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Phialomyces macrosporus REDUCES *Cercospora coffeicola* SURVIVAL ON SYMPTOMATIC COFFEE LEAVES

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ABSTRACT: Brown eye spot is among the most important coffee diseases, it is caused by a necrotrophic fungal *Cercospora coffeicola*. Saprobe fungi have potential in reducing the survival of necrotrophic pathogens and can act through competition of nutrients, mycoparasitism, antibiosis and resistance induction. We have screened saprobe fungi for the ability to reduce *C. coffeicola* sporulation and viability and determined the possible mechanisms involved in the biocontrol. The selected saprobe fungus, *Phialomyces macrosporus*, reduced the germination of *C. coffeicola* conidia by 40%. *P. macrosporus* produced both volatile and non-volatile compounds that inhibited *C. coffeicola* growth, sporulation and viability. The production of antimicrobial substances was the main mode of action used by the saprobe fungi. Therefore, *P. macrosporus* is a promising biological agent for the integrated management of brown eye spot.

Index terms: *Coffea arabica*, competition, biological control, brown eye spot, antibiosis.

Phialomyces macrosporus REDUZ A SOBREVIVÊNCIA DE *Cercospora coffeicola* EM FOLHAS DE CAFÉ SINTOMÁTICAS

RESUMO: A cercosporiose é uma das mais importantes doenças do cafeeiro e tem como agente etiológico o fungo necrotrófico *Cercospora coffeicola*. Os fungos sapróbicos têm potencial na redução da sobrevivência de patógenos necrotróficos e podem atuar por meio da competição de nutrientes, micoparasitismo, antibiose e indução de resistência. Nós selecionamos fungos sapróbios para a capacidade de reduzir a esporulação e viabilidade de *C. coffeicola* e determinamos os possíveis mecanismos envolvidos no biocontrole. O fungo saprobio selecionado, *Phialomyces macrosporus*, reduziu a germinação de conídios de *C. coffeicola* em 40%. *P. macrosporus* produziu compostos voláteis e não voláteis que inibiram o crescimento, esporulação e viabilidade de *C. coffeicola*. A produção de substâncias antimicrobianas foi o principal modo de ação utilizado pelos fungos sapróbicos. Portanto, *P. macrosporus* é um potencial agente biológico que pode ser usado no manejo da cercosporiose do cafeeiro.

Termos para a indexação: *Coffea arabica*, competição, controle biológico, mancha de olho pardo, antibiose.

1 INTRODUCTION

Coffee (*Coffea arabica* L.) is an important commodity worldwide. However, diseases are responsible for many losses in this crop. Brown eye spot (BES), caused by the necrotrophic fungus *Cercospora coffeicola* Berkeley & Cooke is considered one of the most important coffee diseases and affects both leaves and berries, causing yield losses of approximately 50% (Zambolim et al., 2016).

Although effective fungicide-based alternatives are available for the disease management (Patricio et al., 2008), it should not be the exclusive tool but part of the integrated brown eye spot management such as plant nutrition (Cardoso et al., 2013) and biological control (Sirinunta and Akarapsan, 2015).

Considering that conidia of *C. coffeicola* remain viable for up to nine months in coffee stubble (Zhang and Bradley, 2014) until favorable

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conditions for germination, the reduction of the initial inoculum should be taken into account as a strategy for the integrated management of the disease. This can be achieved by compost-accelerating products which encompasses both fast-growing fungi and cell wall degrading enzymes (Bellotte et al., 2009; Hauptman, 2014).

Because *C. coffeicola* is a necrotrophic fungus, its suppression by microbial antagonists may occur through a combination of mechanisms, such as the production of antimicrobial compounds, mycoparasitism and/or competition for nutrients. Competition for nutrients is likely to occur since hydrolytic enzymes produced by both pathogen and antagonist target the saprophytic survival on decaying leaves (Jurado et al., 2015; Alvarez-Rodriguez et al., 2016). Among these enzymes are pectinases, which are composed by an heterogeneous group of enzymes that degrades pectin (Falmy et al., 2008).

One such pectinase producers group are saprobic fungi. They play a significant role in the self-maintenance of a balanced ecosystem, mostly through the decomposition of organic matter, using a system of digestive enzymes that are secreted from the cells to the environment (Lin et al., 2015). Furthermore, they can withstand sudden variations in temperature and humidity and sustain biological control in such environment, which turn them promising biological control agents (Köhl et al., 1995). Currently, saprobe fungi have been used as a biological agent for coffee disease control (Botrel et al., 2018; Rodríguez et al., 2016). Therefore, we hypothesize that saprobe fungi might inhibit *C. coffeicola* growth in crop residues and its production antifungal compounds and cell-wall degrading enzyme may play a role in the pathogen displacement.

2 MATERIALS AND METHODS

2.1. Culture condition

The isolate LFP 37 of *Cercospora coffeicola* was obtained from the Culture Collection of *Cercospora coffeicola* at the Plant Pathology Department, Federal University of Lavras, Minas Gerais State, Brazil. Isolate LFP 37 was grown for 10 days on solid V8 medium (V8S) (200 mL of V8 juice, 800 mL of distilled water and 20 g of agar) at 25°C. A modified version of the methodology proposed by Souza et al. (2005) was used to sporulate the isolate LFP 37. The pathogen spore suspension was adjusted to 3×10^4 conidia mL⁻¹.

Three saprobe fungal strains were used in this study: *Phialomyces macrospores* (0053/07), *Curvularia inaequalis* (0005/06), and *Curvularia eragrostidis* (0047/06), obtained from the Culture Collection of the Microorganisms from Bahia, State University of Feira de Santana, Bahia, Brazil.

The strains were grown for 7 days in a medium of carrot maize agar (CMA) at 27°C. After incubation, one disk (7 mm) containing conidia and mycelium was suspended in 100 mL of CM liquid medium, grown in an orbital shaker at 120 rpm and 27°C. The suspension was shredded, homogenized in a blender, and adjusted to 3.4×10^7 CFU x mL⁻¹. Antagonistic fungi were inoculated by spraying the leaves until runoff, unless otherwise stated.

2.2. Coffee seedlings

Coffee seeds (Cultivar Mundo Novo IAC 376-4) were purchased from Agricultural Research Company of Minas Gerais (EPAMIG) and were seeded in plastic trays containing white sand for pre-germination. Two-month old seedlings were transferred to polyethylene bags (250 x 90mm) filled with substrate made from a dystroferic Red Latosol (700 L m⁻³) taken from a gully, and fertilize was by the addition of natural phosphate (5 kg m⁻³) and potassium sulfate (0.5 kg m⁻³) (MATIELLO et al., 2010). Plants were kept in a greenhouse at 25 °C –28 °C during the entire experiment.

2.3. Screening test

Pathogen inoculation consisted of spraying the inoculum suspension on the abaxial side of the coffee leaves (7-month-old) until runoff. Dark wet plastic bags were placed during 3 days to simulate a moist micro-chamber. At 15 days after inoculation (dai), when BES symptoms appeared, the three abovementioned antagonists were sprayed onto the leaves until runoff. Soil Set® (0.75 mL L⁻¹) associated with compost Aid® (0.75 mL L⁻¹) were used as positive control, as described by Bellotte et al. (2009). Distilled water was applied as a negative control. After saprobe application, 10 leaves from each treatment were stored in plastic bags at 28°C for 7 and 14 d. Number and germination of LFP 37 conidia were determined.

To determine the number of conidia, lesions of each leaf were excised and transferred to 10 mL tubes with 1 mL of distilled water with 300 µL of Tween 20% (v/v). Aliquots of each suspension

were counted using a haemocytometer. For the viability spore test, a 400 µL aliquot of the suspension of each treatment was transferred to 90mm Petri dishes containing 15 mL of water-agar at 2% (w/v). The plates were incubated at 25°C for 6 h, and the number of germinated spores was counted under light microscope at 40×. Conidia were considered germinated when the germ tube was equal to or greater than half of the spore length (Beckman and Payne, 1983). The experiment was performed twice, and the fungus with the highest potential to reduce pathogen survival was selected to continue the study. The experimental design was a randomized block with five treatments (*C. inaequalis*, *C. eragrostidis*, *P. macrosporus*, Compost Aid+ Soil Set and water), five plants per experimental unit and three replications.

2.4. Assessment of antagonist drought-tolerance

Drought tolerance was assessed to study the ability of *P. macrosporus* to reduce *C. coffeicola* isolate LFP 37 survival in plant lesions under humidity variation to simulate field conditions (Köhl et al., 1995). Fungal growth, plant cultivation, and fungal spray were performed as described above. Immediately after antagonist application, wetness was interrupted. Thereafter, 10 leaves per treatment were placed on two layers of sterile dry filter paper in open Petri dishes in laminar flow cabinet for 9 h. The leaves were incubated for two different periods of interrupted wetness (0 and 61 h) after the antagonist application. (Köhl et al., 1995). After the dry period, the filter paper was wetted with 2 mL of sterile tap water. Then, the Petri dishes were closed and kept under the same conditions, as described above. After 7 days, number and germination of pathogen conidia were determined, as previously described. The experiment was set up in a completely randomized block design, using a factorial scheme (1 x 2) with *P. macrosporus* fungus and two different periods of interrupted wetness (0 and 61 h). Each treatment was represented by three replicates and each replicate was composed with 10 leaves per plot.

2.5. Antibiosis and volatile production test

A method of measuring the direct activity of antagonists against pathogens was used in the *in vitro* antibiosis and volatile production test (Prasad et al., 2011). These assays were performed by cultivating the fungi on regular and bipartite Petri for antibiosis and volatile production, respectively.

The putative ability of *P. macrosporus* to produce different molecules or enzyme toxic to the pathogen was assessed by the confrontation assay. Plates were incubated at 25°C and 12 h in the light for 5 days. The assay was evaluated by cultivating *P. macrosporus* on one side of the Petri dish and *C. coffeicola* on the opposite side. The fungi were cultivated on Potato Dextrose Agar (PDA, 39 g L⁻¹), CMA and V8 media. The negative control contained only the pathogen in one side of the plates. The effect of toxic molecules or enzyme produced by *P. macrosporus* on *C. coffeicola* was quantified by comparing the mycelia growth and number of conidia. The experiment was distributed in a randomized block design with four replicates and was performed twice.

The production of fungitoxic volatiles compounds by *P. macrosporus* was evaluated by cultivating *P. macrosporus* in one side of a bipartite Petri and *C. coffeicola* in the opposite side. Plates were incubated at 25°C and 12 h in the light for 5 days. Saprobe fungi were cultivated on PDA, CMA and V8. The negative control contained only the pathogen in one side of the plates. The effect of fungitoxic volatiles compounds by *P. macrosporus* on *C. coffeicola* was quantified by comparing the mycelial growth, number and germination (%) of conidia. The experiment was distributed in a randomized block design with four replicates and was performed twice.

2.6. Pectinase production

The ability to produce hydrolytic enzyme in solid medium was assessed by growing *P. macrosporus* on mineral medium (pH 7.2) (2.0 g L⁻¹ KH₂PO₄; 7.0 g L⁻¹ K₂HPO₄; 1.0 g L⁻¹ (NH₄)₂SO₄; 1.0 g L⁻¹ MgSO₄·7H₂O; yeast extract 0.06% (p/v); and 13 g L⁻¹ agar). The medium was supplemented with 3 g L⁻¹ citrus pectin and incubated at 27°C for 5 days. Agar discs (7 mm diameter) containing mycelia from the colonies were transferred to Mac McIlvaine buffer (0.2 M NaHPO₄, 0.1 M citric acid, 13 g L⁻¹ agar-pH 6.0) with 0.25% (w/v) citrus pectin and incubated at 40°C for 48 h. A solution of I₂/KI (1 g of I₂ and 5 g of KI in 330 mL of H₂O) was added to detect the clearing zones.

2.7. Fungal cultivation for Polygalacturonase (PG) assays

P. macrosporus was cultivated on solid minimum medium containing 6,0 g L⁻¹ NaNO₃; 1,5 g L⁻¹ KH₂PO₄; 0,5 g L⁻¹ KCL; 0,5 g L⁻¹ MgSO₄·7H₂O; 0,01 g L⁻¹ FeSO₄; 0,01 g L⁻¹ ZnSO₄;

10,0 g L⁻¹ glucose; 15 g L⁻¹ agar at 6,8 pH for 7d, to a final concentration of 10⁶ esporos mL⁻¹. Then, one disk (7 mm) containing conidia and mycelium was inoculated into erlenmeyer flasks (125 mL) containing 50 mL of liquid mineral medium (6.8 g K₂HPO₄; 3.8 g KH₂PO₄; 1.0 g (NH₄)₂SO₄; 1.1 g MgSO₄·7H₂O; 0.6 g yeast extract, 3 g pectin 2% in one liter of distilled water) with one disk (7 mm) of mycelium and shaken at 150 rpm at 27°C for 5 days. The culture filtrates were used to directly estimate PG activity and the mycelia were collected daily to quantify total mycelial dry mass.

2.8. Assessment of Fungal growth and PG activity

The fungal growth was quantified by dry weight for 5 days. For this, the mycelium was collected by filtration through nylon cloth under vacuum and dried at 60°C for 48 h. The dried mycelium mass was measured using a precision balance (0.01 g precision).

The PG activity was determined by mixing 1 mL of culture filtrate in 1 mL of substrate solution (100 mM sodium acetate buffer; [pH 4.5]; 0.2 mM NaCl, 1.2% polygalacturonic acid [w/v]) and incubated at 40°C for 20 min. The reducing sugars released in the medium were measured by using the 3,5-dinitrosalicylic acid methods with galacturonic acid as a control. One PG unit was defined as micromoles of galacturonic acid released per minute per millilitre.

2.9. Assessment of each fungal derivate to pathogen viability reduction

To determine the ability of each fungal-derived product or enzyme to displace the pathogen from lesions were carried out *in vitro* and *in vivo* assays. For the *in vitro* assay, coffee leaf samples naturally infected by isolate LFP 37 were collected from field-grown fungicide-free plants. The leaves were distributed in a randomized-block design with four replicates and five to eight injured leaves per treatment per plot. Treatments were: i) water; ii) Conidia suspension (10⁶ spores mL⁻¹) of *P. macrosporus*; iii) polygalacturonase enzyme produced by the saprobe iv) conidia +supernatant of the saprobe and v) the supernatant of the saprobe growth.

In every case, the fungi were grown for the previously determined minimum time necessary for the maximum activity of polygalacturonase, 120 hours. After 7 days of incubation, the number of conidia of LFP 37 and leaf weight were assessed.

For the *in vivo* assay, coffee seedlings cv. 'Mundo Novo IAC 376-4' were inoculated with isolate LFP 37 and from the onset of symptoms (25 dai), each fungal-derived product or enzyme mentioned above was sprayed onto the leaves until runoff. Then, 10 leaves from each treatment were used to determine the pathogen conidia count and initial germination. After 7 days of incubation, the number and final germination of conidia were assessed. The plants were distributed in a randomized-block design with four replicates and one seedling per plot. The treatments were the same used for the *in vitro* assay. The *in vivo* assay was performed twice.

2.10. Experimental design and statistical analysis

Data from different experiments were tested for normality and submitted to an analysis of variance (ANOVA), Tukey and Scott-Knott multiple range tests (*P*=0.05). Data related to Polygalacturonase activity and dry mycelial weight of *P. macrosporus* were submitted to regression equations and subjected to a parallelism (*F*-test) test. The goodness of fit of the models was evaluated by coefficient of determination (*R*²). Data analysis was performed in the R software version 3.1.1.

