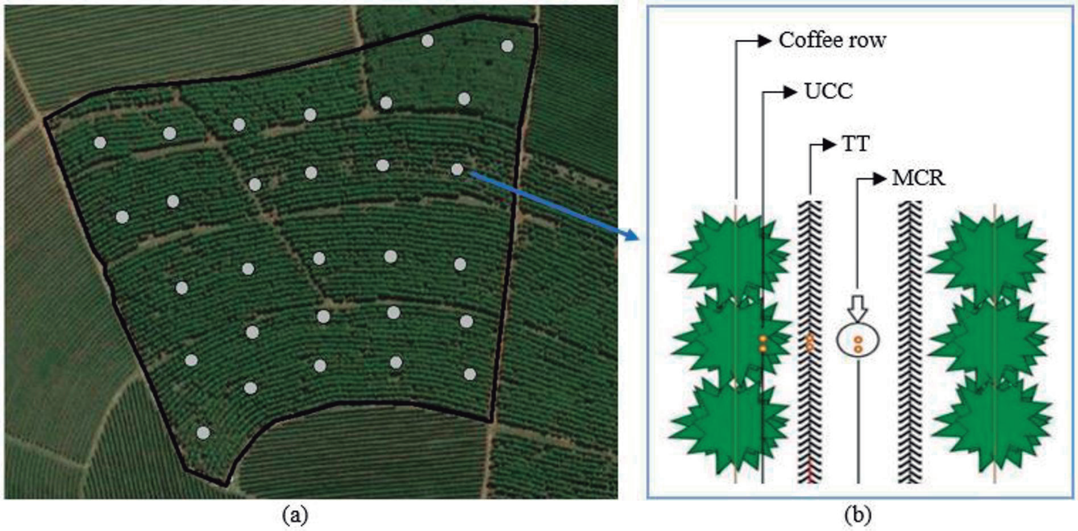

FIGURE 1 - Grid sample of the area (a) and schematic sampling drawing (b).

TABLE 1 - Classes of soil penetration resistance.

Class	Penetration resistance (MPa)
Extremely low	< 0.01
Very low	0.01 - 0.1
Low	0.1 - 1.0
Moderate	1.0 - 2.0
High	2.0 - 4.0
Very high	4.0 - 8.0
Extremely high	> 8.0

Source: Adapted from Soil Science Division Staff (2017).

3 RESULTS AND DISCUSSION

Descriptive analysis of soil penetration resistance (SPR) data is presented in Table 2. Regarding the coefficient of variation (CV), it can be observed values ranging from 13.2% to 71.6%. According to Gomes and Garcia (2002), the variability of a property can be classified according to the magnitude of its CV, which can be: low, when lower than 10%; moderate, between 10 and 20%; high, between 20 and 30%; and very high, when higher than 30%.

Thus, it can be noted that the variable studied fits into the variability classes from moderate to very high. Moreover, high CV values are indicators of data heterogeneity.

Referring to the average, minimum and maximum value, a variation is observed in the SPR data. However, according to Carvalho et al. (2013), only the knowledge of these measures cannot be used as the only way to identify the expression of the variable in an area. To this end, the geostatistical analysis aims to verify the spatial variability of a variable and, whether identified, thematic maps are generated in order to aid in understanding of its behavior in the field.

By analyzing the estimated parameters of experimental semivariogram for SPR (Table 3), it is verified that the range varied from 34.78 to 181.56 m between the studied positions and depths, demonstrating a good structural continuity of the soil. This result differs from that found by Kamimura et al. (2013), in which the range was 3.4 m for SPR in the layer from 0.25 to 0.28 m, using the spherical model. Silva and Lima (2013), aiming to analyze the spatial distribution of soil physical properties and coffee productivity, found values for the range varying from 11 to 95 m, adjusting the spherical and the gaussian models.

The spatial dependence index (SDI) was strong for most positions and depths, being that the SDI was moderate only in MCR and TT, both at depths from 0.10 to 0.20 m. This indicates that there is spatial dependence for SPR data, and geostatistics might be used to describe the variable behavior in the studied area.

SDI results are similar to those found by Palma et al. (2013), which obtained the SDI classification as moderate for SPR in the layers from 0.10 to 0.20 and 0.40 to 0.50 m, whereas was strong for the other layers. On the other hand, Bottega et al. (2011) did not find much satisfactory results, since SDI of SPR was considered as moderate for all studied soil depths.

Once the semivariogram of the variable and its structure of spatial dependence by kriging are known, it is possible to interpolate values at any point in the study area, without tendency and with minimum variance (KAMIMURA et al., 2013). Thus, the SPR maps were generated for the positions UCC, MCR and TT in the layers from 0 to 0.10 m, 0.10 to 0.20 m, 0.20 to 0.30, and 0.30 to 0.40 m (Figure 2). The maps were generated based on the SPR classes, being possible to distinguish each area and their framing to a specific SPR class.

TABLE 2 - Descriptive statistics of the soil penetration resistance (MPa) in different positions and layers.

Positions	Layers	Descriptive statistics								
		Min	Max	Md	Avg	Var	SD	C_s	C_K	CV
UCC	0-10 cm	0.00	2.41	0.58	0.62	0.20	0.44	1.52	4.15	71.6
	10-20cm	0.48	2.26	1.50	1.48	0.14	0.38	-0.34	0.29	25.6
	20-30cm	0.59	2.62	1.74	1.74	0.19	0.43	-0.25	-0.10	24.8
	30-40cm	0.25	2.43	1.64	1.62	0.19	0.43	-0.41	0.71	26.8
MCR	0-10 cm	0.49	3.44	2.00	2.01	0.34	0.58	-0.01	-0.01	28.9
	10-20cm	1.16	2.62	1.86	1.93	0.10	0.32	0.08	-0.17	16.6
	20-30cm	1.16	2.67	1.93	1.93	0.12	0.35	-0.11	-0.66	17.9
	30-40cm	1.11	2.74	1.85	1.89	0.14	0.38	0.17	-0.39	20.1
TT	0-10 cm	0.30	3.40	1.53	1.55	0.23	0.48	0.62	3.28	30.80
	10-20cm	1.93	3.66	2.71	2.64	0.16	0.40	0.12	-0.26	15.10
	20-30cm	1.56	2.84	2.13	2.17	0.08	0.29	0.42	-0.20	13.20
	30-40cm	0.73	2.60	1.71	1.74	0.10	0.31	0.03	1.38	18.00

UCC - Under coffee canopy; MCR - Midway between coffee rows; TT - Tractor trail; Min - Minimum value; Max - Maximum value; Md - Median; Avg - Average; Var - Variance; SD - Standard deviation; Cs - Coefficient of skewness; Ck - Coefficient of kurtosis; CV - Coefficient of variation.

TABLE 3 - Estimated parameters of experimental semivariogram for soil penetration resistance (MPa).

Position	Depth	C_0	C	a	C_0+C	SDI	
UCC	0 – 0.10 m	6124.27	118241.81	34.78	124366.08	0.05	Strong
	0.10 – 0.20 m	0.82	121955.96	86.15	121956.78	0.00	Strong
	0.20 – 0.30 m	11061.88	115968.84	120.42	127030.71	0.09	Strong
	0.30 – 0.40 m	7289.06	124276.04	61.40	131565.10	0.06	Strong
MCR	0 – 0.10 m	0.00	191696.25	71.95	191696.25	0.00	Strong
	0.10 – 0.20 m	42917.72	40040.00	155.96	82957.72	0.52	Moderate
	0.20 – 0.30 m	0.00	59876.51	60.48	59876.51	0.00	Strong
	0.30 – 0.40 m	0.00	70192.26	59.10	70192.26	0.00	Strong
TT	0 – 0.10 m	7289.06	124276.04	61.40	131565.10	0.06	Strong
	0.10 – 0.20 m	52109.79	109835.14	181.56	161944.92	0.32	Moderate
	0.20 – 0.30 m	1979.49	55613.04	53.50	57592.53	0.03	Strong
	0.30 – 0.40 m	7461.50	59635.41	37.95	67096.91	0.11	Strong

UCC - Under coffee canopy; MCR - Midway between coffee rows; TT - Tractor trail; C_0 - nugget effect; C - contribution; a - range; C_0+C - sill; SDI - spatial dependence degree.

In the UCC position, the layer from 0 to 0.10 m showed SPR in the low class for 98.2% of the area. This condition was already expected due to the presence of a layer of organic material underneath the plant, as well as the machinery does not travel in that region after the crop establishment. In the layers from 0.10 to 0.20 and 0.30 to 0.40 m, the SPR was predominantly framed in the moderate class, where these values reflect intrinsic soil characteristics.

In the layer from 0.20 to 0.30 m, 19.9% of the area of the UCC position was framed in the high SPR class. This fact can be explained by the high concentration of roots in this soil layer, since the penetrometer may have shocked with the roots, increasing the measured values of penetration resistance. Palma et al. (2013) showed high SPR results for layers ranging between 0.20 and 0.60 m depth, in the coffee growing row with mechanized management system.

In the MCR position, 50% of area of the layer from 0 to 0.10 m exhibited SPR class classified as high, the other half area was classified into the moderate SPR class. For the layer from 0.10 to 0.20 m, 36% of the area was classified as high SPR and the remainder as moderate. These SPR values for coffee interlines were higher than expected. However, this result corroborates to those found by Martins et al. (2012) studying the effect of mechanized operations on soil compaction in the coffee growing, where they observed that the layer from to 0.03 m underwent higher compaction than the layer from 0.15 to 0.18 m. The lack of vegetation mulch and the low soil moisture in this position may have influenced this result.

Another justification for these results in the MCR would be the compaction generated in the superficial layers of the soil by the mechanized operations for weed control, sweeping and windrowing of coffee. For the layers from 0.20 to 0.30 and 0.30 to 0.40 m, the SPR was framed as moderate in approximately 70% of the area.

In the TT position, the layers from 0 to 0.10 m and 0.30 to 0.40 m displayed predominantly values classified as moderate SPR. The layers from 0.10 to 0.20 and 0.20 to 0.30 m were framed as high SPR class, result expected due to the compaction generated by the traffic of machines in the crop. Similar results were found by Beutler et al. (2001), where an increasing SPR gradient was verified with increasing depth, reaching maximum resistance between 0.15 and 0.30 m in a dystrophic red latosol under no-till systems.

Nevertheless, Bottega et al. (2011) analyzed the spatial variability of SPR in a dystrophic red latosol under no-till system for soybean and maize crops, and found different results than those presented here. The authors emphasize that the increase in depth resulted in decrease of values of variance and standard deviation, demonstrating that the compaction variability degree is higher in more superficial layers, showing that the compaction effect by machine traffic is reflected in these regions.

Palma et al. (2013) state that in the coffee growing is verified higher RSP to greater depth in relation to annual crops, which is justified by the traffic of machines always occurring parallel to the planting row of the crop.

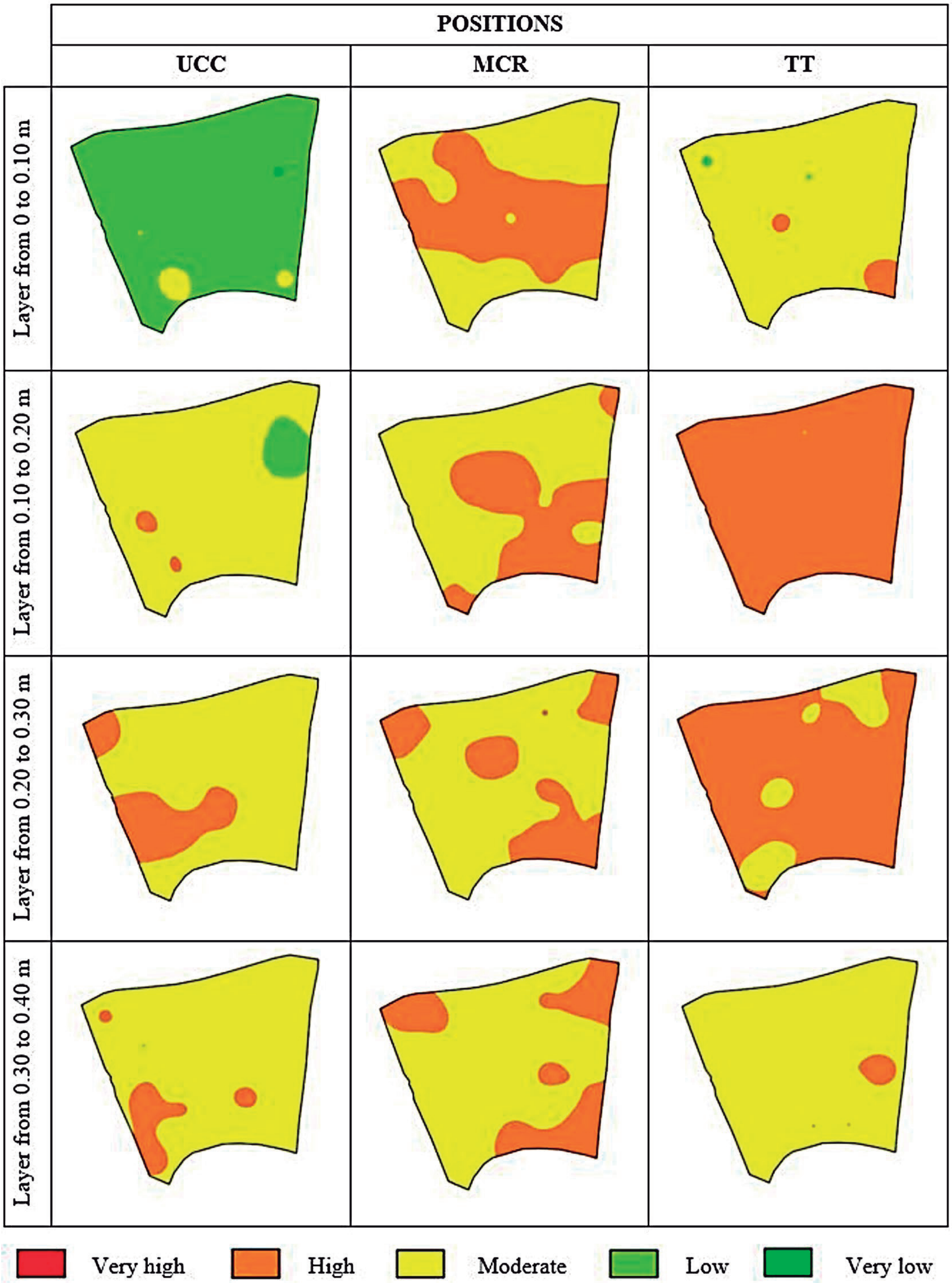

FIGURE 2 - Spatial distribution maps of soil penetration resistance (SPR) in the study area, as a function of the positions (under coffee canopy - UCC; Midway between coffee rows - MCR; tractor trail - TT) and studied soil layers.

4 CONCLUSIONS

Soil penetration resistance showed spatial dependence, being possible to use geostatistical tools to generate thematic maps based on classes of soil penetration resistance.

In the tractor trail, the soil penetration resistance obtained in deep from 0.1 to 0.30 m was classified as high, indicating soil compaction.

The generated maps have important and useful information to proceed with the subsoiling operation, aiming to reduce soil compaction.

5 REFERENCES

BEUTLER, A. N. et al. Resistência à penetração e permeabilidade de Latossolo Vermelho distrófico típico sob sistemas de manejo na região dos cerrados. **Revista Brasileira de Ciência do Solo,** Viçosa, v. 25, n. 1, p. 167-177, Jan/Mar. 2001.

BOTTEGA, E. L. et al. Variabilidade espacial da resistência do solo à penetração em um Latossolo Vermelho distroférrico. **Revista Brasileira de Ciências Agrárias,** Recife, v. 6, n. 2, p. 331-336, Apr/June. 2011.

CAMBARDELLA, C. A. et al. Field scale variability of soil properties in Central Iowa soils. **Soil Science Society of America Journal**, Madison, v. 58, n. 5, p. 1501-1511, Sept/Oct. 1994.

CARMO, D. L. et al. Propriedades físicas de um latossolo vermelho-amarelo cultivado com cafeeiro em três sistemas de manejo no sul de minas gerais. **Revista Brasileira de Ciência do Solo,** v. 35, n. 3, p. 991-998, May/June. 2011.

CARVALHO, L. C. C. et al. Variabilidade espacial de atributos físicos do solo e características agronômicas da cultura do café. **Coffee Science,** Lavras, v. 8, n. 3, p. 265-275, July/Sept. 2013.

CUNHA, J. P. B.; SILVA, F. M.; DIAS, R. E. B. A. Eficiência de campo em diferentes operações mecanizadas na cafeicultura. **Coffee Science,** Lavras, v. 11, n. 1, p. 76-86, Jan/Mar. 2016.

EMBRAPA - EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. **Sistema brasileiro de classificação de solos**. Centro Nacional de Pesquisa de solos: Rio de Janeiro, 2013.

FERNANDES, A. L. T.; SANTINATO, F.; SANTINATO, R. Utilização da subsolagem na redução da compactação do solo para produção de café cultivado no cerrado mineiro. **Enciclopédia Biosfera, Centro Científico Conhecer,** Goiânia, v. 8, n. 15, p. 1648, 2012.

FERRAZ, G. A. S. et al. Variabilidade espacial e temporal do fósforo, potássio e da produtividade de uma lavoura cafeeira. **Engenharia Agrícola,** Jaboticabal, v. 32, n. 1, p. 140-150, Jan/Feb. 2012.

GOMES, F. P.; GARCIA, C. H. **Estatística aplicada a experimentos agronômicos e florestais**. Piracicaba: FEALQ, 2002. 305 p.

KAMIMURA, K. M. et al. Variabilidade espacial de atributos físicos de um latossolo vermelho-amarelo, sob lavoura cafeeira. **Revista Brasileira de Ciência do Solo,** Viçosa, v. 37, n. 4, p. 877-888, July/Aug. 2013.

MARTINS, P. C. C. et al. Compaction caused by mechanized operations in a Red-Yellow Latosol cultivated with coffee over time. **Ciência e Agrotecnologia,** Lavras, v. 36, n. 4, p. 391-398, July/Aug. 2012.

NAWAZ, M. F.; BOURRIÉ, G.; TROLARD, F. Soil compaction impact and modelling. A review. **Agronomy for Sustainable Development**, Paris, v. 33, n. 2, p. 291-309, Apr/June. 2013.

PALMA, M. A. Z. et al. Resistência do solo à penetracao em cafezais cultivados com sistema mecanizado e manual. **Coffee Science,** Lavras, v. 8, n. 3, p. 364-370, July/Sept. 2013.

RIBEIRO JÚNIOR, P. J.; DIGGLE, P. J. GeoR: a package for geostatistical analysis. **R-News**. New York, v. 1, n. 2, p. 14-18, May/Aug. 2001.

SALVADOR, N.; BENEZ, S. H.; MION, R. L. Demanda energética na subsolagem realizada antes e depois de diferentes sistemas de preparo periódico do solo. **Ciência Rural**, Santa Maria, v. 39, n. 9, p. 2501-2505, Dec. 2009.

SILVA, F. M. et al. Variabilidade espacial de atributos químicos e de produtividade na cultura do café. **Ciência Rural**, Santa Maria, v. 37, n. 2, p. 401-407, Mar/Apr. 2007.

SILVA, S. A.; LIMA, J. S. S. Atributos físicos do solo e sua relação espacial com a produtividade do café arábica. **Coffee Science**, Lavras, v. 8, n. 4, p. 395-403, Oct/Dec. 2013.

SOIL SCIENCE DIVISION STAFF. **Soil survey manual**. USDA Handbook 18. Washington: Government Printing Office, 2017, 605 p.

SOUZA, J. M. et al. Atributos físicos do solo em lavoura de cafeeiro conilon submetida à subsolagem. **Revista Engenharia na Agricultura**, Viçosa, v. 22, n. 5, p. 413, Sept/Oct. 2014.

ST-MARTIN, A.; BOMMARCO, R. Soil compaction and insect pollination modify impacts of crop rotation on nitrogen fixation and yield. **Basic and Applied Ecology**, Uppsala, v. 17, n. 7, p. 617-626, Nov. 2016.

INCIDENCE AND SEVERITY OF COFFEE LEAF RUST, CERCOSPORIOSIS AND COFFEE LEAF MINER IN COFFEE PROGENIES

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ABSTRACT: Coffee leaf rust is the main disease of this crop, however cercosporiosis and coffee leaf miner can also cause significant damage when they reach high levels of infestation. Plant genetic improvement for resistance is one of the best tools for controlling plant diseases. The objective in this work was to identify F_3 progenies of *Coffea arabica* with resistance to coffee leaf rust, which present a lower incidence and severity of cercosporiosis and coffee leaf miner. The treatments were constituted by 10 progenies, besides two cultivars coffee leaf rust susceptible, used as a control. The experimental design was a randomized block design (RBD), with two replicates, each block consisting of 12 plots randomly distributed, each corresponding to one treatments. The following characteristics were evaluated: coffee leaf rust intensity and severity, cercosporiosis and coffee leaf miner, plants vegetative vigor, grain maturity uniformity and plants height. The progeny averages were grouped by the Scott & Knott test at 5% probability. Progenies 27, 30 and 15 were selected, since they presented low incidence in relation to coffee leaf rust, cercosporiosis and coffee leaf miner, and will be used to continue the breeding program.

Index terms: *Coffea arabica*, genetic breeding, diseases.

INCIDÊNCIA E SEVERIDADE DE FERRUGEM, CERCOSPORIOSE E BICHO MINEIRO EM PROGÊNIES DE CAFÉ

RESUMO: A ferrugem do cafeeiro é a principal doença da cultura, contudo a cercosporiose e bicho mineiro também podem causar danos significativos quando atingem altos níveis de infestação. O melhoramento genético de plantas visando a resistência é uma das melhores opções para o controle de doenças de plantas. Objetivou-se com este trabalho identificar progênies F_3 de *Coffea arabica* portadoras de resistência durável à ferrugem, que apresentem menor incidência e severidade de cercosporiose e bicho mineiro. Os tratamentos foram constituídos por 10 progênies, além de duas cultivares suscetíveis à ferrugem, utilizadas como testemunha. Utilizou-se o delineamento em blocos casualizados (DBC), com duas repetições, cada bloco constituído por 12 parcelas distribuídas ao acaso, cada uma destas correspondendo a um tratamento. Foram avaliadas as seguintes características: incidência e severidade de ferrugem, cercosporiose e bicho mineiro, vigor vegetativo das plantas, uniformidade de maturação dos grãos e altura das plantas. As médias das progênies foram agrupadas pelo teste de Scott & Knott a 5% de probabilidade. As progênies 27, 30 e 15 foram selecionadas por apresentar baixa incidência em relação à ferrugem, cercosporiose e ao bicho mineiro, e serão utilizadas para dar continuidade ao programa de melhoramento.

Termos para indexação: *Coffea arabica*, melhoramento genético, doenças.

1 INTRODUCTION

Coffee is one of the most consumed beverages in the world. Brazil is the largest producer and exporter and the second largest consumer, generating an important source of wealth for the country. It is one of the main crops of Brazilian agribusiness, mainly in the state of Minas Gerais, where the South of Minas Gerais stands out in the activity (CONAB, 2018).

During crop management farmers may face several problems, including phytosanitary problems. In this context, we can highlight the coffee leaf rust caused by the fungus *Hemileia vastatrix* Berkeley & Broome, which is the main disease of the crop, and can cause serious financial damage to coffee growers (REZENDE et al., 2013; TALHINHAS et al., 2016; ZAMBOLIM, 2016). Other diseases and pests can cause significant damage, such as cercosporiosis, caused by the fungus *Cercospora coffeicola* Berkeley & Cooke (POZZA; CARVALHO; CHALFOUN, 2010) and the known as coffee leaf miner (*Leucoptera coffeella* Guérin-Mèneville & Perrottet) (MAGALHAES et al., 2010).

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Cercosporiosis is found in the majority of coffee growing regions of Brazil, causing injuries in the leaves and fruits (SANTOS et al., 2008; PEREIRA et al., 2011). The disease symptoms in leaves are circular in format, with a dark brown colored spot encircled by a light yellow halo. This fungi presence stimulates the plant to produces ethylene which can cause intense defoliation, creating quantitative losses, reducing the yield and productivity of the culture, even in reduced severity (CUSTÓDIO et al., 2011). According to Botelho et al. (2017), there is genetic variability to *C. coffeicola* resistance among the coffee tree in different genotypes and genetic improvements could be obtained by selection.

The coffee leaf miner is an exotic African pest and it is considered monophagous. High attack levels reduce the photosynthetic capacity due to the leaf area reduce and their occurrence is strongly tied to meteorological factors (ZAMPIROLI et al., 2017). It may cause up to 70% defoliation (SCALON; MATEUS; ZACARIAS, 2013).

Brazilian Arabic coffee production is based on a set of highly productive cultivars susceptible to coffee leaf miner, and most of them are also susceptible to coffee leaf rust (MENDONÇA et al., 2016). Among the control methods for coffee leaf rust, chemical control is the most used. Although it is efficient if carried out indiscriminately and incorrectly it can bring diverse environmental and phytosanitary problems, besides the high cost and probability of resistance of phytopathogens (ZAMBOLIM et al., 2005). In this context, the use of varieties that are resistant or tolerant to pests and diseases is presented as a more economical, viable and sustainable alternative (BOISSEAU et al., 2009; CARVALHO et al., 2017; CARVALHO et al., 2012).

The use of cultivars without coffee leaf rust tolerance is widespread in the production systems and may differ in the degree of susceptibility, since resistant cultivars may become susceptible over time to new breeds of fungus originated by genetic mutations (FAZUOLI et al., 2007). This fungus has a very large genome (about 797 Mbp) and hides great pathological diversity. Currently, more than 50 physiological races of coffee leaf rust have been identified by the CIFC (Center for the Research of Coffee Rust) (TALHINHAS et al., 2017). In the improvement of the coffee tree, the cultivars with durable resistance are mainly obtained.

In breeding programs, it is desirable that new cultivars under development should be superior to their predecessors, which have, besides pest and disease resistance, improvements of other agronomic characteristics (MATIELLO et al., 2008; RAMALHO et al., 2012), aiming to reconcile cultivars with high yield, resistance to coffee leaf rust and less incidence of cercosporiosis (CARVALHO et al., 2017). The objective in this study was to identify *Coffea arabica* L. F_3 progenies with durable resistance to coffee leaf rust, with lower incidence and severity of cercosporiosis and coffee leaf miner, and to evaluate the height, maturity uniformity and vegetative vigor.

2 MATERIAL AND METHODS

Progenies of coffee trees (*C. arabica* .) at two and a half years were evaluated in F_3 generation. The methodology used to obtain these progenies was adaptation of recurrent selection model to the coffee tree. The initial population was obtained by means of artificial hybridization carried out in 2009, involving five groups: Catuaí (LCH-2077-2-5-02, LCH-2077-2-5-10, LCH-2077-2-517, LCH-2077-2-5-62, LCH-2077-2-5-99), Icatu (MG-3282, MG-4040, MG-4042, MG-2942, MG-2944, MG-4040-179, MG-4042-222), Topázio (MG-5002), Rubi (MG-1192) and Acaiá (LCP-474-19). The cultivars were crossed according to a diallel scheme, in which each parental was crossed twice. The cultivar Acaiá LCP-474-19 was crossed in another diallel with Catuaí (LCH-2077-2-5-17, LCH-2077-2-5-62, LCH-2077-2-5-99) and Rubi MG -1192. 40 progenies were obtained, and the control cultivars were added for the selection of families within each selected progeny (Catuaí vermelho LHC-2077-2-5-15, LHC-2077-2-5-44, LHC-2077-2 LCP-379-19, LCP-388-17, Acaiá LPC-474-19, Icatu MG-3282, MG-2942, MG-2944 and Rubi MG-1192). The F_1 seeds obtained from this hybridization were used for production of seedlings. Of these 40 progenies, there was an advance of one generation, and from this new generation the 10 best progenies were selected. The control cultivars (Mundo Novo and Topázio), susceptible to coffee leaf rust, were added to the selection of families within each selected progeny (Table 1). The selected F_3 seeds were used to produce seedlings, following the usual production system of the region.

The trial was carried out in Lavras, MG, at the campus of the Federal University of Lavras (UFLA), in the Department of Agriculture, latitude south 21°14’06”, longitude west 44°59’00” and altitude of approximately 910m.

TABLE 1 - *Coffea arabica* L. F_3 progenies implanted in experiment in Lavras, MG

Progenies F_3	Genealogy
4	119 (Icatu 4042 x Catuaí 99)
12	117 (Icatu 2944 x Catuaí 17)
15	101 (Catuaí 10 x Icatu 4040)
26	127 (Icatu 3282 x Catuaí 99)
27	141 (Icatu 2944 x Catuaí 62)
30	108 (Catuaí 02 x Icatu 4042)
31	142 (Icatu 2942 x Catuaí 62)
35	128 (Icatu 3282 x Catuaí 62)
39	Acauã normal 1365
41	116 (Icatu 2944 x Rubi)
MN	Mundo Novo
T	Topázio

The climate of region according to Koppen is humid temperate with hot summer and dry winter (OMETTO, 1981), the annual average temperature is 19.4° C, the annual average total rainfall is 1,590 mm and the average annual relative humidity is 76%. The soil of the experimental area is classified as red-dystroferric oxisol.

In 2012, F_3 seeds were collected from this material. In March 2013, seedlings were formed and in December of this year the seedlings were planted in the 3.0m x 0.8m spacing on the UFLA campus.

The experimental design was in randomized blocks, with two replicates, each block consisting of 12 randomly distributed plots, each corresponding to a different progeny. Each experimental plot contains 10 plants.

The vegetative vigor was evaluated through notes using an arbitrary scale of 10 points, where note 1 refers to the worst plants, with very low and marked vigor, which characterizes depletion and note 10 to plants with excellent vigor, more leafy and with marked vegetative growth of productive branches (SHIGUEOKA et al., 2014).

For the maturation uniformity, an arbitrary scale of 100 points was used, where note 1 refers to the plants with less maturity uniformity, and note 100 to plants with excellent maturity uniformity.

The height in centimeter of the plants was measured using a ruler, from the base of stem to the top of canopy.

Each of plants was evaluated as follows: leaves of the middle third of the plants were sampled, being five leaves of each side, always having as reference the third or fourth pair of leaves.

The incidence and severity of infestation of coffee leaf rust (*H. vastatrix*), cercosporiosis (*C. coffeicola*) and coffee leaf miner (*L. coffeella*) were evaluated. For coffee leaf rust, the incidence was estimated by counting the number of leaves with sporulated pustules, and dividing by the total number of leaves of the sample, and multiplying the value found by 100. For severity, the incidence was estimated by the number of pustules per leaf, dividing this value by the number of infected leaves, being expressed in average of pustules per infected leaf (CARDOSO et al., 2016; RIBEIRO; BERGAMIM FILHO; CARVALHO, 1981). The same methodology was used for cercosporiosis and coffee leaf miner.

The collection and evaluation were carried out in June 2016, three weeks before harvest. Throughout the harvest, the cultural treatments were made according to the recommendations for the crop, without the chemical control of coffee leaf rust, cercosporiosis and coffee leaf miner.

Data were submitted to analysis of variance (Test F) and the means of the progenies were grouped by the Scott & Knott test at 5% probability.

3 RESULTS AND DISCUSSION

For the Vegetative Vigor (VV), it can be observed that there was no significant difference between the progenies (Table 2), which presented the same note as the controls, recognized by the high vigor (ANDRADE et al., 2013; SILVA et al. 2016).

TABLE 2 - Average vegetative vigor, height, maturation uniformity, severity and incidence of coffee leaf rust, cercosporiosis and coffee leaf miner.

Progenies	VV	HEIGHT	MU	RS	RI	CS	CI	LMS	LMI
4	8.20 a	126.50 a	77.145 b	2.95 a	31.90 b	1.15 b	9.30 a	0.50 a	0.70 a
12	8.45 a	145.00 a	87.000 a	5.00 b	21.60 b	1.05 b	5.65 a	0.50 a	6.45 c
15	8.65 a	149.00 a	87.915 a	3.80 a	8.70 a	1.10 b	15.00 b	1.00 b	3.35 b
26	8.65 a	138.50 a	86.500 a	1.30 a	20.00 b	1.40 c	16.35 b	0.50 a	1.65 a
27	8.95 a	130.00 a	87.125 a	1.65 a	5.00 a	1.00 b	3.25 a	0.50 a	1.00 a
30	9.05 a	135.50 a	88.615 a	2.40 a	5.55 a	0.65 a	3.90 a	1.00 b	0.55 a
31	8.55 a	107.50 a	89.645 a	3.60 a	29.00 b	0.50 a	2.15 a	0.50 a	2.15 a
35	8.50 a	131.00 a	87.500 a	5.30 b	23.35 b	1.35 c	15.00 b	0.50 a	0.65 a
39	8.10 a	119.00 a	79.835 b	5.00 b	6.85 a	1.65 c	5.50 a	1.25 b	1.65 a
41	8.65 a	141.50 a	87.680 a	6.15 b	26.20 b	1.15 b	6.45 a	1.00 b	3.80 b
MN	7.35 a	168.00 a	84.165 a	8.85 c	64.80 d	1.10 b	4.10 a	2.55 c	7.45 c
T	8.45 a	131.50 a	86.135 a	6.80 b	43.00 c	0.70 a	2.50 a	1.10 b	6.50 c

Averages followed by the same letter in the same column belong to the same group by the Scott & Knott test at 5% probability. Caption: VV = Vegetative vigor; MU = Maturation uniformity; RS. = Rust severity; RI = Rust incidence; CS = Cercosporiosis severity; CI= Cercosporiosis incidence; LMS = Leaf miner severity; LMI. = Leaf miner incidence.

Coffee plants with high VV present lesser impoverishment, higher yield and consequently greater tolerance to pests and diseases. The VV is related to the adaptation capacity of the cultivars in the different edaphoclimatic conditions in which they are grown (CARVALHO et al., 2017). All the progenies presented a good performance in relation to the VV, characterizing a good adaptability to the environment.