3 RESULTS AND DISCUSSION

To the best of our knowledge, for the first time a biological control option for BES targeted its saprophytic phase was best achieved by the use of *P. macrosporus* (Figure 1). In the screening assay, there was no significant interaction between the treatments and the time when the experiment was carried out for either the conidia count (*P*=0.46) or the viability of LFP 37 conidia (*P*=0.14). Regarding LFP 37 conidial germination, these *in vivo* assays showed that the application of *P. macrosporus* to the injuries caused by the pathogen reduced the viability of LFP 37 by 40% in 7 days (*P*=0.0003) (Figure 1). This effect persisted for at least 14 days after application of the antagonist under study (Figure 1), showing that not only the saprobe was able to reduce the viability of the pathogen, but this effect also occurs at the earliest considered time point (7 days after treatment). Odile-Mathieu and David Daniel (2006) studied isolates of *Microsphaeropsis ochracea* in the lesions of onion leaves during senescence and reported a reduction in the sporulation of *Botrytis squamosa* from 10 days after the application of the antagonist.

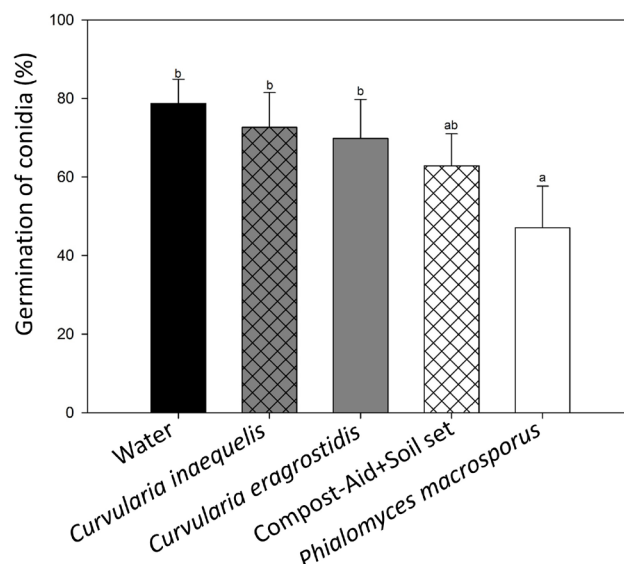


FIGURE 1 - Germination of *Cercospora coffeicola* conidia evaluated at 0, 7, and 14 days after the application of water, *Curvularia inaequalis*, *Curvularia eragrostidis*, Compost-Aid+Soil set and *Phialomyces macrosporus*. Bars with the same letter are similar at the 5% level according to Tukey multiple range test. CV=24.85%. The line on each bar represents \pm SE.

In our study, the contribution of the reduced inoculum of *Cercospora* to the occurrence of BES in the field was not evaluated, but a similar study by Galletti et al (2008) showed that when *Trichoderma* sp. was applied to the leaves of sugar beet infected with *Cercospora beticola*, the viability of the pathogen spores decreased, resulting in a delay of the epidemic in the subsequent crop.

The performance of antagonists under field conditions is determined mainly by their ecological competence. Regardless water stress condition ($P=0.82$ and $P=0.95$), there was a reduction of conidial viability of up to 37.72%. This result is in agreement with Köhl et al. (1995), who showed suppression of sporulation of *Botrytis aclada* in dead onion tissue treated with the antagonist *Ulocladium atrum* in bioassays with repeated wet-dry-cycles. In addition to evaluating the potential of the saprobe to control the pathogen regardless of the stress to which it is exposed, which aims to determine the sustainability of the control technique, it is necessary to evaluate the action mechanisms of the biocontrol agent. One of the most common mechanisms of reduced survival of pathogens is antibiosis. Hiradate et al. (2002) observed a decrease in the survival of *Colletotrichum dematium* on mulberry leaves infected when treated with *Bacillus amyloliquefaciens*, confirming that the

production of antifungal compounds was the primary mechanism of action that was used by the antagonists under study.

For the antibiosis test, interaction was observed between medium and treatments ($P=0.0090$). A positive interaction was observed between culture media CMA ($P=0.0018$), V8 ($P\leq 0.001$), and production of non-volatile compounds, resulting in greater pathogen control (Figures 2 and 3).

Reduction in the mycelium growth, sporulation and spore germination was observed when *C. coffeicola* was cultured in the presence of the *P. macrosporus* (Figure 4 A, B e C). Thus, it is suggested that such culture media induce the production of a greater amount or range of compounds with an inhibitory action on isolate LFP 37. Similar result was obtained by Abe et al. (2015) who isolated a class of secondary metabolites, polyketides from the fungus *P. macrosporus*, which is known to provide survival advantages to microbial hosts. Some of the benefits could be the production of antibiotics, as reported by Zhang et al. (2015) who studied the antibiosis functions during interactions of *Trichoderma* spp. with plant pathogenic *Rhizoctonia* and *Pythium*. The authors confirmed that polyketide positively correlated to the production of antibiotics.

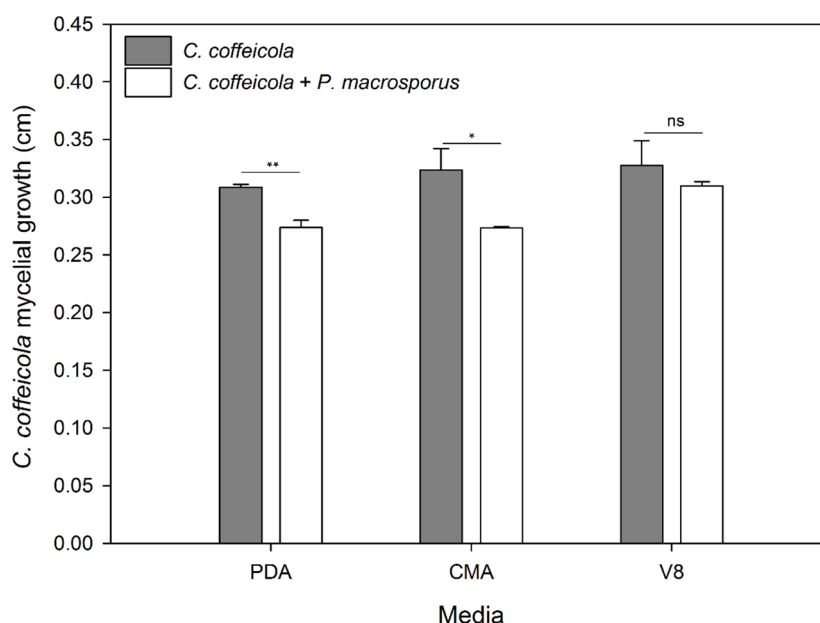


FIGURE 2 - *Cercospora. coffeicola* mycelial growth in confrontation test against *P. macrosporus* on PDA, CMA, and V8 media. Related graphic analysis of the unfolding of the treatments (*C. coffeicola* and *C. coffeicola* + *P. macrosporus*) in each culture medium. ^{ns}Non significant by Student's t-test. CV=10.60%. Each bar represents the mean of four replicates (Petri dishes) per treatment per culture medium.

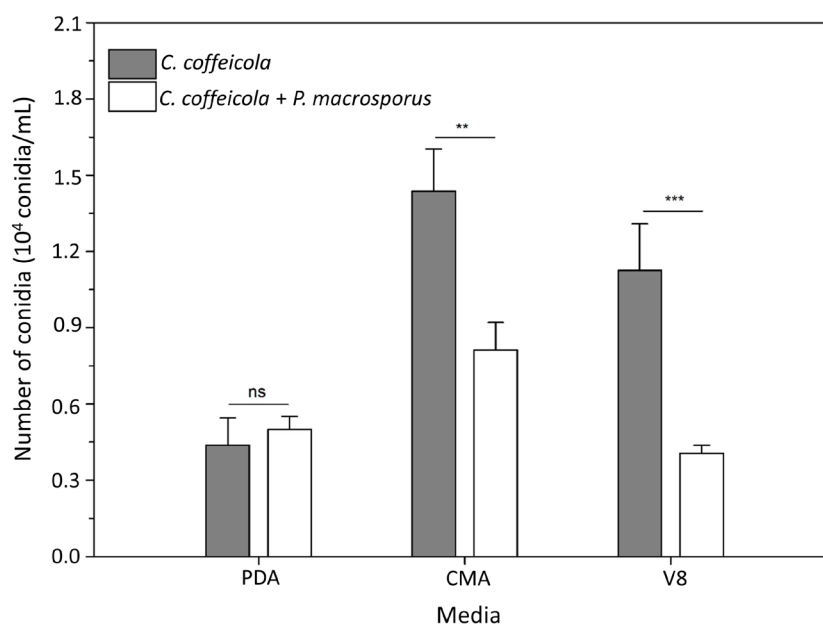


FIGURE 3 - Number of conidia of *Cercospora coffeicola* in volatile test against *P. macrosporus* on PDA, CMA, and V8 media. Graphic analysis of the unfolding of the treatments (*C. coffeicola* and *C. coffeicola* + *P. macrosporus*) within each culture medium. Significantly different according to the Student's t-test **($P \leq 0.01$) or ***($P \leq 0.001$). ^{ns}Non significant by the Student's t-test. CV=30.66%. Each bar represents the mean of four replicates (Petri dishes) per treatment per culture medium.

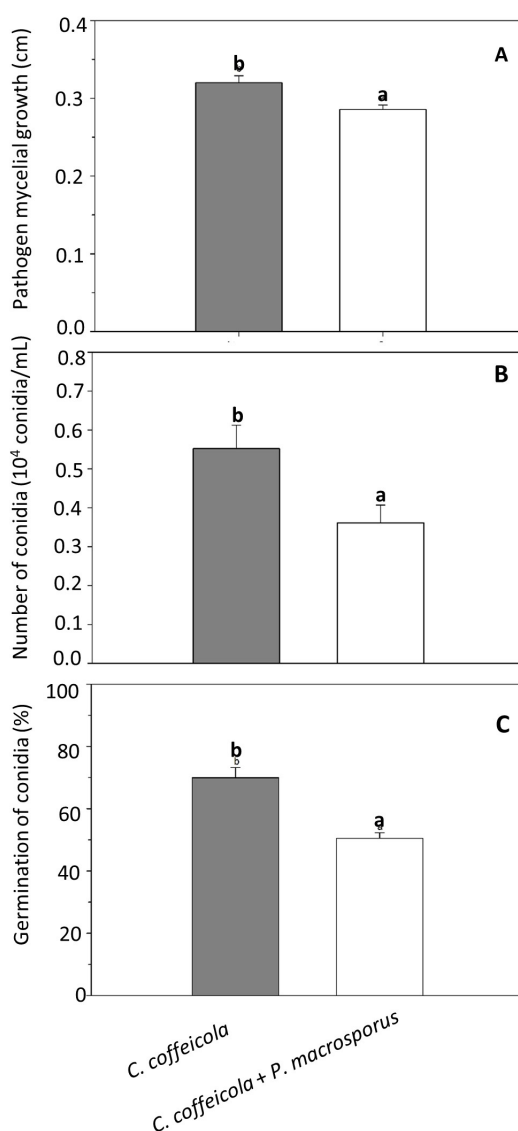


FIGURE 4 - Effect of volatile production by *Phialomyces macrosporus* on mycelium growth (A), sporulation (B) and spore germination (C) of *Cercospora coffeicola*. The bars with the same letter are similar at the 5% level according to the Student's t-test ($P \leq 0.05$). CV = 13.23%, 39.11%, and 7.80% for graphs A, B, and C, respectively. The line on each bar represents \pm SE.

The largest dry micelial weight was observed at 120 hours (Figure 5 A). The enzymatic activity of PG, detected in the culture supernatant, showed a gradual increase over the growing time, with the highest activity in 120 hours (Figure 5B). The competition mechanism between microorganisms in a settling necrotic area was tested by determining and quantifying pectinolytic enzymes. These enzymes degrade dead plant tissue and release nutrients for both the pathogen and saprobe. Thrane et al. (2000) monitored GFP-

tagged *Trichoderma* spp. saprobes in cucumber plants infected with *Pythium ultimum* and found that the biocontrol isolates were able to take up the nutrients in the plant tissue more efficiently than was the pathogen, confirming that competition for nutrients is an important mechanism in this biocontrol pathosystem. Rodríguez et al. (2016) recently confirmed this hypothesis by determining that the niche overlap index between *P. macrosporus* and *Colletotrichum gloeosporioides* was 100%, i.e., all nutrients the

pathogen uses, the biocontrol agent also does and surviving in the necrotrophic tissue provoked by the pathogen.

Furthermore, in addition to externally colonizing plants, one important feature of fungi is the colonization of live plant organs and exerting benefits to the plant over the long term (Barahona et al., 2011). Reports by Ahmad et al. (2003) showed that *P. macrosporus* colonizes coffee berries of robusta and arabica. Therefore, similar to *Cladosporium cladosporioides* isolates, *P. macrosporus* growth may acts as a barrier to the entry of other harmful fungi quality of coffee. Furthermore, *C. cladosporioides* is characterized as a saprophyte fungus and PG enzyme producer (Rezende et al., 2013).

In this study, we identified the production of PG on solid medium through the formation of the corresponding halo enzyme production. The average diameter of the formed halo was 0.93 cm. However, when we compared the *P. macrosporus* activity of PG (744.2685 U/mL) with microorganisms that have the ability to secrete large amounts of this protein, such as *Penicillium griseoroseum* (1600 U/mL), we cannot consider this saprobe a significant PG producer (Minussi et al. 1998).

Although, we suggest a synergistic activity of this enzyme and antibiosis act on the pathogen displacement from the lesion hence in reduction of its survival on detached leaves and in vivo test. Further studies are necessary to investigate the role of PG in the observed biocontrol.

Contribution of each fungal derivate, such as conidia and any metabolite, in reducing the number of pathogen conidia and lesion degradation were evaluated by *in vitro* and *in vivo* assays. From the *in vitro* experiment, this supernatant was not effective in the degradation of lesion tissue in the short-term evaluation (7 days after treatment) ($P=0.91$), suggesting longer cultivation time to achieve a significant decrease in weight of leaf and therefore treatment effectiveness. Additionally, there was no difference between the treatments for the number of conidia of LFP 37 ($P=0.44$). On the other hand, *P. macrosporus* consistently reduced LFP 37 conidia viability in detached leaves and in coffee plants. For the *in vivo* assay, not only the conidia of the saprobe fungus but also all of the fungal-derived products or enzymes reduced the germination rate by 40% ($P=0.00003$ and 0.00) (Figure 6).

Since there was no significant difference between these treatments, either one could be used as a control, which gives more versatility to the use of this fungus as a future biological product. In addition, other authors have reported the use of *P. macrosporus* as a biocontrol agent in coffee diseases. Botrel et al. (2018) observed a reduction in the severity of coffee halo blight of up to 72% when the seedlings were previously treated with the fungus *P. macrosporus*. Rodríguez et al. (2016) observed similar results in the control of *C. gloeosporioides* in coffee seedlings treated with *P. macrosporus*.

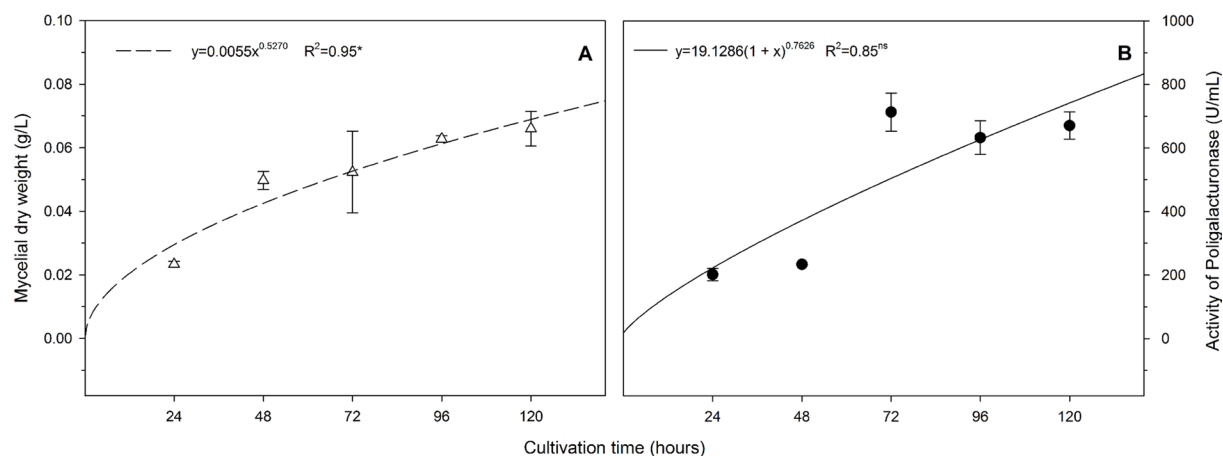


FIGURE 5 - Dry mycelial weight (A) and polygalacturonase activity in the culture supernatant (B) of *Phialomyces macrosporus* at 24, 48, 72, 96, and 120 h of cultivation in liquid medium with pectin. The line represents \pm SE. *Angular coefficients differ significantly by *F*-test.

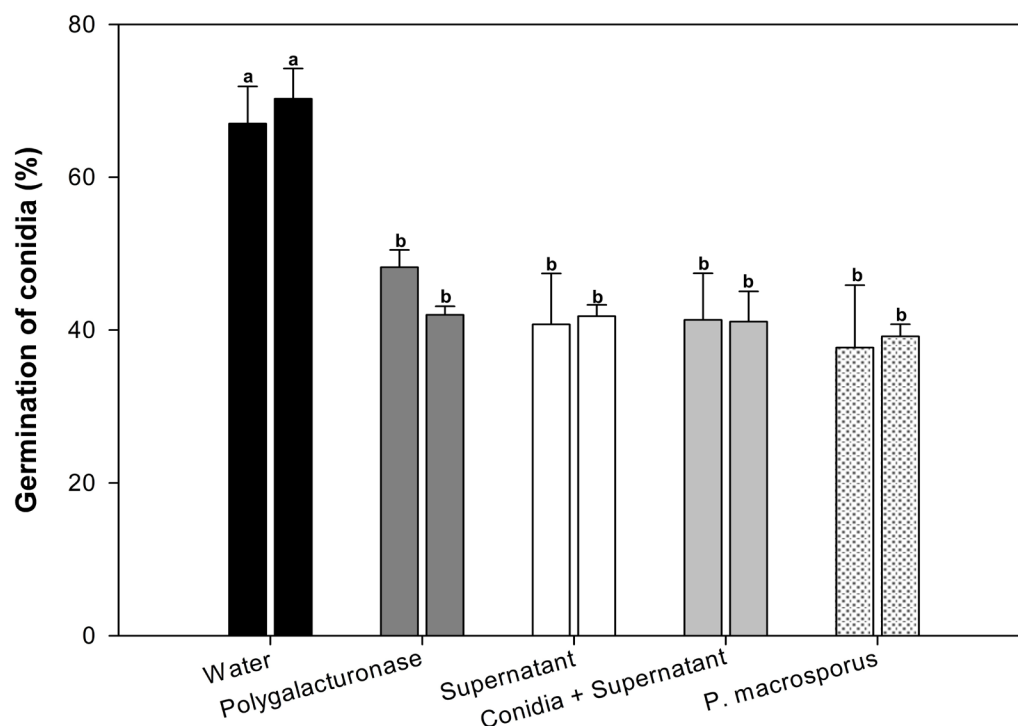


FIGURE 6 - Germination of *Cercospora coffeicola* conidia after treatment with water, polygalacturonase, supernatant alone, conidia+supernatant, *Phialomyces macrosporus* conidia (10^7 conidia mL⁻¹). The graph represents two assays conducted in different periods. The bars with the same letter are similar at the 5% level according to Scott-Knott's multiple range test. CV=14.48% and 12.01%, respectively. The line on each bar represents \pm SE.

4 CONCLUSIONS

P. macrosporus inhibited *C. coffeicola* growth, sporulation and viability. *P. macrosporus* produced antimicrobial substances and is a promising biological agent for the integrated management of brown eye spot.

5 ACKNOWLEDGEMENTS

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6 REFERENCES

ABE, H. et al. Total synthesis of the proposed structure of a polyketide from *Phialomyces macrosporus*. **Chemical Communication**, Murdoch, v. 51, n. 17, p. 3586-3589, Jan. 2015.

AGROFIT. **Sistema de agrotóxicos Fitossanitários**. Available on :< http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons> Accessed Nov 26, 2017.

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

BARAHONA, E. et al. *Pseudomonas fluorescens* F113 mutant with enhanced competitive colonization ability and improved biocontrol activity against fungal root pathogens. **Applied and environmental microbiology**, Bayreuth, v. 7, n. 77, p. 5412-5419. June 2011.

BECKMAN, P. M.; PAYNE, G. A. Cultural techniques and conditions influencing growth and sporulation of *Cercosporazeae-maydis* and lesion development in corn. **Phytopathology**, Athens, v. 73, n. 2, p. 286-289. Aug. 1983.

BELLOTTE, J. A. M. et al. Acceleration of the decomposition of Sicilian lemon leaves as an auxiliary measure in the control of citrus black spot. **Tropical Plant Pathology**, Brasília, v. 34, n. 2, p. 71-76. Apr. 2009.

- BOTREL, D. A. et al. Saprobiic fungi as biocontrol agents of halo blight (*Pseudomonas syringae* pv. *garcae*) in coffee clones. **Coffee Science**, Lavras, v. 13, n. 3, p. 283 - 291, July/Sept. 2018.
- CALVO GARRIDO, C. et al. Suppression of *Botrytis cinerea* on necrotic grapevine tissues by earlyseason applications of natural products and biological control agents. **Pest management science**, New Jersey, v.70, n. 4, p. 595-602. July 2014.
- CARDOSO, R. M. D. L. et al. Efficiency of green manures for *Cercospora* leaf spot management in coffee plants. **Tropical Plant Pathology**, Brasília, v.38, n. 2, p. 122-127. Apr. 2013.
- DE CARVALHO. et al. Modeling spatial variability and pattern of rust and brown eye spot in coffee agroecosystem. **Journal of Pest Science**, Berlin, v. 82, n. 1, p. 137. May 2009.
- CERDA, R. et al. Primary and secondary yield losses caused by pests and diseases: Assessment and modeling in coffee. **PloS one**, San Francisco v.12, n. 1, p. 133-169. Jan. 2017.
- FALMY, A. S. et al. Characterization of an exopoly galacturonase from *Aspergillus niger*. **Applied Biochemistry and Biotechnology**, Calgary, v.149, n. 3, p. 205-217. June 2008.
- GALLETTI, S. et al. *Trichoderma* as a potential biocontrol agent for *Cercospora* leaf spot of sugar beet. **BioControl**, Delémont, v. 53, n. 3, p. 917-930. Sept. 2008.
- GARG, G. et al. Microbial pectinases: an ecofriendly tool of nature for industries. **3 Biotech**, Berlim, v. 6, n. 47, p. 1-13. Feb. 2016.
- HAUPTMAN, T. et al. Application of fungicides and urea for control of ash dieback. **Journal of Biogeosciences and Forestry**, Basilicata, v.8, n. 2, p. 1-7. Aug. 2015.
- HIRADATE, S. et al. Mulberry anthracnose antagonists (iturins) produced by *Bacillus amyloliquefaciens* RC-2. **Phytochemistry**, San Diego, v.61, n. 6, p. 693-698. Nov. 2002.
- KÖHL, J. et al. Effect of interrupted leaf wetness periods on suppression of sporulation of *Botrytis-allii* and *Botrytis-cinerea* by antagonists on dead onion leaves. **European Journal of Plant Pathology**, Utrecht, 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. **Biol Control**, Delémont, v.57, n. 1, p. 1-12. Apr. 2011.
- LIN, Y. et al. Priority colonization of *Cinnamomum camphora* litter by endophytes affects decomposition rate, fungal community and microbial activities under field conditions. **Pedobiologia**, Jena. v. 58, n. 6, p. 177-185. Nov. 2015.
- MATIELLO, J. B. et al. **Cultura de café no Brasil: manual de recomendações**. Edição 2010. Rio de Janeiro: MAPA: Procafé; Varginha: Fundação Procafé, 2010.
- MINUSSI, R. C. et al. Sugar-cane juice induces pectin lyase and polygalacturonase in *Penicillium griseoroseum*. **Revista de microbiologia**, São Paulo, v. 29, n. 4, p. 246-250. Dec. 1998.
- ODILE, C.; DANIEL, R.; DAVID-MATHIEU, T. Effect of *Microsphaeropsisochracea* on production of sclerotia-borne and airborne conidia of *Botrytis squamosa*. **Biocontrol**, Delémont, v. 51, n. 1, p. 107-126. Feb. 2006.
- PRASAD, B. N., KUMAR, M.R. Effect of non-volatile compounds produced by *Trichoderma* spp. on growth and sclerotial viability of *Rhizoctonia solani*, incitant of sheath blight of rice. **Indian Journal of Fundamental and Applied Life Science**, Rajasthan v.1, n. 2, p. 37-42. June 2011.
- REZENDE, E. F. et al. Potencial enzimático e toxigênico de fungos isolados de grãos de café. **Coffee Science**, Lavras, v. 8, n. 1, p. 69-77, Mar. 2013.
- RODRÍGUEZ, G. A. A. et al. *Phialomyces macrosporus* decreases anthracnose severity on coffee seedlings by competition for nutrients and induced resistance. **Biological Control**, Amsterdam, v. 103, v.1. n. 119-128. Dec. 2016.
- SIRINUNTA, A., AKARAPISAN, A. Screening of Antagonistic Bacteria for Controlling *Cercospora coffeicola* in Arabica Coffee. **Journal of Agricultural Technology**, Tehran v.11, n. 5, p. 1209-1218. July 2015.
- SOUZA, A. G. C., MIZUBUTI, E.S.G., MAFFIA, L.A. Esporulação *in vitro* de *Cercospora coffeicola*. **Fitopatologia Brasileira**, Brasília, v.30, n 116. June 2005.
- THRANE, C.; JENSEN, D.F.; TRONSMO, A. Substrate colonization, strain competition, enzyme production in vitro, and biocontrol of *Pythium ultimum* by *Trichoderma* spp. isolates P1 and T3. **European Journal of Plant Pathology**, Utrecht, v.106, n. 3, p. 215-225. Mar. 2000.

ZAMBOLIM, L.; VALE, F. X. R.; ZAMBOLIM, E. M. Doenças do cafeeiro (*C.arabica* e *C. canephora*). In: KIMATI, H.; AMORIM, L.; BERGAMIN FILHO, A (Ed.), **Manual de fitopatologia: doenças das plantas cultivadas**. São Paulo: Agronômica Ceres Ltda, 2016. 193 p.

ZHANG, X. Antibiosis functions during interactions of *Trichoderma afroharzianum* and *Trichoderma gamsii* with plant pathogenic *Rhizoctonia* and *Pythium*. **Functional and Integrative genomics**, Berlin, v.15, n. 5, p. 599-610. Sept. 2015.

ANALYSIS OF THE COFFEE PEEL APPLICATION OVER THE SOIL-CEMENT BRICKS PROPERTIES

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ABSTRACT: The development of materials that use less polluting manufacturing techniques and can be manufactured from agricultural and/or industrial waste is an increasingly explored field of research. The objective of this work was to study the effects of the coffee peel particles (*Coffea arabica* L.) incorporation in partial replacement of cement, on the physical, mechanical and thermal properties of bricks, at different ages. For this purpose, soil corrected with sand, Portland cement type CP II F-32 and coffee peel particles were used. After the initial percentage of cement in the mixture was defined as 10%, coffee peels were added in relation to cement, equal to 5%, 10%, 15% and 20%. The bricks were submitted to simple compression tests, at the ages of 14, 28 and 56 days, thermal insulation at 28 days. The increase in peel content caused increases in water absorption, and reduction of compressive strength and apparent specific dry mass. The interaction between the shell content and the age of the specimens was significant only for the variable resistance to compression. The thermal conductivity found for the material showed its insulating potential, with values below those determined for ceramic bricks. In general, it can be concluded that the material produced did not fit in the normative requirements for soil-cement bricks, however, the mechanical characteristics found in there, indicate the possibility of use in rustic constructions, such as those ones made with adobe bricks.

Index terms: Compressive strength, durability, thermal properties, vegetable particles.

ANÁLISE DA APLICAÇÃO DE CASCA DE CAFÉ NAS PROPRIEDADES DE TIJOLOS DE SOLO-CIMENTO

RESUMO: O desenvolvimento de materiais que utilizem técnicas de fabricação menos poluidoras e que possam ser fabricados a partir de rejeitos agrícolas e/ou industriais é um campo de pesquisa cada vez mais explorado. O presente trabalho teve como objetivo estudar os efeitos da incorporação de partículas de casca de café (*Coffea arabica* L.), em substituição parcial ao cimento, sobre as propriedades físicas, mecânicas e térmicas de tijolos em diferentes idades. Para tanto, foi utilizado solo corrigido com areia, cimento Portland do tipo CP II F-32 e partículas de casca de café. Definido o percentual inicial de cimento na mistura em 10%, foram inseridos percentuais de casca de café, em relação ao cimento, iguais a 5%, 10%, 15% e 20%. Os tijolos foram submetidos aos ensaios de compressão simples, nas idades de 14, 28 e 56 dias, isolamento térmico, aos 28 dias. O aumento no teor de casca provocou acréscimos na absorção de água e redução da resistência à compressão e massa específica aparente seca. A interação entre o teor de casca e a idade dos corpos de prova foi significativa apenas para a variável resistência à compressão. A condutividade térmica encontrada para o material evidenciou seu potencial isolante, com valores abaixo dos determinados para tijolos cerâmicos. De um modo geral, pode-se concluir que o material produzido não se enquadrou nas exigências normativas para tijolos de solo-cimento, entretanto, as características mecânicas encontradas, indicam a possibilidade de utilização em construções rústicas, como as realizadas com tijolos de adobe.

Termos para indexação: Resistência à compressão, durabilidade, propriedades térmicas, partículas vegetais.

1 INTRODUCTION

The development of the construction industry brings with it some adversities that contribute to the environmental degradation. In this context, we are increasingly seeking to manufacture materials by less polluting methods, as well as the use of waste, which, in days before, would be incorrectly discarded in nature.

Among the techniques considered sustainable, the use of materials produced from the use of raw earth, is again gaining its space,

highlighting the use of soil-cement, which basically consists of mixing appropriate dosages of soil, cement and water. With the mixture, it is possible to manufacture bricks that are obtained from their pressing, which do not require burning and are therefore, considered environmentally friendly. In addition, soil-cement brick is an interesting alternative for housing construction, especially in developing countries (SEGANTINI; WADA, 2011; RODRIGUES; HOLANDA, 2015).

Regarding the reuse of waste, in the last years, many researches have been developed

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around the world, from the combination of the most varied types of residues of industrial and agricultural origin, in the manufacture of soil and cement-soil bricks. In general, plant fibers and particles tend to reduce the bricks density, improve thermal insulation, and may increase the material ductility, as observed by some authors, such as Chaib et al. (2015), Sutas et al. (2012) and Zak et al. (2016).

Among the agricultural residues produced in Brazil, the coffee peel is prominent, mainly in the state of Minas Gerais, in particular, in the southern region of Minas Gerais, which implies in the exacerbated generation of this residue. Most of the time, this waste is disposed inappropriately in the environment, which can cause ecological imbalances.

The concern with the energetic efficiency of the constructions has considerably increased in the last years, considering that the majority of energy consumed comes from the residential buildings, with predominance of heating and cooling systems, which culminated with an effective increase in the adoption of air conditioners (DOUKAS et al., 2006).