Plant height is a very important feature in the recommendation of cultivars, as this is directly related to the ease of handling of the crop and harvesting operations. For this characteristic, it is observed that although there is variation among progeny averages, there was no significant difference (Table 2). This fact may be related to the variation between the plants within the progenies. In the genetic improvement of the coffee tree, plants in the F_3 generation show segregation, which may have contributed to the fact that the progenies still do not present uniformity and there is significant difference between the means of the progenies.

For maturation uniformity, two distinct groups were formed statistically, and the lowest averages observed were of progenies 4 and 39. Among the other progenies there was no significant difference. Fruit uniformity is an important and desirable feature, since coffee beans harvested at the cherry maturation stage present better beverage quality (LEROY et al., 2006).

It was possible to identify a significant difference in the incidence and severity of coffee leaf rust, cercosporiosis, and coffee leaf miner. According to Ivoglo et al., 2008, this fact may indicate the presence of genetic variability among the progenies.

Regarding the leaf rust severity (RS), the formation of three distinct groups was statistically significant, where the lowest averages were of the progenies 26, 27, 30, 4, 31 and 15, and the highest RS was observed in cultivar Mundo Novo, who is one of the witnesses and is highly susceptible. The cultivar Acauã (progeny 39), recognized for resistance to this pathogen (CARVALHO et al., 2012; GROSSI et al., 2013), was in the intermediate group for severity, evidencing the potential of the hybrids under study.

Progenies 27, 30, 39 and 15 presented lower leaf rust incidence and cultivars Mundo Novo and Topázio presented higher RI. Most progenies are derived from Icatu, which originates from the artificial cross of *Coffea canephora* Pierre with the cultivar Bourbon Vermelho of *C. arabica*.

The cultivar Icatu presents rusticity, high vegetative vigor, good yield and variability for resistance to coffee leaf rust, both specific and non-specific (SERA et al., 2010).

The progenies 27, 30 and 15 that obtained good results for RI and RS were highlighted. In the case of coffee leaf rust resistance of the non-specific or polygenic type, it is desirable because it is durable, while the specific resistance can be broken by new genotypes of the pathogen (COSTA et al., 2013). Most of the hybrids studied presented low RI and RS, a fact that is desired by researchers because they are sources of durable and efficient resistance against multiple races of a specific pathogen (REZENDE et al., 2017), not hiding the expression of horizontal resistance (BOTELHO et al. al., 2010).

According to Carvalho et al. (2017), cercosporiosis is a disease that has increased its incidence in coffee crops in recent years and has gained great importance due to the economic damage caused in the crop. Regarding the severity and incidence of this disease, progenies 31, 30 and Topázio worse mean values, while progenies 26 and 35 remained in the group with the highest values for the disease showing less promising.

Three different groups were statistically significant for leaf miner severity. The best means were from progenies 27, 26, 35, 31, 4 and 12, being the highest severity observed in the cultivar Mundo Novo. Regarding to leaf miner incidence, the best averages were those of 30, 35, 4, 27, 39, 26 and 31, and the worse averages were found in Mundo Novo, Topázio and 12.

Some progenies stood out with good averages in several evaluated characters, and this was observed to rank the best ones. Progenies 30, 27 and 15 obtained the best means in relation to RI and RS. At 30 and 27, they presented the best means in six of the seven characters evaluated, where progeny 30 was classified in the second best mean group in relation to LMS and progeny 27 in the second best mean group in relation to CS. And the progeny 15 obtained a relatively good performance in the other characters, being present in the second group of better means of these characters.

4 CONCLUSIONS

Variability was observed among progenies considering the vegetative vigor and the susceptibility of progenies to coffee leaf rust, cercosporiosis and coffee leaf miner.

Progenies 27, 30 and 15 have been indicated to be more tolerant to coffee leaf rust, cercosporiosis and coffee leaf miner, and may be used to continue the breeding program.

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6 REFERENCES

ANDRADE, V. T. et al. Interação genótipo x ambiente em genótipos de cafeeiro Mundo Novo por modelos não lineares e multiplicativos. **Bragantia**, Campinas, v. 72, n. 4, p. 338-345, Oct/Dec. 2013.

BOISSEAU, M. et al. Resistance to *Meloidogyne paranaensis* in wild *Coffea arabica*. **Tropical Plant Pathology**, Brasília, v. 34, n. 1, p. 38-41, Jan/Feb. 2009.

BOTELHO, C. E. et al. Seleção de progênies F4 de cafeeiro obtidas pelo cruzamento de Icatu com Catimor. **Revista Ceres**, Viçosa, MG, v. 57, n. 3, p. 274-281, May/June. 2010.

BOTELHO, D. M. S. et al. Cercosporiosis resistance in coffee germplasm collection. **Euphytica,** v. 213, n. 6, p. 117, June, 2017.

CARDOSO, D. A. et al. Seleção de progênies f4 de cafeeiros com resistência à ferrugem na região sul de minas gerais. **Coffee Science**, Lavras, v. 11, n. 4, p. 555-566, Oct/Dec. 2016.

CARVALHO, A. M. et al. Desempenho agronômico de cultivares comerciais de café resistentes à ferrugem no Estado de Minas Gerais. **Bragantia**, Campinas, v. 71, n. 4, p. 481- 487, Oct/Dec. 2012.

CARVALHO, A. M. et al. Comportamento de cultivares de cafeeiro sob a incidência das doenças da ferrugem e cercosporiose em dois ambientes de cultivo. **Coffee Science**, Lavras, v. 12, n. 1, p. 100-107, Jan/Mar. 2017.

COMPANHIA NACIONAL DE ABASTECIMENTO (CONAB). **Acompanhamento da safra brasileira de café, safra 2018, primeiro levantamento. 2018**. Available on: <http://www.conab.gov.br/>. Access in: 28 jan. 2018.

COSTA, J. C. et al. Field performance of coffee progenies and cultivars with specific resistance to rust. **Coffee Science**, Lavras, v. 8, n. 2, p. 183-191, Apr/June. 2013.

CUSTÓDIO, A. A. P. et al. Comparação e validação de escalas diagramáticas para cercosporiose em folhas de cafeeiro. **Ciência e Agrotecnologia**, Lavras, v. 35, n. 6, p. 1067-1076, Nov/Dez. 2011.

FAZUOLI, L. C. et al. A ferrugem alaranjada do cafeeiro e a obtenção de cultivares resistentes. **O Agronômico**, Campinas, v. 59, n. 1, p. 48-53. 2007.

GROSSI, L. et al. Rust resistance in arabic coffee cultivars in northern Paraná. **Brazilian Archives of Biologyand Technology**, Curitiba, v. 56, n. 1, p. 27-33, Jan/Feb. 2013.

IVOGLO, M. G. et al. Divergência genética entre progênies de café robusta. **Bragantia**, Campinas, v. 67, n. 4, p. 823-831, Oct/Dec. 2008.

LEROY, T. et al. Genetics of coffee quality. **Brazilian Journal or Plant physiology**, Londrina, v. 18, n. 1, p. 229-242, Jan/Mar. 2006.

MAGALHÃES, S.T. et al. Leaf Alkaloids, Phenolics, and Coffee Resistance to the Leaf Miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). **Journal of Economic Entomology**, Annapolis, v. 103, n. 4, p. 1438-1443, July/Aug. 2010.

MATIELLO, J. B. Critérios para a escolha de cultivar de café. In: CARVALHO, C. H. S. de (Ed.). **Cultivares de café: origem, características e recomendações**. Brasília: EMBRAPA Café, 2008. v. 1, p. 157-226.

MENDONÇA, A. P. N. et al. *Coffea arabica* clones resistant to coffee leaf miner. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 16, n. 1, p. 42-47, Jan/Mar. 2016.

OMETTO, J. C. **Bioclimatologia Vegetal**. São Paulo: Ceres, 1981. 425p.

PEREIRA, R. B. et al. Potential of essential oils for the control of brown eye spot in coffee plants. **Ciência e Agrotecnologia**, Lavras, v. 35, n. 1, p. 115–123, Jan/Feb. 2011.

POZZA, E. A; CARVALHO, L. C; CHALFOUN, S. M. Sintomas de injúrias causadas por doenças em cafeeiro. In: GUIMARÃES, R.J; MENDES A.N.G; BALIZA D.P. (eds). **Semiologia do cafeeiro**: **Sintomas de desordens nutricionais, fitossanitárias e fisiológicas**. Lavras: Editora UFLA, 2010. p. 68-106.

RAMALHO, M. A. P. et al. **Aplicações da genética quantitativa no melhoramento de plantas autógamas**. Lavras: Editora UFLA, 2012. 522 p.

REZENDE, R. M. et al. Resistance of C*offea arabica* progenies in field conditions infested by *Meloidogyne exigua*. **Nematropica**, Bradenton, v. 43, n. 2, p. 233-240, July/Dec. 2013.

REZENDE, R. M. et al. Agronomic characterization and responses to coffee leaf rust in coffee progenies resistant to the gall nematode *Meloidogyne exigua*. **Semina: Ciências Agrárias**, Londrina, v. 38, n. 2, p. 671-682, Mar/Apr. 2017.

RIBEIRO, I. J. A.; BERGAMIM FILHO, A.; CARVALHO, P. C. T. Avaliação da resistência horizontal a *Hemileia vastatrix* Berket Br. em cultivares de *Coffea arabica* L. em condições naturais de epidemia. **Summa Phytopathologica**, Piracicaba, v. 7, n. 1-2, p. 80-95, Apr/June. 1981.

SANTOS, F. da S. et al. Progress of brown eye spot (*Cercospora coffeicola* Berkeley and Cooke) in coffee trees in organic and conventional systems. **Summa Phytopathologica**, Botucatu, v. 34, n. 1, p. 48-54, Jan/Feb. 2008.

SCALON, J. D.; MATEUS, A. L. S. S.; ZACARIAS, M. S. Análise espaço-temporal do nível de infestação do bicho-mineiro *Leucoptera coffeella*, (Guérin-Menèville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) em cafezal orgânico (*Coffea arabica* L.). **Coffee Science**, Lavras,v. 8, n. 3, p. 347-353, Jul/Set. 2013.

SERA, G. H. et al. Resistência à ferrugem alaranjada em cultivares de café. **Coffee Science**, Lavras, v. 5, n. 1, p. 59-66, Jan/Apr. 2010.

SHIGUEOKA, L. H. et al. Selection of Arabic coffee progenies with rust resistance. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 14, n. 2, p. 88-93, Apr/June. 2014.

SILVA, V. A. et al. Recuperação de cultivares de café submetidas ao esqueletamento aos quatro anos e meio de idade. **Coffee Science**, Lavras, v. 11, n. 1, p. 55-64, Jan/Mar. 2016.

TALHINHAS, P. et al. The coffee leaf rust pathogen *Hemileia vastatrix*: one and a half centuries around the tropics. **Molecular Plant Pathology**, v. 18, n. 8, p. 1039-1051, Oct/Nov. 2016.

ZAMBOLIM, L. Current status and management of coffee leaf rust in Brazil. **Tropical Plant Pathology**, Brasília, v. 41, n. 1, p. 1-8, Feb. 2016.

ZAMBOLIM, L.; VALE, F.X.R.; ZAMBOLIM, E.M. Doenças do cafeeiro (*Coffea arabica* e *Coffea canephora*). In: KIMATI, H.; AMORIM, L.; REZENDE, J.A.M.; BERGAMIM FILHO, A.; CAMARGO, L.E.A. (Ed**.). Manual de fitopatologia: doenças das plantas cultivadas. 4.ed**. São Paulo: Agronômica Ceres, 2005. 663p.

ZAMPIROLI, R. et al. Application technology for chemically controlling coffee leaf miner in the cerrado of Minas Gerais State. **Revista de Ciências Agrárias/Amazonian Journal of Agricultural and Environmental Sciences**, Belém, v. 60, n. 3, p. 256-262, July/Sept. 2017.

EARLY GROWTH OF COFFEE PLANTS AND SOIL FERTILITY PROPERTIES IN RESPONSE TO COFFEE HUSK APPLICATION

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ABSTRACT: Coffee processing generates large amounts of husk, which can be used as organic fertilizer if technical criteria are considered. This study investigated the effect of coffee husk, applied to or incorporated into the soil, on soil fertility properties, early crop growth and nutrient accumulation in coffee plants. The experiment analyzed coffee plants in a greenhouse in pots, in randomized blocks, in a 5x2 factorial arrangement plus a control treatment, with four replicates. The treatments consisted of the combination of five coffee husk rates (3.5; 7; 14; 28, and 56 t ha^{-1}), applied in two forms: spread on the surface or incorporated into the soil, plus the control treatment, without husk application. Portions of 7 dm^3 soil were blended with lime, phosphate fertilizer, as well as coffee husk rates in the treatments with residue incorporation, and incubated for 30 days. Thereafter, one coffee seedling per plot was planted, the coffee husk rates were applied on the soil surface for the treatments without residue incorporation, and the plants were left to grow for 180 days. Coffee husk applied to or incorporated into the soil surface increases the K and organic matter contents of the soil, intensifies the early growth of coffee plants and accelerates N and K accumulation in the plant shoots. The application of coffee husk on the surface is more indicated than its incorporation into the soil, and the best rate at coffee planting is equivalent to 20 t ha^{-1}.

Index terms: *Coffea arabica*, organic fertilization, organic waste, nutrient, potassium.

CRESCIMENTO INICIAL DO CAFEEIRO E ATRIBUTOS DE FERTILIDADE DO SOLO COM APLICAÇÃO DE CASCA DE CAFÉ

RESUMO: No beneficiamento do café são geradas grandes quantidades de casca, e o seu uso como adubo orgânico deve seguir critérios técnicos. Objetivou-se avaliar o efeito da casca de café, aplicada na superfície ou incorporada ao solo, em atributos de fertilidade do solo, no crescimento inicial e no acúmulo de nutrientes no cafeeiro. O experimento foi conduzido em casa-de-vegetação, em vasos, em delineamento em blocos casualizados, em esquema fatorial 5x2, um tratamento controle, e 4 repetições. Os tratamentos foram constituídos pela combinação de 5 doses de casca de café, equivalentes a 3,5; 7; 14; 28 e 56 t ha^{-1}, aplicadas de 2 formas diferentes: na superfície ou incorporada no solo, além do controle, sem aplicação da casca. Porções de 7 dm^3 de solo receberam calcário e adubo fosfatado, além das doses de casca de café, nos tratamentos com a incorporação do resíduo, e foram submetidas à incubação por 30 dias. A seguir, foi efetuado transplantio de uma muda de cafeeiro por vaso, aplicação das doses de casca de café na superfície do solo, e o experimento foi conduzido por 180 dias. A aplicação da casca de café na superfície ou incorporada aumenta os teores de K e de matéria orgânica do solo, o crescimento inicial do cafeeiro e o acúmulo de N e K na parte aérea das plantas. A aplicação da casca de café na superfície é mais indicada do que a incorporação desse resíduo orgânico no solo, e a melhor dose no transplantio do cafeeiro é equivalente a 20 t ha^{-1}.

Termos para indexação: *Coffea arabica*, adubação orgânica, resíduo orgânico, nutriente, potássio.

1 INTRODUCTION

Brazil is the world's leading coffee producer, and the crop is essential for the Brazilian economy, especially for the Southern region of the state of Minas Gerais, which has the highest domestic output (CONAB, 2017).

Coffee processing generates high residue quantities, one of which is the coffee cherry husk, a spin-off from the manufacturing of dry-processed coffees (ZOCA et al., 2014; GARCIA and BIANCHI, 2015; MENEGHELLI et al., 2016).

The husk: grain proportion in terms of weight, is approximately 1:1, i.e., in each growing season an equal quantity of processed coffee and of husk is generated (MATIELLO et al., 2010; ASSIS et al., 2011; FERNANDES et al., 2013). Thus, in the growing seasons of 2017, the production of 44,970,000 bags of processed coffee in Brazil (CONAB, 2017) may have generated approximately 2.7 million tons of coffee husk, of which approximately 820 thousand tons in the South and Midwest regions of Minas Gerais. Accordingly, there is a need to find alternative uses for this organic waste, to prevent environmental pollution problems (ZOCA et al., 2014; PESSOA et al., 2015).

Coffee husk can be used as animal feed (CARVALHO et al., 2011; OLIVO et al., 2017); to

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extract antioxidant compounds, used in the food, cosmetic and pharmaceutical industries (GARCIA and BIANCHI, 2015); as bedding in confinement facilities of animals (MATIELLO et al., 2010); and for power generation by direct burning, in industrial furnaces or in coffee bean dryers (SATER et al., 2011). However, for being a source of nutrients, in particular of K (HIGASHIKAWA; SILVA; BETTIOL, 2010), it is largely used in agriculture, as a substrate component for seedling production (ASSIS et al., 2011), in organic compost (SILVA et al., 2015), or mainly as organic fertilizer of coffee plants (FIDALSKI and CHAVES, 2010; FERNANDES et al., 2013).

However, for coffee cultivation, particularly at transplanting, there is no consensus on neither adequate coffee husk rates and about the best form of applying this residue, be it applied on or incorporated into the soil. The official recommendations of liming and fertilization for the state of São Paulo and Minas Gerais mention that coffee husk should be applied in the planting furrow of coffee (RAIJ et al., 1997; CFSEMG., 1999); while there is also information that organic residues should be spread on the soil surface (MATIELLO et al., 2010).

This study aimed to evaluate the effect of coffee husk, applied on the soil surface or incorporated into it, on soil fertility properties and on the early growth and nutrient accumulation of coffee plants.

2 MATERIAL AND METHODS

The experiment was carried out in a greenhouse, with coffee cultivated in pots, in Alfenas, Minas Gerais, from September 2014 through July 2015. A soil sample of approximately 400 dm^3 with clay texture from the surface layer (0-20 cm) was analyzed, and the results of the initial routine chemical (SILVA, 2009) and particle-size analysis (CAMARGO et al., 2009) are presented in Table 1.

The experiment was arranged in randomized blocks, in a 5x2 factorial arrangement, with one control treatment and four replicates, resulting in a total of 44 experimental units (pots). The treatments consisted of combinations of five coffee husk rates (12.3; 24.6; 49.2; 98.4; 196.6 g pot^{-1}), equivalent, based on surface area of each pot (352.39 cm^2) and in the area corresponding to 1 ha (10.000 m^2), to 3.5; 7; 14; 28; and 56 t ha^{-1}, with two application forms: spread on the soil surface or mixed with the soil volume of each pot, simulating an application in the planting furrow. In the control treatment, no organic fertilization with coffee husk was applied. The coffee rusk rates were defined according to the CFSEMG (1999). The pots used in the experiment had a capacity of 8 dm^3.

The coffee husk, a spin-off product of the processing of dry-processed coffees, was previously air-dried in the shadow and sampled to determine the moisture, pH and chemical composition, on a dry basis, as described by Tedesco et al. (1995) (Table 2).

Soil portions of 7 dm^3 were weighed, treated with dolomitic lime (CaO = 39%; MgO = 13%; PRNT = 91%), to raise the initial base saturation of the soil to 60% (CFSEMG, 1999); phosphate fertilizer, at a rate of 100 mg P dm^{-3}, as simple superphosphate; and with coffee husk rates, in the treatments with organic waste incorporation. After blending these inputs with each soil portion, these were filled in 8 dm^3 pots, moistened with distilled water to about 70% of the water-holding capacity, and incubated for 30 days. During incubation, the soil moisture of each pot was maintained at 70% of water-holding capacity by periodic weighing of the pots and replacement of the lost water.

At the end of incubation, the soil portions were removed from the pots, air-dried, and a sample of 0.2 dm^3 per pot was collected for routine analysis of the chemical properties (SILVA, 2009) and electrical conductivity, in a soil:distilled water extract at a 1:5 (v:v) ratio (RAIJ et al., 2001). After sampling, 6.8 dm^3 of soil was refilled into the pots, and one seedling of coffee cultivar Mundo Novo IAC 376-4 was planted per pot in Dezember 2014. At transplanting, the seedlings had the following mean characteristics: height 21 cm; stem diameter 0.25 cm; and 12 leaves.

After planting the coffee seedlings the coffee husk rates were applied to the surface of the pots, in the treatments of surface residue application. Thereafter, the soil of each pot was watered to 70% water-holding capacity, and the plants were left to grow for 180 days.

During the experiment, the soil moisture was maintained at approximately 70% of the water-holding capacity. At 30, 70 and 100 days after planting of the coffee seedlings N fertilization in the form of urea was applied. At each application, 100 mL solution containing 20 mg N dm^{-3} was applied on the surface of all pots. No K was fertilized in the experiment.

TABLE 1 - Chemical and particle-size characterization of the soil used in the experiment.

OM	pH $CaCl_2$	P-Mehlich	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	T	V	m	Sand	Silt	Clay
g dm^{-3}		mg dm^{-3}	——— $mmol_c$ dm^{-3} ———							— % —		——— g kg^{-1} ———		
30	5,3	6	3,1	20	11	1	38	34	72	47	3	384	93	523

OM - Organic matter; H+Al - potential acidity; SB - sum of bases; T - potential cation exchange capacity; V - base saturation; m - Al^{3+} saturation

TABLE 2 - Moisture, pH and chemical composition on a dry basis of the coffee husk used in the experiment.

Moisture	pH	C-org.	N	C/N ratio	P		K
%		——— g kg^{-1} ———			——— g kg^{-1} ———		
8,8	4,5	360,2	22,1	16/1	0,7		27,3
Ca	Mg	S	B	Cu	Fe	Mn	Zn
——— g kg^{-1} ———				——— mg kg^{-1} ———			
3,4	1,1	1,7	27	31	238	42	13

At crop, the plant height from the soil surface to the base of the apical bud was measured with a tape measure. Thereafter, the coffee plants were cut at the ground level of each pot, washed, dried to constant weight in a forced air circulation oven, ground, and the macronutrient and micronutrient contents were determined (TEDESCO et al., 1995). Nutrient accumulation in the coffee shoots was calculated as the product of the nutrient contents by the shoot dry matter yield of the coffee trees.

After the crop, in the treatments with coffee husk spread on the soil surface, the remaining husk was removed with a spatula, dried in a forced air circulation oven and weighed to determine the decomposition rate of this organic waste. Then, with a spatula and a ruler, soil was taken from the layers 0-5, 5-10 and 10-20 cm of each pot for routine chemical analysis (SILVA, 2009).

The results of soil chemical attributes and parameters evaluated in the plants were subjected to analysis of variance, the Tukey test for mean comparison ($p < 0.05$) and to polynomial regression. In the treatments of residue surface application, the statistical analysis of the results regarding K and organic matter contents were carried out for each layer, since a comparison between layers was not the objective of this study. For these analyses, we used the statistical program AgroEstat (BARBOSA; MALDONADO JÚNIOR, 2015).

3 RESULTS AND DISCUSSION

There was interaction between the factors studied (coffee husk rates and application forms) on the chemical attributes evaluated in soil. The incorporation of coffee husk rates into the soil altered ($p < 0.01$) the K, organic matter contents and values of electrical conductivity of the soil.

There was a linear increase in K^+ and organic matter contents and in the values of electrical conductivity (EC) of the soil after incorporation of coffee husk rates (Figure 1A, 1B and 1C). In relation to K^+, the soil content ranged from 3.3 to 20.4 $mmol_c$ dm^{-3}, and was six times higher in the treatment with the highest coffee husk rate than in the control.

Soil K^+ contents above 5 $mmol_c$ dm^{-3} are considered very high for coffee, and under this condition, no K fertilization is required at seedling planting, nor in the first and second year after planting (CFSEMG, 1999). Thus, soil incorporation of an amount of 56 t ha^{-1} of coffee husk increased the soil K content 4-fold in relation to the lower limit of the class of very high content, established by the soil fertility commission of the state of Minas Gerais CFSEMG (1999).

On average 94% of the K supplied to the soil with the incorporation of the doses of coffee husk were detected by soil analysis, in the samples collected 30 days after incubation of the organic waste with soil, demonstrating the rapid K release from coffee husk. This can be explained by the fact that K does not participate in the structure of organic compounds, and is maintained in the ionic form in plant tissues and in organic waste (SODRÉ et al., 2012).

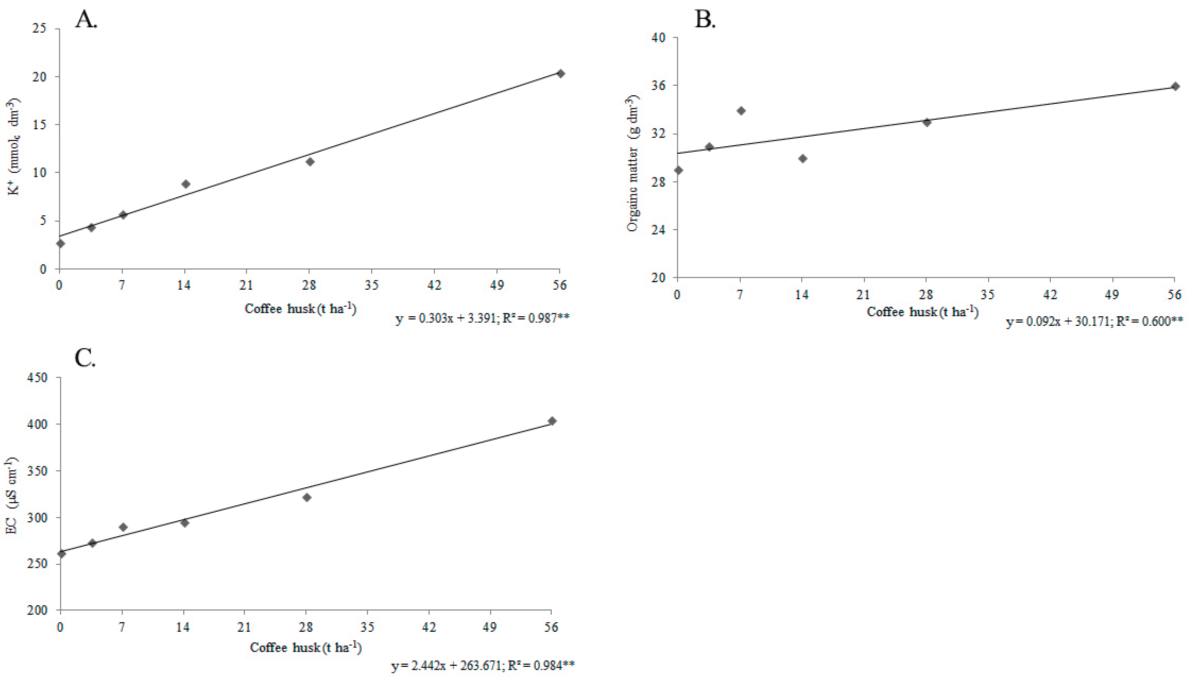

FIGURE 1 - Contents of K (A), organic matter (B) and electrical conductivity of the soil (C) in response to incorporated coffee husk rates.

Similar results were reported by Zoca et al. (2014), in a greenhouse experiment using soil columns and rates of different residues from coffee processing, including the husk of coffee cherry. The authors found that K release from these residues was high (> 90%), and that they could be used as a substitute for mineral K fertilizer. In a greenhouse experiment, Sodré et al. (2012) also found a linear increase in the soil K^+ content after application extract rates of cocoa pod husk. For producing coffee plants, Fernandes et al. (2013) found that annual applications of 20 t ha^{-1} of coffee husk can offset mineral K fertilization by up to 100%.

Very high nutrient contents in the soil can induce nutritional imbalances. In the case of K^+, the result is a decrease in the plant uptake of other cations such as Ca^{2+} and Mg^{2+}, due to competition for absorption sites, aside from favoring the displacement of Mg^{2+} from the exchange complex, thereby aggravating K losses by leaching (DAMATTO JÚNIOR et al., 2006). The incidence of diseases such as brown eye spot and rust in coffee plants is also favored by nutritional imbalance, with very high K contents in soil and plant leaves, due to coffee husk applications (SANTOS et al., 2008). Excessive K^+ contents in the soil can also promote the dispersion of clay particles, reducing the porosity and affecting other soil physical properties (UYEDA et al., 2013).

The contents of soil organic matter ranged from 30.2 to 35.3 g dm^{-3} in the treatments in which coffee husk was incorporated into the soil, and the resulting increase was 17% compared to the treatments with highest and lowest applications (Figure 1B). Of the quantity of organic C added to the soil in the coffee husk rates, a mean 32% was found to remain in the soil as a soil organic matter component, where it was detected by chemical analysis. This result can be explained by the high lignin content contained in this organic waste (ZOCA et al., 2014), which hampers decomposition and humus formation in the soil.

Souza et al. (2014) found no changes in the organic matter content of the 0 -20 cm soil layer after five years of surface applications of rates up to 36 t ha^{-1} of waste generated by the guava processing industry, consisting basically of seeds of the fruit. In the 0 -5 cm layer of an area of producing coffee plants, Silva et al. (2015) found a linear increase in organic C content, and consequently of soil organic matter, in response to surface applications of organic compound containing coffee husk.

After incorporating coffee husk rates into the soil, the EC ranged from 264 to 400 µs cm^{-1}, and in the treatment fertilized with the highest organic residue rate the soil EC was 1.5 times higher than in the control treatment. The high ion concentrations, e.g., of K^+, in coffee husk explain the increase in soil EC by the application of this organic waste (CARMO et al., 2016). Similar results were reported by Higaskikawa, Silva and Bettiol (2010); Pinheiro, Silva and Lima (2014) and Carmo, Lima and Silva (2016), who also observed an increase in the soil EC in response to coffee husk application.

In view of the increase in the EC of the soil, Higaskikawa, Silva and Bettiol (2010) warned that the use of coffee husk in agriculture requires a careful management of the rates to be applied to crops. However, in this study the soil EC values were below the critical range of 750 -3.490 µs cm^{-1}, mentioned by Carmo, Lima and Silva (2016) as detrimental to plant growth.

The pH values and potential acidity (H+Al), and the contents of P-Mehlich, Ca^{2+} and Mg^{2+} in the soil were not altered ($p > 0.05$) with the incorporation of coffee husk rates into the soil. The mean values of the pH in $CaCl_2$ and H+Al in the experiment were 5.8 and 24.7 mmolc dm^{-3}, respectively. The mean nutrient contents were, respectively, 31 mg dm^{-3}; 36 and 15 $mmol_c$ dm^{-3}, which are classified as, respectively, medium, good and good values, based on CFSEMG (1999), for the implementation of a coffee plantation on clayey soil. These results can be explained by the low P, Ca and Mg contents of coffee husk (Table 2). Similar results were found by Fidalski and Chaves (2010) and by Fernandes et al. (2013).

In a field experiment with producing coffee plants, Fidalski and Chaves (2010) found that two surface applications of 20 t ha^{-1} of coffee husk did not alter the contents of P-Mehlich, Ca^{2+} and Mg^{2+} in the soil in the 0-5; 5-10; 10-20 and 20-40 cm layers. In a coffee plantation treated with surface applications of coffee husk for three consecutive growing seasons, Fernandes et al. (2013) found no alterations in the pH and P-resin, Ca^{2+}, and Mg^{2+} contents in the soil surface layer (0-20cm).

The application of coffee husk rates on the surface altered ($p < 0.01$) the K and organic matter contents of the soil, but did not influence ($p > 0.05$) the pH and H+Al values, and the P, Ca and Mg contents in the 0-5, 5-10 and 10-20 cm layers.

There was an increase in K contents in the soil in the 0-5, 5-10, and 10-20 cm layers, with the application of coffee husk rates on the surface (Figure 2A). The K contents in the 0-5, 5-10 and 10-20 cm layers were 0.8 - 19.8, 0.6 - 13.4, and 0.9 - 7.9 mmolc dm^{-3}, respectively. With this, after 180 days of coffee husk applications to the surface, the increases in K contents in these layers were, respectively, 24.8; 22.3 and 8.8-fold, compared to the control treated with the highest organic waste rate (equivalent to 56 t ha^{-1}).

Induced by the application of residues from coffee processing, Zoca et al. (2014) also stated an increase in the soil K content in the different layers. According to these authors, the potassium applied in these residues did not prevent K leaching losses.

The contents of soil organic matter, in the 0-5 cm layer, increased linearly with the surface application of the coffee husk rates, and the resulting increase was 1.1-fold compared to the extreme treatments with applications of 0 and 56 t ha^{-1} (Figure 2 B). In the other evaluated layers (5-10 and 10-20 cm) the organic matter content of the soil fertilized with coffee husk on the surface remained unchanged, with mean contents of 26 and 25 g dm^{-3}, respectively.

After 180 days of organic residue applications on the soil surface, the decomposition of coffee husk was, on average, 70% and 50%, respectively, in the soils treated with the two lowest and the two highest coffee husk rates. Zoca et al. (2014) reported decomposition of 20 to 60% of the coffee cherry husk applied to the soil surface, and the lowest decomposition rates were also observed in response to the highest residue applications.

No effect was observed of interaction between the factors studied (coffee husk rates and application forms) on the parameters evaluated in coffee plants: plant height; dry matter yield and nutrients accumulated in shoots. With this, both the surface application and the incorporation of coffee husk rates in the soil resulted in a quadratic effect in height and dry matter yield of coffee shoots, and the highest values were obtained in response to the estimated rates of 42 and 49 t ha^{-1}, respectively (Figure 3A and 3B).

The application of a rate of 20 t ha^{-1} would result in coffee plants with a height of 60 cm and shoot dry matter of 60 g, corresponding to 94 and 89% of the maximum values, and 18 and 31% higher than the control, respectively. Thus, rates above 20 t ha^{-1} of coffee husk induced practically no increase in the early growth of coffee plants.

In producing coffee plants, Fidalski and Chaves (2010) observed an increase in plant height, number of branches, canopy volume, and yield in response to coffee husk applications.

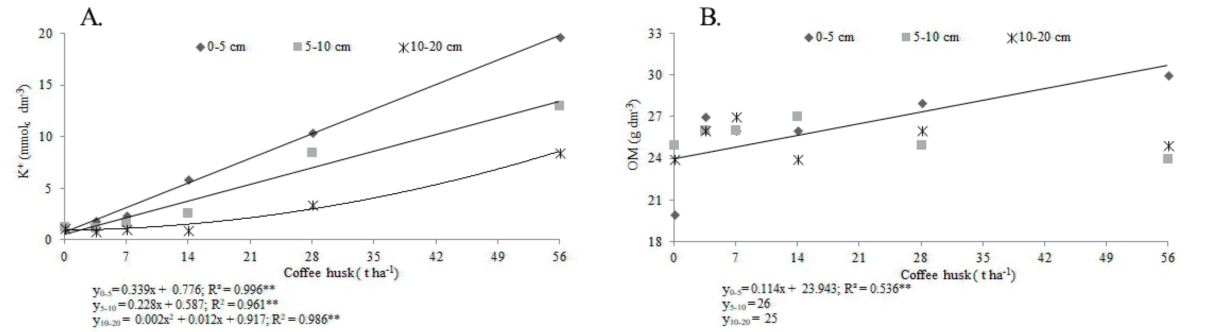

FIGURE 2 - Contents of K (A), and organic matter (B), in the 0-5; 5-10; 10-20 cm layers, in response to the application of coffee husk rates on the soil surface.

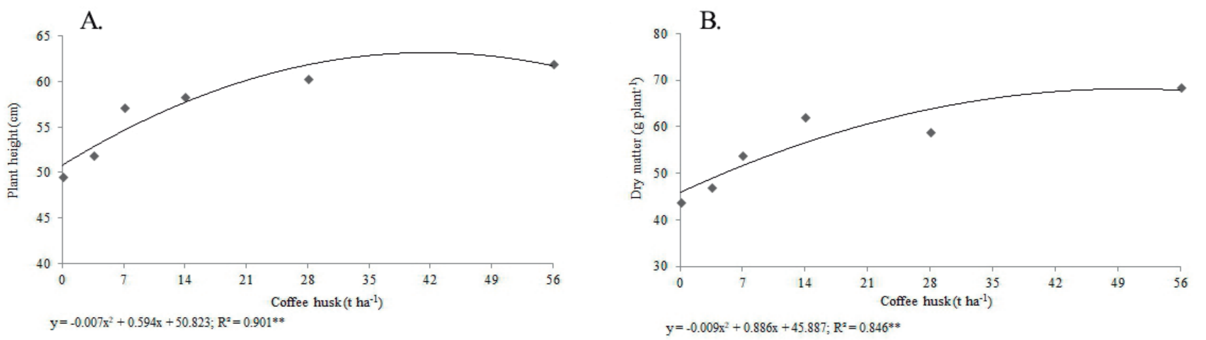

FIGURE 3 - Height (A) and shoot dry matter production (B) of coffee plants in response to coffee husk rates.

An increase in coffee yield was also reported by Fernandes et al (2013) due to the use of organic waste.

In relation to the husk application forms, the soil surface application resulted in coffee plants with taller height and higher shoot dry matter yield, with mean increases of 20 and 14%, respectively (Figures 4A and 4B). The immobilization of soil N and the higher temperature and soil moisture variations, which probably occurred in the treatments with coffee husk incorporation, possibly explain these results. Thus, the coffee husk application of the surface may possibly contribute to the maintenance of soil moisture and, consequently, favor the growth of the coffee plants.

In an experiment with coffee plants cultivated in pots, Santos, Souza and Alves (2003) found that the incorporation of crop residues of maize shoots in the soil reduced the leaf area and plant height of coffee trees, whereas in the treatments where these residues were applied to the soil surface, the early growth of coffee plants was intensified. The authors used the same explanations as in this study to justify these results. In a coffee area intercropped with tree species and fruit trees, Souza et al. (2017) found lower temperature and higher humidity, compared to the treatment in which this practice was not used (monoculture, under full sun), where the former conditions favored the plant development.

There was an increase in the cumulative N and K quantities in coffee shoots in response to the application of coffee husk rates, both on the soil surface or incorporated into it, and the increases were 1.5 and 2.1-fold when compared with the control treated with the highest organic waste rate (Figures 5A and 5B).

The cumulative Ca and Mg coffee shoot contents were not altered ($p>0.05$) by coffee husk application, with mean contents of 310 and 108 mg plant^{-1}, respectively (Figure 5C). Therefore, in this experiment no decrease in Ca and Mg plant uptake was observed, due to the presence of very high K contents in the soil. The initial Ca and Mg contents in the soil (20 and 11 mmol$_c$ dm^{-3} respectively), classified as good content, according to CFSEMG (1999), and the use of dolomitic lime (CaO = 39%; MgO = 13%) may explain the absence of competitive inhibition among the cations K^+, Ca^{2+} and Mg^{2+}.

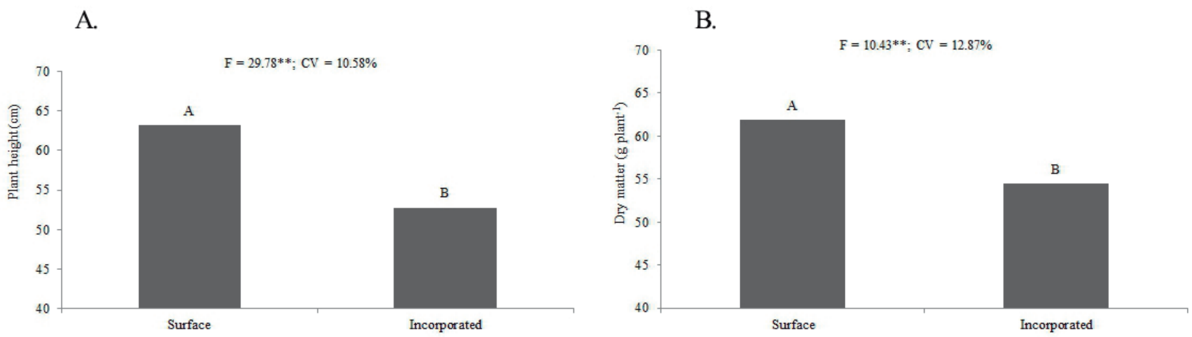

FIGURE 4 - Plant height (A) and shoot dry matter production (B) of coffee plants in response to coffee husk application on the soil surface or incorporated into it.

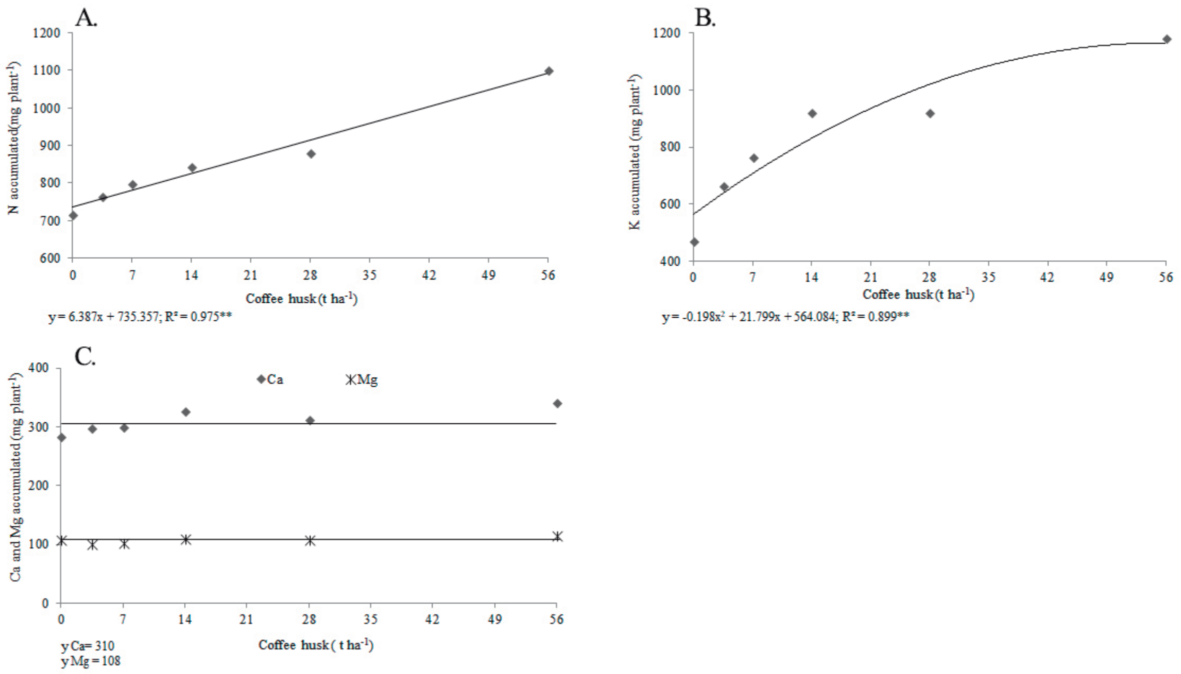

FIGURE 5 - Accumulated quantities of N (A) (B), K, Ca, and Mg (C) in coffee shoots in response to coffee husk rates.

The coffee husk rates did not change ($p> 0.05$) the quantities of P, S, B, Cu, Fe, Mn, and Zn accumulated in the coffee shoots, and the mean values were, respectively, 56; 41; 1.4; 0.3; 4.1; and 2.4 mg $plant^{-1}$. The low contents of these nutrients in coffee husk explain this lack of response.

The cumulative quantities of N and K in the coffee shoots were not influenced ($p>0.05$) by the application form of coffee husk, be it spread on or incorporated into the soil (Figure 6). In the case of K, the release of the nutrient from coffee husk is independent of the application form of the residue. In relation to N, the possible immobilization by soil microorganisms, in the treatments of coffee husk incorporation, probably occurred only during the first weeks after coffee planting, and was followed by N mineralization. Therefore, at the end of the experiment, N accumulation in the plant shoots was similar to that of the treatments in which the organic residue was applied on the surface.

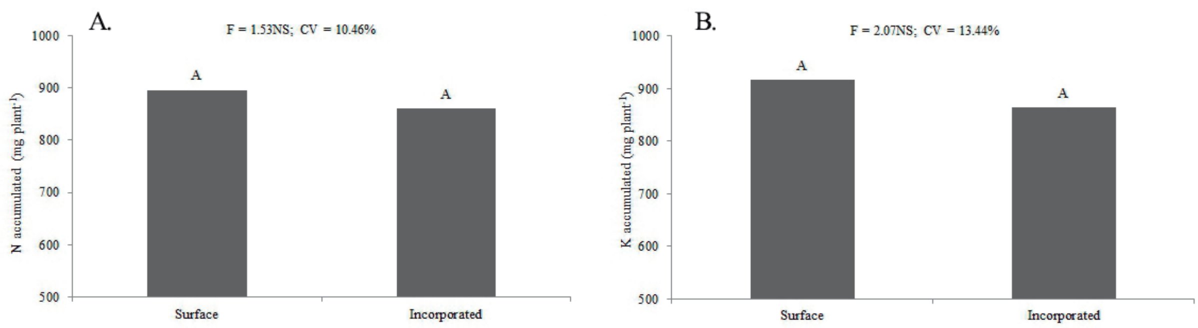

FIGURE 6 - Accumulated quantities of N (A) and K (B) in coffee shoots in response to coffee husk application on the soil surface or incorporated into it.

4 CONCLUSIONS

Coffee husk applied to or incorporated into the soil surface increases the contents of K and organic matter, the early growth of coffee plants and N and K accumulation in the plant shoots;

The application of coffee husk on the soil surface is more indicated than the incorporation of organic waste into the soil, and the best rate at coffee planting is equivalent to 20 t ha^{-1}.

5 ACKNOWLEDGMENTS

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6 REFERENCES

ASSIS, A. M. et al. Cultivo de orquídea em substratos à base de casca de café. **Bragantia**, Campinas, v. 70, n. 3, p. 544-549, Apr/June. 2011.

BARBOSA, J. C.; MALDONADO JÚNIOR, W. **Experimentação agronômica e AgroEstat**: sistema para análises estatísticas de ensaios agronômicos. Jaboticabal: Multipress, 2015. 396p.

CAMARGO, O. A. et al. **Métodos de análise química, mineralógica e física de solos do Instituto Agronômico de Campinas**. Campinas: Instituto Agronômico, 2009. 77p. Boletim Técnico, 106.

CARMO, D. L.; LIMA, L. B.; SILVA, C. A. Soil fertility and electrical conductivity affected by organic wastes and nutriente inputs. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 40. 2016.

CARVALHO, P. L. O. et al. Sticky coffee hull silage on the feeding of growing and finishing pigs. **Revista Brasileira de Zootecnia**, Viçosa, v. 40, n. 2, p. 343-351, Feb. 2011.

COMISSÃO DE FERTILIDADE DO SOLO DO ESTADO DE MINAS GERAIS – CFSEMG. **Recomendações para o uso de corretivos e fertilizantes em Minas Gerais**, 5ª aproximação. Viçosa: Ed. UFV, 1999. 359p.

COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira café safra 2017**: quarto levantamento, dezembro/2017. Brasília, 2017.

DAMATTO JUNIOR, E. R. et al. Alterações em propriedades de solo adubado com doses de composto orgânico sob cultivo de bananeira. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 28, n. 3, p. 546-549, Sept/Dec. 2006.

FERNANDES, A. L. T. et al. Redução da adubação mineral do cafeeiro arábica com a utilização de palha de café. **Coffee Science**, Lavras, v. 8, n. 3, p. 324-336, July/Sept. 2013.

FIDALSKI, J.; CHAVES, J. C. D. Resposta do cafeeiro (*Coffea arabica* L.) IAPAR-59 à aplicação superficial de resíduos orgânicos em um latossolo vermelho distrófico típico. **Coffee Science**, Lavras, v. 5, n. 1, p. 75-86, Jan/Apr. 2010.

GARCIA, L. R. P.; BIANCHI, V. L. D. Capacidade antioxidante em resíduos da indústria cafeeira. **Brazilian Journal of Food Technology**, Campinas, v. 18, n. 4, p. 307-313, Oct/Dec. 2015.

HIGASHIKAWA F. T.; SILVA, C. A.; BETTIOL, W. Chemical and physical properties of organic residues. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 34, n. 5, p. 1743-1752, 2010.

MATIELLO, J. B. et al. Cutura do café no Brasil: manual de recomendações. Rio de Janeiro; Varginha: Fundação PROCAFÉ, 2010. 542 p.

MENEGHELLI, C. M. et al. Resíduo da secagem dos grãos de café como substrato alternativo em mudas de café conilon. **Coffee Science**, Lavras, v. 11, n. 3, p. 329-334, July/Sept. 2016.

OLIVO, P. M. et al. Assessing the nutritional value of agroindustrial co-products and feed through chemical composition, in vitro digestibility, and gas production techinique. **Acta Scientiarum Animal Science**s, Maringá, v. 39, n. 3, p. 289-295, July/Sept. 2017.

PESSOA, R. S. et al. Solubilization of potassium from alternative rocks by humic and citric acids and coffee husk. **Ciência e Agrotecnologia**, Lavras, v. 39, n. 6, p. 553-564, Nov/Dec. 2015.

PINHEIRO, G. L.; SILVA, C. A.; LIMA, J. M. Soluble carbon in oxisol under the effect of organic residue rates. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 38, n. 3, p. 810-820, May/June. 2014.

RAIJ, B. V. et al. (Ed.). **Recomendações de adubação e calagem para o Estado de São Paulo**. 2 ed. rev. atual. Campinas: Instituto Agronômico/Fundação IAC, 1997. 285p. Boletim Técnico, 100.

RAIJ, B. V. et al. **Análise química para avaliação da fertilidade de solos tropicais**. Campinas: Instituto Agronômico, 2001. 285p.

SANTOS, C. C.; SOUZA, I. F.; ALVES, L. W. R. Efeitos de restos culturais de milho sobre o crescimento de plantas de cafeeiro (*Coffea arabica* L.). **Ciência e Agrotecnologia**, Lavras, v. 27, n. 5, p. 991-1001, Sept/Oct. 2003.

SANTOS, F. S. et al. Adubação orgânica, nutrição e progresso de cercosporiose e ferrugem-do-cafeeiro. **Pesquisa Agropecuária Brasileira**, Brasília, v. 43, n. 7, p. 783-791, July. 2008.

SATER, O. et al. Estudo comparativo de carbonização de resíduos agrícolas e florestais visando à substituição de lenha no processo de secagem de grãos de café. **Revista Ceres**, Viçosa, v. 58, n. 6, p. 717-722, Nov/Dec. 2011.

SILVA, F. C. **Manual de análises químicas de solos, plantas e fertilizantes**. 2ed. rev atual. Brasília: Embrapa Informação Tecnológica, 2009. 627p.

SILVA, V. M. et al. Estoques de carbono e nitrogênio e densidade do solo em sistemas de adubação orgânica de café conilon. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 39, n. 5, p. 1436-1444, Sept/Oct. 2015.

SODRÉ, G. A. et al. Extrato da casca do fruto do cacaueiro como fertilizante potássico no crescimento de mudas de cacaueiro. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 34, n. 3, p. 881-887, July/Sept. 2012.

SOUZA, G. S. et al. Soil phisico-hydraulic properties under organic conilon coffee intercropped with tree and fruit species. **Pesquisa Agropecuária Brasileira**, Brasília, v. 52, n. 7, p. 539-547, July. 2017.

SOUZA, H. A. et al. Uso fertilizante do subproduto da agroindústria processadora de goiabas. I – Atributos químicos do solo. **Revista Brasileira de Fruticultura**, Jaboticabal, v. 36, n. 3, p. 713-724, July/Sept. 2014.

TEDESCO, M. J. et al. **Análise de solo, plantas e outros materiais**. 2.ed. Porto Alegre: Universidade Federal do Rio Grande do Sul, 1995. 147p.

UYEDA, C. A. et al. Influence of vinasse application in hydraulic conductivity of three soils. **Engenharia Agrícola**, Jaboticabal, v. 33, n. 4, p. 689-698, July/Aug. 2013.

ZOCA, S. M. et al. Coffee processing residues as a soil potassium amendment. **Journal of Recycling of Organic Waste in Agriculture**, Isfahan, v. 3, n. 1, 155-165, Jan/Mar. 2014.

MAGNESIUM IN THE DYNAMICS OF CARBOHYDRATES AND ANTIOXIDANT METABOLISM OF COFFEE SEEDLINGS IN TWO IRRADIANCE LEVELS

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ABSTRACT: The aim of this study was to verify the physiological impacts and the carbohydrate dynamics of *Coffea arabica*, seedlings subjected to increasing concentrations of magnesium (Mg) and two irradiance levels. Methods: The experiment was carried out in growth chambers with nutrient solution. The treatments were five concentrations of Mg (0, 48, 96, 192, and 384 mg L^{-1}) and two irradiance levels (80 and 320 µmol of photons m^{-2} s^{-1}). The coffee seedlings were under the treatments for 90 days. Results: The leaves with deficiency or excess of Mg exposed to the irradiance of 320 µmol of photons m^{-2} s^{-1} accumulated more carbohydrates than those exposed to 80 µmol of photons m^{-2} s^{-1}. The accumulation of carbohydrates in the leaves increased the activity of antioxidant enzymes due to the increased production of reactive oxygen species (ROS). Leaves exposed to 320 µmol of photons m^{-2} s^{-1} exhibited symptoms of scald by the sun caused by photo-oxidation. The scald was more intense in plants with abnormal concentrations of Mg. The antioxidant system of the coffee tree is closely relates to the Mg supply and irradiance levels. Concentrations of Mg between 48 and 96 mg L^{-1} functioned as a mitigating agent of oxidative stress under stressful conditions caused by high irradiance level.

Index terms: Oxidative stress, enzyme activity, scald, coffee nutrition.

MAGNÉSIO NA DINÂMICA DE CARBOIDRATOS E NO METABOLISMO ANTIOXIDANTE DE CAFEEIROS SUBMETIDOS A DOIS NÍVEIS DE IRRADIÂNCIA

RESUMO: O objetivo deste trabalho foi verificar os impactos fisiológicos e a dinâmica de carboidratos em mudas de *Coffea arabica*. cultivadas em solução nutritiva, com doses crescentes de magnésio (Mg) sob o efeito de dois níveis de irradiância. O experimento foi conduzido em câmaras de crescimento com mudas de café da cultivar Mundo Novo IAC 379/19. Os tratamentos foram cinco doses de Mg (0; 48; 96, 192 e 384 mg L^{-1}) e dois níveis de irradiância (80 e 320 µmol fóton m^{-2} s^{-1}) aos quais as mudas foram expostas, em delineamento em blocos casualizados, em arranjo fatorial 5x2, com 6 repetições, e uma planta por unidade experimental (n=60). As plantas supridas com concentrações de Mg menores ou maiores do que a recomendada para a cultura (48 mg L^{-1}) expostas à irradiância de 320 µmol de fótons m^{-2} s^{-1} acumularam mais carboidratos do que as expostas a 80 µmol de fótons m^{-2} s^{-1}. A acumulação de carboidratos nas folhas aumentou a atividade de enzimas antioxidantes devido ao aumento da produção de espécies reativas de oxigênio. As folhas expostas a 320 µmol de fótons m^{-2} s^{-1} apresentaram sintomas de escaldadura de sol causada por foto-oxidação. A escaldadura foi mais intensa em plantas com concentrações anormais de Mg. O sistema antioxidante das mudas de café está intimamente relacionado às concentrações de Mg nas folhas. As concentrações de Mg entre 48 e 96 mg L^{-1} funcionaram como agente atenuante do estresse oxidativo em condições estressantes de alto nível de irradiância.

Termos para indexação: Estresse oxidativo, atividade enzimática, escaldadura, nutrição do cafeeiro.

1 INTRODUCTION

Magnesium (Mg) is an important nutrient for plants because compose the chlorophyll molecules, which contribute to the energy metabolism of the plants (photosynthesis). Consequently, Mg deficiency affects many biochemical and physiological processes that decreasing the growth and yield of crops (CAKMAK; YAZICI, 2010).

Magnesium has a primary role in the transport of carbohydrates, especially sucrose. The accumulation of carbohydrates in leaves seems to be one of the first symptoms of Mg deficiency (SILVA et al., 2014). This elevated concentration of carbohydrates in leaves of plants deficient in Mg, accompanied by an increase in the ratio of the mass of the aerial part by the root is indicative of severe inhibition in the export of photoassimilates (sugars) in the phloem (CAKMAK; KIRKBY, 2008; CAKMAK; YAZICI, 2010). The reduction of transportation of carbohydrates and, consequently, the growth of the roots, decrease the water and nutrients absorption and thus decreasing the productivity of the crops.

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Additionally, the accumulation of carbohydrates in the leaves could stimulate the production of reactive oxygen species (ROS) which are toxic to plant species (MARSCHNER, 2012).

The irradiance levels also influence the biochemical and physiological processes in the plants, influencing severely the transport of carbohydrates and the growth of roots. Typical visual symptoms of Mg deficiency are clearly note at high irradiance level (CAKMAK; KIRKBY, 2008).

The antioxidant skill of plants is considers an important factor in their protection against different environmental stresses (GILL; TUTEJA, 2010). The fundamental difference between the sensitive plants and those resilient to oxidative stress is the capability to reduce the damage caused by free radicals produced during the environmental stress (SÁNCHEZ-RODRIQUEZ et al., 2010).

Different stresses suffered by plants increase the production of ROS, for example, superoxide, hydrogen peroxide, and hydroxyl radicals that become harmful to the plant's organism when their production is higher than the antioxidant agent's production, thus resulting in oxidative stress (HUSSAIN et al., 2011).

An efficient degradation of ROS requires the joint action of enzymes of the antioxidant system of the plants — mainly superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) (NEILL et al., 2008). Despite the presence of an effective antioxidant system, oxidative damage still occurs in the plant cells due to either the uncontrolled production or inefficient removal of ROS.

The climate change, especially in the last decade, may modify the areas of expansion of agriculture, and it could influence the plant's growth, mainly in areas like Brazilian Cerrado, where plants would have to grow under a much higher irradiance than the light saturation point of the photosynthetic apparatus. Thus, a detailed study of Mg nutrition in coffee as a function of irradiance is required.

Therefore, the objective in this work was to determine the physiological impacts and carbohydrate dynamics in *Coffea arabica* L. seedlings with increasing concentrations of Mg and different irradiance levels.

2 MATERIAL AND METHODS

The experiment was carried out in growth chambers, under controlled conditions and with nutrient solution by Hoagland and Arnon (1950). The treatments were five concentrations of Mg (0, 48, 96, 192, and 384 mg L^{-1}) and two irradiance levels (80 and 320 µmol of photons m^{-2} s^{-1}); the standard concentration of Mg is 48 mg L^{-1} (HOAGLAND; ARNON, 1950), and the irradiance level of 80 µmol of photons m^{-2} s^{-1} simulating the conditions of low incident radiation, such as in shaded coffee trees within the plant canopy or in coffee trees in high-density plantations, whereas 320 µmol of photons m^{-2} s^{-1} simulated normal conditions of photosynthetic activity.

Six repetitions of three-liter pots were used as growth chambers, with one coffee seedling per growth chamber, organized in randomized blocks of a 5 X 2 factorial arrangement (n=60).

Coffee seedlings of Mundo Novo IAC 379/19 cultivar with four pairs of true leaves cultivated in substrate not limed were transfer to trays containing deionized water for 10 days until the growth of new roots. The plants were transfer to three-liter pots containing 50% ionic strength of the Hoagland and Arnon (1950) solution, but excluding Mg, where they remained under constant aeration for 15 days.

After this period, the nutrient solution was replaced by 100% ionic strength of the Hoagland and Arnon (1950) solution and Mg was added at the concentrations of 0, 48, 96, 192, and 384 mg L^{-1}. The volume of the pots were fill with deionized water daily, pH was adjusted to 5.0–5.5 by addition of HCl 0.1 mol L^{-1} or NaOH 0.1 mol L^{-1}, and the pots were kept aerated continually.

The lighting was provides by special daylight tubular fluorescent bulbs (Osram 20W). The levels of irradiance were controlled using shelves to regulate the distance between the plants and the light source. The irradiance levels at the different heights were measured using a quantum sensor (Licor LI-190SA, Li-Cor Biosciences Inc., Lincoln, USA). The plants were subjected to a photoperiod of 12 h of light and 12 h of darkness simulating a natural condition of tropical regions.

Fully expanded leaves were collected ninety days after the application of the treatments to performing the physiological analyses. After the material was collects, it was immediately preserved in liquid nitrogen and then stored at -80°C in a deep freezer. Subsequently, the plants were exposed to full sun for three days, in an irradiance of 1500 µmol of photons m^{-2} s^{-1} and one more fully expanded leaf was sampled for the physiological analyses. The plants were then harvest and divided into leaves, stems and roots that were wash in deionized water, packed in paper bags, and dried at 60 °C until constant weights.

The dried leaves, stems, and roots were milled and a portion of the dry-milled material was sample for nutritional analysis following EMBRAPA (2009). Samples of dry-milled leaves were take for carbohydrate analysis.

2.1 Carbohydrates

The carbohydrates were extract from 25 mg of the dry mass of leaves, homogenized in 100 mM potassium phosphate buffer, pH 7.0, followed by a water bath at 40 °C for 30 min (ZANANDREA et al., 2010). Subsequently, 1.0 mL of water was extracts through ultrasonication at 60 °C for 15 min. An aliquot of 500 µL was transfers to 1.5 mL tube and centrifuged at 5,500 rpm for 5 min. The supernatant was diluted and filtered through a PES membrane with 0.22 µm pores (adapted from MELLINGER, 2006).

The following standards were use: for glucose and galactose analysis — Sigma-Aldrich (St. Louis, MO, USA); for sucrose and fructose analysis— Fluka (St. Louis, MO, USA). The mobile phase was prepared from 50–52% NaOH solution sourced from Sigma-Aldrich (St. Louis, MO, USA). All solutions were prepared using water obtained from a Milli-Q Biocel system from Millipore (Billerica, MA, USA).

The samples were analysed using an HPLC system (Shimadzu, Kyoto, Japan) coupled to an Antec DECADE II detector (Zoeterwoude, Netherlands) — the electrochemical cell was equipped with a gold electrode. The HPLC system, controlled by CBM-20A and with PEEK piping, was set to the isocratic flow of the 20 mM NaOH solution — which had been previously degassed in DGU-A_5 — supplied at 0.2 ml.min^{-1} by an LC-10Ai pump. A volume of 20 µL of the samples was injects (model 7725i Rheodyne injector) — the separation was performed by a 250 x 4 mm internal diameter DIONEX CarboPac PA1 column (Sunnyvale, CA) equipped with pre-column and maintained at 44 °C. The saccharides were analysed by pulsed amperometric detection, with the following detection conditions: E_1 = +0.05 V, t_1= 500 ms, and t_s = 60 ms; E_2 = + 0.75V and t_2 = 130 ms; and E_3 = -0.80 V and t_3= 120 ms (MARTINS et al., 2005).

The total soluble sugar (TSS) content was calculates by summing the sucrose, fructose, glucose, and galactose contents.

2.2 Antioxidant enzymes

The enzyme extract was obtained through the maceration in liquid nitrogen of 0.1 g of leaves, to which was added 1.5 ml of the extraction buffer containing 1.47 ml of 0.1 M potassium phosphate buffer (pH 7.0), 15 µL of 0.1 M EDTA (pH 7.0), 6 µL of 0.5 M DTT, 12 µL of 0.1 M PMSF, 0.001 M ascorbic acid, and 22 mg of PVPP. The extract was centrifuged at 12,000 g for 10 min at 4 ºC, and the supernatant was collected, stored at -20 °C and used in the following enzymatic analyses: catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) (BIEMELT et al., 1998).

a) Catalase

CAT activity was evaluated using a 5 µL aliquot of the enzyme extract, which was added to 950 µL of the incubation medium containing 200 mM potassium phosphate (pH 7.0) and 12.5 mM H_2O_2 and incubated at 28 °C. The activity was determines by the decrease in the absorbance at 240 nm every 15 seconds for 3 min, monitored by the consumption of the hydrogen peroxide (HAVIR; MCHALE, 1987).

b) Superoxide dismutase

SOD activity was evaluated by the ability of the enzyme to inhibit the photoreduction of nitroblue tetrazolium (NBT) (MAROUANE et al., 2011) in an incubation medium consisting of 50 mM potassium phosphate at pH 7.8, 14 mM methionine, 0.1 µM EDTA, 75 µM NBT, and 2 µM riboflavin. The tubes with the reaction medium and the sample were illuminate for 7 min using a 20 W fluorescent lamp. The same reaction medium without the sample was illuminates for the control and the blank was kept in the dark. The readings were taken at 560 nm, and the concentration of SOD was calculated using the following equation:

$$\%\ Inhibition = (A_{560}\ sample\ with\ enzime\ extract - A_{560}\ control\ without\ enzime)/(A_{560}\ control\ without\ enzime)$$

One unit of SOD corresponds to the amount of enzyme capable of inhibiting the photoreduction of NBT by 50% under the assay conditions.

c) Ascorbate peroxidase

APX activity was determined by monitoring the oxidation rate of the ascorbate at 290 nm every 15 s for 3 min. An aliquot of 5 µL of the enzyme extract was added to 950 µL of incubation buffer consisting of 500 µL of 200 mM potassium phosphate (pH 7.0), 50 µL of 10 mM ascorbic acid, and 50 µL of 2 mM hydrogen peroxide (NAKANO; ASADA, 1981). The molar extinction coefficient used was 2.8 mM^{-1}cm^{-1}.

2.3 Statistical analyses

Regression equations were estimate for the Mg concentrations. Mean tests (Scott-Knott) were complete to examine the differences in the irradiance levels at each Mg concentration. All analyses were performed using the Sisvar software (Ferreira, 2011), and graphs were built using the SigmaPlot 11.0 software. The maximum and minimum points of the quadratic functions were get through making the first order derivative equal to zero.

3 RESULTS AND DISCUSSION

3.1 Nutrient content

The content of Mg in leaves increased while K in leaves decreased with the concentration of Mg applied (Figure 1), fitted by quadratic functions for both irradiance levels. This reduction in the K leaf contents is due to the antagonistic effect of these nutrients (Figure 1b). In general, increasing the amount absorbed from one cation can result in the reduction of the absorption of another cation (MARSCHNER, 2012).

3.2 Carbohydrate content

The levels of sucrose, reducing sugars fructose, glucose, and galactose and total soluble sugars (TSS) were significantly influence by the concentrations of magnesium (Mg) and by the applied irradiance levels, as well as by the interactions between these factors (Figure 2). The interaction between the Mg concentrations and the irradiance levels was significant for sucrose (Figure 2A), fructose (Figure 2B), glucose (Figure 2C), galactose (Figure 2D), and TSS (Figure 2E).

The leaves of coffee seedlings with 0 mg of Mg L^{-1} (control) showed sucrose accumulation (Figure 2A). One of the first symptoms of Mg deficiency is the accumulation of sucrose (HERMANS et al., 2010; SILVA et al., 2014). The accumulation of sucrose in the leaves of plants deficient in Mg is related to the reduction in its transport to the roots via phloem, which causes a drastic reduction in root growth (CAKMAK et al., 1994; CAKMAK; YAZICI, 2010; HERMANS et al., 2010; SILVA et al., 2014) and decreasing absorption of water and nutrients, thus hampering the growth and productivity of the coffee plants.

Sucrose contents decreased with increasing concentrations of Mg up to 96 and 48 mg L^{-1} for irradiance levels of 80 and 320 µmol of photons m^{-2} s^{-1}, respectively. The influence of the reduction in photosynthesis on the slower transport of sucrose via the phloem is questionable because the decrease in the photosynthesis rate occurs in stages following Mg deficiency (CAKMAK; KIRKBY, 2008). Accumulation of carbohydrates in the leaves in low Mg content seems to result in adverse effects on photosynthetic gene activity, such as the gene responsible for the coding of chlorophylls *a* and *b* (HERMANS et al., 2010) which is partially responsible for the decline in both the chlorophyll contents and the photochemical performance in more advanced stages of Mg deficiency (CAKMAK et al., 1994; HERMANS et al., 2010).