The soil itself, as a construction material, has good thermal transmittance characteristics, considering the energy efficiency, in addition, the possibility of allying it to plant residues may result in an increase in the insulation capacity of the material developed, providing greater comfort for the occupants of the building, thus (ASHOUR et al., 2015).

In this context, the objective of this work was to evaluate the effects of the substitution of cement by coffee peels on the physical, mechanical and thermal properties of soil-cement bricks at different ages.

2 MATERIAL AND METHODS

Soil characterization and coffee peels

The coffee peel belonging to the *Coffea arabica* L. species was obtained in the city of Machado-MG, from the drying of the grains in mechanical dryers. The residues were immersed in a solution of hydrated lime CH III, concentrated at 5% for a period of 24 hours (SERRANO; CASTRO, 1985). Subsequently, the materials were washed and oven dried for a period of 48 hours at a temperature of 80 °C. This procedure was necessary to avoid chemical incompatibility between the plant biomass and the mineral binder.

After drying, the vegetal materials were crushed and classified, using the residues that passed in the 40-mesh sieve (0.420 mm mesh opening) and were retained in the 60-mesh sieve (0.250 mm mesh opening), aiming at a better particles homogenization with the other mixture components. The characterization tests took place in the Experimental Unit of Wood Panels - UEPAM of the Federal University of Lavras, as described in Table 1.

The soil, extracted in the campus of the Federal University of Lavras, was previously smashed, sieved and conditioned in a place free of the weather action. The characterization tests were carried out at the Geotechnics Laboratory at the Federal University of Lavras, as described in Table 1.

The basic density of the particles was determined using the Direct Volume Measurement by Graduated Cylinder method, with samples immersed in water in six replicates. After saturation, about 10 g per sample were weighed and placed in graduated beakers. The sample volume was given by the difference between the final volume and the initial volume. Afterwards, the samples were oven dried at 103 ± 2 °C until reaching constant weight. The basic density is given by the quotient between the dry mass of the particle and the volume of the sample.

Bricks production

In the essays and in the tests, the bricks with particles of coffee peel, with four different proportions, in relation to dry mass, replacing the cement, besides a control treatment, free of particles, were evaluated, according to Table 2. The amount of cement to be added to the mixture was defined, from the characterization of the soil to be used and its classification, according to the HRB (1961), as recommended in ET-35 published by ABCP (1986).

The bricks were produced in the Experimental Unit of Wood Panels - UEPAM of the Federal University of Lavras. After the components homogenization, the mixture was pressed by a manual press adaptation, within one hour, in maximum, according to ABCP (2000). The bricks obeyed the established dimensions, for Type II, according to the standard NBR 8491 (ABNT, 2012a). The cure occurred in the first seven days of age with wet cycles three times a day. After curing, the bricks were stored in a covered place until the test date.

TABLE 1 - Characterization tests of soil and coffee peels.

Type of material	Test	Standards
Soil	Granulometry	NBR 7181 (ABNT, 1984b)
	Specific grain mass	NBR 6508 (ABNT, 1984a)
	Liquidity and Plasticity Limit	NBR 6459 (ABNT, 2016b) NBR 7180 (ABNT, 2016c)
	Humidity	NBR 6457 (ABNT, 2016a)
	Proctor Normal Compression	NBR 7182 (ABNT, 1986)
Coffee peels	Lignin content	NBR 7989 (ABNT, 2010a)
	Extraction content	NBR 14583 (ABNT, 2010b)
	Ashes	NBR 13999 (ABNT, 2003)
	Holocellulose	H (%) = 100 – Lignin - % Total Extracts - % Ashes

TABLE 2 - Experimental Design

Treatment	Soil (%)	Cement (%)	Coffee peel (%)	Peel /cement (%)
T0	90	10	0	0
T1	90	9.5	0.5	5
T2	90	9.0	1.0	10
T3	90	8.5	1.5	15
T4	90	8.0	2.0	20

Bricks characterization

The bricks produced were subjected to the described tests, to determine their properties, as shown in Table 3.

The bricks thermal properties were evaluated in three replicates of each treatment at the 28 days age. To determine the thermal conductivity, a chamber was used, composed of reconstituted wood panels and, for sealing, styrofoam plates of 15 mm thickness, model Fresh foil Premium aluminized blanket, consisting of five layers (aluminum / polyethylene / reinforcement / polyethylene / aluminum), with approximate reflection of 90% and aluminized adhesive tape. All treatments were evaluated using the same heat source, an incandescent lamp with controllable temperature and variable up to 90 °C. The temperatures were recorded by thermocouples Data Collector, model

IM DC 100-01E, manufactured by Yokogawa. The samples were assayed alone, and the material was exposed at room temperature, around 50°C. The heating rate was 1°C/min. and the test cycle for each treatment was approximately 3 hours. The thermal conductivity was calculated when the system reached a steady state of conduction of heat, from the temperatures obtained in the base and the top of the specimen and the heat flow (W/m²) measured by means of a radiation-measuring device. The information obtained was used to calculate the properties established in the NBR 15220 (ABNT, 2005a).

The equations below were used to calculate the thermal conductivity (Equation 1), thermal resistance (Equation 2), thermal transmittance (Equation 3), thermal delay (Equation 4) and solar gain factor (Equation 5).

TABLE 3 - Bricks characterization tests.

Test	Procedure	Age (days)	Bricks / Treatment
Dimensional analysis	NBR 8492 (ABNT, 2012b)	14, 28, 56	7(total 105)
Compressive strength	NBR 8492 (ABNT, 2012b)	14, 28, 56	4(total 60)
Water absorption	NBR 8492 (ABNT, 2012b)	14, 28, 56	3(total 45)

$$K = \frac{qk/A}{dT/dx}$$

(1)

$$R = \frac{e}{h}$$

(2)

$$U = \frac{1}{Rt}$$

(3)

$$\phi = 1.382 \times e \times \sqrt{\frac{\rho \times c}{3.6 \times e}}$$

(4)

$$FS_o = 4 \times U \times \alpha$$

(5)

Where K represents the thermal conductivity (W / (mK)), qk is the heat flux (W), A is the area perpendicular to the heat flux (m), dT is temperature variation (° C or K), dx is the heat flux length (m), R is the thermal resistance (m².K / W), U is thermal transmittance (m².K) / W), φ is the thermal delay, and is the thickness of the (m), ρ is the apparent density of the material, c is the specific heat of the material, FS_o is the solar factor in opaque elements (%), α is solar radiation absorptivity - color function.

The experimental data were evaluated considering a completely randomized design, in a 5 x 3 factorial scheme (five percent cement replacement by coffee husk particles - 0, 5, 10, 15 and 20%, and three cure ages - 14, 28 and 56 days). The results were submitted to variance analysis and, when significant, the linear regression analysis was performed and, for the treatments differentiation, the cluster test Scott Knott (SCOTT; KNOTT, 1974) at the 5% level of significance was done.

3 RESULTS AND DISCUSSION

Soil characterization

The results obtained from the soil characterization are shown in Table 4.

According to NBR 10833 (ABNT, 2012c), the maximum percentage of through-grains in the 200-mesh sieve should be equal to 50%. Thus, to use the chosen soil it was necessary to increase the grains percentage with diameters greater than 0.075mm, thus reducing the amount of fines. This correction was made by adding sand in proportions calculated as a function of the initial percentage of the grain in the soil. After correction, the soil presented a percentage of through-grains in the 200-mesh sieve equal to 43.79%, lower than the maximum allowed by standards, making its use possible in soil-cement mixtures.

According to studies developed by the Center for Research and Development (CEPED, 1999), the ideal sand percentages in the mixture should be between 45% and 90%, the silt + clay content between 10 and 55% and a clay content less than 20%. Considering these parameters, it is evident, when analyzing the results presented in Table 3, the success obtained in the applied granulometric correction.

In relation to the specific mass of the soil grains, a slight reduction is observed between the value reached for the natural soil and the value found for the corrected soil, which may be associated with the substitution of denser clay minerals for lighter minerals, since the clay quantity was quite reduced, in detriment of the addition of sand.

NBR 10833 (ABNT, 2012c) establishes that the value of the liquidity limit for soils for use in soil-cement mixtures is less than 45%. According to the results found, the soil, in its natural condition, had a liquidity limit equal to 51%, slightly above the standard allowed.

TABLE 4 - Natural and corrected soil characteristics.

Soil Characteristic	Natural soil	Corrected soil
Boulder (%)	0	0
Sand (%)	28.58	74.91
Silt (%)	26.68	7.02
Clay (%)	44.74	18.07
% of material passing through # 4 sieve (4.8 mm)	100	100
% of the material passing through the # 200 sieve (0.075mm)	86.09	43.79
Specific soil grains (g/cm ³)	2.75	2.55
Liquidity limit (%)	51	27
Plasticity Limit (%)	40	25
Plasticity Index (%)	11	2

This situation can be explained by the high presence of fine particles in its composition, which ratifies the need of applied granulometric correction. According to the correction adopted, the value obtained for the liquidity limit was 27%, within the limits of the standard.

NBR 10833 (ABNT, 2012c) does not establish limit values for the plasticity limit, only for the plasticity index, which should not exceed 18%. From the data in Table 3, it can be observed that both the plasticity index for the natural soil and for the corrected soil are within the norm (IP <18%).

According to the characteristics showed, the soil used was classified as type A-4, the recommended percentage of cement, for this type of soil, is equal to 10% (ABCP, 1986).

Table 5 shows the values found for optimum moisture and maximum specific apparent dry mass as consequence of the compositions studied.

It can be inferred that the insertion of the coffee peel particles into the soil-cement mixture tends to reduce the specific apparent dry mass while increasing the optimum moisture. When comparing the treatments with 0% and 20% of coffee peel respectively, it was observed that there was a reduction of 10% in the maximum specific apparent dry mass and an increase equal to 9.78% in the optimum moisture of the mixture. One of the points that contribute to this conclusion is the difficulty found in compaction of the mixture for high coffee peel contents, which causes the volume of voids to increase in the compacted system (MILANI; FREIRE, 2006).

Characterization of coffee peels particles

Table 6 shows the results of the chemical analysis and basic density of the coffee peel particles before and after the applied treatment. It is noticed that there was significant variation, for the different chemical components evaluated, due to the application of the treatment in the particles of coffee peel.

The ash, lignin and extractives contents suffered considerable reduction of their values. It is observed a considerable reduction in the total extractive content, present in the *in natura* material, which tends to be beneficial, since the starches, sugars, phenols and other components present in the extractive content are mainly responsible for the inhibition of the cement hydration reaction, which directly influences the physical and mechanical properties of the material (WEATHERWAX; TARKOW, 1964; FRYBORT et al., 2008; IWAKIRI; PRATA, 2008).

The increase in the observed holocellulose content can be attributed to the reduction of the other components in proportional terms. Asasutjarit et al. (2007), when studying the chemical composition of green coconut fibers with different treatments also verified the increase in percentage terms of holocellulose.

Regarding density, the values found were compatible with other studies, such as that developed by Vale et al. (2007), which obtained densities equal to 0.166 g / cm³ and 0.144 g / cm³, for the dry and wet coffee peel of the variety (*Coffea arabica* L.). It is also observed that the density of the peel after treatment, grinding and sieving was much higher than that of the peel *in natura*.

TABLE 5 - Values obtained for the specific apparent maximum dry mass and optimum humidity for the compositions adopted.

Coffee peel / Cement	Composition			W_{ot} (%)	$\gamma_{dm\acute{a}x}$ (g/cm ³)
	Corrected soil (%)	Cement (%)	Coffee peel (%)		
0%	90%	10%	0%	16.60%	1.80
5%	90%	9.5%	0.5%	18.00%	1.73
10%	90%	9.0%	1%	18.20%	1.72
15%	90%	8.5%	1.5%	18.70%	1.69
20%	90%	8.0%	2.0%	18.40%	1.62

Note: W_{ot} : optimum humidity; $\gamma_{dm\acute{a}x}$: specific apparent maximum dry mass.

TABLE 6 - Results of chemical analysis and basic particle density.

Coffee peel	Ashes (%)	Lignin (%)	Extracts (%)	Holocellulose (%)	Density (g/cm ³)
No treatment	5.89±0.28A	50.70±0.39A	11.47±0.82A	31.93±0.53A	0.139±0.02A
With treatment	2.47±0.04B	24.30±2.08B	7.38±0.49B	65.85±1.63B	0.298±0.08B

Note: Mean values followed by the same capital letter in the column belong to the same cluster by Scott-Knott test at 5% significance.

This situation occurred due to the increase of the specific surface and consequent reduction of the number of voids, which left the material less porous. Another hypothesis is the possible existence of lime residues, which can mask the results by increasing the density value, since the lime is much denser than the particle.

Specific apparent dry mass and water absorption of bricks

Based on the statistical analysis, it was verified that the factors age of evaluation and type of treatment were significant, for the apparent specific dry mass variable and water absorption, however the interaction between them was not significant. Figure 1 shows the average values obtained for the specific dry mass and bricks water absorption, as a function of the percentage of cement replacement per coffee peel used in each treatment.

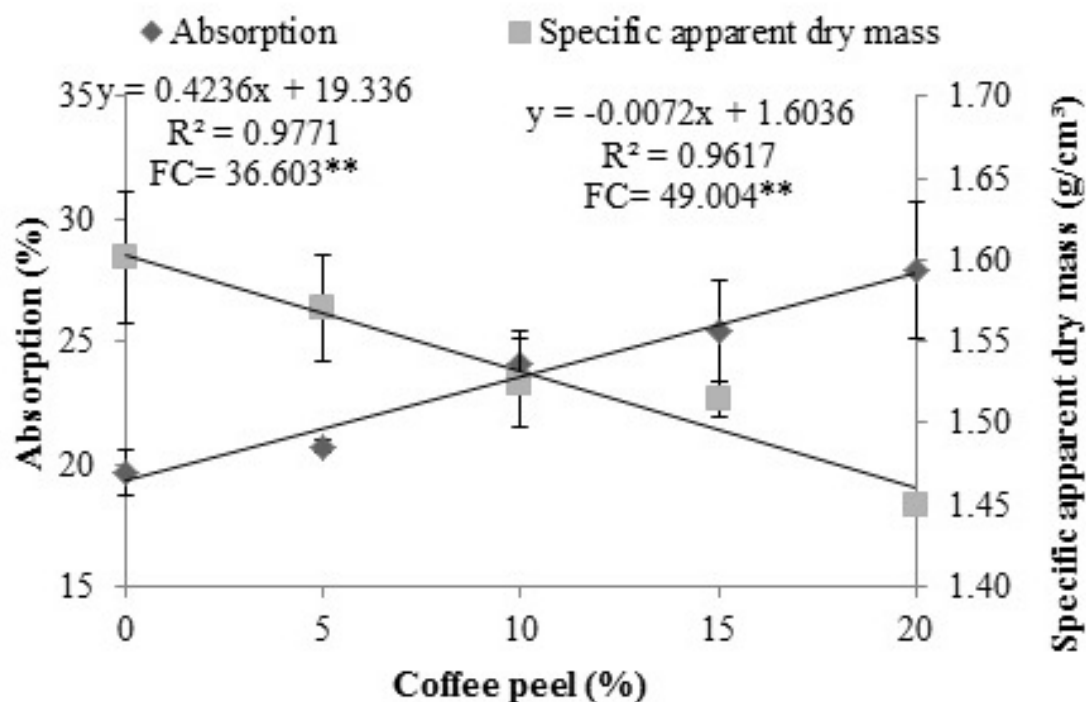
It can be seen from the graph that the increase in the coffee peel content in the mixture causes the reduction in the specific dry mass of the brick, and in contrast, increases water absorption of the material. When comparing the values found for the control treatment and the treatment containing 20% of coffee peel, there was a decrease of 9.06% in the apparent specific dry mass and a 41.79% increase in water absorption. These results can be

explained by the lack of interaction between the soil-cement system and peel and by the presence of chemical components, such as lignin, extractives, cellulose, hemicellulose, among others, present in the coffee peel. They tend to delay or even impede the cement hydration process, which promotes an increase in the volume of voids, leaving the material more porous, consequently lighter and with a greater capacity to absorb water, as the increase in the content of particles.