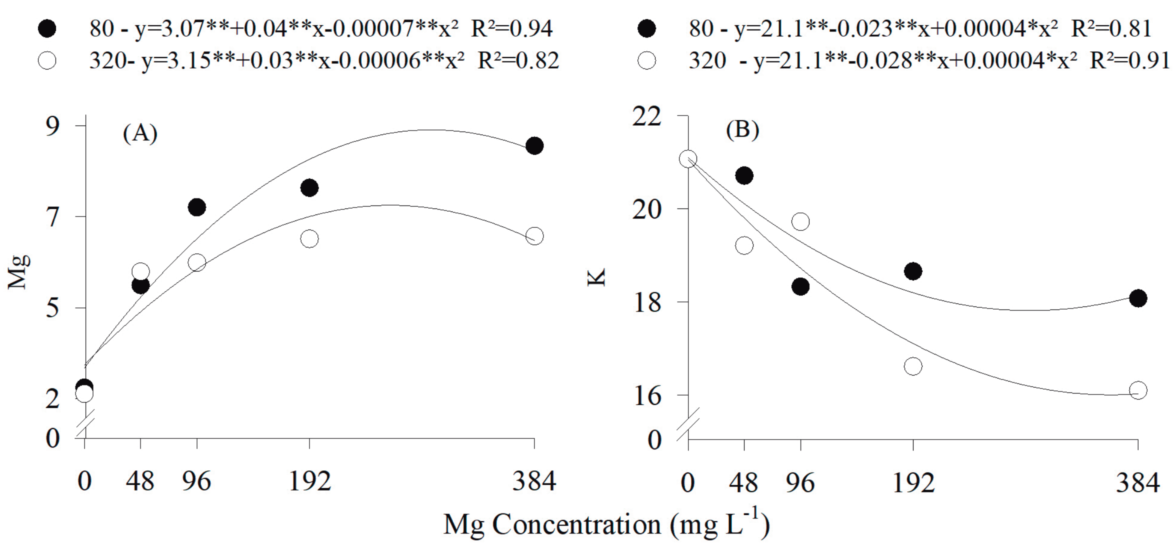

FIGURE 1 - Contents of Mg (a) and K (b) in leaves of coffee seedlings as a function of the application of different concentrations of Mg at two irradiance levels, 80 (closed circle) and 320 (empty circle) µmol of photons m^{-2} s^{-1}. Significance according to t-test is indicated at 5% (*) and 1% (**)

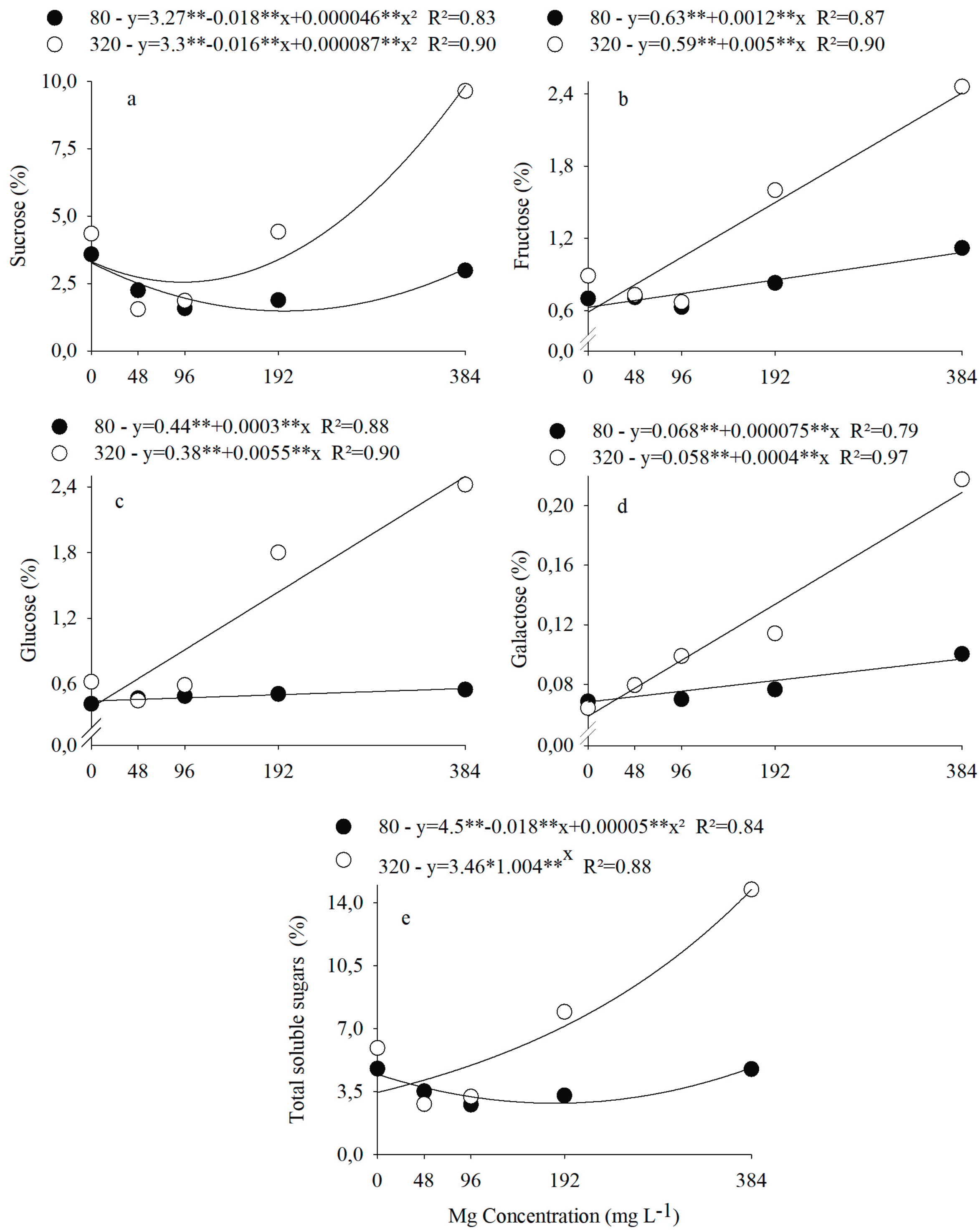

FIGURE 2 - Levels of (a) sucrose, (b) fructose, (c) glucose, (d) galactose, and (e) TSS in leaves of coffee seedlings as a function of the application of different concentrations of Mg at two irradiance levels (80 and 320 µmol of photons m^{-2} s^{-1}). Significance according to t-test is indicated at 5% (*) and 1% (**) .

Thus, Mg has a direct effect on the transport of carbohydrates, especially sucrose, via the phloem (CAKMAK et al., 1994; CAKMAK; KIRKBY, 2008).

The function that Mg performs in the transport of carbohydrates could be relates to the decrease in the metabolic activity of the source organs (WARAICH, et al., 2011). However, it is most likely relates to the decrease in the Mg-ATP concentration in the transport locations of the phloem (CAKMAK; KIRKBY, 2008; HERMANS et al., 2010). Depending on the species that transport may occur actively and selectively (symplastic pathway), thus requiring energy in the form of ATP for co-transport via the plasma membrane (H+) (TAIZ; ZEIGER, 2009).

The increases in the sucrose levels from the previously mentioned concentrations are probably relates to the reduced absorption of K due to the excess Mg in the solution (figure 1). The higher sucrose accumulations were observe at the 320 µmol of photons m^{-2} s^{-1} of irradiance and the highest rate of photosynthesis was also relates to the lower K levels observed.

K deficiency causes a reduction in the transport and use of photoassimilates (MARSCHNER, 2012). Cakmak et al. (1994) observed higher concentrations of sucrose and reducing sugars in the leaves of bean plants deficient in Mg and K. In addition to the accumulation of carbohydrates in the leaves, the role of K in the transport of carbohydrates is supported by the lower concentrations of sucrose in the roots of plants deficient in K compared with the roots of plants with adequate supply of this nutrient (CAKMAK et al., 1994). Therefore, K is required for the efficient transport of carbohydrates (sucrose particularly) via the phloem (PILOT et al., 2003).

In plants deficient in K, soluble carbohydrates and soluble nitrogen compounds accumulate, and the starch content decreases (MARTINEZ et al., 2014). These changes are related to the high requirement of K for the functioning of certain regulatory enzymes, in particular, pyruvate kinase and phosphofructokinase (MARSCHNER, 2012). According to Hermans et al. (2010) these reductions in photosynthesis rates observed in plants deficient in K may occur as a consequence of sucrose accumulation in the leaves due to their role in the synthesis and transport of carbohydrates. Moreover, K is extremely important in the activation of the carboxylase function of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (PRADO, 2008). Increases in photosynthetic rates due to the adequate supply of K have been attributed to these functions (CATUCHI et al., 2011; JIA et al., 2008;).

At the higher Mg concentrations, there was greater accumulation of sucrose (Figure 2a), fructose (Figure 2b), glucose (Figure 2c), galactose (Figure 2d), and TSS (Figure 2e), especially in the plants subjected to the higher irradiance level (320 µmol of photons m^{-2} s^{-1}). This likely indicates that plants grown under high irradiance levels require a greater supply of K to maintain transport of carbohydrates via the phloem at normal rates.

Fructose and glucose (sucrose precursors) were present at higher levels than galactose. This behavior was linear for the fructose, glucose, and galactose contents at the two studied irradiance levels (Figures 2b, 2c, and 2d).

The levels of TSS resulting from the Mg concentrations behaved quadratically at the lower irradiance level and exponentially at 320 µmol of photons m^{-2} s^{-1} of irradiance.

Seedlings in 0 mg L^{-1} of Mg, showed higher contents of fructose (Figure 2b) and glucose (Figure 2c) at the highest irradiance level, and TSS (Figure 2e) increased at both irradiance levels. That indicates that Mg also plays a role in the transport of these sugars and/or in the sucrose transformation reactions. Silva et al. (2014) observed increases in the levels of sucrose, reducing sugars, and TSS as a function of Mg deficiency in coffee seedlings; however, the effect of excess Mg on the levels of reducing sugars (fructose, glucose, and galactose) — see Figures 2b, 2c, and 2d — was more significant. Perhaps K has a more important role in the transport and/or transformation of these sugars. The synthesis of carbohydrates is dependent on sucrose-cleaving enzymes, sucrose synthase (SuSy), and invertases, which also influence the velocity of transport in the phloem in the source/drain direction — some of these enzymes are activated by K (TAIZ; ZEIGER, 2009).

In general, there was a greater accumulation of carbohydrates at the extremes of Mg supply (0 and 384 mg L^{-1}), at the highest irradiance level, due to the higher photosynthesis rate probably (Dias 2015).

3.3 Relative distribution of carbohydrates

The Mg concentrations also altered the relative distribution of carbohydrates in the coffee leaves; that is, the ratio of the content of each carbohydrate relative to the total soluble sugar (TSS) – see Figure 3 – fit a decreasing quadratic function for the sucrose and increasing quadratic for the reducing sugars.

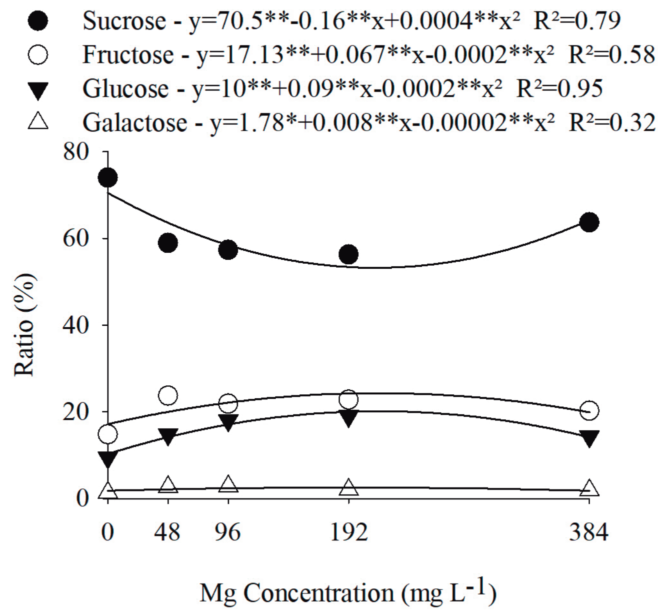

FIGURE 3 - Relative distribution of carbohydrates in leaves of coffee seedlings as a function of the application of different concentrations of Mg and two irradiance levels. Significance according to t-tests is indicated at 5% (*) and 1% (**)

The highest percentage of sucrose in relation to the other sugars was observed in the control, indicating that Mg deficiency has more influence on the accumulation of sucrose proportionally.

Regardless of the Mg concentration, sucrose was the sugar with the highest concentration in the leaves, followed by fructose, glucose, and then galactose, which had values below the others. According to Ding and Xu (2011) sucrose and starch are the main photosynthetic products in almost all of the higher plants.

Irradiance levels had no effect on the relative distribution of carbohydrates. On the other hand, there were changes in the carbohydrate content ratios with the different Mg concentrations. The proportions of sucrose: fructose: glucose: galactose were 74: 15: 10: 1% with 0 g L^{-1} of Mg to the plants. A supply of 48 g L^{-1} of Mg resulted in 59: 24: 15: 3% of sucrose, fructose, glucose and galactose, while 96 g L^{-1} of Mg resulted in 57: 22: 18: 3%, respectively. The concentration of 192 g L^{-1} of Mg resulted in 56: 23: 19: 2% of sucrose, fructose, glucose and galactose and of 384 g L^{-1} of Mg in 64: 20: 14: 2% sucrose, fructose, glucose and galactose.

Sucrose has a fundamental role in plant metabolism, acting as a source of carbon and energy, especially for non-photosynthetic tissues (BASSON et al., 2010). Reduction in the sucrose synthesis rate or increase in its degradation affects plant physiology, root development, and fruit quality (KÜHN; GROF, 2010).

The main enzymes that catalyze the sucrose dynamics reactions are sucrose synthase (SuSy), sucrose phosphate synthase (SPS), and the invertases (alkaline invertase and acid invertase). SPS controls the biosynthesis and accumulation of sucrose and it plays an important role in the translocation and distribution of photoassimilates in higher plants (TAIZ; ZEIGER, 2009; WANG et al., 2013). SuSy and alkaline invertase are primarily located in the cytosol, where as acid invertase is mainly associated with cell walls and vacuoles, where pH is approximately 5 (TAIZ; ZEIGER, 2009).

The catalyzed equations are:

Sucrose synthase: Sucrose + UDP ⟷ Fructose + UDP-Glucose

Sucrose phosphate synthase: Sucrose + UDP ⟷ Fructose + UDPG

Invertase: Sucrose + H_2O *⟷ Fructose + Glucose*

In addition to the reactions shown, SPS could also irreversibly convert sucrose-6-phosphate into sucrose (PARK et al., 2009).

The importance of these enzymes depends on where sucrose is being metabolizes. SuSy is likely the main enzyme that degrades sucrose in organs that store starch (e.g., developing seeds or tubers) and in tissues undergoing rapid growth, which require translocated sucrose in the respiration process for the production of energy and carbon skeletons. However, when the unloading of the phloem occurs passively (via apoplast), the acid invertase — because it is present in the cell wall — can convert the sucrose into hexoses (fructose and glucose) before entering the cell. In mature cells, cytosolic invertase may be important in the degradation of sucrose, supplying glucose and fructose for respiration (HOPKINS, 2000).

SPS is a key enzyme in the carbohydrate dynamics of plants — it is primarily responsible for the participation of sucrose in the physiological processes of different metabolisms. Previous studies of different crops have found a correlation between sucrose accumulation and increases in SPS activity or decreases in invertase activity (HIROTSU et al., 2007; ISHIMARU et al., 2008; ZHANG et al., 2010).

The relationships between sucrose and fructose and between sucrose and glucose were inversely proportional, suggesting that Mg and/or K participate in the activities of these enzymes. K has an established connection to SuSy activity (TAIZ; ZEIGER, 2009). Mg is possibly relates to invertase activity because the activity of these enzymes is generally inversely proportional to sucrose accumulation. However, more research is required regarding the role of these nutrients in the activity of these enzymes.

3.4 Antioxidant metabolism

The increase in Mg concentrations in the solution decreased the enzyme activity of the plant antioxidant system (SOD, CAT, and APX) — see Figures 4 and 5. For the SOD activity, the interaction between the Mg concentrations and the irradiance was not significant — the activity of this enzyme as a function of the concentrations was represent by a decreasing quadratic function (Figure 4). Increasing Mg concentrations up to 245 mg L^{-1} caused a reduction in SOD activity, and from this point onward, the activity of this enzyme increased. SOD is responsible for the dismutation of O_2^- to form H_2O_2 and O_2 and it is consider the first line of defense against ROS (KARUPPANAPANDIAN et al., 2011). CAT and APX are enzymes that catalyze the conversion of H_2O_2 to water and O_2 (ABEDI et al., 2010).

Increases in the activity of the antioxidant system resulting from Mg deficiency have been observed in the following crops: rice (CHOU et al., 2011), bean (CAKMAK; YAZICI, 2010), and coffee (SILVA et al., 2014).

Silva et al. (2014) observed the greater activity of the SOD, CAT, and APX enzymes in coffee seedlings deficient in Mg compared with those with an adequate supply of Mg.

Before the appearance of visual Mg deficiency symptoms, the activity of the enzymes from the antioxidant complex of the plant reduces the photooxidative damage caused by ROS and the inactivation of photosynthetic enzymes, which results in decreased photosynthetic activity only at more advanced stages of deficiency (KAISER, 1976).

The interaction between the Mg concentrations and the irradiance levels was significant for both APX and CAT activities. The activity of these enzymes as result of the Mg concentrations fit a descending quadratic function within each irradiance level, for both the growth chamber samples and the samples exposed to full sunlight (Figure 5). The significant interaction indicates that the activity of these enzymes as a function of the Mg concentrations depends on the irradiance.

The CAT and APX activities were both greater at lower Mg concentrations. According to Cakmak and Yazici (2010), high levels of components from the antioxidant metabolism are a physiological response of the plants to the effects of Mg deficiency.

The activation of antioxidant metabolism, under Mg deficiency, likely occurs in chloroplasts, the location of a reduction in O_2^- and H_2O_2 as a result of the restricted consumption of reducing potential in the fixation of CO_2^- (Silva et al., 2014).

Excessive Mg also caused an increase in the enzyme activity of the antioxidant system (Figures 3 and 4) likely due to the physiological disorders caused by the excess of this nutrient itself, the reduction in the absorption of other nutrients, especially K, and the increase in the saline concentration of the solution.

Several studies have demonstrated the role of enzymatic antioxidant mechanisms in protecting against oxidative stress induced by salinity (RUBIO et al., 2009). The increase in the activity of enzymes such as SOD, APX, and CAT is associated with maintaining levels of lipid peroxidation under salt stress (ASHRAF, 2009).

K deficiency can increase the production of ROS in conditions of environmental stress particularly, for example, drought, high light intensity, heat, and nutrient limitation, and the improvement in potassium nutrition could greatly reduce the production of ROS through the reduction of NADPH oxidase activity and the maintenance of electron transport (Cakmak et al., 1994). K deficiency causes a reduction in the photosynthetic fixation of CO_2 and losses in the transport and use of photoassimilates. Such disorders can result in an excess of electrons, thereby stimulating the production of ROS (MARSCHNER, 2012).

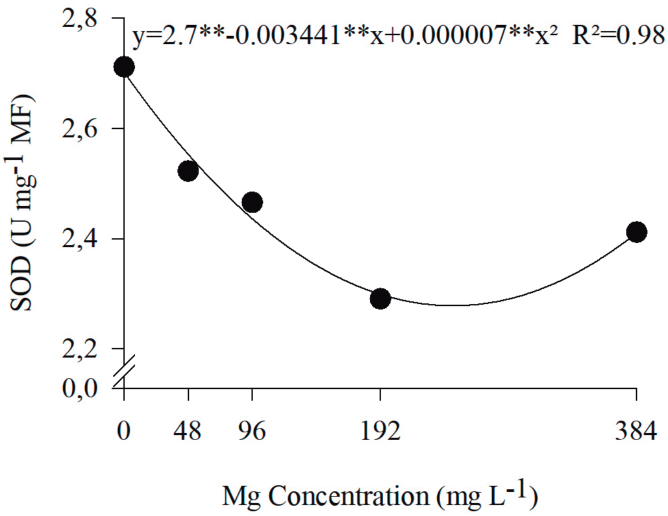

FIGURE 4 - Activity of the superoxide dismutase (SOD) enzyme resulting from the application of different concentrations of Mg. Significance according to t-tests indicated at 5% (*) and 1% (**).

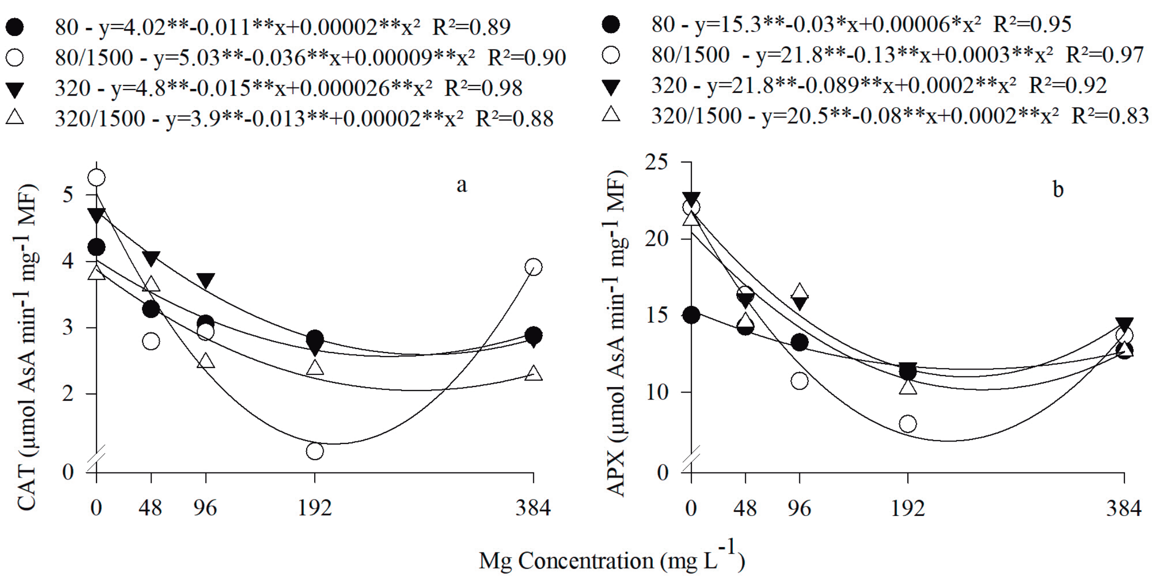

FIGURE 5 - Activity of (a) CAT and (b) APX enzymes in coffee seedlings as a function of the application of different concentrations of Mg and different irradiance levels in samples collected inside and outside the growth chamber after full sun exposure (/1500). Significance according to t-test is indicated at 5% (*) and 1% (**)

In addition to the Mg content, light intensity also influences biochemical and physiological processes in which Mg is involved, causing visual symptoms typical of deficiency, the reduction of the transport of carbohydrates, root growth (CAKMAK; KIRKBY, 2008) and reductions in crop productivity consequently.

The saturation irradiances of photosynthesis in coffee trees are relatively low (300 to 700 µmol of photons m^{-2} s^{-1}) (DAMATTA et al., 2004). Irradiances higher than those needed to saturate the photosynthetic complex can cause photoinhibition of photosynthesis. Additionally, they often lead to decreases in the rate of electron transport through photosystem II (PSII) and to increases in the spin rate of D1, the main polypeptide of the PSII reaction centers (NISHIYAMA,et al., 2011).

In general, during sampling within the chamber where the irradiance was controlled, the highest irradiance level (320 µmol of photons m^{-2} s^{-1}) triggered greater CAT and APX activities at 0 mg L^{-1} of Mg. With increasing Mg concentrations, the activity of these enzymes at the higher (320 µmol of photons m^{-2} s^{-1}) and lower (80 µmol of photons m^{-2} s^{-1}) irradiance levels tended to be equal (Figure 4). The effect of irradiance antioxidants enzyme activity became even more evident in the plants in the lower irradiance (80 µmol of photons m^{-2} s^{-1}) that were exposed to full sun, where the irradiance reached 1500 µmol of photons m^{-2} s^{-1}. This change in irradiance expose caused stress, which increased the CAT and APX activities of the control; however, less activity occurred due to the increase in the Mg concentrations.

Cakmak and Yazici (2010) observed a rapid increase in the antioxidant mechanisms in bean plants with Mg deficiency, especially those exposed to high light intensities.

Increases in the formation of ROS occur when the absorption of light energy captured by the plant exceeds the utilization capacity during photosynthesis (Murchie and Niyogi, 2011). These excess ROS are toxic to the cells and destroy chlorophyll, the membranes, DNA, and other organelles. Under normal growth conditions, the accumulation of ROS in cells is low. However, adverse environmental factors that disturb cellular homeostasis induce the production of ROS, thus leading to oxidative stress (MILLER, et al., 2010).

Additionally, even for the control, the coffee seedlings did not display the typical visual symptoms of deficiency in the growth chamber. After being exposes to full sun for three days, the typical visual symptoms of deficiency appeared, together with symptoms of scald, which appeared more intensely in the control plant and at the highest concentration of Mg due to the restriction in the absorption of K.

The oldest part of the leaf, which was shade by the younger leaf, remained green, whereas the portion that was exposes to full irradiance exhibited symptoms of scald (Figure 6). Note also that the newest leaf that was fully expose to irradiance, even with less physical protection, remained green (Figures 6 and 7). This occurred because the scald is the effect of photooxidative damage and is not a purely physical process. Mg and K, which are mobile in the plant, most likely have an important role in protecting newer leaves against scald.

At high light intensities, the development of chlorosis increases, together with some reddish spots on the leaf blade, and the parts of the leaves that did not receive the full luminosity were asymptomatic (Cakmak and Kirkby, 2008). Plants growing in high-intensity light conditions appear to have higher Mg demands than plants grown under low light intensity.

The increased oxidative stress observed as a function of the Mg deficiency, which was more evident in the plants exposed to higher irradiance levels, can be link to several factors:

–With the onset of the stress caused by Mg deficiency, carbohydrates accumulated in the leaves (Figure 1), as has been previously observed by several authors (CAKMAK et al., 1994; SILVA et al., 2014). This accumulation of carbohydrates changes the photosynthetic metabolism and reduces the use of the light energy absorbed in photosynthesis, leading to a saturation of the electron transport chain with the accumulation of NADPH (HERMANS et al., 2010). High levels of reducing equivalents and components of the saturated electron chain offer favorable conditions for the formation of ROS (BIEMELT et al., 1998; MITTLER, 2002).

–Mg also affects the activity of Rubisco, and the binding of this enzyme to Mg increases the affinity for CO_2 and doubles the maximum reaction velocity (SUGIYAMA et al., 1968). Therefore, under Mg deficiency, the photosynthetic rate will decrease, thereby generating the accumulation of oxygen and reducing equivalents, which leads to the generation of ROS and, consequently, triggers oxidative stress. This process is more serious in high irradiance conditions because the generation of reducing equivalents and water absorption are both higher.

The results demonstrated that a relationship exists between the antioxidant complex of the coffee tree and the Mg supply as a function of the irradiance to which the plants are subject. Mg appears to work as a mitigating agent of oxidative stress under stress conditions caused by increased irradiance.

The results indicate the need for regional field studies, given that coffee is cultivated in various regions of Brazil and other countries with different irradiance levels, and thus, the requirements for Mg should not be the same for these different conditions.

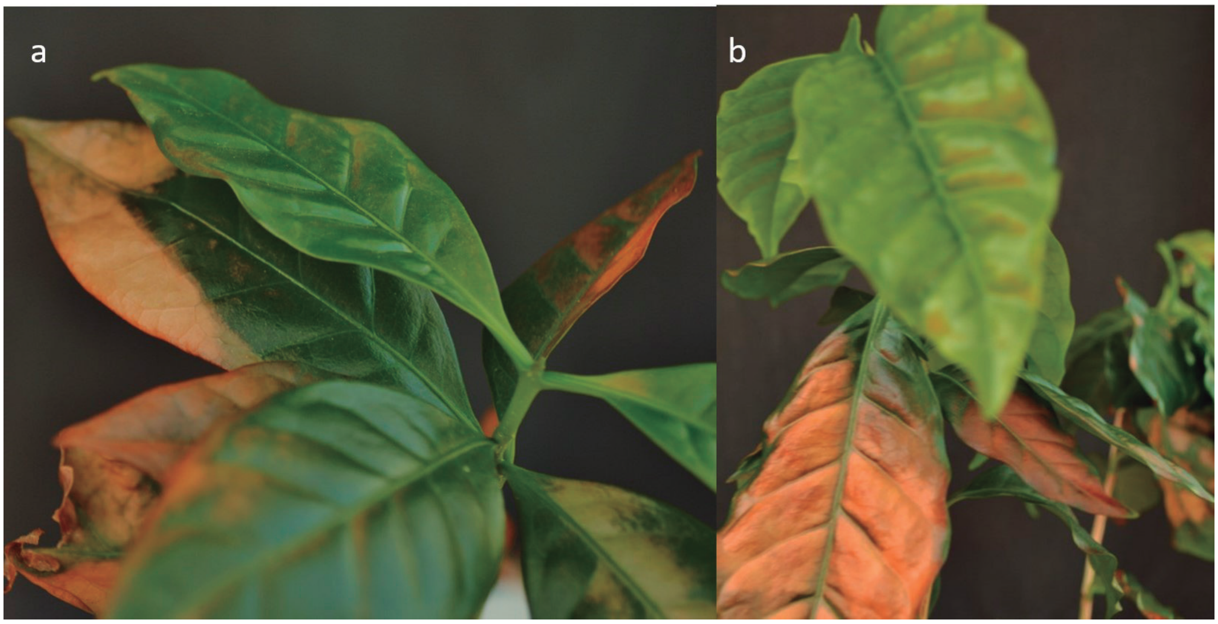

FIGURE 6 - Coffee seedlings cultivated in nutrient solution with no Mg supplied and exposed to full sun for three days.

FIGURE 7 - Coffee seedlings cultivated in nutrient solution with a high level of Mg supplied (low foliar content of K) and exposed to full sun for three days.

4 CONCLUSIONS

Available Mg levels influence the carbohydrate dynamics in the leaves of coffee trees. Both the deficiency and excess of Mg cause increases in their carbohydrate content, especially sucrose in the coffee leaves. Coffee seedlings leaves are highest in sucrose content, followed by fructose and glucose, which are in turn greater than the amount of galactose present in the leaves.

The accumulation of carbohydrates in coffee tree leaves is dependent on the irradiance to which the plants are subject. Under conditions of deficiency or excess of Mg, leaves exposed to higher irradiances accumulate more carbohydrates.

The accumulation of carbohydrates in coffee tree leaves caused increases in antioxidant enzyme activity due to the greater production of ROS.

Deficiency or excess of Mg under high radiation conditions leads to intense photooxidation and symptoms of sun scalding in the coffee leaves.

There is a close relationship between the antioxidant complex of coffee trees and the Mg supply due to the irradiance to which the plants are subject where Mg acts as a mitigating agent of oxidative stress caused by increased irradiance.

5 REFERENCES

ABEDI, T. et al. Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape (Brassica napus L.). **Czech Journal of Genetics and Plant Breeding**, Prague, v. 46, n. 1, p. 27-34, Jan/Mar. 2010.

ASHRAF, M. Biotechnological approach of improving plant salt tolerance using antioxidants as markers. **Biotechnology advances**, v. 27, n. 1, p. 84-93, Jan/Feb. 2009.

BASSON, C. E. et al. Sugar and acid-related quality attributes and enzyme activities in strawberry fruits: invertase is the main sucrose hydrolysing enzyme. **Food Chemistry**, London, v. 121, n. 4, p. 1156–1162, Aug. 2010.

BIEMELT, S.; KEETMAN, U.; ALBRECHT, G. Re-aeration following hypoxia or anoxia leads to activation of the antioxidative defense system in roots of wheat seedlings. **Plant Physiology**, Washington, v. 116, n. 2, p. 651–658, Feb. 1998.

CAKMAK, I.; HENGELER, C.; MARSCHENER, H. Changes in phloem export of sucrose in leaves in response to phosphorus, potassium and magnesium deficiency in bean plants. **Journal of Expert Botany**, London, v. 45, n. 9, p. 1251–1257, Sept. 1994.

CAKMAK, I.; KIRKBY, E. A. Role of magnesium in carbon partitioning and alleviating photooxidative damage. **Physiologia Plantarum**, Copenhagen, v. 133, n. 4, p. 692-704, Aug. 2008.

CAKMAK, I.; YAZICI, A.M. Magnesium: a forgotten elemento in croop production. **Better Crops with Plant Food**, Norcross, v. 94, n. 2, p. 23-25, 2010.

CATUCHI, T. A. et al. Tolerance to water deficiency between two soybean cultivars: transgenic versus conventional. **Ciência Rural**, v. 41, n. 3, p. 373-378, Mar. 2011.

CHOU, T. S. et al. Effect os magnesium deficiency on antioxidante status and cádmium toxicity in rice seedlings. **Journal of Plant Physiology**, New York, v. 168, n. 10, p. 1021–1030, July 2011.

DAMATTA, F. M. et al. Ecophysiological constraints on the production of shaded and unshaded coffee: a review. **Field Crops Research**, Amsterdam, v. 86, n. 2, p. 99-114, Mar. 2004.

DIAS, K. G. L. **Nutrição, bioquímica e fisiologia de cafeeiros supridos com magnésio**. 2015. 80 p. Tese (Doutorado em Solos) - Universidade Federal de Lavras, Lavras, 2015.

DING, Y.; XU, G. Low magnesium with high potassium supply changes sugar partitioning and root growth pattern prior to visible magnesium deficiency in leaves of Rice (Oryza sativa L.). **American Journal of Plant Sciences**, Amsterdam, v. 2, n. 4, p. 601–608, Oct. 2011.

EMPRESA BRASILEIRA DE PESQUISA AGROPECUÁRIA. **Manual de analises químicas de solos, plantas e fertilizantes.** 2. ed. Brasília: Embrapa Informação Tecnológica, 2009.

FERREIRA, D. F. SISVAR: a computer statistical analysis system. **Ciência e Agrotecnologia**, Lavras, v. 35, n. 6, p. 1039-1042, Nov/Dec. 2011.

GILL, S. S, TUTEJA, N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. **Plant Physiology and Biochemistry**, v. 48, n. 12 p. 909-930, Dec. 2010.

HAVIR, E. A.; MCHALE, N. A. Biochemical and developmental characterization of multiple forms of catalase in tobacco leaves. **Plant Physiology**, Washington, v. 84, n. 2, p. 450–455, June. 1987.