The sugars from lignin hydrolysis and hemicellulose solubilization are mainly responsible for the drop in temperature and consequent delay, in the process of cement hydration, according to some authors as Bilba, Arsene and Ouensanga (2007), Sudin and Swamy (2006) and Vaickelionis and Vaickelioniene (2006).

Zak et al. (2016), in their studies with mixtures of soil, cement, gypsum and flax and hemp fibers, also observed the decrease of the specific mass, from the introduction of vegetal matter. The authors attribute this effect to the low density of plant fibers and the need for higher water contents to achieve the ideal molding consistency.

When only the age factor of the test specimens was evaluated, it can be seen from Figure 2 that the means reached for the ages of 14, 28 and 56 days were statistically different from each other for the specific apparent dry mass variable.



** Significant regression analysis at the level of 5%.

FIGURE 1- Regression analysis, mean values and respective standard deviation, for the apparent specific dry mass and water absorption, in the different cement substitution levels per coffee peel.

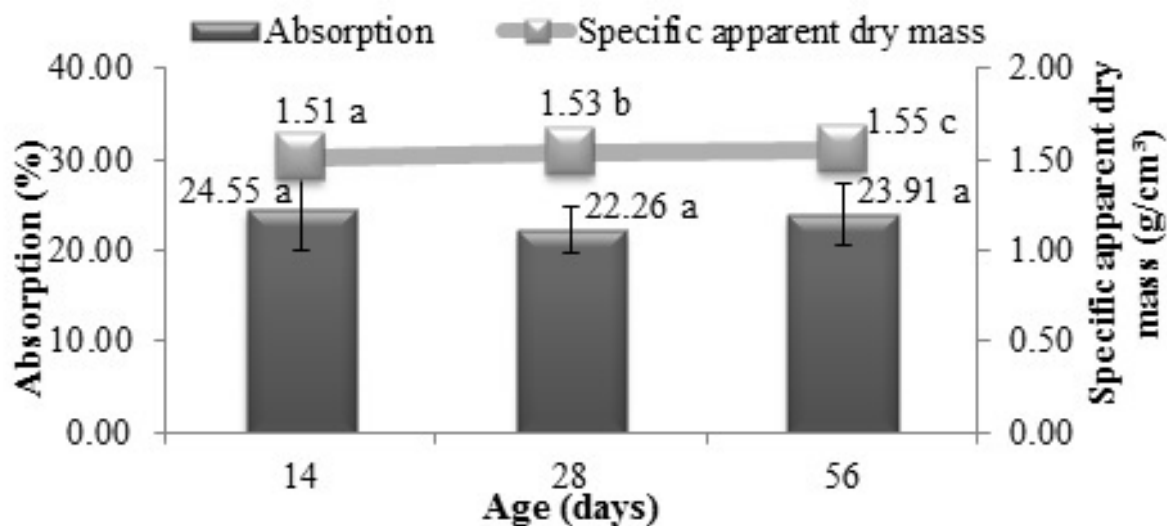


FIGURE 2 - Average values and standard deviation, for the apparent specific dry mass and water absorption, according to the evaluation age of the bricks.

It should be pointed out that the value of the specific dry mass was higher, according to the advancement in the evaluation age, this behavior may be associated to the cement hydration process, since the empty spaces occupied, initially by water, are gradually filled by the products hydration, resulting in the material densification over time (TAYLOR, 1998).

However, it is observed that the increase in the density of the material with the advancement of the age was not enough to promote the reduction of the absorbed water content, once the treatments presented statistically equal values for the variable water absorption.

Regarding the water absorption, the values reached in this work were above that determined by the NBR 8492 (ABNT, 2012b), which establishes the average limit value for water absorption equal to 20%. Thus, regardless of age, only the treatment with 0% of coffee peel falls within the normative requirement.

Compressive strength

Based on the statistical analysis, it was verified that the type of treatment, that is, the percentage of peel, used in partial replacement to the cement, was significant, as well as its interaction with the age evaluation factor. Table 7 shows the mean values found with their respective standard deviation, as well as the result of the statistical analysis for the variable resistance to compression.

When comparing the treatments T0 (0% of coffee peel) and T4 (20% of coffee peel in relation to the cement), a decrease in the resistance of approximately, 52% at 14 days, 73% at 28 days e 72% at 56 days, was observed. Only the T0 treatment (0% of coffee peel) evaluated at 56 days of age, was able to reach the minimum resistance of 2.0 MPa, requested by the norm NBR 8491 (ABNT, 2012a), which indicates that other factors interfered in the gain of the material.

The degree of compaction obtained was lower than 95%, indicating that the specific apparent dry mass reached after the production process was significantly lower than that obtained in the compaction essay. The lower the value of the specific dry mass, the greater the presence of voids in the material, which interferes negatively in the soil-binder system, leading to lower resistance values.

The presence of chemical compounds from the vegetal residue, such as extractives, lignins, hemicelluloses, among others; significantly

interfere in the hydration cement reaction. Sedan et al. (2008), when evaluating the chemical compatibility between hemp fibers and cement, found that the incorporation of the fibers caused a delay in the initial handle of the cement. The authors also observed the presence of OH⁻ generated ions from the solubilization of pectin present in the fibers. These ions reacted with calcium present in the cement, forming crystals of Ca(OH)₂. Thus, the silicon calcium reactions were impaired, hindering the calcium silicate (C-S-H) formation, main product of the cement hydration and responsible for the composite strength.

Despite the fact that the used particles in the study have undergone a washing process in lime solution, and the amount of extractives and lignin present in the waste has considerably decreased and, even so, the presence of chemical compounds, even in reduced amounts, may have negatively influenced the cement hydration reaction, which may have contributed to the low values of compressive strength achieved in this work.

Regarding the age of evaluation of the bricks, from the data contained in Table 6, it is verified that, for the control treatment, the compressive strength was higher, according to the advance in the age of the specimens. A resistance gain of 13.54% was observed from 14 to 28 days and 44% from 28 to 56 days. This resistance gain is related to the pores filling by the components formed, mainly, by the hydration of the dicalcium silicates (C₂S) and (C₃S), which are responsible for the resistance growth over time.

For a percentage of 5% of coffee peel, all ages were statistically the same, indicating, once again, that the vegetal residue affected the cement hydration process, preventing the resistance gain as the passing of years.

The behavior, verified for percentages of cement substitution per peel of 10% and 15%, was similar. For both, no significant differences were found at the ages of 14 and 28 days, while the evaluated bricks at 56 days had significantly lower averages. For the incorporation content equal to 20%, the lowest resistance value was found at 28 days, which presented a statistically different mean of the ages of 14 and 56 days, which were the same as each other. The reduction of the resistance at 56 days for the percentages of 10% and 15% may be related to the alkaline attack suffered by the plant particles, which mainly degrades the lignin present in the particles, promoting the separation of cellulose fibrils (AGOPYAN et al., 2005).

TABLE 7 - Mean values with their respective standard deviations and mean test, for the compressive strength, in MPa.

Age (days)	Percentages of coffee peels				
	0%	5%	10%	15%	20%
14	1.32±0.13Aa	1.42±0.09Aa	1.15±0.04Ba	0.75±0.07Ca	0.68±0.01Ca
28	1.50±0.11Ab	1.37±0.15Aa	1.18±0.06Ba	0.73±0.07Ca	0.41±0.03Db
56	2.16±0.27Ac	1.32±0.16Ba	0.91±0.09Cb	0.51±0.09Db	0.60±0.03Da

Note: Mean values followed by a same capital letter, on the line, belong to the same cluster by the Scott-Knott test at 5% significance, relative to the age of evaluation.

Mean values followed by a same lowercase letters in the same column belong to the same cluster by Scott-Knott test at 5% significance, relative to the age of evaluation.

Although the average values of bricks compressive strength made with vegetal residue were lower than 2 MPa, required by NBR 8491 (ABNT, 2012a), bricks produced with up to 10% of coffee peels in substitution for cement, could be used in constructions with a rustic style, only as sealing masonry without any type of structural function.

Thermal Properties

The thermal conductivity was calculated considering the radiation emitted by the heat source, obtained with the aid of a radiation measuring device, specimens thickness measurements, as well as temperature variation, calculated from the last thirty measurements recorded by the thermocouples at the base and at the top of the bricks. The results are shown in Table 8.

It is observed that the temperature variations, for the percentages of 0% and 5%, presented statistically the same averages. However, these treatments were statistically different from the treatments with the percentages of 10%, 15% and 20%, which obtained statistical equality and the highest of temperature variations. Although the temperature variation increased significantly, according to the insertion of particles of coffee peel in the bricks, it is perceived that it was not sufficient to cause a reduction in the thermal conductivity result, since, for all treatments evaluated the value obtained was equal to 0.20 W/ m°C. The other data of the thermophysical properties of the soil-cement bricks are presented in Table 9.

The NBR 15220-2 (ABNT, 2005b) establishes values for the thermal conductivity of ceramic materials produced with clay, such as bricks and tiles ranging from 0.70 to 1.05 W/m.K, for materials with specific mass of 1000 to 2000 kg/m³, respectively. The bricks developed in this study had values of specific mass, ranging from 1480 to 1816.67 kg/m³, within the norm, but the thermal conductivities achieved were lower than the normative values. From the point of view of thermal comfort, this reduction is even more favorable, considering that buildings constructed with materials with lower thermal conductivity values imply in milder temperatures inside the environments.

By the values obtained for thermal transmittance and solar gain factor, it is verified that they fit in the determinations of the NBR 15220-3 (ABNT, 2005c) for external wall-type fences, which require that the thermal transmittance be less than 3 W/m².K and the lowest solar gain factor equal to 5.0%. This type of fence is recommended for bioclimatic zones 1 e 2. These zones correspond to parts of the South and Southeast regions of the country and some cities belonging to bioclimatic zoning 1 are: Curitiba-PR, Campos do Jordão-SP, Poços de Caldas-MG, São Joaquim-SC, Caxias do Sul- RS. For bioclimatic zoning 2, we have the following cities: Pelotas-RS, Piracicaba-SP, Ponta Grossa-PR, São João Del Rei-MG, Nova Friburgo-RJ and Laguna-SC.

TABLE 8 - Temperature gradients of each treatment.

% Coffee peel	ΔT (K)	Thickness (m)	Radiation (W/m ²)	Thermal Conductivity (W/m.K)
0	17.25±1.20a	0.1027	570	0.20
5	16.64±0.45a	0.1026	570	0.20
10	20.94±0.35b	0.1028	570	0.20
15	20.80±0.56b	0.1027	570	0.20
20	20.60±0.67b	0.1035	570	0.20

Note: ΔT - temperature variation (K).

TABLE 9 - Main thermophysical properties of bricks.

% Coffee peel	ρ	K	Rt	Ut	FSe
0	1816.67	0.20	0.51	1.95	5.06
5	1640.00	0.20	0.51	1.95	5.07
10	1580.00	0.20	0.51	1.95	5.06
15	1513.33	0.20	0.51	1.95	5.06
20	1480.00	0.20	0.52	1.93	5.02

Note: ρ - apparent specific mass (kg/m³); K- thermal conductivity (W/m.K); Rt- thermal resistance (m.K/W); Ut- Thermal transmittance (W/m.K); FSe- solar gain factor (%).

4 CONCLUSIONS

The partial replacement of cement by coffee peels in the soil-cement mix for the manufacture of bricks had a direct impact on the physical, mechanical and thermal properties of the material. The increase in the particle content promoted the fall, in the specific dry bulk density and compressive strength, as well as an increase in the water absorption. The increase in the age of the bricks had a significant effect on the mechanical performance, observing a decrease in the strength achieved, from levels above 10%.

The thermal conductivity value presented by all treatments bricks was equal to 0.20 kJ/W.m, quite below that that one determined in the NBR 15220, for ceramic materials produced with soil with densities correlated to the bricks densities manufactured in the study, evidencing the potential use of the material in improving the thermal comfort of buildings.

Although the resistance values obtained for the bricks with percentage of coffee husks inserted in their composition do not reach the minimum normative values, the possibility of using them in simpler constructions, such as masonry of fence, should not be emphasized. Therefore, it is essential that new studies should be carried out.

5 REFERENCES

- AGOPYAN, V. et al. Developments on vegetable fibre-cement based materials in São Paulo, Brazil: An overview. **Cement & Concrete Composites**, 27(5):527-536, 2005.
- ASASUTJARIT, C. et al. Development of coconut coirbased lightweight cement board. **Construction and Building Materials**, 21(2):277-288, 2007.
- ASHOUR, T.; KORJENIC, A.; KORJENIC, S. Thermal conductivity of unfired earth bricks reinforced by agricultural wastes with cement and gypsum. **Energy and Buildings**, 104(1):139-146, 2015.
- ASSOCIAÇÃO BRASILEIRA DE CIMENTO PORTLAND. **Dosagem das misturas de solo-cimento**: normas de dosagem e métodos de ensaio. São Paulo: ABCP, 1986. 51 p. ET-35.
- ASSOCIAÇÃO BRASILEIRA DE CIMENTO PORTLAND. **Fabricação de tijolos de solo-cimento com a utilização de prensas manuais**. São Paulo: ABCP, 2000. 16 p.
- ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 6457**: solo: determinação do teor de umidade do solo. Rio de Janeiro: ABNT, 2016a. 8 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 6459**: solo: determinação do limite de liquidez. Rio de Janeiro: ABNT, 2016b. 5 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 6508**: grãos de solos que passam na peneira de 4,8 mm: determinação da massa específica. Rio de Janeiro: ABNT, 1984a. 8 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 7180**: solo: determinação do limite de plasticidade. Rio de Janeiro: ABNT, 2016c. 3 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 7181**: solo: análise granulométrica. Rio de Janeiro: ABNT, 1984b. 12 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 7182**: solo: ensaio de compactação. Rio de Janeiro: ABNT, 1986. 9 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 7989**: pasta celulósica e madeira: determinação de lignina insolúvel em ácido. Rio de Janeiro: ABNT, 2010a. 6 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 8491**: tijolo de solo-cimento: requisitos. Rio de Janeiro: ABNT, 2012a. 5 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 8492**: tijolo de solo-cimento: análise dimensional, determinação da resistência à compressão e da absorção da água: método de Ensaio. Rio de Janeiro: ABNT, 2012b. 4 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 10833**: fabricação de tijolo e bloco de solo-cimento com a utilização de prensa manual ou hidráulica: procedimento. Rio de Janeiro: ABNT, 2012c. 3 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 13999**: papel cartão, pasta celulósica e madeira: determinação do resíduo (cinza) após incineração a 525 °C. Rio de Janeiro: ABNT, 2003. 5 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 14853**: determinação do material solúvel em etanol-tolueno e em diclorometano e em acetona. Rio de Janeiro: ABNT, 2010b. 3 p.

ASSOCIACAO BRASILEIRA DE NORMAS TECNICAS. **NBR 15220**: desempenho térmico de edificações: parte 1, definições, símbolos e unidades. Rio de Janeiro: ABNT, 2005a. 8 p.

ASSOCIACAO BRASILEIRA DE NORMAS TECNICAS. **NBR 15220**: desempenho térmico de edificações: parte 2, método de cálculo de transmitância térmica, da capacidade térmica, do atraso térmico e do fator solar de elementos e componentes de edificações. Rio de Janeiro: ABNT, 2005b. 34 p.

ASSOCIAÇÃO BRASILEIRA DE NORMAS TÉCNICAS. **NBR 15220**: desempenho térmico de edificações: parte 3, zoneamento bioclimático brasileiro e diretrizes construtivas para habitações unifamiliares de interesse social. Rio de Janeiro: ABNT, 2005c. 30 p.