HERMANS, C. et al. Systems analysis of the responses to long term magnesium deficiency and restoration in Arabidopsis thaliana. **New Phytologist**, v. 187, n. 1, p. 132-144, July. 2010.

HIROTSU, N.; KASHIWAGI T.; MADOKA, Y. Strategies to improve plant height for the production of biomass energy in rice. **Japanese Journal of Crop Science**, Tokyo, v. 76, n. 4, p. 501-507, Oct/Dec. 2007.

HOAGLAND, D.; ARNON, D. I. The water culture method for growing plants without soil. Berkeley, **Califórnia Agricultural Experiment Station**, v. 347, n. 2ed. 1950. 32p.

HOPKINS, W. G. **Introduction to plant physiology**. 2nd ed. New York: John Wiley & Sons, 2000.

HUSSAIN, S. S. et al. Polyamines: natural and engineered abiotic and biotic stress tolerance in plants. **Biotechnology advances**, v. 29, n. 3, p. 300-311, May/June. 2011.

ISHIMARU, K.; HIROTSU, N.; KASHIWAGI, T. Over-expression of a maize SPS gene improves yield characters of potato under field conditions. **Plant Production Science**, Oxford, v. 11, n. 1, p. 104-107, Jan. 2008.

JIA, Y. et al. Effects of potassium deficiency on chloroplast ultrastructure and chlorophyll fluorescence in inefficient and efficient genotypes of rice. **Journal of Plant Nutrition**, v. 31, n. 12, p. 2105-2118, Nov. 2008.

KAISER, M. W. The effect of hydrogen peroxide on CO2 fixation of isolated chloroplast. **Biochimica Biophysica Acta**, Amsterdam, v. 440, n. 3, p. 476–482, Sept. 1976.

KARUPPANAPANDIAN, T. et al. Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. **Australian Journal of Crop Science**, v. 5, n. 6, p. 709-725, June. 2011.

KÜHN, C.; GROF, C. P. Sucrose transporters of higher plants. **Current Opinion in Plant Biology**, Amsterdam, v. 13, n. 3, p. 288–298, June. 2010.

MAROUANE, W. et al. The protective effect of Malva sylvestris on rat kidney damaged by vanadium. **Lipids in health and disease**, v. 10, n. 1, p. 65, Aug. 2011.

MARSCHNER, H. **Mineral nutrition of higher plants**. 3. ed. London: Academic Press, 2012.

MARTINEZ, H. E. P. et al. Nutrição mineral do cafeeiro e qualidade da bebida. **Revista Ceres**, Viçosa, v. 61, n. suppl, p. 838-848, Nov/Dec. 2014.

MARTINS, M. C. M. et al. Carboidratos na bebida do café preparado sob diferentes processos. **Ciência e Tecnologia de Alimentos**, Campinas, v. 25, n. 2, p. 382-386, Apr/June. 2005.

MELLINGER, C. G. **Caracterização estrutural e atividade biológica de carboidratos de Phyllanthus niruri (quebra-pedra)**. 2006. 138 p. Tese (Doutorado em Ciências) - Universidade Federal do Paraná, Curitiba, 2006.

MILLER, G. A. D. et al. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. **Plant, cell & environment**, v. 33, n. 4, p. 453-467, Apr. 2010.

MITTLER, R. Oxidative stress, antioxidants and stress tolerance. **Trends Plant Science**, Oxford, v. 7, n. 9, p. 405–410, Sept. 2002.

MURCHIE, E. H.; NIYOGI, K. K. Manipulation of photoprotection to improve plant photosynthesis. **Plant physiology**, v. 155, n. 1, p. 86-92, Jan. 2011.

NAKANO, Y.; ASADA, K. Hydrogen peroxide is scavenged by ascorbato-specific peroxidase in spinach chloroplasts. **Plant and Cell Physiology**, Kyoto, v. 22, n. 5, p. 867–880, Aug/Sept. 1981.

NEILL, S. et al. Nitric oxide, stomatal closure, and abiotic stress. **Journal of Experimental Botany**, Oxford, v. 59, n. 2, p. 165–176, Feb. 2008.

NISHIYAMA, Y. et al. Protein synthesis is the primary target of reactive oxygen species in the photoinhibition of photosystem II. **Physiologia Plantarum**, v. 142, n. 1, p. 35-46, May. 2011.

PARK, J. Y. et al. Sucrose phosphate synthase expression influences poplar phenology. Tree Physiology, v. 29, n. 7, p. 937-946, July. 2009.

PILOT, G. et al. Regulated expression of Arabidopsis Shaker K+ channel genes involved in K+ uptake and distribution in the plant. Plant Molecular Biology, Dordrecht, v. 51, n. 5, p. 773–787, Mar. 2003.

PRADO, R. M. **Nutrição de plantas**. São Paulo: Editora da Unesp, 2008. 408 p.

RUBIO, M. C. et al. Effects of salt stress on the expression of antioxidant genes and proteins in the model legume Lotus japonicus. **New Phytologist**, Cambridge, v. 181, n. 4, p. 851-859, Mar. 2009.

SÁNCHEZ-RODRÍGUEZ, E. et al. Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. **Plant science**, v. 178, n. 1, p. 30-40, Jan. 2010.

SILVA, D. M. et al. Physiological and biochemical impacts of magnesium-deficiency in two cultivars of coffee. **Plant and Soil**, The Hague, v. 382, n. 1-2, p. 133-150, Sept. 2014.

SUGIYAMA, T.; NAKYAMA, N.; AKASAWA, T. Structure and function of chloroplast proteins. V. Homotropic effect of bicarbonate in RuBP carboxylase relation and the mechanism of activation by magnesium ions. **Archives of Biochemistry and Biophysics**, New York, v. 126, n. 3, p. 734-745, Sept. 1968.

TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 4. ed. Porto Alegre: Artmed, 2009.

WANG, L. et al. Research advance of sucrose phosphate synthase (SPS) in higher plant. **International Journal of Agriculture & Biology**, Faisalabad, v. 15, n. 6, p. 1221–1226, June. 2013.

WARAICH, E. A. et al. Role of mineral nutrition in alleviation of drought stress in plants. **Australian Journal of Crop Science**, v. 5, n. 6, p. 764-777, June. 2011.

ZANANDREA, I. et al. Tolerance of Sesbania virgata plants to flooding. **Australian Journal of Botany**, v. 57, n. 8, p. 661-669, Nov/Dec. 2009.

ZHANG, X. M. et al. Cloning and expression of pineapple sucrose phosphate synthase gene during fruit development. **African Journal of Biotechnology**, Bowie, v. 9, n. 49, p. 8296-8303, Dec. 2010.

RELATIVE IMPORTANCE AND INTERACTION OF ROASTING VARIABLES IN COFFEE ROASTING PROCESS

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ABSTRACT: This work describes a study in which levels of variables that may control the coffee roasting process were set in an experimental matrix that aimed at measuring their relative importance and the interaction between variables. Each control variable was set in two levels and the combination of these levels elicited 32 different roasting procedures. The physical responses were determined for a specific roaster. Experimental planning allowed the determination of the relative influence of each control variable in each response variable for this roaster. This led to a primary quantification of the major factors that contribute to the roasting process and the relative importance of roast parameters that influence the quality of the coffee beverage. Moreover, these results indicated what interactions could occur between these parameters. The characterization of the relative influence of control variables is a first approach to model the roaster response and the coffee quality that each roasting can achieve.

Index Terms: Coffee roasting, multivariate experimental planning, coffee roasting control variables, coffee roasting response variables.

IMPORTÂNCIA RELATIVA E INTERAÇÃO DAS VARIÁVEIS DE TORRA NO PROCESSO DE TORREFAÇÃO DE CAFÉ

RESUMO: Este trabalho descreve o estudo no qual níveis de variáveis que podem controlar o processo de torrefação de café foram estabelecidos em uma matriz experimental com o objetivo de medir a importância relativa e a interação dessas variáveis. Cada variável de controle assumiu dois níveis e a combinação desses níveis resultou em 32 processos de torra diferentes. As respostas físicas foram determinadas para um torrefador específico. O planejamento experimental permitiu a determinação da influência relativa de cada variável de controle em cada variável de resposta para o torrefador estudado. Isto permitiu uma quantificação básica dos fatores que contribuíram para o processo de torrefação e a medida da importância relativa dos parâmetros de torra que influenciam a qualidade da bebida café. Além disso, estes resultados indicaram que interações podem ocorrer entre esses parâmetros. A caracterização da importância relativa das variáveis de controle é uma primeira abordagem para modelar a resposta do torrefador e a qualidade do café que pode ser alcançada em cada torra.

Termos para indexação: Torrefação de café, planejamento experimental multivariado, variáveis de controle da torra, variáveis de resposta da torra**.**

1 INTRODUCTION

Roasting description and its modelling in scientific literature

Raw coffee without roasting cannot achieve the taste and the flavor that every corner in the world knows. However, the roasting process is a complex process to control, since it involves mechanical, thermal and chemical changes that affect each other as well as the final taste and flavor of the brew. The roasting itself deeply affects the final quality of the coffee drink and the price that the final beverage can achieve (GLOESS et al., 2014; WIELAND et al., 2012; YERETZIAN et al., 2002).

The world total coffee consumption has been increasing since 2012 (ICO (1), 2016). However, the total production by all exporting countries decreased in the same period (ICO (2), 2016). The migration to better quality coffee is also a tendency detected in the USA market, despite the higher price to the final customer (VELLUCI, 2015).

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The final quality of the coffee beverage evolves from the constitution of the roasted beans, which depends on the characteristics of the raw material, as well as the post-harvest treatment conditions: processing, drying, reprocessing, storage, roasting and grinding (GIOMO, 2012).

Several steps must happen from planting to the moment that someone drinks the beverage that achieves the well-known flavor and aroma of the coffee brew that traditionally marks its quality. All post-harvest processing steps exert marked influence on the final quality of the coffee beverage, but the roasting process is the major contributor to its quality, since there is no coffee flavor without roasting. However, an improperly executed roast can mask all the characteristics preserved during the other post-harvest processing steps.

Several complex chemical reactions occur inside the coffee bean during the roasting process, leading to various physical and chemical changes in the original composition and consequent alterations in coffee flavor and aroma (MWITHIGA; JINDAL, 2003; GLOESS et al., 2014; KARYADI et al., 2009).

This process is still quite empirically controlled and the knowledge of the roaster master is one of the most important assets. Nevertheless, depictions of coffee roasting usually divide it in three stages:

The first stage (the drying phase) is the endothermic phase of the roasting, which is characterized by the drying of the green beans due to the vaporization process (GLOESS et al., 2014). Once the beans enter the roaster, the temperature of the roaster drum drops and reaches the lowest temperature achieved during roasting. The difference between the temperature of the bean inserted in the roaster and the higher temperature of the roaster itself causes the drop;

The second stage is the pyrolysis phase, when other kinds of reactions occur (GLOESS et al., 2014). In this stage, there is a major production of volatile (VOC) and semi-volatile organic compounds due to these pyrolytic reactions. The pressure increases inside the coffee bean, since gas release can occur but only when the gas is able to permeate through the bean walls. When this pressure increase overcomes the mechanical resistance of the bean walls, they crack and the beans emit a popping sound known as the first pop or first crack (YERETZIAN et al., 2002; WILSON, 2014).

The third stage is the end of the roasting process, in which the coffee beans must cool quickly (GLOESS et al., 2014; YERETZIAN et al., 2002). In this phase, the beans produce heat by themselves through exothermic reactions, and they must exit the roaster to cool immediately by exposure to ventilation or water to prevent overcooking.

Even though the roasting process occurs in stages, it can also variegate according to the quantity of beans in relation to the total volume of the roaster and to the characteristics of the product, such as species, variety and origin of the coffee. Thus, the operational conditions of roasting (time, rotation and temperature programs) are not fixed and the operator adjusts them according to the characteristics of the raw material and the quality of flavor that is attainable.

Several authors have already tried to model the roasting process, notwithstanding all its inherently complexity. Most of the works tried theoretical and semi empirical models, many of them based in the assumptions concluded by Schwartzberg and republished in 2013.

Hernandez et al. (2007) experimentally measured and modeled heat and mass transfer – moisture decrease – in coffee beans during batch roasting. Their mass transfer results deviate from the model in the exothermic step, but their modeled heat-transfer agreed very well in any isotherm below 250 ºC and the moisture model described the mass transfer with good agreement before the exothermic step. Heyd et al. (2007) tried a similar experiment but with a different mathematical approach for modelling. A thermocouple inside the coffee bean helped measure inner bean temperature. They also measured input and output air temperatures as well as bean moisture at each minute. They modeled the bean as a sphere, which could introduce sensible deviations from the measured bean behavior.

Fabbri et al. (2011) modelled heat and water loss during the roasting and compared their model to practical results. Their model was purely endothermic, and thus they reasonably modeled only the first step of roasting. Botazzi et al. (2012) numerically modelled water loss and heat transfer. They took into account more physical-chemical attributes, such as the heat produced by the beans, which increased the modelling complexity. Each attribute had its own independent differential equation without cross-linked terms and they modelled the beans as perfect spheres. They tested their models against (i) previous results of Schenker (2000) and (ii) results from an industrial coffee roaster. The authors credited the observed deviations between the modeled and measured values to the long sampling period of the data logger and to the radiation that emanates from the burner.

Putranto and Chen (2012) modeled barley and coffee roasting using another kind of modelling that started with a very simple time dependent differential equation. The determination of some thermal dependent constants was necessary for the model and they tested isothermal roastings, where each temperature generated a distinct equation. The model seemed to fit the experimental data very well for barley applications.

Romani et al. (2012) used an electronic nose (EN) and neural network (NN) computation to trace weight loss, density, moisture content, and surface color. They used the same Arabica coffee samples for different roasting times in the same roasting conditions of 200 ºC as initial temperature and with constant fuel input. They sealed each roasted sample into a glass vial for 20 hours at room temperature and the combined PCA (Principal Component Analysis) and NN analysis of the sensor responses showed a good prediction capacity.

Alonso-Torres et al. (2013) modeled heat and mass transfer in individual coffee beans with computational fluid dynamics (CFD) using a three-dimensional simulation of the bean placed in a glass tube where hot air flowed. Thousands of small geometrical domains known as mesh or grid composed the modeled bean, in which they solved differential equations using model parameters for usual roasting conditions. Higher temperatures led to some deviation from literature results, and the authors credited it to the lack of exothermic and physical expansion parameters in the model.

Chiang et al. (2017) modeled air and heat flow in a roaster oven, and took into account roaster dimensions, rotation and materials. They also modeled coffee bean heat exchange along the roasting process. They used computational fluid dynamics and numerical equations with finite volume technique. However, they modeled only the empty oven and not the effect of the bean presence. The modeled coffee bean heat exchange also ignores the exothermic contribution of the bean at the end of the roasting.

Oliveros et al. (2017) took into account porosity changes inside the bean in their coffee roasting modeling .This increased the mathematical complexity of their equations, though their model still does not incorporate bean expansion and the late exothermic step into the roasting. They compared their results with literature data and their own experiment and concluded that their premise of considering the porous void as only air was one of the reasons for model deviation from experimental data. They incorporated geometrical simulations of previous publications in their roasting modeling.

With all these limitations of model in mind, this work examined the coffee roasting process through the analysis of the roaster variables that influence the roast to build a solely empirical model and study variable importance. Multivariate experimental planning helped concoct this model and its implication can be paired with those found in previous theoretical modelling. Although this model only applies as a whole to the same conditions of this experiment, it allowed establishing correlations between roaster variables and predictions of variable effects on the roaster operation. The use of multivariate experimental planning, which generates as few experiments as possible, also helped reduce working time and experimental cost.

Experimental Planning

Experimental planning is a chemometric strategy that allows the determination of the control variables that exerts the major influence on the measured responses of a system (CALADO; MONTGOMERY, 2003; MONTGOMERY, 2013; NETO et al., 2010). This strategy helps extract more information with a lower number of experiments, which leads to time and operational cost reductions (PATIENCE, 2013).

Among the different kinds of experimental planning, the complete factorial planning is very reliable for scanning the true significance of control variables in only two setting levels. In this kind of planning, r^k usually represents the number of experiments. The number of levels is ‘r’, and ‘k’ is the number of variables. Setting levels are also usually represented by (+) and (–). This planning allows the simultaneous changing of all chosen control variables and the combination of all their setting levels. It yields not only the evaluation of control variables but can also show the occurrence of interactions between these control variables through the analysis of the measurements of response variables as well as the elimination of control variables that show no influence in responses (CALADO; MONTGOMERY, 2003; MONTGOMERY, 2013). An eventual development using three levels with the variables that showed significance can model the control/response interactions in a mathematical function.

Factorial planning (FP) helps establish correlations between control variables that alter roast response and the response variables that the operator cannot control. FP also allows establishing whether there are secondary correlations between control variables themselves. These correlations aimed at furthering the understanding of the roasting process, since the characteristic taste and aroma of coffee develop along this process. The correlation with the sensorial (or cupping) quality response of this beverage will be the subject of another work.

2 MATERIAL AND METHODS

The line of commercial roasters from Atilla Ltda. offers roasters from 2 to 60 kg. Their smallest model for 2 kg suits the trials for perfecting the roasting of specialty coffees, while it mirrors the conditions for larger roasters. This small roaster works in batches and burns gas fuel to heat both its horizontal rotating drum and the air that crosses its bean layer, while the rotation of its internal pallets permanently force the mixing of beans. Its digital controls adjust drum rotation as well as air flow speeds. Its bean cooler uses forced-air flow from top to bottom and is located in front of the drum exit to receive coffee beans at the end of the roasting time (Atilla, 2018).

Thorough discussions with the roaster manufacturers helped set the significant control variables and their meaningful ranges as well as the levels to measure within these ranges. Moreover, the discussion elicited what should be the sensible response variables that would support the aim of verifying the relationship with control variables and an attainable coffee quality.

The results obtained using complete factorial planning helped establish correlations between the control variables that may determine the roasting profile and affect coffee quality and the response/operational variables that may vary with roasting conditions. The roast manufacturing company helped in all procedures. Table 1 shows control variables and their levels.

The factorial design combination of five variables at two levels elicited 32 (2^5) combinations or 32 different roasting conditions that followed the experimental matrix (Table 2).

These numbers of variables and levels – even though they do not allow the full modelling of the relation between control and response variables – determine a mathematical model in which the coefficients show the relative importance of each variable and of each interaction of two or more variables (Equation 1). Response variable results allow the calculation of these coefficients.

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{14}x_1x_4 + b_{15}x_1x_5 + b_{23}x_2x_3 + b_{24}x_2x_4 + b_{25}x_2x_5 + b_{34}x_3x_4 + b_{35}x_3x_5 + b_{45}x_4x_5 + b_{123}x_1x_2x_3 + b_{124}x_1x_2x_4 + b_{125}x_1x_2x_5 + b_{134}x_1x_3x_4 + b_{135}x_1x_3x_5 + b_{145}x_1x_4x_5 + b_{234}x_2x_3x_4 + b_{235}x_2x_3x_5 + b_{245}x_2x_4x_5 + b_{345}x_3x_4x_5 + b_{1234}x_1x_2x_3x_4 + b_{1235}x_1x_2x_3x_5 + b_{1245}x_1x_2x_4x_5 + b_{1345}x_1x_3x_4x_5 + b_{2345}x_2x_3x_4x_5 + b_{12345}x_1x_2x_3x_4x_5$$

However, other six roastings aside the roasting conditions of the experimental matrix in Table 2 allowed the evaluation of experimental error and robustness. These control roastings occurred at the beginning, in the middle and at the end of each workday. Their conditions (Table 3) were different from any set of conditions employed in the experimental matrix.

The 2 kg Atilla Gold Plus roaster performed all 38 roastings in two days. Each roasting used 500 g of the same homogeneous coffee with all beans above 16 sieve. A coffee producer – Sitio Bela Vista – naturally processed (without peeling off coffee fruit) coffee beans in an open drier. This producer cultivated this coffee in high altitude – 1100 m – and kindly selected and provided the Arabica coffee of variety red catucaí 785 (Catucaí Vermelho 785) that he harvested in the previous harvest season.

It is worth to emphasize that bean sieving is a common procedure that helps (i) classify that parcel of coffee in relation to the size distribution of the beans and (ii) obtain a more homogeneous and uniform roast containing beans of roughly the same size. High size beans (larger than sieve 16) usually come from higher altitudes and have better cupping performance. The correlation between size and quality is not mandatory, since there are other factors that can affect coffee quality before roasting, but larger and homogeneous sizes are preferred (ICO (3), 2016).

Mathematical calculations in Excel established the correlation between control variables – and their levels – (Table 1 and Table 5) with operational/physical variables, which are mass reduction (%), minimum temperature along the roasting (°C), cracking temperature (°C), cracking time (min:sec), and final temperature (°C).

TABLE 1 - Control variables and their chosen levels.

Control variables	Superior Level (+)	Inferior Level (-)
Roaster starting temperature (x_1)	160 °C	200 °C
Vent opening (x_2)	25-50-100 (%)	25-75-100 (%)
Rotation speed (x_3)	40-65 rpm	65 rpm
Time after the first cracking (x_4)	1 min	2 min
Fuel flow increase (x_5)	0.5 L min^{-1}	1 L min^{-1}

TABLE 2 - Experimental Matrix.

Experiment	T_0 (°C) $\mathbf{X_1}$	Vent (%) $\mathbf{X_2}$	Rotation speed (rpm) $\mathbf{X_3}$	Fuel flow increase (mbar/min) $\mathbf{X_4}$	Time after cracking (min) $\mathbf{X_5}$
1	160	25-50-100	40-65	0,5	1
2	200	25-50-100	40-65	0,5	1
3	160	25-75-100	40-65	0,5	1
4	200	25-75-100	40-65	0,5	1
5	160	25-50-100	65	0,5	1
6	200	25-50-100	65	0,5	1
7	160	25-75-100	65	0,5	1
8	200	25-75-100	65	0,5	1
9	160	25-50-100	40-65	1	1
10	200	25-50-100	40-65	1	1
11	160	25-75-100	40-65	1	1
12	200	25-75-100	40-65	1	1
13	160	25-50-100	65	1	1
14	200	25-50-100	65	1	1
15	160	25-75-100	65	1	1
16	200	25-75-100	65	1	1
17	160	25-50-100	40-65	0,5	2
18	200	25-50-100	40-65	0,5	2
19	160	25-75-100	40-65	0,5	2
20	200	25-75-100	40-65	0,5	2
21	160	25-50-100	65	0,5	2
22	200	25-50-100	65	0,5	2
23	160	25-75-100	65	0,5	2
24	200	25-75-100	65	0,5	2
25	160	25-50-100	40-65	1	2
26	200	25-50-100	40-65	1	2
27	160	25-75-100	40-65	1	2
28	200	25-75-100	40-65	1	2
29	160	25-50-100	65	1	2
30	200	25-50-100	65	1	2
31	160	25-75-100	65	1	2
32	200	25-75-100	65	1	2

TABLE 3 - Control Roasting Conditions.

Parameters	Conditions				
Starting temperature	185 °C				
Vent opening	100%				
Rotation speed	65 rpm				
Air flow	**Initial**			**From 6,5 min on**	
	1740			1800	
Pressure (Fuel flow)	**0.00 – 4.00 min**	**4.50 – 7.00 min**	**7.50 – 9.00 min**	**9.50 -10.50 min**	**11.00 min - end**
	3.5 mbar	4.5 mbar	8.0 mbar	9.5 mbar	0.5 mbar

The measurement of the above-mentioned operational variables during the roasting helped evaluate how the operational – or response – variables changed with alterations in control variables. Thermocouples type k located inside the roaster's drum in two positions allowed the measurements of oven temperature and coffee bean temperature during roasting. The mass reduction was measured by weighing the coffee beans before and after the roast in room temperature and using a scale (Urano UDC 15/5 POP). The hearing of the cracking sound determined the cracking time and the chronometer of a cell phone Samsung measured the cracking time.

3 RESULTS AND DISCUSSIONS

During each roast, the temperature was recorded every half minute and the roasting/temperature profiles were established as a function of time (Figure 1).

Confidence Intervals for roasting profiles

Control roastings apart from the roasting set of experiments and developed by the roast master helped determine the error for each experiment and the robustness of the equipment. The roast master performed this kind of roast in the same way and following the same parameters a few times throughout the two days of work (Table 3). In total, six control roastings were performed (three on the first and three on the second day). Based on these roasts, the standard deviations and confidence intervals (CI) were calculated (i) for the roast profiles obtained during the experiment and (ii) for the results obtained for the response variables.

Confidence intervals calculated from these data, represented the deviation of each control roasting from the average roast (Table 4). Figure 1 shows that the variation between control roastings is small when compared to all roastings. Thus, although there were significant variations in room temperature between control roastings, the stability of the measurements showed that the roaster was robust and only small variations between profiles occurred.

Confidence Intervals for roasting control variables

The coefficients calculated using the model of equation 1 allowed the determination of the relative influence of control variables upon each operational variable. The interaction coefficients of three or more control variables on each operational variable allowed the calculation of confidence intervals (CI) for each response function and the elimination of coefficients that were inside the confidence interval, since these interactions are consistently considered as errors (MYERS et al., 2016; NATRELLA, 2005; NETO et al., 2010; BOX et al., 2005). Table 5 exhibits the retained coefficients that remained after the evaluation of confidence intervals.

The analysis of results showed that 'time after cracking' exerts the largest and positive influence on mass reduction, that is, the increment of its time increases the mass loss. On the other hand, 'vent' exerts a negative influence on mass loss that decreases when it increases, possibly because of temperature reduction.

The initial roasting temperature (T_o) has a major and positive influence on the lowest temperature along roasting. Thus, the higher the initial temperature, the higher the lowest temperature along roasting. The rotation exerts a negative influence on the lowest temperature, so that the increase of rotation lowers the lowest temperature of the roasting process.

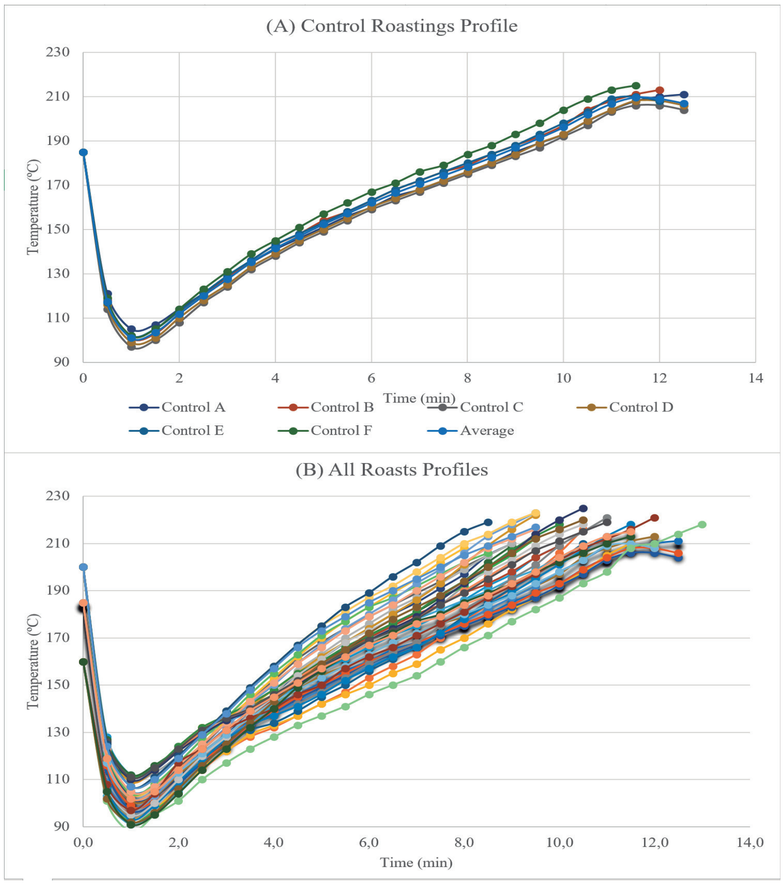

FIGURE 1 - (A) Roast profiles of control roastings and the average roasting. (B) Profiles of all roasts executed in the experimental planning.

TABLE 4 - Confidence intervals obtained from control roasting profiles.

	Control A	Control B	Control C	Control D	Control E	Control F
Standard deviation	0,47	0,28	0,71	0,45	0,27	1,06
n	26	25	26	26	25	24
t (n,α)	2,056	2,060	2,056	2,056	2,060	2,064
CI	0,19	0,12	0,29	0,18	0,11	0,45

TABLE 5 - Control variables and one interaction versus operational/response variables.

	T_0	Vent	Rotation	Fuel increase	Time after cracking	$T_0 \times$ Rotation	CI*
Mass reduction		-0.56			1.56		0.385
Lowest temperature	14.12		-3.12			-2.5	1.514
Cracking temperature				-1.19			0.804
Cracking time	-1.35	0.53	-1.11				0.241
Final temperature		-2.75	-2	2	10.12		1.652

*Following the model of BOX et al., 2005. See supplementary material for calculation details.

The interaction between T_o and rotation exerts a negative influence on the lowest temperature. The fuel flow increase is the only variable that exerts negative influence in cracking temperature, e.g. if the fuel flow increases, the cracking temperature lowers.

Three variables exert influence on the time after cracking or cracking time. Initial temperature (T_o) has the highest but negative influence: it dwindles the cracking time when it rises. The rotation also exerts a negative influence on cracking time, so that the use of the higher rotation all the time dwindles the cracking time. The only minor positive influence is vent overture that increases the cracking time when the rise in vent overture is steeper.

The roasting time after cracking exerts the most positive influence on final temperature, although fuel flow also show a positive influence on this variable. Vent and rotation exert negative influence on this variable in this order of importance.

The results obtained evaluating the control roastings to each response variable as a function of the alterations in control variables helped determine the confidence interval (CI) of response variables. The coefficient of variation (CV) establishes a better comparison between measurements, since it is a percentage, which allows the comparison of dispersions even though they result of different measurements and have different units and ranges. Table 6 presents these results for CI and CV. The CV range was from 0.74 to 5.21 %, which is a low range for this kind of measurement.

4 CONCLUSIONS

Our study elucidated the positive and negative influence of control variables and the occurrence of correlations between them. This led to a primary quantification of the major factors that contribute to the roasting process and eventually to the quality of the coffee beverage. Even though these results showed the common knowledge among roast masters and professionals in the area, they also provided an in-depth knowledge about the roaster operation, since it was possible to quantify the relative importance of roast parameters. Besides, these results indicated the interaction between parameters.

The study of response variables elicited some specific conclusions:

*the initial temperature exerts a strong influence in the lowest oven temperature;

*vent and rotation alone do not show a strong influence in any response variable;

*only the increase of fuel speed influences the cracking temperature;

*the time after cracking exerts the largest influence on mass reduction and final temperature; and

*the interaction between initial temperature and rotation influences the lowest oven temperature.

The analysis of control roasts showed that the equipment used is robust regardless external variations of temperature and humidity. The control roasts were similar to each other in the roasting profiles and in the results obtained for the response variables.

TABLE 6 - Confidence intervals for response variables in control roastings.

Controls	Mass Reduction (%)	Lowest Temperature (°C)	Cracking Temperature (°C)	Cracking Time (min)	Final Temperature (°C)
A	17	105	202	10,75	211
B	16	101	200	10,32	208
C	15	97	203	11,00	204
D	16	99	204	11,00	205
E	15	102	204	10,55	205
F	15	102	203	9,90	215
Average	15,67	101,00	202,67	10,59	208,00
Standard Deviation	0,82	2,76	1,51	0,43	4,29
CI	0,86	2,89	1,58	0,45	4,50
CV (%)	5,21	2,73	0,74	4,04	2,06

Our study highlighted the importance and influence of a set of main variables upon the roasting process and as far as we are concerned this is the first approach to this process using multivariate planning tools.

5 ACKNOWLEDGEMENTS

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6 REFERENCES

ATILLA. **Manual do usuário, Atilla linhas Gold e Gold Plus**. Available on: <http://www.atilla.com.br/wp-content/uploads/2017/06/manual-torrador-atilla-gold-e-gold-plus.pdf>. Access in: 4 July 2018.

ALONSO-TORRES, B. et al. Modeling and Validation of Heat and Mass Transfer in Individual Coffee Beans during the Coffee Roasting Process Using Computational Fluid Dynamics (CFD). **CHIMIA International Journal for Chemistry**, v. 67, n. 4, p. 291–294, Apr. 2013.

BOTTAZZI, D. et al. A numerical approach for the analysis of the coffee roasting process. **Journal of Food Engineering**, v. 112, n. 3, p. 243–252, Oct. 2012.

BOX, G. E.; HUNTER, W. G.; HUNTER, J. S. **Statistics for Experimenters: Design, Innovation and Discovery**. 2nd. ed. 2005. John Wiley & Sons, Inc., Hoboken, New Jersey.

CALADO, V. Montgomery, D. C. 2003. **Planejamento de experimentos usando o Statistica**. E-Papers Serviços Editoriais, Rio de Janeiro.

CHIANG, C. C.; WU, D. Y.; KANG, D. Y. Detailed Simulation of Fluid Dynamics and Heat Transfer in Coffee Bean Roaster: Simulation of Coffee Bean Roaster. **Journal of Food Process Engineering**, v. 40, n. 2, p. e12398, Mar/Apr. 2017.

FABBRI, A. et al. Numerical modeling of heat and mass transfer during coffee roasting process. **Journal of Food Engineering**, v. 105, n. 2, p. 264–269, jul. 2011.

GIOMO, G. S. 2012. **Café-Uma boa pós-colheita é segredo para qualidade**. A Lavoura. Available on: <http://sna.agr.br/wp-content/uploads/alav688_cafe.pdf>. Access in: 8 May 2017.

GLOESS, A. N. et al. Evidence of different flavour formation dynamics by roasting coffee from different origins: On-line analysis with PTR-ToF-MS. **International Journal of Mass Spectrometry**, v. 365–366, p. 324–337, May. 2014.

HERNÁNDEZ, J. A. et al. Analysis of the heat and mass transfer during coffee batch roasting. **Journal of Food Engineering**, v. 78, n. 4, p. 1141–1148, Feb. 2007.

HEYD, B. et al. Physical Model of Heat and Mass Transfer in a Spouted Bed Coffee Roaster. **Drying Technology**, v. 25, n. 7–8, p. 1243–1248, Aug. 2007.

ICO (1), International Coffee Organization. **Trade in Statistics Tables – World Coffee Consumption.** Available on: <http://www.ico.org/prices/new-consumption-table.pdf>. Access in: 19 dez. 2016.

ICO (2), International Coffee Organization. **Trade in Statistics Tables – Total Production by Exporting Countries**. Available on: < http://www.ico.org/prices/po-production.pdf>. Access in: 19 dez. 2016.

ICO (3), International Coffee Organization. **Grading and Classification of Green Coffee**. Available on: <http://www.ico.org/projects/Good-Hygiene-Practices/cnt/cnt_en/sec_3/docs_3.3/Grading%20&%20class.pdf>. Access in: 19 dez. 2016.

KARYADI, J. N. W.; RAHAYOE, S.; MELIALA, E. A. Effect of Time Temperature History on Coffee Aroma during Roasting with Heat Conduction. **International Agricultural Engineering Conference**, Dec. 2009.

MONTGOMERY, D. C. **Design and analysis of experiments**. Eighth edition ed. Hoboken, NJ: John Wiley & Sons, Inc, 2013.

MWITHIGA, G.; JINDAL, V. K. Physical Changes during Coffee Roasting in Rotary Conduction-Type Heating Units. **Journal of Food Process Engineering**, v. 26, n. 6, p. 543–558, Dec. 2003.

MYERS, R. H.; MONTGOMERY, D. C.; ANDERSON-COOK, C. M. **Response surface methodology: process and product optimization using designed experiments**. 4ed. Hoboken, New Jersey: Wiley, 2016.

NATRELLA, M. G. **Experimental statistics**. Mineola, N. Y: Dover Publications, 2005.

NETO, B. B.; SCARMINIO, I. S.; BRUNS, R. E. **Como fazer experimentos: Aplicações na Ciência e na Indústria**. [s.l.] Bookman, 2010.

OLIVEROS, N. O. et al. Experimental study of dynamic porosity and its effects on simulation of the coffee beans roasting. **Journal of Food Engineering**, v. 199, p. 100–112, Apr. 2017.

PATIENCE, G. S. **Experimental methods and instrumentation for chemical engineers**. Waltham, Mass.: Elsevier, 2013.

PUTRANTO, A.; CHEN, X. D. Roasting of Barley and Coffee Modeled Using the Lumped-Reaction Engineering Approach (L-REA). **Drying Technology**, v. 30, n. 5, p. 475–483, Apr. 2012.

ROMANI, S. et al. Evaluation of Coffee Roasting Degree by Using Electronic Nose and Artificial Neural Network for Off-line Quality Control. **Journal of Food Science**, v. 77, n. 9, p. 960–965, Sept. 2012.

SCHWARTZBERG, H. Batch Coffee Roasting; Roasting Energy Use; Reducing That Use. In: YANNIOTIS, S. et al. (Eds.). **Advances in Food Process Engineering Research and Applications**. Boston, MA: Springer US, 2013. p. 173–195.

VELLUCI, M. 2015. **The Continued Rise of Premium Coffee in the U.S.: Will It De-Commoditize Coffee?** Brown Brothers Harriman | Commodity Markets Update, December 2015. Available on: <https://www.bbh.com/blob/10968/7d4a3ed1cd7a5ab6c0eb637164cff9d1/the-continued-rise-of-premium-coffee-in-the-u-s---will-it-de-commoditize-coffee--pdf-data.pdf>. Access in: 8 May 2017.

WIELAND, F. et al. Online monitoring of coffee roasting by proton transfer reaction time-of-flight mass spectrometry (PTR-ToF-MS): towards a real-time process control for a consistent roast profile. **Analytical and Bioanalytical Chemistry**, Heidelberg, v. 402, n. 8, p. 2531–2543, Mar. 2012.

WILSON, P. S. Coffee roasting acoustics. **The Journal of the Acoustical Society of America**, v. 135, n. 6, p. 265–269, June. 2014.

YERETZIAN, C. et al. From the green bean to the cup of coffee: investigating coffee roasting by on-line monitoring of volatiles. **European Food Research and Technology**, v. 214, n. 2, p. 92–104, Feb. 2002.

ZIMMERMANN, R. et al. Application of Laser Ionization Mass Spectrometry for On-line Monitoring of Volatiles in the Headspace of Food Products: Roasting and Brewing of Coffee. **Rapid Communications in Mass Spectrometry**, v. 10, n. 15, p. 1975–1979, Dec. 1996.

COFFEE LEAF MINER INCIDENCE AND ITS PREDATION BY WASP IN COFFEE INTERCROPPED WITH RUBBER TREES

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ABSTRACT: The coffee leaf miner (CLM) *Leucoptera coffeella* has a wide distribution and causes significant losses in coffee plantations (*Coffea* spp.) in Brazil. Its occurrence can be mitigated in intercropped systems, with the rubber tree (*Hevea brasiliensis*) adapting well to the consortium, while also providing extra income to the producer. We aimed to determine whether the afforestation influences the microclimate and affects the leaf miner incidence and its predation by wasp in coffee plants intercropped with rubber trees. The study was undertaken in state of Paraná, Brazil, using coffee plants intercropped with rubber trees planted in double rows (alleys) spaced at 13, 16 and 22 m between alleys, and compared to sole cropping coffee plots. From January 2008 to November 2010, the presence of CLM's lesions including those with signs of wasp predation was monitored in coffee plants intercropped with rubber trees and in the non-consorted coffee. A higher CLM's incidence was verified in monoculture coffee plots, while coffee plants located under and two meters away from rubber trees had the lowest incidences. CLM's incidence in intercropping system got higher as it increased distance from the trees. The number of lesions with signs of predation by wasps was positively correlated with the number CLM's lesions, indicating a density-dependent predator-prey relationship. The coffee plants intercropped with alley rubber trees reduce the CLM's occurrence and can be a management's tactic for this economically important pest.

Index terms: *Coffea arabica*, *Leucoptera coffeella, Hevea brasiliensis*, afforestation of coffee plants, shading.

INCIDÊNCIA DE BICHO-MINEIRO E SUA PREDAÇÃO POR VESPAS EM CAFEEIRO CONSORCIADO COM SERINGUEIRA

RESUMO: O bicho-mineiro do cafeeiro *Leucoptera coffeella* possui ampla distribuição e causa perdas significativas nas plantações de café (*Coffea* spp.) no Brasil. Sua ocorrência pode ser atenuada em sistemas consorciados, com a seringueira (*Hevea brasiliensis*) adaptando-se bem a consorciação, além de fornecer renda extra ao produtor. O trabalho objetivou determinar se a arborização influencia o microclima afetando a incidência do bicho-mineiro e sua predação por vespas em cafeeiro consorciado com seringueira. O estudo foi conduzido no estado do Paraná, Brasil, sobre plantas de café consorciadas com seringueiras plantadas em filas duplas (aleias) espaçadas a 13, 16 e 22 m entre aleias, e comparadas a parcelas de café em monocultivo. Entre janeiro de 2008 e novembro de 2010, a presença de lesões ocasionadas pelo bicho-mineiro, incluindo aquelas com sinal de predação por vespas, foi monitorada nas parcelas de café consociado com seringueira e nas parcelas de café em monocultivo. Maior incidência de bicho-mineiro foi verificada nos cafeeiros em monocultivo, enquanto plantas de café sob as seringueiras e a dois metros destas exibiram as menores incidências. A incidência do bicho-mineiro do cafeeiro no sistema consorciado foi maior com o aumento da distância das árvores de seringueira. O número de lesões com sinal de predação por vespas foi positivamente correlacionado com o número de lesões do bicho-mineiro, indicando uma relação predador-presa denso-dependente. Plantas de café, consorciado com seringueira em aléias, reduz a ocorrência do bicho-mineiro e pode ser uma tática de manejo para esta praga de importância econômica.

Termos para indexação: *Coffea arabica*, *Leucoptera coffeella*, *Hevea brasiliensis*, arborização dos cafezais, sombreamento.

1 INTRODUCTION

Coffee cultivation (*Coffea* spp.) plays an important role in the economy of Brazil, which is the world's largest producer and exporter, and the second largest consumer of the product (International Coffee Organization - ICO, 2018). Coffee plantations take up an estimated 2.16 million hectares distributed across 10 states, which correspond to 99% of the national production (Companhia Nacional de Abastecimento - CONAB, 2018).

Coffee plants in Brazil are predominantly cultivated in monocultures, due to the ease of managing and implementing mechanized cultivation techniques (CONCEIÇÃO; GUERREIRO-FILHO; GONÇALVES, 2005), which resulted in systems with greater spacings between the plants to accommodate the traffic of machines. However, greater spacings in monocrop

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coffee plantations expose the crop to climate risks such as frost, excessive solar radiation, and heat (WALLER; BIGGER; HILLOCKS, 2007). Moreover, plants spaced further apart are also vulnerable to wind and lower relative ambient humidity, factors that favor the incidence of the coffee leaf miner (CLM) *Leucoptera coffeella* (Guérin-Mèneville, 1842) (PARRA; REIS, 2013).

The CLM is the one of the main coffee pests in Brazil (CONCEIÇÃO; GUERREIRO-FILHO; GONÇALVES, 2005; PARRA; REIS, 2013). Its larvae consume the palisade parenchyma, causing: necrosis of the leaf blade, reduction of photosynthesis, and premature leaf fall (GRAVENA, 1983b; WALLER; BIGGER; HILLOCKS, 2007). One of the main control strategies has been the application of insecticides (CONCEIÇÃO; GUERREIRO-FILHO; GONÇALVES, 2005), but this solution results in a dependency on future insecticide use to manage the population level of the pest. In addition, intensive use of insecticides has caused problems, like insect resistance (FRAGOSO et al., 2002; RIBEIRO; MAGALHÃES; GUEDES, 2003).

In organic production systems the CLM can be mitigated by plant extracts (VENZON et al., 2005; ALVES et al., 2013), lime sulfur (VENZON et al., 2013), organic fertilization (THEODORO; GUIMARÃES; MENDES, 2014), and vegetational diversification with "key" plants (AMARAL et al., 2010). The crop diversification is desirable due to a sustainable pest control, based on system resilience (LIN, 2011).

The coffee crop diversification with other tree species is a promising alternative to reduce CLM's occurrence and make the management system less dependent on insecticide use. This is because coffee plants intercropped with tree species present lower insolation, air temperature, and wind intensity, as well as higher relative air humidity (CARAMORI et al., 2004; MORAIS et al., 2007), unfavorable conditions to the CLM's development (GUHARAY; MONTERROSO; STAVER, 2001; LOMELÍ-FLORES; BARRERA; BERNAL, 2010).

Natural enemies are more abundant in coffee systems intercropped with tree species resulting in reduced leaf miner populations (DE LA MORA; LIVINGSTON; PHILPOTT, 2008; REZENDE et al., 2014). Predatory wasps are considered the main agents of biological control for the CLM in Brazil, as they are responsible for the majority of leaf miner's mortality (TOZATTI; GRAVENA, 1988; PEREIRA et al., 2007). Intercropping with tree species has the potential to favor wasps by increasing shelter availability and nesting sites (SOUZA et al., 2014).

Rubber trees (*Hevea brasiliensis* Müell. Arg.) are a good choice for a producer to implement an intercropping coffee system. In addition of improving the microclimate for the coffee plants, rubber trees are well adapted to intercropped systems (RIGHI; BERNARDES, 2008), providing an alternative source of income (PEREIRA; PEREIRA; JUNQUEIRA, 1996). However, it's essential to understand how the proposed agroforestry intercropping will affect the main pest of the coffee plants. The simple diversification does not always reduce the pest occurrence (TSCHARNTKE et al., 2016). It was verified that coffee plants intercropped with banana trees (*Musa* spp.) or even the rubber trees (*H. brasiliensis*), in the tested design did not reduce the CLM occurrence (AMARAL et al., 2010; RIGHI et al., 2013). The selected tree's species or its spatial arrangement within intercropped systems, result in different microclimate changes (PARTELLI et al., 2014; ARAÚJO et al., 2015).

The coffee-rubber trees intercropped system in a design that decreases temperature and increases the humidity inside the system, as well as enhances the predatory wasp's establishment, probably will reduce the population densities of CLM. On the other hand, the trees can provide shelter to CLM's eggs and larvae against raindrop impacts, which cause great mortality (SILVA et al., 2003). This study aimed to determine whether the coffee intercropped with rubber trees at different densities, influences the microclimate (temperature, humidity and sun radiation) or provides protection against raindrop impacts, affecting the coffee leaf miner occurrence and its predation by wasps.

2 MATERIAL AND METHODS

The study was carried out from January 2008 to November 2010 in Londrina, Paraná state, Brazil (23°21′38.22″S, 51°10′04.15″W, altitude 581 m). The soil at the Experimental Station is Rhodic Ferralsol with a very clayey texture (Food and Agriculture Organization of the United Nations - FAO, 1997). The climate is humid subtropical (Cfa, according to the Köppen classification) with an average annual precipitation of 1,608 mm and average annual temperature of 21.1°C (Instituto Agronômico do Paraná - IAPAR, 2014). During the experimental period, records of cumulative monthly rainfall were obtained from a meteorological station located 100 m from the experimental plots.

The experimental area consisted of coffee plants of the cultivar IAPAR 59, planted in the year 2000 in a 2.5 × 1.0 m grid, with two plants per hole and rows arranged in a north-south direction. In the east-west direction, there were double rows (alleys) of rubber trees, Clone PB235, planted in 1999 with a spacing of 4 m between alley rows and 2.5 m between plants within the row (Figure 1).

This study used a randomized block split-plot design (3 x 5 + 1), with five repetitions. Each plot tested three different spacings (13, 16, and 22 m) between rubber tree alleys. For each alley spacing, coffee plants were sampled at five positions relative to the rubber tree alleys: between rubber trees within the alley (BRT), 2 m to the south and north of the alley (2 m south and 2 m north), and in the middle between the focal alley and the next alley to the south (middle south) and to the north (middle north) (6.5, 8.0, or 11.0 m from the focal alley, depending on alley spacing). All these designs were compared with coffee plants in the center of a monocrop system (Figure 1).

Coffee leaf miners were sampled on coffee plants in all intercropped and sole cropping systems. In each sampling point, intact leaf miner lesions and those with signs of predation by wasps were quantified using visual analysis of two pairs of leaves (third and fourth pair from the apex of the branch) on two branches (of upper half of plants) on eight plants, totaling 64 leaves on each evaluation.

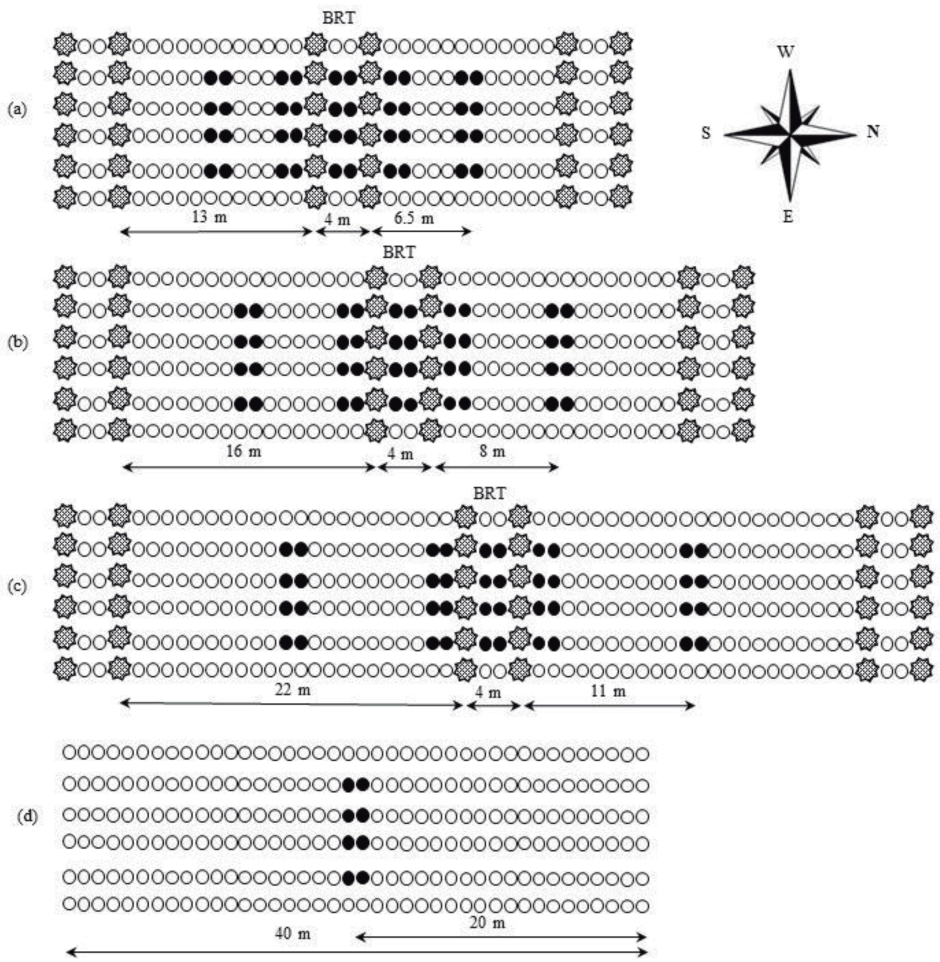

FIGURE 1 - Schematic diagram of the planting configurations used in this study. Dashed stars, empty circles and dark circles represent, rubber trees, coffee plants and evaluated coffee plants, respectively. Panels (a)-(c) describe coffee plots intercropped with rubber trees alleys (double rows) spaced 13, 16 and 22 m apart, respectively. Panel (d) describes the monoculture coffee plots. Compass rose indicates the planting orientation and arrows show distances in meters. BRT = between rubber trees. Londrina, PR.

Over the study period, 35 monthly leaf miner evaluations were performed, giving a total of 2,240 leaves examined at each sampling point.

The following parameters were measured: number of coffee leaves with lesions produced by *L. coffeella* (percentage of leaves with at least one lesion), total number of lesions on the evaluated leaves (indicating the severity of the infestation), and total number of *L. coffeella* lesions with signs of predation by wasps.

In order to characterize microclimatic conditions, data on temperature and relative humidity were obtained from five automated meteorological stations installed at the following points in the experimental area: one in the center of the monocrop coffee plot, two 2 m away from the alley of rubber trees, and two in between the alleys spaced 16 m apart. The four meteorological stations in the intercropped plots were evenly distributed to the north and south of the alley. The sensors were installed at a height equivalent to the mean height of a coffee plant (approximately 85 cm above ground level). Data were obtained during the period April 16th–19th, 2010. To better understand the results, data from the north and south sides were pooled and averaged for each distance in relation to the rubber tree alleys.

The rubber trees' shading over the coffee plants was estimated using illuminance (lux) measured with a digital lux meter. Readings were taken every half hour from sunrise to sunset on May 31st, 2010. These values were integrated over the day to determine the total daily illuminance and this value was used as a percentage relative to the value obtained in the monocrop coffee plots.

Before analysis, the data was tested for homoscedasticity and normality. In order to meet the assumptions for a parametric analysis, the data from the system intercropped with rubber trees were examined using variance analysis for subdivided plots and the outcomes for each configuration were compared using Tukey's test. Subsequently, the monoculture coffee plants were compared to those intercropped with rubber trees through a contrast analysis using the Scheffé test. For both tests, the significance threshold was 5%. These statistical analyses were performed by using the statistical program SISVAR 5.3.

To examine the possibility of a density-dependent relationship between the pest and its predator, the association between the total number of *L. coffeella* lesions and the total number of lesions with signs of wasp predation was tested using Spearman's correlation analysis, using the statistical program BioEstat 5.0. Spearman's correlation analysis was also used to assess the association between leaf miner incidence (percentage of leaves with lesions and the total lesions on the third leaf pair) and monthly rainfall two months prior to the evaluation. Previous work has demonstrated that rainfall correlates with *L. coffeella* incidence in this time interval (ANTUNES, 1986), since the effects of rain on the incidence of lesions are become visible later.

3 RESULTS AND DISCUSSION

Pest incidence and microclimates

Coffee plants positioned between the rubber trees within the alley and at a distance of 2 m from them, showed lower *L. coffeella* incidence than coffee plants situated in between the alleys, both in terms of the number of leaves with lesions and the total number of lesions (Table 1 and 2). Moreover, the spacing between the rubber tree alleys influenced infestation intensity (number of leaves with lesions and total number of lesions) in the coffee plants positioned between the alleys. For coffee plants in this configuration, infestation was lower when rubber tree alleys were spaced at 13 m and greater when spaced at 22 m (Table 1 and 2).

The direction of the coffee plants from the rubber tree alleys did not influence the number of leaves with lesions or the total number of lesions, with similar results from plants on the south and north sides (Table 1 and 2). Regardless of the distance between the rubber tree alleys and the distance of the coffee plants to these alleys, all coffee plants intercropped with rubber trees showed a lower number of leaves with lesions and a lower total number of lesions compared to the monoculture coffee (Table 1 and 2).

The CLM's incidence was lowest in coffee plants within the alley and at 2 m of distance from it, and remained below the control level (30% of leaves with lesions - see REIS; SOUZA; ZACARIAS, 2006) throughout the assessment period (Figure 2). The most intense infestation was found in the monoculture coffee plants followed by coffee plants situated between rubber tree alleys, with nine and six of the 35 monthly evaluations exceeding the control level, respectively. It is notable that when the percentage of leaves with lesions exceeded control levels, values for coffee plants in between alleys spaced 13 m apart remained close to 30%; this was not the case for the other alley spacings (Figure 2).

TABLE 1 - Percentage (mean ± standard deviation; n=175) of leaves with *Leucoptera coffeella* (Guérin-Mèneville, 1842) lesions from coffee plants in different planting configurations, calculated across 35 monthly evaluations performed between January 2008 and November 2010. Londrina, PR.

Position of coffee plants [(a)]	Spacing between rubber tree alleys (m)		
	13	16	22
BRT	4.0 ± 1.0Ba*	4.2 ± 0.5Ba*	4.8 ± 0.8Ba*
2 m south	4.6 ± 0.7Ba*	4.6 ± 1.0Ba*	4.9 ± 0.7Ba*
2 m north	4.3 ± 0.5Ba*	4.5 ± 1.0Ba*	4.3 ± 0.6Ba*
Middle south	11.9 ± 0.9Ab*	13.4 ± 1.3Aa*	14.4 ± 0.9Aa*
Middle north	10.7 ± 1.1Ac*	12.5 ± 1.3Ab*	13.9 ± 0.9Aa*
Monocrop coffee	18.4 ± 0.8		

[(a)] Between rubber trees within an alley (BRT), 2 m away from the alley (2 m), and in the middle between two alleys (Middle). Values followed by the same letter did not differ significantly (Tukey test, α=5); the upper-case letter denotes column values and the lower-case line values. The * indicates a significant difference (Scheffé test, α=5) compared with monocrop coffee.

TABLE 2 - Number (mean ± standard deviation; n=5) of *Leucoptera coffeella* (Guérin-Mèneville, 1842) lesions across 2,240 leaves from coffee plants in different planting configurations, gathered from 35 monthly evaluations performed between January 2008 and November 2010. Londrina, PR.

Position of coffee plants [(a)]	Spacing between rubber tree alleys (m)		
	13	16	22
BRT	132.0 ± 27.7Ba*	144.4 ± 10.8Ba*	161.0 ± 29.5Ba*
2 m south	150.2 ± 20.9Ba*	153.0 ± 37.6Ba*	165.2 ± 27.2Ba*
2 m north	141.4 ± 7.7 Ba*	157.2 ± 30.4Ba*	143.4 ± 25.5Ba*
Middle south	375.8 ± 46.1Ac*	421.0 ± 28.4Ab*	470.6 ± 49.4Aa*
Middle north	334.2 ± 37.6Ac*	412.4 ± 30.0Ab*	465.2 ± 27.8Aa*
Monocrop coffee	646.8 ± 39.7		

[(a)] Between rubber trees within an alley (BRT), 2 m away from the alley (2 m), and in the middle between two alleys (Middle). Values followed by the same letter did not differ significantly (Tukey test, α=5); the upper-case letter denotes column values and the lower-case line values. The * indicates a significant difference (Scheffé test, α=5) compared with sole cropping coffee.

The microclimate (temperature and relative air humidity) differed between monoculture coffee plants and intercropped ones with rubber trees. From 10:00 to 16:00, coffee plants located two meters from the rubber tree alley experienced lower air temperatures than the monoculture coffee plants and those in between the alleys 16 m apart, with greater differences in the hottest times of the day. In contrast, from 18:00 to 07:00, monoculture coffee plants experienced a slightly lower air temperature (Figure 3a). The relative air humidity demonstrated an opposite pattern compared to the temperature. From 10:00 to 16:00, relative humidity values for coffee plants 2 m from the rubber tree alley were greater than for monoculture coffee, while from 18:00 to 07:00, humidity was higher for monoculture plants (Figure 3b). Coffee plants under the canopy of rubber trees thus experienced a lower range of temperature and relative air humidity than monoculture coffee plants.

The microclimate of forested systems is different to non-forested systems. The trees act as a windbreak and are a physical barrier to high insolation and water loss from evapotranspiration in the lower strata, resulting in greater humidity and lower air temperature in the hottest periods of the day (LIN, 2007, 2010).

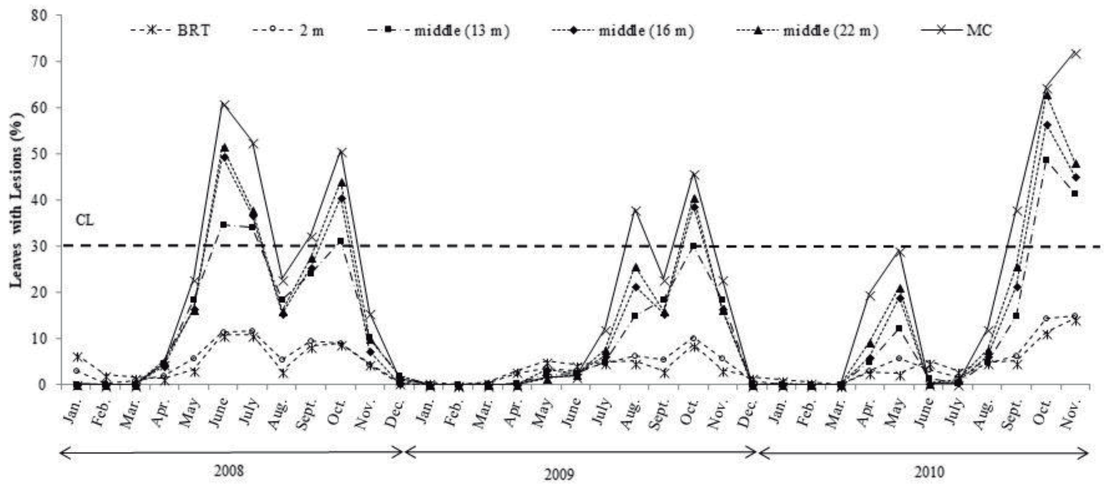

FIGURE 2 - Average percentage (n = 5 replicates) of leaves with *L. coffeella* lesions in different planting configurations: monoculture coffee (MC), between rubber trees within an alley (BRT), 2 m away from the alley (2 m), and in the middle between two alleys (middle) spaced 13, 16, or 22 m apart, during the years 2008 (a), 2009 (b) and 2010 (c). Horizontal dashed lines indicate the control level (CL) for *L. coffeella*, Londrina, PR.

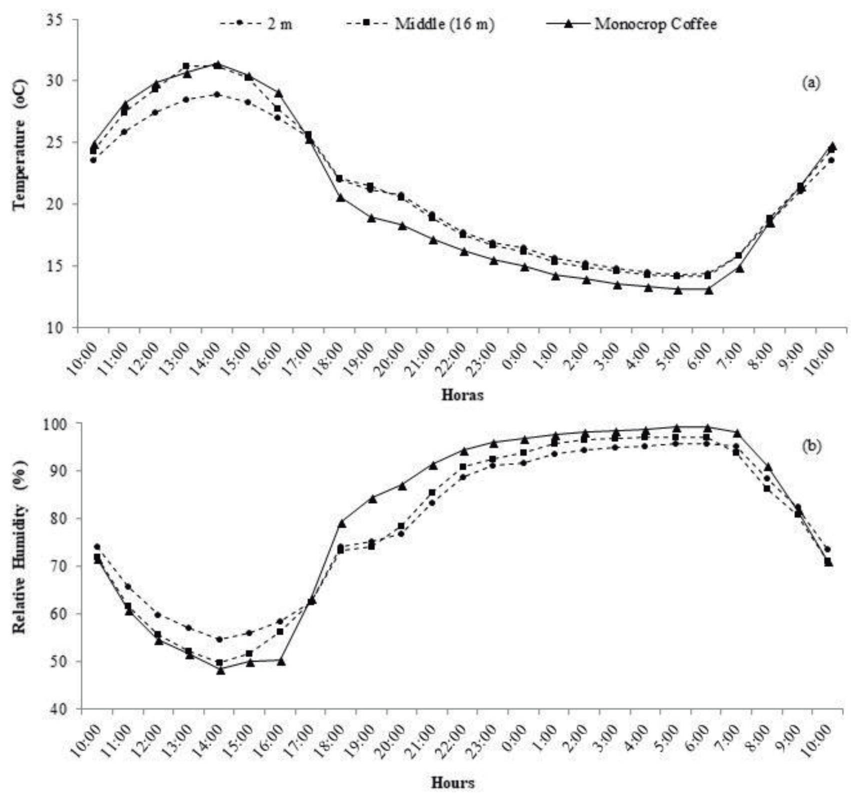

FIGURE 3 - Average values (n = 3 days) for temperature (a) and relative air humidity (b) measured in three locations: in the center of the monoculture coffee plot, coffee plants 2 m away from rubber tree alleys and in the middle of two alleys spaced 16 m apart. Gray areas indicate the night period. Londrina, PR.

These microclimate changes are likely to be linked to the lower incidence of *L. coffeella* in coffee plants near rubber trees. Studies indicate that a greater relative air humidity reduces the total number leaves with *L. coffeella* (GRAVENA, 1983a; GUHARAY; MONTERROSO; STAVER, 2001; TEODORO; KLEIN.; TSCHARNTKE, 2008). In addition, the decrease of temperature in the day's hottest periods, disfavor the CLM's incidence. Lomelí-Flores; Barrera; Bernal (2010) observed that the temperature influences not only the survival rate of *L. coffeella*, which is higher at higher maximum air temperatures, but also oviposition by leaf miner females, with no oviposition on nights with temperatures below 20ºC. Moreover, reduced temperatures increase the time required for the *L. coffeella* caterpillar-miners to hatch, as well as the duration of the caterpillar phase (PARRA; GONÇALVES; PRECETTI, 1981). This prolongs the biological cycle of the pest, reducing the number of generations per year and hence contributing to reduce the potential damage.

Overall, coffee plants situated under the rubber trees canopy had on average 61.6% shading (ranging 22.3% to 78.7%), while those in between the rubber tree alleys had on average 29.5% shading (ranging 10.7% to 43.7% of shading) (Figure 4). The greater shading occurred in coffee plants situated on the south side of the alley (70.8% to 78.7%).

This is probably due to the declination of the sun on the day when the measurements were taken due the latitude of the study site, thus the solar declination was slightly shifted to the north, resulting in a greater projection of shade towards the south side of the alley. Coffee plants beyond the rubber tree canopy showed the least shading (10.7% to 20.1%) when rubber tree alleys were spaced furthest apart (22 m).

Coffee plants shaded by rubber tree alleys probably have higher water availability compared to coffee plants under direct sunlight farther away from the rubber trees. According to Neves et al. (2007), intercropping coffee plants in agroforestry systems entails water savings, and coffee transpiration has shown to be significantly reduced when it is grown in shade conditions (MORAIS et al., 2007). Righi et al. (2013) studying coffee in a crop production system with rubber trees, found a higher CLM's incidence after periods with greater soil water deficiency, the same occurred in the present study. Coffee plants under lower water stress are less susceptible to attacks from CLM (MEIRELES; CARVALHO; MORAES, 2001; ASSIS et al., 2012), given that plants under water stress show greater levels of nitrogen and lower levels of secondary metabolites, which are attractive to the pest (LAWTON; MCNEILL, 1979).

The shading provided by the rubber tree may also impair the CLM due leaf's anatomy alteration of coffee plants. The shading of the coffee leaves result in a thickening of the epidermal cells and a narrowing of the mesophyll cells (VOLTAN; FAHL; CARELLI, 1992). Thus, the caterpillar will cross the epidermis less readily and will have a smaller mesophyll area in which to develop, which may result in lower survival rates for leaf miners in the shaded areas.

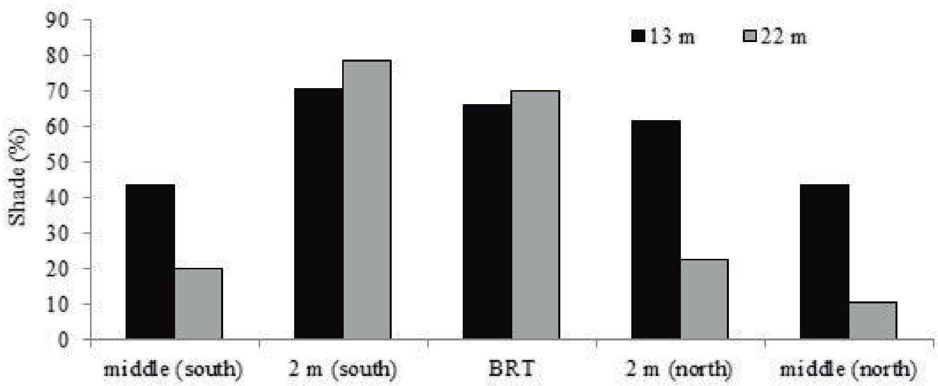

FIGURE 4 - Average values (n = 5) for percentage shading relative to monocrop coffee, at the top of coffee plants in different planting configurations: between rubber trees in the alley (BRT), two meters away from the alley (2 m), and in the middle between two alleys (middle) spaced 13 or 22 m apart. Londrina, PR.