BILBA, K.; ARSENE, M. A.; OUENSANGA, A. Study of banana and coconut fibers: botanical composition, thermal degradation and textural observations. **Bioresource Technology**, 98(1):58-68, 2007.

CENTRO DE PESQUISAS E DESENVOLVIMENTO - CEPED. **Manual de construção com solo-cimento**. Camaçari: CEPED, 1999. 116 p.

CHAIB, H.; KRIKER, A.; MEKEHERMECHE, A. Thermal study of earth bricks reinforced by date palm fibers. **Energy Procedia**, 74:919-925, 2015.

Doukas, H. et al. Renewable energy sources and rationale use of energy development in the countries of GCC: Myth or reality. **Renewable Energy**, 31(6):755–770, 2006.

FRYBORT, S. et al. Cement bonded composites: a mechanical review. **Bioresources**, 3(2):602-626, 2008.

HIGHWAY RESEARCH BOARD - HRB. **Soil stabilization with Portland Cement**. Washington: HRB, 1961. 212 p. Bulletin 292.

INSTITUTO DE LA CONSTRUCCIÓN Y GERENCIA. **NTE E.080**: regulamento. Lima: Nacional Construcciones, 2000. 17 p.

IWAKIRI, S.; PRATA, J. G. Utilização da madeira de *Eucalyptus grandis* e *Eucalyptus dunnii* na produção de painéis cimento-madeira. **Cerne**, 14(1):68-74, 2008.

MILANI, A. P. da S.; FREIRE, W. J. Características físicas e mecânicas de misturas de solo, cimento e casca de arroz. **Engenharia Agrícola**, 26(1):1-10, 2006.

RODRIGUES, L. P.; HOLANDA, J. N. F. Recycling of Water Treatment Plant Waste for Production of SoilCement Bricks. **Procedia Materials Science**, 8:197-202, 2015.

- SCOTT, A. J.; KNOTT, M. A. Cluster analysis methods four grouping, means in the analysis of variance. **Biometrics**, 30(3):507-512, 1974.
- SEDAN, D. et al. Mechanical properties of hemp fibre reinforced cement: Influence of the fibre/matrix interaction. **Journal of Europe Ceramic Society**, 28(1):183-192, 2008.
- SEGANTINI, A. A.; WADA, H. P. Estudo de dosagem de tijolos de solo-cimento com adição de resíduos de construção e demolição. **Acta Scientiarum Technology**, 33(2):179-183, 2011.
- SERRANO, J. S.; CASTRO, J. V. Materiales de construcción com propiedades aislantes a base de cascara de arroz. **Informes de la Construcción**, 37(372):53-64, 1985.
- SUDIN, R.; SWANY, N. Bamboo and wood fibre cement composites for sustainable infrastructure regeneration. **Journal Material Science**, 41(21):6917-6924, 2006.
- SUTAS, J.; MANA, A.; PITAK, L. Effect of rice husk and rice husk ash to properties of bricks. **Procedia Engineering**, 32:1061-1067, 2012.
- TAYLOR, H. F. W. **Cement chemistry**. 2. ed. London: T. Telford, 1998. 459 p.
- VAICKELIONIS, G.; VAICKELIONIENE, R. I. T. A. Cement hydration in the presence of wood extractives and pozzolan mineral additives. **Journal Ceramics Silikaty**, 50(2):115-122, 2006.
- VALE, A. T. et al. Caracterização energética e rendimento de carbonização e resíduos de grãos de café (*Coffea arabica*) e madeira (*Cedrelinga catenaeformis*). **Revista Cerne**, 13(4):416-420, 2007.
- WEATHERWAX, R. C.; TARKOW, H. Effect of wood on setting of Portland cement. **Forest Product Journal**, 14:567-570, 1964.
- ZAK, P. et al. The influence of natural reinforcement fibers, gypsum and cement on compressive strength of earth bricks materials. **Construction and Building Materials**, 106(1):179-188, 2016.

COFFEE SILVERSKIN AND EXPIRED COFFEE POWDER USED AS ORGANIC FERTILIZERS

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ABSTRACT: The coffee industry produces a wide range of organic wastes, some in large amounts, and most of them do not have a well-defined final disposal. The agricultural use of these wastes can be based on the recycling of nitrogen (N), but their chemical characterization and evaluation with soil under controlled conditions are required. The aim in this work was to evaluate the use of coffee silverskin and expired coffee powder as organic fertilizers. The wastes were chemically characterized according to CONAMA'S resolution No. 375 and passed through Brazil's Agriculture Ministry normative for organic fertilizers. A greenhouse experiment with Rhodic Ferralsol soil and maize as a model plant was carried out to obtain the agronomic efficiency index (AEI) for nitrogen. Three treatments were considered: one control (without waste), ammonium nitrate as a mineral reference, and both organic wastes, at a dose of 450 mg of Kjeldahl nitrogen per pot, in triplicate. The characterization results were favorable to the wastes as nitrogen sources for agriculture. However, the AEIs obtained were low (0.5 and 7.9% for the expired coffee powder and the coffee silverskin, respectively) compared to that of the mineral reference (92%). Although coffee silverskin and expired coffee powder showed nitrogen levels were well above the minimum require by normative (0.5 % m/m), its use as organic fertilizers (as exclusive sources of N) is not recommended since less than 10% of N was available as showed by the AEI index.

Index terms: Agricultural use, chemical characterization, waste management.

USO DA PELÍCULA E DO PÓ DE CAFÉ VENCIDO COMO FERTILIZANTES ORGÂNICOS

RESUMO: A indústria de café produz uma série de resíduos, sendo alguns em grande quantidade, a maioria dos quais sem uma disposição final bem definida. O uso agrícola destes resíduos pode se basear na reciclagem do nitrogênio (N), porém a caracterização química e a avaliação destes materiais em condições controladas no solo são obrigatórias. Portanto, o objetivo desse trabalho foi avaliar o uso agrícola da película de café e pó de café vencido como fertilizantes orgânicos. A caracterização química foi realizada segundo a resolução 375 do CONAMA e os resultados comparados com a legislação para fertilizantes do Ministério da Agricultura. Um experimento em casa de vegetação com amostras de um Latossolo Vermelho distrófico, utilizando o milho como planta teste, foi realizado para obtenção do Índice de Eficiência Agronômica (IEA) para nitrogênio. Três tratamentos foram utilizados: controle (sem resíduo), nitrato de amônio como referência mineral e os ambos os resíduos, na dose de 450 mg de nitrogênio Kjeldahl por vaso, em triplicata. Os dados da caracterização química calcularam-se os desvios padrões. Os resultados de caracterização foram favoráveis ao uso agrícola desses resíduos como fonte de nitrogênio, porém os IEAs obtidos foram baixos (0.5 e 7.9% para o pó e película de café respectivamente) quando comparados com a referência mineral (92%). Embora a película e o pó de café apresentem teores de nitrogênio acima do mínimo exigido pela instrução normativa (0,5% m/m), o uso como fertilizantes orgânicos (como fonte exclusiva de N) não é recomendada, visto que menos de 10% do N estava disponível como mostrado pelo IEA.

Termos para indexação: Uso agrícola, caracterização química, gerenciamento de resíduos.

1 INTRODUCTION

The presence of plant nutrients, mainly nitrogen, is associated with a high content of organic matter in many organic wastes. For this reason, its use as nitrogen source in agriculture are particularly interesting, as it can be both environmentally and economically valuable (PAULA et., 2013). Based on this relationship, organic fertilizers must have at least 0.5 % (m/m) of total Nitrogen in its content, according to the Brazilian normative N°25 from the Agriculture Ministry. Also, a minimum content of 15% (m/m)

of carbon is required. If the minimum amount of N is not reached, organic wastes with potential use in agriculture might be registered as soil conditioners. For this use, only its carbon content and cation exchange capacity are regulated by the Brazilian normative N°35 (BRASIL, 2006). The presence of any other mineral nutrient at material used as organic fertilizer requires minimum contents addressed also by Brazilian normative N°25.

Brazil is the largest coffee producer, being responsible for 30.1% of coffee beans grown worldwide, and has a trade value of more than

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20.6 billion Brazilian reais (CONCEIÇÃO et al., 2017). However, within the coffee bean agricultural chain, approximately 50% of the volume of products generated are wastes, such as the coffee husks, pulp, parchment and coffee silverskin (MATIELLO, 1991; ESQUIVEL; JIMÉZEZ, 2012). Several of these wastes may be used in agriculture as fertilizers or soil conditioners if meet the normative requirements listed above. The reuse of coffee silverskin, the main waste product of the coffee-roasting industry, could be also a significant alternative to its environmental disposal (TOSCHI et al., 2014).

In contrast to mineral fertilization, the use of organic materials as fertilizers depends on organic matter mineralization for the plant's nutrient release (ECKHARDT et al, 2016). For this reason, nitrogen availability in the form of inorganic nitrogen is often lower than that in mineral fertilizers. These characteristics can protect the environment and meet the plant's needs. Organic matter mineralization is affected by several factors, such as pH, C/N ratio and water availability (PAULA et al., 2013). For this reason, only the presence of the minimum amounts of nitrogen, according to the Brazil's Agriculture Ministry normative cited earlier, in new fertilizers based on recycling of organic wastes may not be enough, and the evaluation of nitrogen availability in tests with soils by means of the agronomic efficiency index (AEI) is advisable.

In addition, besides to the agronomic efficiency index (AEI) testing for nitrogen, the chemical characterization of organic wastes that shall be used as new organic fertilizers is mandatory as a first step in its evaluation. The chemical characterization of organic wastes is the basis for the agronomic recommendation and is usually performed following the requirements described in the CONAMA'S resolution No. 375. The characterization of the wastes is important not only to identify the concentration of macro and micronutrients but also to indicate other attributes and relationships among attributes in the waste composition. Furthermore, it can also show the presence of heavy metals, that limits are regulated by the Brazilian normative instruction N°7 (BRASIL, 2016). After the characterization, the data was compared with Brazil's Agriculture Ministry normative for organic fertilizers to verify if it is in accordance.

The aim of this work was to evaluate the use of coffee silverskin and expired coffee powder as organic fertilizers.

2 MATERIAL AND METHODS

Coffee silverskin and the expired coffee powder were obtained from a sewage sludge treatment plant where these materials are used for sewage sludge composting. The samples were air-dried, homogenized and sieved through 2-mm. All analyses were run in triplicate. Chemical characterization followed the standard established by the National Environment Council resolution No. 375 (CONAMA, 2006). Moisture was determined according to EPA-SW 846, with drying to constant weight at 65°C. The pH was measured using 2 g of moist sample and 20 ml of deionized water, and the mixture was stirred for 5 min at 220 rpm and rested for 30 min. For inorganic N, 5-g samples was distilled with 50 ml of 1.0 mol/L KCl, 0.2 g of MgO, and 0.2 g of Devarda alloy. The distillate was collected in 5 mL of 20 g/L H₃BO₃ and titrated with 0.0025 mol/L H₂SO₄ (BREMNER, 1996). Nitrite and nitrate were determined according to Mulvaney (1996). For organic N (N-Kjeldahl), 0.05 g of oven-dried samples were mixed with 3 mL of concentrated H₂SO₄ and placed in a digester block (360°C) for 3 h; the sample was then distilled with 20 mL of 10 mol/L NaOH. The distillate was also collected in 20 mL of 20 g /L H₃BO₃ and then titrated with 0.0025 mol/L H₂SO₄ (APHA, 2017). Total solids and volatile solids were obtained by drying samples at 105°C and then combusting at 550°C for 4 h (APHA, 2017). Organic carbon was determined by the K₂Cr₂O₇ method (NELSON; SOMMERS, 1996). Ca, K, P, Mg, S, Cu, Fe, Ni, Mn, Mo, Si, Zn, Al, As, Ba, Cd, Cr, Pb, Hg, and Na were extracted in a microwave oven (EPA, 2018). K and Na were quantified by flame photometry and the other elements by ICP-AES. The cation exchange capacity (CEC) was measured following the procedure described by Normative No. 17 (BRASIL, 2007). The electrical conductivity (EC) was measured in a 1:5 (10 g of waste: 50 mL of water) extract. From triplicate data, the average and standard deviation were calculated. The C:N ratio was obtained considering N-Kjeldahl and organic C.

The experiment was carried out in a greenhouse at the Agronomic Institute of Campinas, State of São Paulo, Brazil (22°53'37.27" S; 47°3'54" W), from December 2016 to February 2017. The soil sample, collected from the 0-20 cm depth of a Rhodic Ferralsol soil, was air-dried, homogenized and sieved through 2-mm. The soil characteristics are shown in Table 1 and were determined according to Raij et al. (2001).

TABLE 1 - Soil fertility attributes.

Soil Organic Matter		pH	H+Al	Ca	Mg	K	BS	CEC
g dm ⁻³		—	mmol _c dm ⁻³					
27.0		4.1	54.0	7.0	5.0	0.7	12.7	66.7
N Kjeldahl	P	S	V	B	Cu	Fe	Mn	Zn
g kg ⁻¹	mg dm ⁻³		%	mg dm ⁻³				
1.4	8.0	11.0	19.0	0.3	4.4	60.0	7.5	0.9

¹Soil organic matter - dichromate oxidation and colorimetry, pH in CaCl₂, P, K, Ca, and Mg by ion exchange resin and H+Al by SMP buffer. Cu, Fe, Mn and Zn-DTPA pH 7.3. V-base saturation, B.S.-sum of basis; CEC- cation exchange capacity

The greenhouse experiment was performed using 2-kg pots vases with maize, as the test plant. Five plants were kept per vase. The treatments were control (without residue), ammonium nitrate as mineral reference, and both organic wastes, at a dose of 450 mg of Kjeldahl nitrogen per pot. The corresponding experimental design was a completely randomized block design, with four treatments, in triplicate, with a total of 12 vases.

The pots were prepared as follows: 1- the soil base saturation was settled to 70% by the addition of 2.7 g of lime per pot, and the moisture was adjusted to 60% of soil water retention, with incubation for 10 days; 2- to each pot, the calculated amount of waste or ammonium nitrate was added and incubated for 15 days; and 3- all pots were fertilized by the addition of 0.45, 0.4, 0.46, 0.27 and 0.17 g per pot of K, P, Ca, Mg and S, respectively, plus micronutrients (2.0, 1.0, 0.28, 1.0, 6.3 and 8.0 mg per pot of B, Mn, Mo, Fe, Zn and Cu, respectively). After fertilization, 10 healthy and homogeneous seeds of maize (AG8676PR02 variety) were sown uniformly distributed on the surface of each pot at a depth of 2 cm. After emergence, five seedlings were kept per pot. Thirty-seven days after seeding, the shoots were measured and cut. Fresh weight was registered, followed by washing with water and oven drying until a constant weight at a 65 °C ± 5 °C. The dry matter was milled, and its N Kjeldahl determined (BREMNER, 1996).

Soil and plant data were submitted to ANOVA and Tukey's test for average comparison of treatments ($p \leq 0.05$). To perform these analyses, the statistical program used was the SISVAR® - 5.6 version.

Shoot nitrogen extraction was calculated as follows (equation 1):

$$SNE = \frac{DM \times N \text{ content}}{1000}$$

Where: SNE is the shoot nitrogen extraction (mg pot⁻¹); DM is dry matter (g pot⁻¹); N content is the nitrogen found in shoots (g kg⁻¹). The agronomic efficiency index (AEI) was calculated as described by Farinelli e Lemos (2010) (equation 2):

$$AEI (\%) = \frac{(SNE1 - SNE0) \times 100}{N \text{ applied}}$$

Where:

SNE0 is the shoot nitrogen extraction in the control; SNE1 is the shoot nitrogen extraction in the nitrogen sources (wastes or ammonium nitrate) and N applied that was 450 mg of N per pot.