Predation by wasps

The greatest predation of leaf miners occurred in the monoculture coffee plants. In an average of 646.8 *L. coffeella* lesions present in this system (Table 2), approximately 111.6 (17.3%) presented signs of wasp predation. In the system intercropped with rubber trees, coffee plants situated within the rubber alley and 2 m away from it showed fewer lesions with signs of wasp predation (from 22.2 to 33.4) than the coffee plants in between alleys (from 42.0 to 59.8). In addition, for coffee plants in between two rubber alleys on the north side, the number of lesions with signs of predation was lower when the alleys were spaced 13 m apart compared to 22 m apart (Table 3).

There was a strong positive correlation (r = 0.80; $p < 0.01$) between number of lesions and the number of lesions with signs of predation, increasing the number of lesions with signs of wasp predation proportional to the incidence of lesions in the different locations, suggesting a density-dependent effect between pest and predator.

Predatory wasps are an important natural enemy in Brazilian coffee plantations, being the main biotic cause of CLM's mortality (PEREIRA et al., 2007). More leaf miner predation was expected in the coffee plants intercropped with rubber trees, since greater numbers of natural enemies are supported when the plant diversity in an agricultural system is greater, resulting in improved biological control of pest (BIANCHI; BOOIJ; TSCHARNTKE, 2006). Moreover, studies indicate that the predatory wasps prefer arboreal plants to set nesting sites (SANTOS; BISPO; AGUIAR, 2009; SOUZA et al., 2014), favoring its permanence in arborized systems.

However, the greatest number of predated mines occurred in the monoculture coffee plants, followed by coffee plants in between two rubber tree alleys, which also showed greater incidence of the leaf miner. The results observed in our study are probably due to the experiment's size and design. Several studies indicate that the main predator wasps' species of the leaf miner that occur in the coffee plants are *Polistes* spp., *Polybia* spp., *Protonectarina sylveirae* (de Saussure, 1854), and *Brachygastra* spp. (GRAVENA, 1983a; TOZATTI; GRAVENA, 1988; PEREIRA et al., 2007; SCALON et al., 2011), where the genera *Polybia* spp. and *Polistes* spp. has been shown to have a foraging range of 75–126 m from its nest (BICHARA FILHO et al., 2010; PREZOTO; GOBBI, 2005; SANTOS et al., 2000). In this study, however, the maximum distance between a double row of rubber trees and the center of the locations considered sole cropped was approximately 20 m (Figure 1), such that there were no coffee plants at a distance greater than the wasps' dispersion capacity. Thus, in terms of wasp foraging behavior, the experiment as a whole probably functioned as a large intercropped system.

In a situation in which there are no environmental limits on the wasps' dispersal ability, they are expected to forage preferentially in locations with greater prey availability, due the wasps' capacity to memorize sites where they had greater success in capturing food (RICHTER, 2000), minimizing energy expenditure when searching for food.

TABLE 3 - Number (mean ± standard deviation; n=5) of *Leucoptera coffeella* (Guérin-Mèneville, 1842) lesions with signs of wasp predation across 2,240 leaves from coffee plants in different planting configurations, gathered from 35 monthly evaluations performed between January 2008 and November 2010. Londrina, PR.

Position of coffee plants [a]	Spacing between rubber tree alleys (m)		
	13	16	22
BRT	25.0 ± 6.6Ba*	26.8 ± 4.1Ba*	33.4 ± 11.8Ba*
2 m south	26.2 ± 4.2Ba*	26.2 ± 5.2Ba*	22.2 ± 6.1Ba*
2 m north	27.0 ± 6.9Ba*	22.8 ± 10.9Ba*	25.0 ± 13.1Ba*
Middle south	42.6 ± 7.1Aa*	42.2 ± 9.9Aa*	55.2 ± 16.2Aa*
Middle north	42.0 ± 5.8Ab*	50.4 ± 8.8Aab*	59.8 ± 7.3Aa*
Monocrop coffee	111.6 ± 12.0		

[a] Between rubber trees within an alley (BRT), 2 m away from the alley (2 m), and in the middle between two alleys (Middle). Values followed by the same letter did not differ significantly (Tukey test, α=5); the upper-case letter denotes column values and the lower-case line values. The * indicates a significant difference (Scheffé test, α=5) compared with sole cropping coffee.

The strong positive correlation between the number of predated mines by wasps and the total number of mines in the leaves indicate a density-dependent relationship between predators and prey, corroborating that which has been previously noted between predatory wasps and *L. coffeella* by Fernandes et al. (2009).

Effect of rainfall on pest incidence

Rainfall correlated moderately and negatively with the percentage of leaves with lesions ($-0.38 \leq r \leq -0.55$; $p \leq 0.03$) and with the total number of lesions ($-0.41 \leq r \leq -0.54$; $p \leq 0.02$) at the different sampling points. To the coffee plants beneath rubber tree canopy the negative correlation ranged between -0.38 and -0.55, while in the monoculture coffee system ranged between -0.44 and -0.42. Thus, the increase in rainfall resulted in the lower number of lesions in the leaves, irrespective of the system of cultivation (monocrop or intercropped with rubber trees) or the distance of the coffee plants from the rubber trees.

The incidence of *L. coffeella* decreased as the rainfall increased, corroborating other studies that have shown a reduction in the leaf miner's survival rate during rainy periods (ANTUNES, 1986; LOMELÍ-FLORES; BARRERA; BERNAL, 2010; RIGHI et al., 2013). Pereira et al. (2007) found that rainfall is one of the primary causes of mortality at the egg and especially larval stages, which occur during the rainy season. Other authors suggest that larval mortality during rainy periods occur because the larvae drown in flooded lesions (NESTEL; DICKSCHEN; ALTIERI, 1994; VEGA; POSADA; INFANTE, 2007).

Coffee plants beneath rubber tree canopy would be protected from rain drops impact and thus avoid larvae drown in flooded lesions, however this was not observed in this study, due to the correlations between leaf miner incidence and rainfall were similar between coffee plants beneath rubber trees and monoculture coffee plants. Probably the alley intercrop system does not provide shelter to the leaf miner against rain impact, since the study area have convective summer rainfall (strong winds with oblique rainfall) (GRIMM, 2009) reaching coffee plants within the rubber alley.

The results indicate that the adoption of coffee intercropped with higher rubber tree densities (13 m between alleys) is more promising to maintain the CLM's population for longer time below the control level, in relation to the other densities tested and to monoculture coffee.

4 CONCLUSIONS

Coffee plant systems intercropped with rubber trees result in a lower incidence of coffee leaf miners when compared to monoculture coffee, especially in coffee plants near the rubber trees.

The lower incidence of coffee leaf miners near the rubber trees was not related to predation by wasps, but to pest's unfavorable microclimate conditions. Intercropped rubber trees had no protective effect on the leaf miner against rainfall ("umbrella effect"), and rain negatively affected leaf miner occurrence.

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6 REFERENCES

ALVES, D. S. et al. Selection of active plant extracts against the coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). **Revista Brasileira de Plantas Medicinais**, Campinas, v. 15, n. 3, p. 352-362, July/Sept. 2013.

AMARAL, D. S. et al. Does vegetational diversification reduce coffee leaf miner *Leucoptera coffeella* (Guérin-Mèneville) (Lepidoptera: Lyonetiidae) attack? **Neotropical Entomology**, Londrina, v. 39, n. 4, p. 543-548, July/Aug. 2010.

ANTUNES, F. Z. Fenômenos adversos para a agricultura. **Informe Agropecuário**, Belo Horizonte, v. 12, n. 138, p. 25-30, 1986.

ARAÚJO, A. V. et al. Microclima e crescimento vegetativo do café conilon consorciado com bananeiras. **Coffee Science**, Lavras, v. 10, n. 2, p. 214-222, Apr/June. 2015.

ASSIS, G. A. et al. Leaf miner incidence in coffee plants under different drip irrigation regimes and planting densities. **Pesquisa Agropecuária Brasileira**, Brasília, v. 47, n. 2, p. 157-162, Feb. 2012.

BIANCHI, F. J. J. A.; BOOIJ, C. J. H.; TSCHARNTKE, T. Sustainable pest regulation in agricultural landscapes: a review on landscape composition, biodiversity and natural pest control. **Proceedings of the Royal Society of London B: Biological Sciences**, London, v. 273, n. 1595, p. 1715-1727, Apr. 2006.

BICHARA FILHO, C. C. et al. Foraging behavior of the swarm-founding wasp *Polybia* (Trichothorax) *sericea* (Hymenoptera, Vespidae): daily resource collection activity and flight capacity. **Sociobiology**, Feira de Santana, v. 55, n. 3, p. 899-907, Jan. 2010.

CARAMORI, P. H et al. Arborização de cafezais e aspectos climatológicos. In: MATSUMOTO, S. N. (Ed.) **Arborização de Cafezais no Brasil.** Vitória da Conquista: UESB, 2004. Cap. 1, p. 1-42.

COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB **Acompanhamento da safra brasileira de café, Safra 2018**. Brasília: CONAB, 2018. 66 p. (v. 5, n. 2). Available on: <https://www.conab.gov.br/info-agro/safras/cafe>. Access in: Aug. 03. 2018.

CONCEIÇÃO, C. H. C.; GUERREIRO-FILHO, O.; GONÇALVES, W. Flutuação populacional do bicho-mineiro em cultivares de café arábica resistentes à ferrugem. **Bragantia**, Campinas, v. 64, n. 4, p. 625-631, Oct/Dec. 2005

DE LA MORA, A.; LIVINGSTON, G.; PHILPOTT, S. M. Arboreal ant abundance and leaf miner damage in coffee agroecosystems in Mexico. **Biotropica**, Hoboken, v. 40, n. 6, p. 742-746, May. 2008.

FERNANDES, F. L. et al. Efeitos de variáveis ambientais, irrigação e vespas predadoras sobre *Leucoptera coffeella* (Guérin-Méneville) (Lepidoptera: Lyonetiidae) no cafeeiro. **Neotropical Entomology**, Londrina, v. 38, n. 3, p. 410-417, May/June 2009.

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS – FAO **Soil map of the world, revised legend, with corrections and updates**. World soil resourses report 60, FAO, Rome. Reprinted with updates as technical paper 20, Wageningen: ISRIC, 1997. 140p.

FRAGOSO, D. B. et al. Insecticide use and organophosphate resistance in the coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae). **Bulletin of Entomological Research**, Cambridge, v. 92, n. 3, p. 203-212, June. 2002.

GRAVENA, S. Táticas de manejo integrado do bicho mineiro do cafeeiro *Perileucoptera coffeella* (Guérin-Méneville, 1842): I Dinâmica populacional e inimigos naturais. **Anais da Sociedade Entomológica do Brasil**, Londrina, v. 12, n. 1, p. 61-71, 1983a.

GRAVENA, S. Táticas de manejo integrado do bicho-mineiro do cafeeiro *Perileucoptera coffeella* (Guérin-Menèville, 1842). II-Amostragem da praga e de seus inimigos naturais. **Anais da Sociedade Entomológica do Brasil**, Londrina, v. 12, n. 2, p. 273-281, 1983b.

GRIMM, A. M. Clima da Região Sul do Brasil. In: CAVALCANTI, I. F.A. et al. (Eds.) **Tempo e Clima no Brasil**. São Paulo: Oficina de Textos, 2009. cap. 17, p. 259-275.

GUHARAY, F.; MONTERROSO, D.; STAVER, C. El diseño y manejo de la sombra para la supresión de plagas en cafetales de América Central. **Agroforestería en las Américas**, Costa Rica, v. 8, n. 29, p. 22-29, 2001.

INSTITUTO AGRONÔMICO DO PARANÁ – IAPAR. **Cartas climáticas do Paraná.** Available on: <http://www.iapar.br/modules/conteudo/conteudo.php?conteudo=677>. Access in: 22 Nov. 2014.

INTERNATIONAL COFFEE ORGANIZATION – ICO. **Historical Data on the Global Coffee Trade**. Available on: <http://www.ico.org/new_historical.asp>. Access in: 22 Nov. 2018.

LAWTON, J. H.; MCNEILL, S. Between the devil and the deep blue sea: on the problem of being a herbivore. In: ANDERSON, R. M.; TURNER, B. D.; TAYLOR, L. R. (Eds.) **Population dynamics**. London: Blackwell Scientific, 1979. p. 223-244.

LIN, B. B. Agroforestry management as an adaptive strategy against potential microclimate extremes in coffee agriculture. **Agricultural and Forest Meteorology**, New Haven, v. 144, n. 1-2, p. 85-94, May. 2007.

LIN, B. B. The role of agroforestry in reducing water loss through soil evaporation and crop transpiration in coffee agroecosystems. **Agricultural and Forest Meteorology**, New Haven, v. 150, n. 4, p. 510-518, Apr. 2010.

LIN, B. B. Resilience in agriculture through crop diversification: adaptive management for environmental change. **BioScience**, Oxford, v. 61, n. 3, p. 183-193, Mar. 2011.

LOMELÍ-FLORES, J. R.; BARRERA, J. F.; BERNAL, J. S. Impacts of weather, shade cover and elevation on coffee leaf miner *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) population dynamics and natural enemies. **Crop Protection**, Amsterdam, v. 29, n. 9, p. 1039-1048, Sept. 2010.

MEIRELES, D. F.; CARVALHO, J. A.; MORAES, J. C. Avaliação da infestação do bicho-mineiro e do crescimento do cafeeiro submetido a diferentes níveis de déficit hídrico. **Ciência e Agrotecnologia**, Lavras, v. 25, n. 2, p. 371-374, Mar/Apr. 2001.

MORAIS, H. et al. Caracterização microclimática de cafeeiros cultivados sob malha de sombreamento e a pleno sol. **Revista Brasileira de Agrometeorologia**, Sete Lagoas, v. 15, n. 2, p. 133-142, 2007.

NESTEL, D.; DICKSCHEN, F.; ALTIERI, M. A. Seasonal and spatial population loads of a tropical insect: the case of the coffee leaf miner in Mexico. **Ecological Entomology**, London, v. 19, n. 2, p. 159-167, Apr/June. 1994.

NEVES, Y. P. et al. Soil moisture and soil fertility of coffee in agroforestry systems. **Revista Árvore**, Viçosa, v. 31, n. 4, p. 575-588, July/Aug. 2007.

PARRA, J. R. P.; GONÇALVES, W.; PRECETTI, A. A. C. M. Flutuação populacional de parasitos e predadores de *Perileucoptera coffeella* (Guérin-Meneville, 1842) em três localidades do Estado de São Paulo. **Turrialba**, San José, v. 3, n. 4, p. 357-364, 1981.

PARTELLI, F. L. et al. Microclimate and development of 'Conilon' coffee intercropped with rubber trees. **Pesquisa Agropecuária Brasileira**, Brasília, v. 49, n. 11, p. 872-881, Nov. 2014.

PARRA, J. R. P; REIS, P. R. Manejo integrado para as principais pragas da cafeicultura, no Brasil. **Revista Visão Agrícola**, Piracicaba, n. 12, p. 47-50, Jan/July. 2013.

PEREIRA, A. V.; PEREIRA, E. B. C.; JUNQUEIRA, N. T. V. **Seringueira em sistemas agroflorestais**. Planaltina: Embrapa-CPAC, 1996. 42p.

PEREIRA, E. J. G. et al. Seasonal mortality factors of the coffee leafminer, *Leucoptera coffeella*. **Bulletin of entomological research**, Cambridge, v. 97, n. 4, p. 421-432, Aug. 2007.

PREZOTO, F.; GOBBI, N. Flight range extension in *Polistes simillimus* Zikán, 1951 (Hymenoptera, Vespidae). **Brazilian archives of Biology and Technology**, Curitiba, v. 48, n. 6, p. 947-950, Nov. 2005.

REIS, P. R.; SOUZA, J. C. de; ZACARIAS, M. S. Alerta para o bicho-mineiro. **Cultivar**, Pelotas, v. 8, p. 13-16, Apr. 2006.

REZENDE, M. Q. et al. Extrafloral nectaries of associated trees can enhance natural pest control. **Agriculture, Ecosystems & Environment**, Amsterdam, v. 188, p. 198-203, Apr. 2014.

RIBEIRO, B. M.; MAGALHÃES, L. C.; GUEDES, R. N. C. Níveis de resistência a inseticidas organo-fosforados em populações de *Leucoptera coffeella* (Guèr-Ménev) (Lepidoptera: Lyonetiidae) de Minas Gerais. **Bioscience Journal**, Uberlândia, v. 19, n. 2, p. 73-77, May/Aug. 2003.

RICHTER, M. R. Social wasp (Hymenoptera: Vespidae) foraging behavior. **Annual Review of Entomology**, Guelph, v. 45, n. 1, p. 121-150, 2000.

RIGHI, C. A.; BERNARDES, M. S. The potential for increasing rubber production by matching tapping intensity to leaf area index. **Agroforestry System**, Dordrecht, v. 72, n. 1, p. 1-13, Jan. 2008.

RIGHI, C. A. et al. Influence of rubber trees on leaf-miner damage to coffee plants in an agroforestry system. **Agroforestry System**, Dordrecht, v. 87, n. 6, p. 1351–1362, Nov/Dec. 2013.

SANTOS, G. D. M. et al. Flying capacity of swarm-founding wasp *Polybia occidentalis occidentalis* Oliver, 1791 (Hymenoptera, Vespidae). **Revista Brasileira de Zoociências**, Juíz de Fora, v. 2, n. 2, p. 33-39, Dec. 2000.

SANTOS, G. M. M.; BISPO, P. C.; AGUIAR, C. M. L. A. Fluctuations in richness and abundance of social wasps during the dry and wet seasons in three phytophysiognomies at the Tropical Dry Forest of Brazil. **Environmental Entomology**, Oxford, v. 38, n. 6, p. 1613-1617, Nov/Dec. 2009.

SCALON, J. D. et al. Spatial and temporal dynamics of coffee-leaf-miner and predatory wasps in organic coffee field in formation. **Ciência Rural**, Santa Maria, v. 41, n. 4, p. 646-652, Apr. 2011.

SILVA, E. M. et al. Tabela de vida para o bicho-mineiro do cafeeiro. In: Simpósio de pesquisa dos cafés do Brasil, 3., 2003, Porto Seguro. **Resumos...** Brasília: Embrapa Café, 2003. p. 354.

SOUZA, M. M. et al. Nesting of social wasps (Hymenoptera: Vespidae) in a riparian forest of Rio das Mortes in southeastern Brazil. **Acta Scientiarum, Biological Sciences**, Maringá, v. 36, n. 2, p. 189-196, Apr/June. 2014.

TEODORO, A.; KLEIN, A. M.; TSCHARNTKE, T. Environmentally mediated coffee pest densities in relation to agroforestry management, using hierarchical partitioning analyses. **Agriculture, Ecosystems & Environment**, Amsterdam, v. 125, n. 1-4, p. 120-126, May 2008.

THEODORO, V. C. A.; GUIMARÃES, R. J.; MENDES, A. N. G. Infestation by *Leucoptera coffeella* and leaf levels of total soluble sugar and protein in organic coffees. **Coffee Science**, Lavras, v. 9, n. 3, p. 300-311, July/Sept. 2014.

TOZATTI, G.; GRAVENA, S. Natural mortality factors of *Perileucoptera coffeella* Guérin- Ménèville (Lepidoptera, Lyonetiidae) on coffee in Jaboticabal State of Sao Paulo, Brazil. **Científica**, v. 16, p. 179-187, 1988.

TSCHARNTKE, T. et al. When natural habitat fails to enhance biological pest control – Five hypotheses. **Biological Conservation**, Amsterdã, v. 204, n. 2, p. 449-458, Dec. 2016.

VEGA, F. E., POSADA, F., INFANTE, F. Coffee insects: Ecology and Control volume II In: PIMENTEL, D. (Ed.). **Encyclopedia of pest management**. New York: Cornell University, 2007, p. 95-98.

VENZON, M. et al. Toxicity of organic farming-compatible products to the coffee leaf miner. **Pesquisa Agropecuária Brasileira**. Brasília, v. 48, n. 3, p. 241-248, Mar. 2013.

VENZON, M. et al. The potential of NeemAzal for the control of coffee leaf pests. **Crop Protection**, United Kingdom, v. 24, n. 3, p. 213-219, 2005.

VOLTAN, R. B. Q.; FAHL, J. I.; CARELLI, M. L. C. Variação na anatomia foliar de cafeeiros submetidos a diferentes intensidades luminosas. **Revista Brasileira de Fisiologia Vegetal**, Campinas, v. 4, n. 2, p. 99-105, Aug. 1992.

WALLER, J. M.; BIGGER, M.; HILLOCKS, R. J. **Coffee pests, diseases and their management**. Wallingford: CABI, 2007. 434p.

A NOVEL TASTING PLATFORM FOR SENSORY ANALYSIS OF SPECIALTY COFFEE

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ABSTRACT: Although there are many good tools to evaluate coffee, such as rigorous cupping protocols, all of them require improvements in order to benefit scientific research. One aspect to highlight is that coffee is a very important product worldwide and has been and is being investigated for its complexity. All research and any improvement in crop or processing ends-up being verified in the coffee cup quality, which is accomplished through the cupping procedures. However, sufficient tools have not been designed in order to manage the cupping procedures, in accordance with the technological level we have available now. Basically, sheets of paper are used to manage the cupping scores, which hinder the subsequent analysis process, making hard to know what happens inside coffee beans with greater precision and thoroughness. Another worrying aspect is that each region, at each country uses a different format with different flavor references to carry out the scoring, which has generated problems of unity of criteria on the analysis and this is not good to the coffee business. This paper presents the design of a web platform to make information storage and results processing of cupping procedures of specialty coffees easier. The main objective is to achieve better managing of the cupping model for the sensory analysis using a digital environment, allowing greater agility in the treatment of results and a more organized management of the information of the specialty coffees.

Index terms: Sensory evaluation of specialty coffee, cupping protocols, quality attribute, information systems.

UM NOVO SISTEMA DE INFORMAÇÃO PARA A ANÁLISE SENSORIAL DE CAFÉS ESPECIAIS

RESUMO: Embora existam diversas boas ferramentas para avaliar a qualidade do café, tais como os rigorosos protocolos de degustação, todas elas exigem melhorias para beneficiar a pesquisa científica. Destaca-se que o café é um produto de grande importância no mundo inteiro e tem sido investigado por sua complexidade. Toda pesquisa e qualquer melhoria na cultura e no processamento se verifica na qualidade da xícara do café por meio dos procedimentos de degustação. Porém, não foram desenvolvidas ferramentas suficientes para a gestão desses procedimentos de acordo com o nível tecnológico disponível atualmente. Basicamente, são utilizadas folhas de papel para armazenar os resultados da degustação, o que dificulta a análise subsequente e torna mais difícil saber o que acontece no interior do grão de café com maior precisão e rigor. Outro aspecto importante é que cada região em cada país utiliza um formato distinto com referências de sabor diferentes para atribuir a pontuação, o qual tem gerado problemas de unidade de critérios na análise, e isso não é bom para o negócio do café. Apresenta-se neste trabalho o desenho de uma plataforma web para facilitar o armazenamento e tratamento da informação dos resultados das análises sensoriais de cafés especiais. O objetivo principal é conseguir uma melhor administração do modelo de testes para a análise sensorial das amostras em um ambiente digital que permite maior agilidade no tratamento dos resultados e um manejo mais organizado da informação do café especial.

Termos para indexação*:* Análise sensorial de café especial, protocolos de degustação, atributos de qualidade, sistemas de informação.

1 INTRODUCTION

Coffee is one of the most popular and consumed drinks in the world, thanks to its organoleptic characteristics and stimulating effects (ALVES; CASAL; OLIVEIRA, 2010; DI BELLA et al., 2014). Due to this, since the 90s, the term "specialty coffee" has been used to refer to the quality of coffee, in order to describe one with excellent and unique flavor (PICCINO et al., 2014). During the last decade, there has been an increase in the knowledge of parameters that define the quality of coffee bean, among them stands out the value of the aromas and differentiating notes in the cup.

Currently coffee is grown in more than 70 countries, with Brazil being the largest producer, followed by Vietnam and Colombia (BICHO et al., 2013; DI BELLA et al., 2014). The Huila region is a pioneer in coffee production in Colombia, with 126 thousand hectares, where 2.5 million bags of 60 kilos were produced during 2016 and also leads the production of specialty coffees in the country.

The Strategic Plan for Science, Technology and Innovation 2010-2032 for the Huila region

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identifies potentially strategic products to improve the economy of the region. It is clear, according to this plan, that the coffee sector occupies the most important place in the economy of Huila, after the oil sector. At this time, Huila is not only recognized worldwide for the large volume of coffee produced annually, but also for the excellent quality of it. Specialty coffees have been an economically important alternative to improve the income of producers and associative groups in the Huila region. However, specialty coffees involve a series of components and production systems designed to produce high quality coffee for international markets, which creates new forms of consumption focused on quality, differentiation and characteristics of coffee (SEPÚLVEDA et al., 2016).

The Specialty Coffee Association - SCA (SCAA, 2018) has established a very rigorous evaluation procedure, known as "cupping protocols" to determine the sensory profile of coffee beans, in order to grant the distinction of special coffees to coffees from different origins on the planet. Currently, the standard for cupping coffee is the most widely used method for sensory coffee evaluation. This procedure is extremely useful for coffee producers, buyers and other actors in the coffee value chain, in order to evaluate coffee quality and defects. The procedure takes into account several quality attributes simultaneously, including aroma/fragrance, flavor, residual flavor, acidity, body, uniformity, balance, clean cup and sweetness. Each attribute receives an assessment on a quantitative scale and the final score corresponds to the sum of the attributes evaluated. Only those coffees beans who get total scores higher than 80 points are considered "specialty coffees". The procedure also allows to include within the evaluation some notes to provide additional information about specific characteristics of each coffee (DI DONFRANCESCO; GUTIERREZ; CHAMBERS, 2014) (OYOLA; TRUJILLO; GUTIERREZ, 2017).

This cupping coffee test is carried out through panels of tasters who have been adequately trained to fully identify, define and understand the sensory characteristics that determine the quality of the coffee. A sensory panel is equivalent to any scientific instrument for measuring the characteristics associated with the quality of a food product (FERIA, 2002) (OYOLA; TRUJILLO; GUTIERREZ , 2017).

The instrument used by panel members of tasters to record the results of the cupping test is the Specialty Coffee Association of Coffee Cupping Form (SCAA, 2018). In some regions and countries this format is partially modified to adapt it to different work styles. According to our experience, all sensory panels agree on using paper formats to record this information manually, which makes the work more tedious and prone to errors. The above because to date a digital tool has not been developed to facilitate this work, as well as the statistical treatment of the information collected.

The objective in this work was to design an information system for the laboratory of the Southcolombian Coffee Research Center - CESURCAFÉ of Surcolombiana University in Colombia to facilitate the storage and processing of information about the results of the cupping protocols carried out daily in this laboratory. The work is aimed at structuring the test model for the sensory analysis of samples in a digital environment, which allows agility in the treatment of results and more organized management of information. The system takes into account and includes the new sensory lexicon created by World Coffee Research - WCR, the largest collaborative work on coffee flavor that has been developed (CHAMBERS et.al, 2016) and also has language support in Spanish and English.

2 MATERIALS AND METHODS

SCAA Metodology

The set of protocols of the SCAA along with the new sensory lexicon from the WCR were the main input for the development of this work. The SCAA Technical Standards Committee recommends following these protocols in order to guarantee the ability to evaluate coffee with the highest quality through coffee cupping tests.

It must be taken into account that the coffee cupping tests are carried out on coffee samples and for three specific purposes:

To determine real sensory differences between coffee samples.

To describe the taste of the samples.

To determine preference for different types of coffee.

The scoring format used in the coffee cupping test allows registering 11 important attributes of coffee flavor: fragrance/aroma, flavor, residual taste, acidity, body, balance, uniformity, clean cup, sweetness, defects and global note. It cans be downloaded from the following URL: http://www.falconcoffees.com/wp-content/uploads/2016/06/SCAA-score-sheet.pdf. These attributes receive positive scores according to the quality they reflect according to the judgment of value of the sensory panel. Defects receive negative scores and denote unpleasant taste sensations. The overall score is based on the taste experience of each individual taster as a personal valuation. These attributes are qualified using a scale including 16 values, which represent quality levels with steps of 0.25 points between each one, and starting at 6 and ending at 9.75. These levels are presented in Table 1.

Total score

The total score is calculated by first adding the individual scores given to each primary quality in the box marked "total score". Then, defects are subtracted from total score. The total score is recorded in a box destined for it. The scoring guide, shown in Table 2 has proven to be a useful way to describe the quality ranks within the final score.

The new sensory lexicon for brewed coffee

The formulation of the WCR Sensory Lexicon as a universal language of sensory qualities of coffee and a tool to measure them is a first step to understand what makes coffee taste, smell and feel as it happens. This lexicon is aimed to achieve better research results and a better coffee. To overcome this challenge, it is also necessary to get good tools, which allow having a reliable and repeatable way to measure the flavors and their relative magnitude. The new lexicon allows to identifying a series of flavors that can be found in coffee and that are related to the production process from planting to cup. The 110 attributes contained in the new coffee lexicon are included in this tasting platform.

Application model

Figure 1 presents the general model of the platform. This platform is based on a multi-layer design, including a data, business and presentation layers. Data layer uses MySQL as database engine (MYSQL, 2018) and Hibernate, a relational object mapping tool for the Java programming language, which provides a framework for mapping an object-oriented domain model to a relational database. Some components of Spring Framework (SPRING FRAMEWORK, 2018) are used in the business layer. Spring is a framework for the development of high level enterprise applications and an open source for the Java platform. In the presentation layer, the Model-View-Controller - MVC was adopted using Spring-webmvc, Html5, CSS3, JS and Bootstrap.

Database

MySQL was chosen as database engine. It is an open source system for relational database management, based on structured query language - SQL. This database manager is multi-threaded and multiuser, which allows it to be used by several people at the same time, and even, make several queries at once. This makes it extremely versatile.

TABLE 1 - Quality levels for coffee cupping test.

Good	Very Good	Excellent	Outstanding
6.00	**7.00**	**8.00**	**9.00**
6.25	**7.25**	**8.25**	**9.25**
6.50	**7.50**	**8.50**	**9.50**
6.75	**7.75**	**8.75**	**9.75**

TABLE 2 - Scoring guide for total score

Total Score Quality	Classification	
90-100	Outstanding	
85-89.99	Excellent	Specialty
80-84.99	Very Good	
<80.0	Below Specialty Quality	Not Specialty

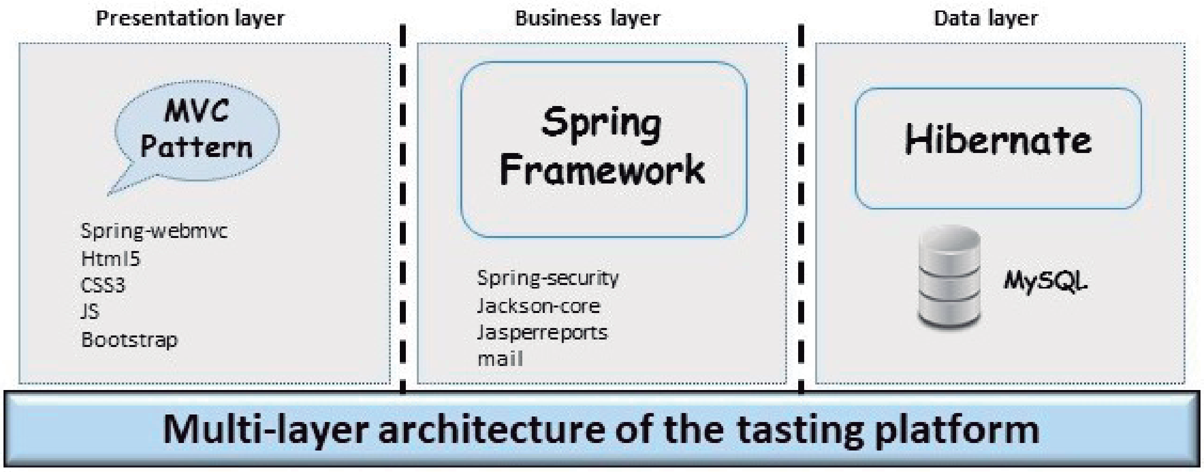

FIGURE 1 - Platform model.

The Figure 2 shows the design of the database application. Since, this is one of the most important parts of the platform, each table is explained below.

user: this table stores the information about each user. Includes the id number, username, password, email, etc.

role: this table stores the types of roles of each user. For this project three types of roles were used: administrator, taster and organizer.

password_reset_token: this table stores the password reset requests.

persistent_login: this table allows to remembering the previous sessions established by users.

panel: this table stores information about sensory panels integrated by the tasters.

sample: this table stores information about coffee samples. It includes information such as meters above sea level of the coffee farm, location, coffee farmer name, results of physical analysis, humidity of the sample, etc.

test: this table stores information about results of sensory analysis test performed by the sensory panel.

attribute: this table stores information about the 110 attributes described by the WCR in the new sensory lexicon documentation.

Controllers and services

The platform is developed in java language using the Spring Framework for the control of the server and Hibernate for the persistence of information in the database. The following describes the technologies used for the control and services into the business layer:

Jasperreports V 6.3.0: for automatic generation of reports in PDF and EXCEL.

Springframework V 4.3.6: spring framework for server control.

Springsecurity V 4.0.4: security framework for application control

Hibernate V 4.3.11: persistence control with the database

Mysql-conector-java: MySQL database connection library with java.

Jackson V 2.8.7: high performance JSON processor for java.

The objects used are mapped from the database using Hibernate. These entities are controlled from the server and with them the editing, deletion, consultation or insertion of the data is performed. The objects receive exactly the same name from the tables in the database that we mentioned earlier.

Design of the views

For the design of the platform, the MVC design model (model - view - controller) was used. The model contains all information or information system status, the controller is responsible for presenting the model to the user through the view or graphical interface. The visual design was implemented in a web interface using the following languages and frameworks:

Used languages

HTML5: used for the labeling of the structure of the views in HTML format.

CCS3: style sheets that controls the appearance of the pages.

JS: interpreted programming language, which controls the functions on the client side.

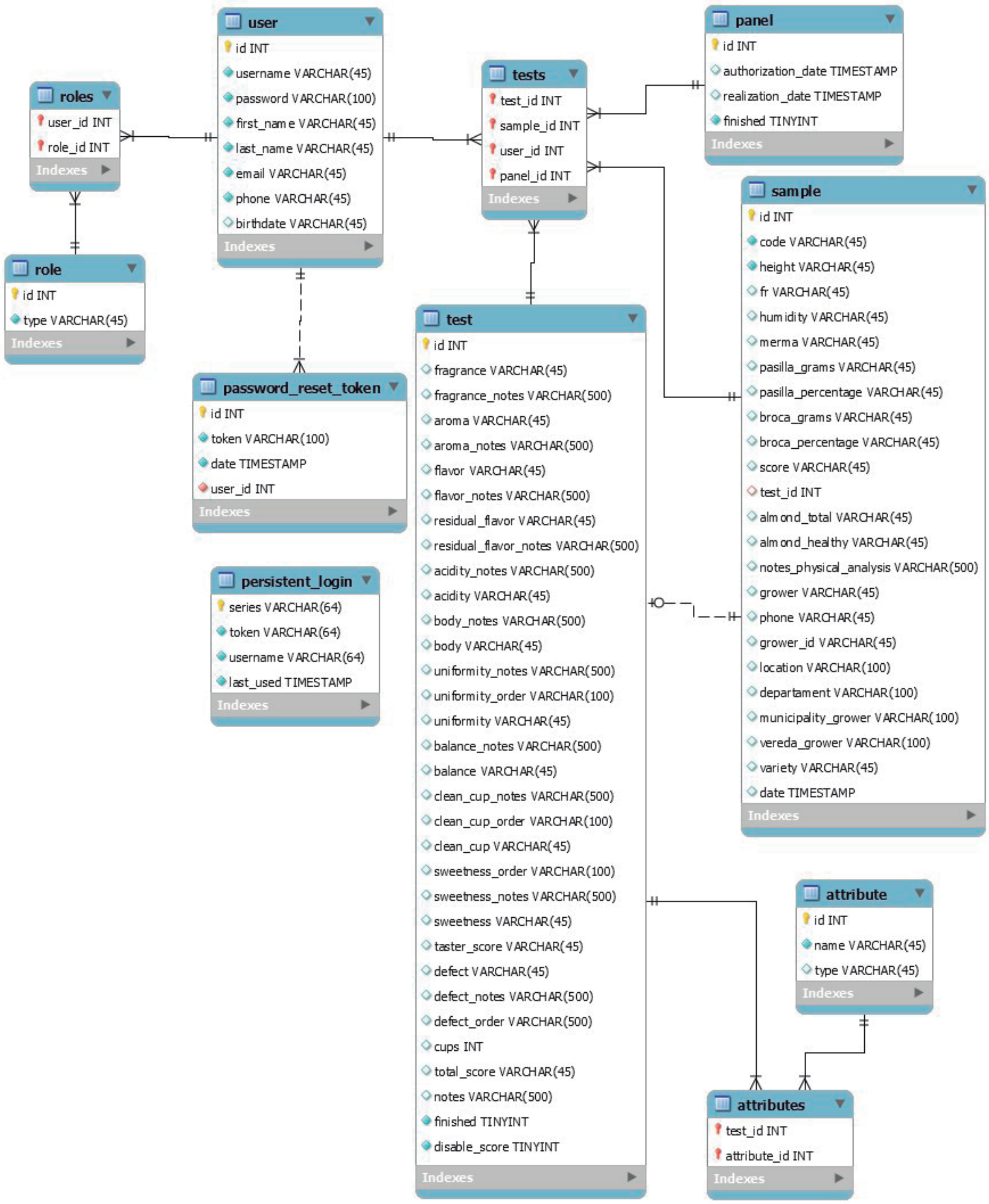

FIGURE 2 -Platform database.

Used languages

Bootstrap v4.0.0-alpha.6: is a set of web design tools with HTML, CSS and JS.

Font Awesome v 4.7.0: It is a typographic font which contains a set of icons for the interpretation and description of functions.

Jquery v 3.2.1: is a Javascript library which simplifies the interaction of the pages with the web client.

3 RESULTS AND DISCUSSION

The project that resulted in the design and implementation of this information system for special coffee tasting was executed by the Signal and Telecommunications Treatment Group - GTST of the Surcolombiana University. It is an application designed for the analysis and treatment of coffee samples. It is aimed at the structuring of the test model for the sensory analysis of samples in a digital environment, which allows to achieving greater speed in obtaining results and a more organized management of information.

The platform can be accessed with the authorization of the administrator through the following URL: http://gtst.usco.edu.co:8080/cesurcafe/. Part of the source code can be downloaded through_https://github.com/albecor/CataCoffeSCAA.

Platform description

Figure 3 shows the start page of the platform. In this, general information about the research center can be seen. The platform has English/Spanish language support, which can be switched from the EN icon in the top bar.

Users have three different roles according to the privileges assigned to them in the platform. These user roles are:

Administrator: users with this role can add, edit or delete users of the platform.

Organizer: users with this role can add tasting role users to the platform. They must also take care of the identification of the samples and the organization of the tasting panels.

Taster: users with this role can perform the sensory analysis tests of the samples.

All users have permission to edit their profile to update their contact information. Figure 4 shows the profile of the Organizer immediately after entering the system. As the figure shows, the functions of the Organizer are divided into three groups.

Users: Through this group of functions the organizer can add, update or delete users.

Tasting even: Through this group of functions, the organizer can create and organize tasting sessions or events, add panels of tasters to tasting sessions, authorize tasting sessions, view the status of any tasting session and generate reports.

Samples: Through this group of functions, the organizer can add, edit or eliminate coffee samples on the platform. The information about the samples can also be consulted.

The panels of tasters are groups of trained people who carry out the cupping or sensory tests of one or several samples of specialty coffee. To add samples, it is necessary to register on the platform information about weight, meters above sea level, variety, humidity, drill bit, performance factor, name of coffee farmer, telephone, location, etc., which requires an analysis and preparation of the samples.

Figure 5 shows the view through which each taster enters the score for each sample; as can be seen in the figure, the test is in accordance with the protocols defined by the SCAA and the new lexicon of the WCR as mentioned above.

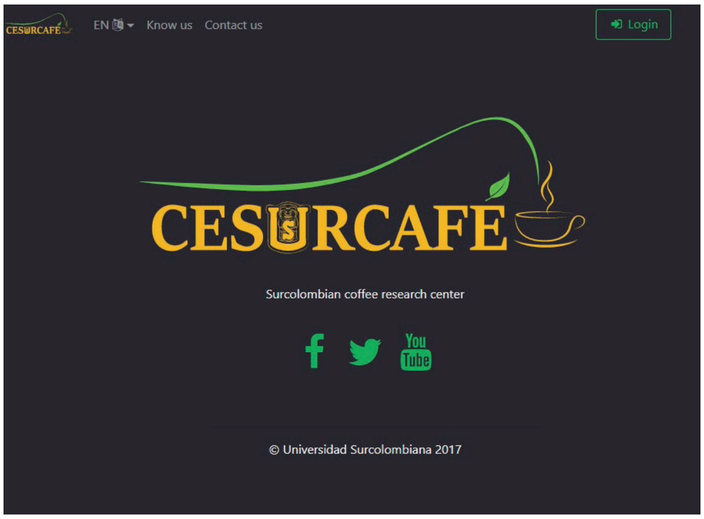

FIGURE 3 - Startig page.

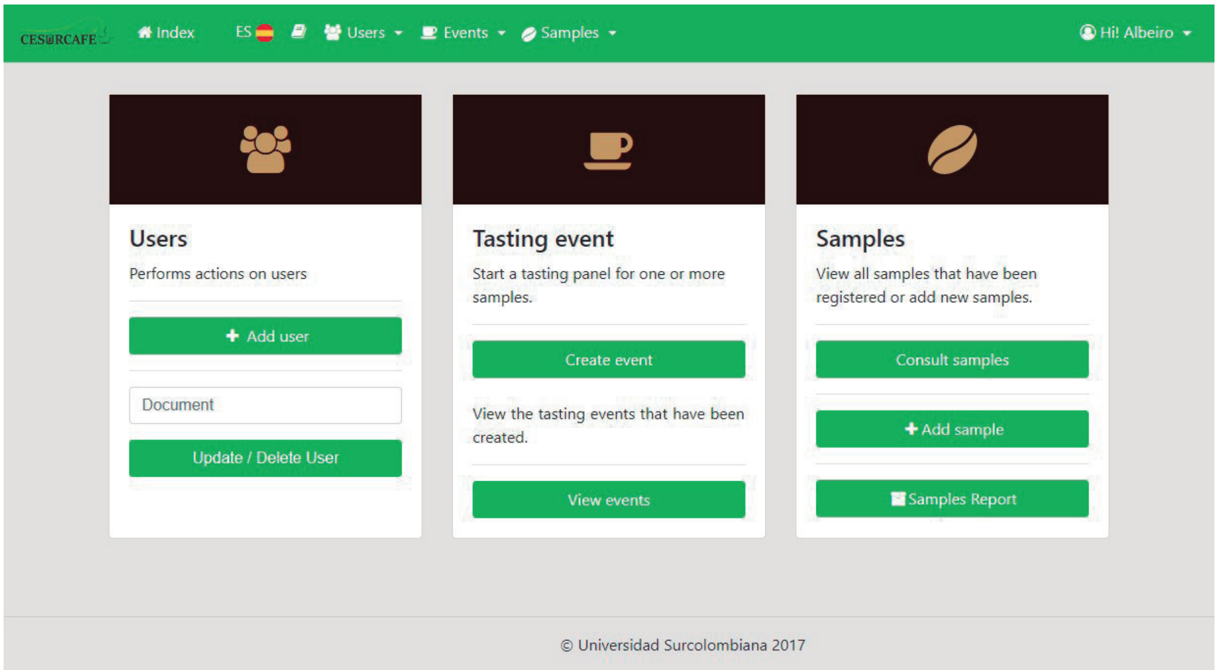

FIGURE 4 - Organizer user profile.

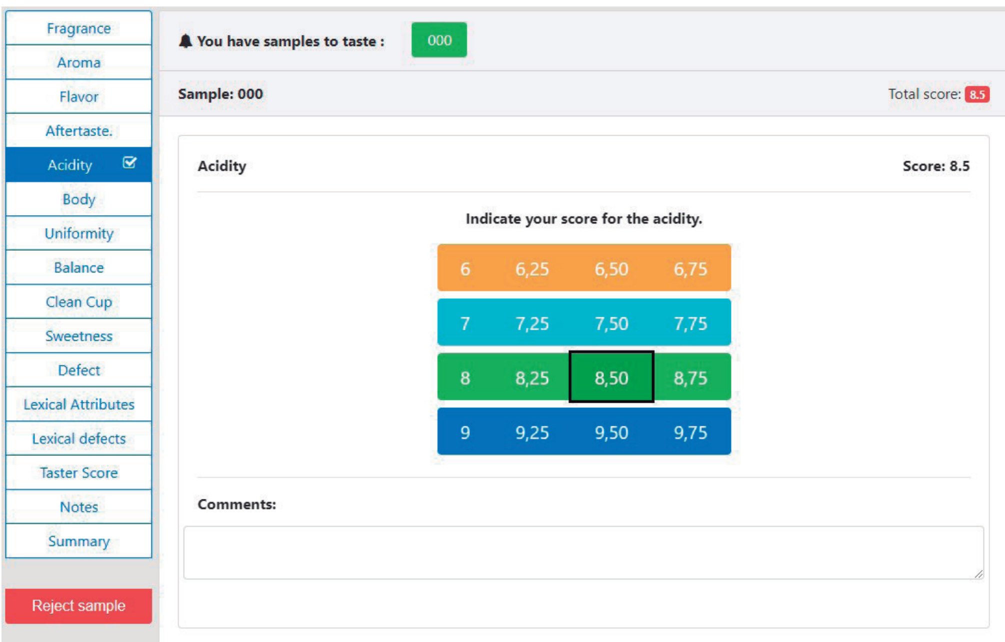

FIGURE 5 - View for samples scoring.

The results of the sensory analysis can be seen with the information from the coffee sample. When several tasters are involved in the sensory test of the sample, then the final result is averaged. The results of each taster's tests can also be seen in the sample information. These results can also be seen on a radar chart from the application as shown in Figure 6.

Once the tasting session is finished, the platform allows you to create multiple reports in HTML, Excel and PDF formats. It is possible to generate reports by sample, by taster, by session, etc.

FIGURE 6 - Radar chart.

4 CONCLUSIONS

Through this article, the design of a web platform was presented to facilitate the storage and processing of information about the results of cupping tests of specialty coffees. The platform was designed by the Signal Processing and Telecommunications Group for the laboratory of the Southcolombian Center for Research in Coffee - CESURCAFÉ of the Surcolombiana University in Colombia, but it can be easily extended to any laboratory in the world.

The work is designed to structure the test model created by the SCAA for the sensory analysis of the samples, but in a digital environment, in such a way that allows greater agility in the treatment of results and a more organized management of information. In addition, the platform takes into account and includes the new sensory lexicon created by World Coffee Research.

This tool was also designed with the aim of being used in current and future research that is being developed in CESURCAFÉ, so it is expected to have a great impact in the Huila region and in coffee growing countries. Finally, it is important to note that as far as is known, this is the first tool developed for this purpose, which makes this project an innovative one.

5 ACKNOWLEDGMENTS

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6 REFERENCES

ALVES, R. C.; CASAL, S; OLIVEIRA, M. B. Tocopherols in coffee brews: Influence of coffee species, roast degree and brewing procedure. **Journal of Food Composition and Analysis,** Maryland, v. 23, n. 8, p. 802-808, Dec. 2010.

BICHO, N. C. et al. Quality assessment of Arabica and Robusta green and roasted coffees – A review. **Emirates Journal of Food and Agriculture**, Abu Dhabi, v. 25, n. 12, p. 945-950, Nov. 2013.

CHAMBERS, E. et al. Development of a "living" lexicon for descriptive sensory analysis of brewed coffee. **Journal of Sensory Studies**, St. Paul, v. 31, n. 6, p. 465-480, Nov/Dec. 2016.

DI BELLA, G. et al. Plasticizer residues by HRGC–MS in espresso coffees from capsules, pods and moka pots. **Food Control**, Reading, v. 41, p. 185-192, July. 2014.

DI DONFRANCESCO, B.; GUTIERREZ, N.; CHAMBERS IV, E. Comparison of results from cupping and descriptive Sensory analysis of Colombian brewed coffee. **Journal of Sensory Studies**, St. Paul, v. 29, n. 4, p. 301-311, Sept/Oct. 2014.

FERIA, A. M. Examining the case of green coffee to illustrate the limitations of grading systems/expert tasters in sensory evaluation for quality control. **Food Quality and Preference**, Harlow, v. 13, n. 6, p. 355-367, Sept. 2002.

MYSQL. **MySql community edition. Version 5.7.** Available on: <https://www.mysql.com/>. Access in: 3 Mar. 2018.

OYOLA, S.; TRUJILLO, D.; GUTIÉRREZ, N. Aplicación del proceso analítico jerarquico AHP para definir la mejor taza en evaluación de cafés especiales. **Coffee Science**, Lavras, v. 12, n. 3, p. 374-380, July. 2017.

PICCINO, S. et al. Aromatic composition and potent odorants of the “specialty coffee” brew “Bourbon Pointu” correlated to its three trade classifications. **Food Research International**, Campinas, v. 61, p. 264-271, July. 2014.

SEPÚLVEDA, W.S.; CHEKMAM, L.; MAZA, M.T.; MANCILLA, N.O. Consumers’ preference for the origin and quality attributes associated with production of specialty coffees: Results from a cross-cultural study. **Food Research International**, Campinas, v. 89, p. 997-1003, Nov. 2016.

SPECIALTY COFFEE ASSOCIATION OF AMERICA. SCAA. **Protocols: coffee cupping standards. Version Feb. 2018**. Available on: <http://scaa.org/?page=resources&d=cupping-standards>. Access in: 2 Mar. 2018.

SPRING FRAMEWORK. **Spring Framework**. Available on: <https://spring.io/>. Access in: 3 Mar. 2018.

NOTA PRÉVIA

NITROGEN FERTILIZERS AND OCCURRENCE OF *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) IN TRANSPLANTED COFFEE SEEDLINGS

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ABSTRACT: The coffee leaf-miner (CLM) *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) is one of the main pests of coffee plants in Brazil. Its occurrence in the crop is directly related to the physiological state and growth characteristics of coffee plants, in turn related to plant nutrition. The present work, therefore, aimed to evaluate the effect of nitrogen sources on the occurrence of CLM in coffee seedlings. The fertilizers used were ammonium sulfate, urea, and organomineral. The number of leaves mined by CLM and the relative contents of chlorophyll and crude protein in the leaves were recorded. Ammonium sulfate and urea favored higher occurrence of leaves mined and organomineral fertilizer provided the lowest incidence of leaves mined by CLM. The three sources of nitrogen increased the chlorophyll content, but only ammonium sulfate caused an increased percentage of crude protein in the leaves of coffee seedlings. For the improvement of management strategies for *L. coffeella* it is fundamental to understand favorable conditions, nutritional management it is one of the pest control mechanisms, thus making it possible to control the insect and prevent the population of CLM from reaching an economic damage threshold.

Index terms: *Coffea arabica*, nitrogen, coffee leaf-miner, infestation, fertilization.

FERTILIZANTES NITROGENADOS E OCORRÊNCIA DE *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) EM MUDAS DE CAFEEIRO TRANSPLANTADAS

RESUMO: O bicho-mineiro-do-cafeiro (BMC) *Leucoptera coffeella* (Lepidoptera: Lyonetiidae) é uma das principais pragas dos cafeeiros no Brasil. Sua ocorrência na cultura está diretamente relacionada ao estado fisiológico e as características de crescimento dos cafeeiros por sua vez relacionados à nutrição das plantas. O presente trabalho, portanto, objetivou avaliar o efeito de fontes de nitrogênio na ocorrência de BMC em mudas de café. Os fertilizantes utilizados foram sulfato de amônio, uréia e organomineral. O número de folhas minadas pelo BMC e o teor relativo de clorofila e de proteína bruta nas folhas foram registrados. Sulfato de amônio e uréia favoreceram o aumento da ocorrência de folhas minadas e o fertilizante organomineral promoveu menor incidência de folhas minadas pelo BMC. As três fontes de nitrogênio aumentaram o teor de clorofila, no entanto apenas sulfato de amônio provocou incremento da porcentagem de proteína bruta nas folhas das mudas do cafeeiro. Para o aprimoramento das estratégias de manejo de *L. coffeella* é fundamental o conhecimento das condições favoráveis, o manejo nutricional é um dos mecanismos de controle de pragas, possibilitando o controle do inseto e impedindo que a população do BMC atinja o nível de dano econômico.

Termos para indexação: *Coffea arabica*, nitrogênio, bicho-mineiro-do-cafeiro, infestação, adubação.

The coffee leaf-miner (CLM) *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) is considered to be the main pest of coffee plants (REIS; SOUZA, 1998). This microlepidopteran is monophagous, i.e. only the coffee plant constitutes a suitable host for CLM development. The moths lay eggs on the leaves, and after hatching, the larvae penetrate in the leaves and consume the parenchyma, causing leaf fall and reducing plant's photosynthetic capacity. Occurrence of the CLM is highest in dry periods of the year, when conditions are favorable for its reproduction and development (REIS; SOUZA, 1998).

Fertilization interferes with the physiological state and growth characteristics of coffee plants, and may be related to higher occurrence of CLM (NESTEL; DICHSCHEN; ALTIERI, 1994). Availability of mineral nutrients usually influences host-plant selection by herbivorous insects as nutrients alter plants' chemical composition, morphology, anatomy and phenology (HAN et al., 2014; OLIVEIRA et al., 2014; RASHID; UESUGI, 2015; JAHAN; ISLAM, 2016).

Nitrogen is important for synthesis of amino acids and proteins, which are limiting nutrients for insect survival (RASHID; JAHAN; ISLAM, 2017). High nitrogen availability in soil increases

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leaf content of amino acids and proteins, as well as improving vegetative growth, delaying maturity and lignification of plant tissues, and making the plant more attractive to insect herbivores (COQUERET et al., 2017). Thus, it is important to investigate the influence of sources of nitrogen fertilizers used in coffee production on CLM occurrence.

The present work reported the effect of nitrogen fertilization on the occurrence of CLM in coffee seedlings. We expect that the preliminary results of this study can provide useful information on appropriate use of nitrogen fertilizers in coffee-producing regions where CLM is the major insect pest.

The experiment was carried out in an experimental area of the University José do Rosário Vellano, UNIFENAS, in Alfenas, Minas Gerais state, Brazil, between 21º 25' 45" S and 45º 56' 50" W, and 881 m altitude. The climate is Cwa according to the Köppen classification. The sub-superficial layer (40 to 80 cm) of the soil (red oxisol) present in the region of Alfenas, Minas Gerais state, was used.

Seedlings of coffee cv. Catuaí Vermelho IAC 144 with four pairs of leaves and without the presence of mines made by CLM were used in the experiment. The seedlings were transplanted in November 2016 into 7-L pots with circular holes (1 cm diameter) on the bottom to allow roots aeration and excess water to drain off. The need for chemical correction with fertilizers was based on soil analysis and recommendations of Novais et al. (1991) for experiments with potted plants. After being transplanted to pots, the seedlings received appropriate irrigation, aiming to keep soil humidity close to field capacity, maximum water capacity that the soil is able to retain. During the experiment, weeds and disease among plants were controlled when necessary, and no insecticides were applied. The products and respective doses used were: Comet® (pyraclostrobin) at 600 ml of commercial product (c.p.) ha^{-1} and Niphokam 10-08-08 at 250 ml c.p. 100^{-1} L of water. The products were applied three times during the experiment.

The nitrogen fertilizers and respective concentrations of N were as follows: ammonium sulfate (21% N and 23% S), urea (45% N), and organomineral fertilizer (20% N). The design used in the experiment was randomized blocks in 4 x 3 factorial scheme, with three nitrogen fertilizers (urea, ammonium sulfate, organomineral fertilizer, and control), and three CLM evaluation periods that were performed in May, June, and July 2017, with four blocks as replicates, totaling 48 experimental plots. Each plot was composed of four plants, making a total of 192 evaluated plants.

All treatments, except the control, received the same amount of N (2 g $plant^{-1}$) that was defined according to Novais et al. (1991), which corresponded in function of the N concentration to 4.4 g of urea, 9.5 g of ammonium sulfate, and 10 g of organomineral per plant. The doses of applied N were divided into equal parts, the first taking place upon transplanting, and the second and third applications at 45 and 90 days after transplanting. At planting-stage fertilization, the fertilizers were mixed into the soil volume in each pot, and for topdressing fertilizations (45 and 90 days) the N sources were applied on soil surface in the pots, followed by irrigation, aiming to incorporate them into the soil and avoid N loss by volatilization.

The experiment was carried out for 240 days after transplanting, and the first evaluation took place in May 2017, when CLM infestations initiated. The second and third evaluations were performed in June and July 2017. All coffee plants (192 potted plants) were inspected for CLM presence in each evaluation period. The evaluations were done by counting the leaves with at least one intact mine of CLM; in other words, without any sign of predation by wasps. Immediately after each evaluation, the leaves with mines were detached from the plants; it was thus possible to determine the CLM infestation, with data expressed as percentage of mined leaves.

At 240 days after transplanting, indirect determination of chlorophyll content was carried out on recently developed leaves using a portable measure device (SPAD-502, Konica Minolta Sensing Americas, Inc). The leaves used for evaluation of chlorophyll content were collected from the plants, stored in bags and taken to the laboratory, where they were washed and dried in an oven with forced air circulation. Next, crude protein analysis was conducted following methodology of Silva and Queiroz (2002).

Data on CLM infestation were submitted to the Shapiro-Wilk test to check for normality of residuals. The data presented normal distribution ($p > 0.05$) and were submitted to analysis of variance. The interactive effects of treatments with N fertilizers and evaluation periods were analyzed. Means of chlorophyll and crude protein contents were compared. The means of treatments were grouped by the Scott-Knott test at 5% significance using the software R, version 3.2.4 (R CORE TEAM, 2016).

There was a significant interactive effect between N fertilization and period of evaluation on the percentage of leaves mined by CLM (df = 6; dfR = 33; $F = 1.85$, $p < 0.05$) (Table 1). In the evaluation of May, no influence was observed for the sources of N on pest infestation. In June and July, ammonium sulfate and urea significantly differed from the organomineral fertilizer and control. These results indicated that the ammonium sulfate and urea sources of N increased CLM infestation.

There was a significant effect on the three evaluation periods from the different sources of nitrogen used, and an increase in the percentage of mined leaves was seen from May onwards (Table 1). These results are similar to those found by Theodoro, Guimarães and Mendes (2014), in a study that observed an increase in the infestation by CLM in those same periods and noticed that these periods are characterized by cold and dry conditions in the south of Minas Gerais state, making it a propitious period for the development of the leaf-miner (REIS; SOUZA, 1998).

In commercial coffee plantations, the percentage of mined leaves is used to determine the economic damage threshold of CLM, which is the recommended timing for insecticide application to prevent the pest from causing economic yield loss. The economic threshold of CLM in Brazil is established at 20% of mined leaves with no signs of predation, mainly in the third and fourth pair of leaves (REIS; SOUZA, 1998). In our study, the percentage of leaves mined in July was higher than 20% in plants treated with urea (22.52%) and ammonium sulfate (23.67%) in older leaves. This information is important for improvement of management strategies for CLM.

Organomineral fertilization led to the lowest incidence of mined leaves, and the percentage of infestation remained below 20%, suggesting a potential benefit this fertilizer can bring to pest management programs in coffee plantations. Theodoro, Guimarães and Mendes (2013) evaluated the use of different sources of organic matter and the interaction between CLM behavior and the production of total soluble sugars (TSS). The authors stated that organic fertilization affected TSS production, which may possibly have competed for an increase in plant resistance to CLM.

High N concentrations reduce the production of lignin, reducing plant resistance to insect pests. Nitrogen also increases concentrations of amino acids in cell apoplast, which favor the incidence of pests. Release of N from the organomineral fertilizer is more gradual than in the other sources. Therefore, gradual release of N from this fertilizer may have provided lower synthesis of amino acids and sugars in the apoplast, and this may explain the lower incidence of CLM in our study (CARDOSO; LUZ; LANA, 2015; CORRÊA et al., 2016).

Regarding the relative chlorophyll content in coffee leaves, there was significant difference among N fertilizers (df = 3; dfR = 9; $F = 5.51$; $p < 0.05$) (Table 2). Ammonium sulfate (68.01), urea (75.66) and organomineral (78.30) fertilizers caused an increase in the chlorophyll content relative to control (54.08) (Table 2). Oliveira et al. (2014) reported that leaf color is important to insect-pests when selecting the host plant, and those insects prefer to lay eggs on greener leaves rather than yellowish ones, presenting a relationship with the better nutritional status of plants and the suitable development of the insect.

There was significant difference among treatments for crude protein content in coffee leaves, with ammonium sulfate (1.87%) causing a higher percentage compared to the other nitrogen fertilizers (df = 3; dfR = 9; $F = 7.43$; $p < 0.05$) (Table 2).

TABLE 1 - Percentage of leaves mined by coffee leaf-miner (mean ± standard error) after fertilization with nitrogen fertilizers.

Fertilizers	May	June	July
Ammonium sulfate	4.64 ± 0.27 aC*	13.97 ± 0.38 aB	23.67 ± 0.52 aA
Urea	1.70 ± 0.48 aC	13.23 ± 0.46 aB	22.52 ± 0.54 aA
Organomineral	2.20 ± 0.25 aC	8.55 ± 0.78 bB	17.88 ± 0.44 bA
Control	2.67± 0.30 aC	8.08 ± 0.42 bB	13.70 ± 0.55 bA

*Means followed by the same uppercase letter in rows and same lowercase letter in columns belong to the same cluster by Scott-Knott test at 5% level of significance.

TABLE 2 - Percentage of crude protein and relative chlorophyll content in leaves of coffee plants (mean ± standard error) fertilized with sources of nitrogen.

Fertilizers	Crude protein (%)	Relative chlorophyll content
Ammonium sulfate	1.87 ± 0.04 a*	68.01 ± 5.43 a
Urea	1.49 ± 0.03 b	75.66 ± 5.65 a
Organomineral	1.58 ± 0.12 b	78.30 ± 1.02 a
Control	1.37 ± 0.08 b	54.08 ± 2.79 b

*Means followed by the same lowercase letters in columns belong to the same cluster by Scott-Knott test at 5% level of significance.

Ammonium sulfate was the only tested fertilizer with sulfur in the formulation, and according to Prado (2008), sulfur acts in protein metabolism in plant tissues, contributing to conversion of non-protein N into protein. Sulfur can also be associated with various enzymatic reactions and synthesis of three essential amino acids, namely cystine, cysteine, and methionine. This may explain the higher crude protein content found in coffee leaves treated with ammonium sulfate.

According to Nestel, Dickschen and Altieri (1994), the population density of CLM during the year is directly related to the physiological state of the plants, considering that egg-laying increases during the period in which plant material is most digestible by the larvae. Thus, the CLM moths probably select plants that are more nutritious for development of their offspring (RASHID; JAHAN; ISLAM, 2017; SANTOS et al., 2017). Another hypothesis that helps explain the increase in leaves mined by CLM is that the nitrogen sources may interact with the plant by affecting metabolic pathways, with release of volatile organic compounds that are attractive to CLM adults (COQUERET et al., 2017; VEROMANN et al., 2013). However, this hypothesis deserves further evaluation.

We conclude that fertilization with ammonium sulfate and urea favors higher CLM infestation, hence higher percentage of mined leaves in coffee plants. All sources of nitrogen herein evaluated increased chlorophyll content, and ammonium sulfate also increased the percentage of crude protein in the leaves.

The preliminary findings of the present study show the importance of correct use of nitrogen fertilization in integrated management of CLM in coffee plantations, given that gradually released fertilizers were shown to slightly reduce the pest infestation. New studies related to the influence of nitrogen fertilization on coffee plant-CLM interaction should be performed, among them the evaluation of plants' release of volatile organic compounds that are attractive to CLM adults.

REFERENCES

CARDOSO, A. F.; LUZ, J. M. Q.; LANA, R. M. Q. Produtividade e qualidade de tubérculos de batata em função do fertilizante organomineral e safras de plantio. **Revista Caatinga**, Mossoró, v. 28, n. 4, p. 80–89, Oct/Dec. 2015.

COQUERET, V. et al. Nitrogen nutrition of tomato plant alters leafminer dietary intake dynamics. **Journal of Insect Physiology**, New York, v. 99, p. 130-138, May/June. 2017.

CORRÊA, J. C. et al. Organic, organomineral, and mineral fertilizers with urease and nitrification inhibitors for wheat and corn under no-tillage. **Pesquisa Agropecuária Brasileira**, Brasília, v. 51, n. 8, p. 916-924, Aug. 2016.

HAN, P. et al. Nitrogen and water availability to tomato plants triggers bottom-up effects on the leafminer *Tuta absoluta*. **Scientific Reports**, London, v. 4, p. 1–8, Mar. 2014.

NESTEL, D.; DICKSCHEN, F.; ALTIERI, M. A. Seasonal and spatial population loads of a tropical insect: the case of the coffee leaf-miner in Mexico. **Ecological Entomology**, London, v. 19, n. 2, p. 159-167, Apr/June. 1994.

NOVAIS, R. F. et al. **Métodos de pesquisa em fertilidade do solo: ensaio em ambiente controlado**. Brasília: EMBRAPA-SEA, 1991. 392 p.

OLIVEIRA, M. D. et al. Reproductive performance of striped mealybug *Ferrisia virgata* Cockerell

(Hemiptera: Pseudococcidae) on waterstressed cotton plants subjected to nitrogen fertilization. **Arthtopod-Plant Interactions**, Berlim, v. 8, n. 5, p. 461–468, Sept/Oct. 2014.

PRADO, R. M. **Nutrição de plantas**. São Paulo: UNESP, 2008. 408 p.

R CORE TEAM. **R**: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2016.

RASHID, M. M.; JAHAN, M.; ISLAM, K. S. Response of adult brown planthopper *Nilaparvata lugens* (Stål) to rice nutrient management. **Neotropical Entomology**, Londrina, v. 45, n. 5, p. 588–596, Sept/Oct. 2016.

RASHID, M. M. D.; JAHAN, M.; ISLAM, K. S. Effects of nitrogen, phosphorous and potassium on host-choice behavior of brown planthopper, *Nilaparvata lugens* (Stål) on Rice Cultivar. **Journal Insect Behavior**, Berlim, v. 30, n. 1, p. 1–15, Jan/Feb. 2017.

REIS, P. R.; SOUZA, J. C. Manejo integrado das pragas do cafeeiro em Minas Gerais. **Informe Agropecuário**, Belo Horizonte, v. 19, n. 193, p. 17-25, 1998.

SANTOS, N. et al. Sulfur fertilization increases defense metabolites and nitrogen but decreases plant resistance against a host-specific_insect. **Bulletin of Entomological Research**, London, v. 108, n. 4, p. 479-486, Aug. 2017.

SILVA, D. J.; QUEIROZ, A. C. **Análise de alimentos: métodos químicos e biológicos**. 3. ed. Viçosa: UFV, 2002. 235 p.

THEODORO, V. C. A.; GUIMARÃES, R. J.; MENDES, A. N. G. Infestação por bicho-mineiro e teores foliares de açúcares solúveis totais e proteína em cafeeiros orgânicos. **Coffee Science**, Lavras, v. 9, n. 3, p. 300-311, July/Sept. 2014.

UESUGI, A. The slow-growth high-mortality hypothesis: direct experimental support in a leafmining fly. **Ecological Entomology**, London, v. 40, n. 3, 221–228, May/June. 2015.

VEROMANN, E. et al. Effects of nitrogen fertilization on insect pests, their parasitoids, plant diseases and volatile organic compounds in *Brassica napus*. **Crop protection**, Amsterdam, v. 43, p. 79–88, Jan. 2013.

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