The relative agronomic efficiency index (RAEI) was calculated as follows (equation 3):

$$RAEI (\%) = \frac{\text{organic waste AEI}}{\text{Ammonium nitrate AEI}} \times 100$$

3 RESULTS AND DISCUSSION

The chemical characterization of the organic wastes is shown in Table 2. The moisture of both materials was quite low (1.2 and 16.6% (m/m) for expired coffee powder and coffee silverskin, respectively) and was consistent with the roasting of the beans to which the wastes were associated. The low moisture of such material is favorable when transport costs for its application are considered. The pH of coffee silverskin was 6.8, and that of expired coffee powder was 5.0. A soil pH in the range 6.0 to 7.0 is considered optimum for plant nutrient availability. Previous studies showed that application of high ratios of expired coffee powder could result in a soil pH below 6.0 (MELO et al., 2008). However, the final soil pH after organic amendment depends on several factors such as the rate of waste application, the waste reaction with the soil and the soil buffering capacity and organic matter content (RONQUIM, 2010).

TABLE 2 - Main characteristics of the organic wastes.

Organic waste ^a	Moisture	pH ^b	Organic Carbon ^c	N Kjeldahl ^c	Inorganic N		C:N
	%	—	g kg ⁻¹	—	NH ₄ ⁺	NO ₃ ⁻ NO ₂ ⁻	—
Coffee silverskin	16.6 ± 0.74	6.8 ± 0.01	433 ± 15.3	23.4 ± 0.23	13 ± 1.8	70 ± 14.0	19:1
Expired coffee powder	1.2 ± 0.12	5.0 ± 0.01	463 ± 28.0	25.6 ± 0.04	99 ± 9.6	390 ± 26.7	18:1
Organic waste	CEC	CEC/OC	Electrical conductivity	P	K	Mg	Ca
	mmol _e kg ⁻¹	—	dS m ⁻¹	—	—	g kg ⁻¹	—
Coffee silverskin	523 ± 15.0	1.21	4.9 ± 0.46	1.7 ± 0.36	34.4 ± 0.00	3.6 ± 0.22	11.6 ± 0.77
Expired coffee powder	354 ± 10.1	0.69	7.5 ± 0.01	2.4 ± 0.08	18.9 ± 0.29	1.4 ± 0.01	2.2 ± 0.14
Organic waste	S	Mo	Cu	B	Zn	Mn	Fe
	g kg ⁻¹	—	—	—	mg kg ⁻¹	—	—
Coffee silverskin	4.4 ± 0.04	<0.9 ^d	44.5 ± 1.74	30.5 ± 1.31	17.1 ± 0.21	44.9 ± 1.27	392 ± 42
Expired coffee powder	44.7 ± 6.54	16.0 ± 2.23	5.8 ± 0.33	7.6 ± 1.46	10.9 ± 0.62	19.8 ± 0.13	111 ± 22

a= Average and standard deviation for three replicates, b= 1:10 (waste:water), c= dry basis, d= lower than the quantification limit

Both wastes presented similar concentrations of organic carbon (43% to 46% of Organic Carbon) and organic nitrogen (2.3 to 2.6% of N- Kjeldahl). Based on these values, the C:N ratio of coffee silverskin and expired coffee powder was quite similar and close to 20. The mineralization of organic nitrogen is favorable over its immobilization in this condition (CANTARELLA, 2007), making the agricultural use of such wastes promising.

In addition to this, either the organic carbon, the N-Kjeldahl of the material tested was in accordance with the organic wastes minimum content established by the Agriculture Ministry at Normative No. 25 of 2009, which corresponds to 150 g kg⁻¹ and 5 g kg⁻¹ for C and N, respectively.

The inorganic nitrogen, in the form of NH₄⁺ and NO₃⁻ + NO₂⁻ ions, was quite different among the wastes, being higher for the expired coffee powder than for coffee silverskin. However, for both wastes, the NO₃⁻ content was higher than NH₄⁺. This can be related to a high oxidation level of the materials since over time, a decrease in NH₄⁺ and increase in NO₃⁻ due to the bacterial nitrification are expected (SANCHEZ-MONEDERO et al., 2001). The agricultural use of wastes with high amounts of NO₃⁻ needs additional caution since NO₃⁻ may be easily leached through the soil profile, causing environmental damage (MELO et al., 2008). Since more than 90% of the nitrogen in the wastes is in the organic form, even the nitrate content in the expired coffee powder was not troublesome. Furthermore, the nitrate can be retained by the positive charges due to the presence of Fe and Al oxides in the soil, principally in the deep layers (OLIVEIRA et al., 2000). The CEC, 354 mmol_c dm⁻³ for expired coffee powder and 523 mmol_c dm⁻³ for coffee silverskin, was above the limit for soil conditioner set by Normative No. 35 (BRASIL, 2006). Considering the typical low organic matter levels of tropical soils (FONTANA et al., 2014), the addition of such wastes could significantly improve the fertility of these soils by means of nutrient retention. Aside from that, the humification degree of the coffee silverskin, based on the CEC/OC, was superior to that of the expired coffee powder. The humification degree also indicates the organic matter maturity, which reflects an increase of the material's nutrient retention capacity (MELO et al., 2008; PAIVA et al., 2013). However, further testing would be need to verify such material impact on soil CEC, for soil conditioning.

The electrical conductivity of the wastes was 4.9 and 7.5 dS m⁻¹ for expired coffee powder and coffee silverskin, respectively. Electrical conductivities in the range from 0.64 to 6.85 dS m⁻¹ are commonly used in agriculture (MELO et al., 2008). Thus, the long-term usage of expired coffee powder grounds in agriculture may require soil solution monitoring in order to prevent salinize.

Overall, higher nutrient concentrations were found in coffee silverskin than in expired coffee powder. It is not clear if this difference may be due to the roasting of the coffee bean or if it is related to the coffee tissue's physiology. It is known, however, that the coffee plant demands high potassium fertilization in leaves, comparable to that of nitrogen (MARTINEZ et al., 2014). This was indeed reflected in the wastes composition, with higher concentration in the coffee silverskin. The presence of a considerable concentration of K in this waste makes its particularly interesting for tropical soils, which are poor in K, and many crops besides coffee itself, where some wastes are traditionally returned to the fields (LIMA, 2014; MOURA, 2016). The use of coffee silverskin or expired coffee powder as part of the potassium fertilization under those conditions might represent a significant advantage since KCl represents the major input of K for the crops (RODRIGUES et al., 2014). However, further testing would be need to verify this. Expired coffee powder also demonstrated considerable S presence. Sulfur in plants is related to protein synthesis, and it is absorbed in the form of sulfate released during organic matter mineralization (FIORINI et al., 2016; SOARES et al., 2017).

The micronutrients Fe and Mn were found in high concentration in both wastes. This can be attributed to the natural high concentration of such elements in Brazilian soils (BIONDI et al., 2011). Although coffee is not a molybdenum-demanding crop (MARTINEZ et al., 2014), a considerable concentration of molybdenum, higher than the required amounts of B, Cu and Zn, was found in the expired coffee powder.

The potentially toxic elements (Pb, Ni and Cd) were below the detection limits of the characterization method (Table 3). For both wastes, low to trace concentrations of selenium (12.4 ± 0.73 and 5.5 ± 0.37 mg/kg for coffee silverskin and expired coffee powder, respectively) and barium (35.7 ± 1.8 and 14.0 ± 0.16 mg/kg for coffee silverskin and coffee silverskin respectively) were found. Only coffee silverskin contained some arsenic (5.0 ± 0.4 mg/kg) and chromium (1.7 ± 0.4 mg/kg). These results are well below those allowed by Normative No. 7 (BRASIL, 2016).

The maize grown in soil treated with the coffee organic wastes, did not differ from that of the control ($p < 0.05$). For all treatments, plants were shorter than those grown in soil treated with ammonium nitrate, where all the nitrogen added in the inorganic form was available to plants (Figure 1). This behavior may indicate that the nitrogen mineralized from coffee silverskin and expired coffee powder was not reflected in plant growth, or if nitrogen mineralization occurred, the presence of another toxic substance (such as tannins) inhibited plant growth. Since chlorosis symptoms were observed, nitrogen deficiency seems to explain the plants' inferior development (COSTA et al., 2015).

After 52 days of the addition of the coffee organic wastes to the soil (15 days of incubation

plus 37 days after maize sowing), the shoot nitrogen content and nitrogen extraction per pot did not differ for treatments that received the coffee organic wastes and the control (Table 4). As observed for the plants height, shoot dry matter weight did not differ. Once more, both the control and treatments with coffee wastes applied differed significantly from the mineral reference treatment. This trend was also reflected by the AEI and RAEI of coffee silverskin and the expired coffee powder

when compared to the ammonium nitrate treatment ($p \leq 0.05$). Considering the costs involved in the high waste volumes needed to be applied in the field and its low nutrient availability, AEI values lower than 60% are considered low and not economically valuable.

TABLE 3 – Potentially toxic elements from the organic wastes.

Organic waste ^a	Pb	Ni	Se	As	Cd	Cr	Ba
	mg kg ⁻¹						
Coffee silverskin	<3.0 ^b	<2.4	12.4 ± 0.73	5.0 ± 0.87	<0.4	1.7 ± 0.25	35.7 ± 1.79
Expired coffee powder	<3.0	<2.4	5.5 ± 0.37	0.0	<0.4	<0.3	14.0 ± 1.6
Normative No. 7	150	70	80	20	3	200	ni ^c

a= Average and standard deviation for three replicates, b= lower than the quantification limit, c= no informed

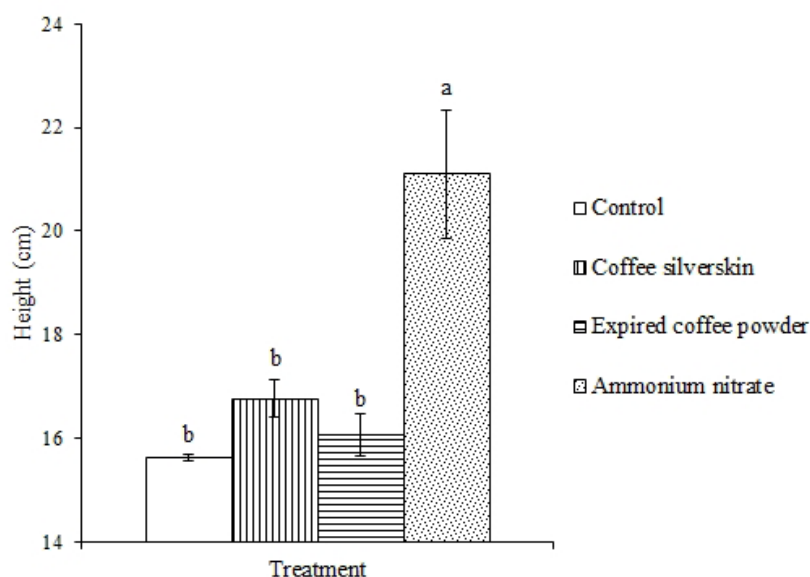


FIGURE 1 - Average height of maize plants in soil treated with coffee organic wastes, and controls. Columns with the same letters do not differ significantly by Tukey test ($p < 0.05$).

TABLE 4 - Nitrogen absorption and efficiency index of nitrogen for maize.

Treatment	Dry matter weight	N Kjeldahl content	Shoot nitrogen extraction	AEI	RAEI
	g	g kg ⁻¹	mg pot ⁻¹	%	
Ammonium nitrate	41.95 a	13.13 a	550.8 a	92.4 a	100.0 a
Coffee silverskin	23.72 b	7.14 b	169.4 b	7.9 b	8.5 b
Expired coffee powder	20.7 bc	7.67 b	158.8 bc	5.5 b	6.0 b
Control	18.7 c	7.16 b	133.9 c	-	-

Different letters indicate a significant difference between treatments at the 5% (P<0.05) level by Tukey's test. AEI = agronomic efficiency index, RAEI = relative agronomic efficiency index

The low nitrogen availability, despite a favorable C:N ratio in coffee silverskin and expired coffee powder, may be due to the organic composition of the wastes associated with the presence of recalcitrant organic compounds, such as cellulose and hemicellulose (SANTOS et al., 2012). Mussato et al. (2011) reported that the presence of fibrous tissues in coffee silverskin was associated with those compounds. Lignin is also related due the high stability of vegetable tissues (BRUM, 2007).

In addition to recalcitrant compounds, the limited nitrogen availability of coffee silverskin and expired coffee powder may be related to the presence of caffeine, tannins and polyphenols. These compounds are toxic to microorganisms and may avoid organic matter mineralization (MUSSATTO et al., 2011; HERMOSA, 2014). Inhibition of extracellular enzymes and direct action on microbial metabolism are some mechanisms of microorganism inhibition due to the presence of tannin (SCALBERT, 1991). Besides that, a protein-tannin complex may be formed in the soil (KRAUS, 2003), that are resistant to N release (HOWARD; HOWARD, 1993).

In addition to this, caffeine and polyphenols are allelopathic to several vegetable species (LIMA et al., 2014). Chandra et al. (2013) found allelopathic activity against *Cicer arietinum* and *Triticum aestivum* caused by coffee bean extract, with a significant reduction of germination for both species.

4 CONCLUSIONS

Although coffee silverskin and expired coffee powder showed nitrogen levels were well

above the minimum require by regulation (0.5 % m/m), its use as organic fertilizers (as exclusive sources of nitrogen) is not recommended since less than 10% of nitrogen was available as showed by the AEI index.

The use of these wastes as soil conditioners is promising due to their high organic matter content and CEC. In addition, these wastes may be used as complimentary sources of potassium. However, further testing is needed to confirm this

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6 REFERENCES

- AMERICAN PUBLIC HEALTH ASSOCIATION (APHA). **Standard Methods for the Examination of Water and Wastewater**, 23rd Edn. Washington, DC: American Public Health Association, 2017.
- BIONDI, C. M.; NASCIMENTO, C. W. A.; NETA, A. B. F.; RIBEIRO, M. R. Teores de Fe, Mn, Cu, e Co em solos de referência de Pernambuco. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 35, n. 3, p. 1057-1066, Nov. 2011.
- BRASIL. **Ministério da Agricultura, Pecuária e Abastecimento**. Aprova os Métodos Analíticos Oficiais para Análise de Substratos e Condicionadores de Solos, na forma do Anexo à presente Instrução Normativa. Instrução Normativa n.17, de 21 de maio de 2007.

BRASIL. **Ministério da Agricultura, Pecuária e Abastecimento**. Aprovar as normas sobre as especificações e as garantias, as tolerâncias, o registro, a embalagem e a rotulagem dos fertilizantes orgânicos simples, mistos, compostos, organominerais e biofertilizantes. destinados à agricultura, na forma dos Anexos à presente Instrução Normativa, n. 25, July 23, 2009.

BRASIL. **Ministério da Agricultura, Pecuária e Abastecimento**. Dispõe sobre as concentrações máximas admitidas para agentes fitotóxicos, patogênicos ao homem, animais e plantas, metais pesados tóxicos, pragas e ervas daninhas em fertilizantes, corretivos, inoculantes e biofertilizantes. Instrução Normativa n.7, May 02, 2016.

BRASIL. **Ministério da Agricultura, Pecuária e Abastecimento**. Fica aprovada as normas sobre especificações e garantias, tolerâncias, registro, embalagem e rotulagem dos corretivos de acidez, de alcalinidade e de sodicidade e dos condicionadores de solo, destinados à agricultura, na forma do Anexo a esta Instrução Normativa. Instrução Normativa n. 35, July 12, 2006.

BREMNER, J. M. Nitrogen total: In: SPARKS, D. L.; PAGE, A. L.; HELMKE, P. A.; LOEPFERT, R. H.; SOLTANPOUR, P. N.; TABATAI, M. A.; JOHNSTON, C. T.; SUMNER, M. E., eds. **Methods of analysis**. Part 3. Chemical methods. Madison, WI, Soil Science Society of America, 1996. p. 1085-1121.

BRUM, S. S. **Caracterização e modificação química de resíduos sólidos do beneficiamento do café para produções de novos materiais**. 2007. 152f. Dissertação (Mestre em Agroquímica) – Universidade Federal de Lavras, Lavras, 2007.

CANTARELLA, H. Nitrogênio. In: NOVAIS, R. F.; ALVAREZ, V. H. V.; BARROS, N. F.; FONTES, R. L. F.; CENTARUTTI, R. B.; NEVES, J. C. L, eds. **Fertilidade do solo**. Viçosa: Sociedade Brasileira de Ciência do Solo, 2007. p. 375-470.

CHANDRA, S.; DEY, P.; CHATTERJEE, P.; BHATTACHARYA, S. Allelopathic activity of coffee against *Cicer arietinum* and *Triticum aestivum*. **Advances in Biological Research**, v. 7, n. 4, p. 129-133, Apr. 2013.

CONAMA (2006). **Conselho Nacional do Meio Ambiente**. Definir critérios e procedimentos, para o uso agrícola de lodos de esgoto gerados em estações de tratamento de esgoto sanitário e seus produtos derivados, e dar outras providências. n.º 375, Aug. 29, 2006.

CONCEIÇÃO, J. C. P. R.; JUNIOR, R. G. E.; CONCEIÇÃO, P. H. Z. **Cadeia agroindustrial do café no Brasil: Uma análise do período recente**. Radar, n. 53, 2017. Available: <http://repositorio.ipea.gov.br/bitstream/11058/8105/1/Radar_n53_cadeia.pdf>. Accessed in: May 05, 2018.

COSTA, R. M. C.; FREIRE, J. L. O.; MACEDO, L. P. M.; SILVA, F. L. S.; BORGES, C. H. A. Manejo de culturas e equilíbrio trofobiótico no perímetro irrigado Cruzeta, RN. **Agropecuária Científica no Semiárido**, Campina Grande, v. 11, n. 4, p. 72-86, Dec. 2015.

ECKHARDT, D. P.; REDIN, M.; JACQUES, R. J. S.; LORENSINI, F.; SANTOS, M. L. D.; WEILER, D. A.; ANTONIOLLI, Z. I. Mineralization and efficiency index of nitrogen in cattle manure fertilizers in the soil. **Ciência Rural**, Santa Maria, v. 46, n. 3, p. 472-477, Mar. 2016.

EPA. SW-846 Test Method 3051A: **Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils**. Available: <<https://www.epa.gov/hw-sw846/sw-846-test-method-3051a-microwave-assisted-acid-digestionsediments-sludges-soils-and-oils>>. Accessed in: Aug 20, 2018.

ESQUIVEL, P.; JIMÉNEZ, V. M. Functional properties of coffee and coffee by-products. **Food Research International**, Ontario, v. 46, p. 488-495, May 2011.

FARINELLI, R.; LEMOS, L. B. Produtividade, eficiência agrônômica, características nutricionais e tecnológicas do feijão adubado com nitrogênio em plantio direto e convencional. **Bragantia**, Campinas, v. 69, n. 1, p. 165-172, Jan. 2010.

FIORINI, I. V. A.; PINHO, R. G. V.; PIRES, L. P. M.; SANTOS, A. O.; FIORINI, F. V. A.; CANCELLIER, L. L.; RESENDE, E. L. Avaliação de fontes de enxofre e das formas de micronutrientes revestindo o NPK na cultura do milho. **Revista Brasileira de Milho e Sorgo**, Sete Lagoas, v. 15, n. 1, p. 20-29, Mar. 2016.

FONTANA, A.; PEREIRA, M. G.; ANJOS, L. H. C.; SANTOS, A. C.; BERNINI, T. A. Matéria orgânica de horizontes superficiais em topolitossequências em ambiente de Mar de Morros, Pinheiral, RJ. **Revista Ciência Agronômica**, Fortaleza, v. 45, n. 2, p. 221-229, Apr./June 2014.

HERMOSA, V. A. B. **Aproveitamento de resíduos do processamento semi-seco do café para a produção de compostos de valor agregado**. 2014. 77f. Dissertação (Mestre em Microbiologia Agrícola) – Universidade Federal de Lavras, Lavras, 2014.

- HOWARD, P. J. A. HOWARD, D. M. 1993. Ammonification of complexes prepared from gelatin and aqueous extracts of leaves and freshly-fallen litter of trees on different soil types. **Soil Biology & Biochemistry**, Leicestershire, v. 25, p. 1249–1256, Sept. 1993.
- KRAUS, T. E. C.; DAHLGREN, R. A.; ZASOSKI, R. J. Tannins in nutrient dynamics of forest ecosystems – a review. **Plant and Soil**, Perth. v. 256, n. 1, p. 41–66, Sept. 2003.
- LIMA, L. K. S. **Desenvolvimento do feijão caupi em função da utilização de resíduo da indústria do café como fonte de potássio**. 2014. 81 p. Dissertação (Mestrado em Agronomia/Fitotecnia) - Universidade Federal do Ceará, Ceará, 2014.
- LIMA, L. K. S.; SANTOS, C. C.; MOURA, M. C. F.; DUTRA, A. S.; FILHO, A. F. O. Utilização de resíduo oriundo da torrefação do café na agricultura em substituição a adubação convencional. **Agropecuária Científica No Semiárido**, Campina Grande, v. 10, n. 1, p. 14–19, Mar. 2014.
- MARTINEZ, H. E. P.; CLEMENTE, J. M.; LACERDA, J. S. NEVES, Y. P.; PEDREOSA, A. W. Nutrição mineral do cafeeiro e qualidade da bebida. **Revista Ceres**, Viçosa, v. 61, n. 4, p. 838–848, Nov/Dec 2014.
- MATIELLO, J. B. **O café do cultivo ao consumo**. São Paulo: Globo, 1991. p. 320
- MELO, L. C. A.; SILVA, C. A.; DIAS, B. O. Caracterização da matriz orgânica de resíduos de origens diversificadas. **Revista Brasileira de Ciência do Solo**, Viçosa, Brasil, v. 32, n.1, p. 101–110, Mar. 2008.
- MOURA, M. C. F. **Resíduos da torrefação do café como fonte de potássio no cultivo do Girassol**. 2016. 79 p. Tese (Doutorado em Agronomia)-Universidade Federal do Ceará, Ceará, 2016.
- MULVANEY, R. L. (1996), Nitrogen – Inorganic forms. In: **Method of Soil Analysis: chemical methods**. Part 3. D. L. Sparks. Madison: Soil Science Society of America, 1996, p. 1123–1184.
- MUSSATO, S. I.; MACHADO, E. M. S.; MARTINS, S.; TEIXEIRA, J. A. Production, composition, and application of coffee and its industrial residues. **Food bioprocess technology**, New York, v. 4, n. 5, p. 661–672, Mar. 2011.
- NELSON, D. W.; SUMMERS, L. E. **Methods of Soil Analysis**. Part 3. Chemical Methods. Madison: Soil Science Society of America, n. 5, 1996, p. 961–1010.
- OLIVEIRA, J. R. A.; VILELA, L.; AYARZA, M. A. Adsorção de nitrato em solos de cerrado do distrito federal. **Pesquisa Agropecuária Brasileira**, Brasília, v. 35, n. 6, p. 1199–1205, June 2000.
- PAIVA, E. C. R.; MATOS, A. T.; BARROS, R. T.; COSTA, T. D. R. Análise comparativa da adequação da relação C/N e do índice CT/COT como parâmetros da evolução da compostagem. In: CONGRESSO BRASILEIRO DE GESTÃO AMBIENTAL, 6., 2013, Salvador, **Anais eletrônicos**...Salvador: Instituto Brasileiro de Estudos Ambientais, 2013. Available: < <http://www.ibeas.org.br/congresso/Trabalhos2013/II-004.pdf>>. Accessed in: Jan. 21, 2019
- PAULA, J. R.; MATOS, A. T.; PEREIRA, M. S.; ANDRADE, C. A. Mineralização do carbono e nitrogênio de resíduos aplicados o solo em campo. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 37, n. 6, p. 1729–1741, Nov./Dec. 2013.
- RAIJ, B. VAN; ANDRADE, J.C.; CANTARELLA, H.; QUAGGIO, J.A. **Análise química para avaliação de fertilidade de solos tropicais**. Campinas: Instituto Agrônomo de Campinas, 2001, 285p.
- RODRIGUES, M. A.; BUZETTI, S.; FILHO, M. C. M. T.; GARCIA, C. M. P. ANDREOTTI, M. Adubação com KCl revestido na cultura do milho no Cerrado. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v. 18, n. 2, p. 127–133, 2014.
- RONQUIM, C. C. **Conceitos de fertilidade do solo e manejo adequado para as regiões tropicais**, Campinas: Embrapa Monitoramento por Satélite, 2010. 30p. (Boletim de Pesquisa e Desenvolvimento, 8).
- SANCHÉZ-MONEDERO, M. A.; ROIG, A.; CEGERRA, J.; BERNAL, M. P. Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. **Bioresource Technology**, Lisbon, v. 78, n.3, p. 301–308, July 2001.
- SANTOS, F. A.; QUIRÓZ, J. H.; COLODETTE, J. L.; FERNANDEZ, S. A.; GUIMARÃES, V. M.; REZENDE, S. T. Potencial da palha de cana-de-açúcar para produção de etanol. **Química Nova**, São Paulo, v. 35, n. 5, p. 1004–1010, Jan. 2012.
- SCALBERT, A. Antimicrobial properties of tannins. **Phytochemistry**, Bari v. 30, n. 12 p. 3875–3883, Dec. 1991.
- SOARES, M. M.; BARDIVIESSO, D. M.; BARBOSA, W. F. S.; BARCELOS, M. N. Adubação de cobertura com enxofre na cultura da rúcula. **Revista de Agricultura Neotropical**, Cassilândia, v. 4, n. 1, p. 49–52, Mar. 2017.

CONTROL OF THE COFFEE ROASTING STAGE USING ARTIFICIAL VISION TECHNIQUES

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ABSTRACT: Artificial vision techniques were used to evaluate its application in the control of the coffee roasting stage. Coffee samples of Colombia and Castillo varieties were obtained and analyzed by comparing images during the roasting stage. A one-way ANOVA analysis exhibited 94.28% of similarity of the coffee varieties studied; a multivariate analysis showed significant differences ($p < 0.05$) for the time factor and its interaction with the variety factor, no differences were observed ($p > 0.05$) for the coffee varieties. Additionally, a Principal Component, with two components demonstrated 90.77% of the variance by differentiating the samples in the different roasting times. Therefore, the proposed technique could be used in the control of the coffee roasting stage.

Index terms: Variety Colombia, variety Castillo, visible spectrum.

CONTROLE DO ESTÁGIO DE TORREFAÇÃO DE CAFÉ ATRAVÉS DE TÉCNICAS DE VISÃO ARTIFICIAL

RESUMO: Para avaliar o controle do estágio de torrefação de café, foram utilizadas técnicas de visão artificial e variedades de café, Colombia e Castillo, as quais foram analisadas através da comparação de imagens durante a torrefação. A análise de variância mostrou similaridade de 94,28% entre as variedades estudadas. A análise multivariada mostrou diferenças significativas ($p < 0,05$) para o fator tempo e sua interação com o fator variedade, não foram observadas diferenças para as variedades de café ($p > 0,05$). E ainda foi realizada uma análise de componentes principais. Com dois componentes principais, 94,23% da variância foi explicada pela discriminação das amostras nos tempos de torrefação. Ao que se conclui que a técnica proposta pode ser uma ferramenta no controle do estágio de torrefação de café.

Termos para indexação: Variedade Colombia, variedade Castillo, espectro visível.

1 INTRODUCTION

Since the late eighties, the introduction of cameras (visible spectrum) in different industrial processes has led to the emergence of control methods, based on the compression of the underlying information in an image using projection techniques on latent structures, known as Multivariate Image Analysis (MIA) (GELADI et al., 2006).

These techniques have been used for more than 20 years in agricultural applications related to robotics, automation, artificial vision and artificial intelligence (GARCÍA-LUNA et al., 2016). Furthermore, these techniques are quite powerful in situations in which images are used to record multitudes of color channels, collected by means of multispectral cameras, in which each channel can contain information of a smaller number of phenomena and therefore, be able to associate the demonstrated color channels with qualities and characteristics of interest to the study (PRATS-MONTALBÁN et al., 2011). Additionally, these techniques are often innovative and precise,

and allow the development of technological and scientific advances.

Practical examples of its application in the control of food processes are: fat classification of Iberian pigs (GIRÓN et al., 2016), analysis in coffee roasting (HERNÁNDEZ et al., 2008), analysis in bread baking (VERDÚ et al., 2017), analysis of shelf life in fish (IVORRA et al., 2013; IVORRA et al., 2016) evaluation of pork meat quality (SUN et al., 2018), among others. Consequently, image analysis techniques demonstrate the effectiveness in predicting the behavior of a product under different conditions.

The expertise of a master roaster in the coffee roasting stage is still utilized, among other artisanal practices in the production of coffee in Colombia. This being said, it is necessary to identify low-cost practices and techniques that enable the processing of coffee to be optimized and controlled, thusly improving the efficiency of production and commercialization.

The analysis techniques that use artificial vision in the visible range provide access to information from which these processes can be

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automated and homogenized. The versatility of these techniques are applicable to the coffee roasting stage given that they allow for the quantification of data related to the changes in color and size that occur due to changes in temperature and time. The objective in this study was to develop and evaluate a procedure based on artificial vision for the control in the coffee roasting stage.

2 MATERIALS AND METHODS

20 kg of coffee (*Coffea arabica*) Castillo and Colombia varieties were harvested at an altitude of 1600 m.a.s.l., only beans with an intense red coloration were collected. The samples were deposited in polystyrene containers with cooling gels to avoid early fermentation and later were transported to CESURCAFÉ at the Universidad Surcolombiana (Neiva, Huila, Colombia) to perform the wet fermentation and drying process. In both varieties, the fermentation began with a pH value of 5.79 ± 0.013 until reaching a value of 4.56 ± 0.105 . Subsequently, the drying process was carried out and the moisture content of the sample was reduced to $11.2\% \pm 0.04$ and $11.0\% \pm 0.06$ for the Colombia and Castillo varieties respectively.

The hulling process was carried out in a laboratory hulling machine (ING-C-200, Ingesec, Bogotá, Colombia), followed by the selection of non-defective coffee beans in accordance with the provisions of the Specialty Coffee Association of America (SCAA, 2009). The coffee beans were passed through a number 13 sieve of 5 mm diameter to eliminate smaller beans. The roasting process was done in a Quantik roaster (TC-150R, Quantik, Colombia); two levels of roasting were defined, medium roast and dark roast, according to luminosity (L^*) measurements, similar to commercially available coffee samples (Craig et al., 2017), the roaster was set to sample entry temperature of 180 °C. For image acquisition, a Canon T3 digital camera (Tokyo, Japan) with 12 megapixels of resolution was adapted. The parameters of focal length, exposure time and white balance were set with an indirect lighting of 7.5 W dichroic LED lamps with 620 Lm (Lumek, Germany). To capture the images, Petri dishes containing the samples were placed 40 cm from the objective, 288 images were obtained in total, 6 images for each sample of 150 g of coffee and 24 captures for each time of roasting. For image analysis, it was developed an algorithm that segmented each captured raw image to eliminate

background and noise (VALA et al., 2013) based on the parametric background subtraction algorithm (PICCARDI, 2004). From each element of interest (beans) a vector of morphological and color characteristics was extracted. Specifically, the average Red, Green, Blue (RGB) and Hue, Saturation, Value (HSV) coordinates values were obtained from a window around the centroid of each bean (LEE et al., 2017). In addition, the morphology was analyzed by textures of Haralick. Afterwards, information filtering with a conditional loop was performed by means of a dynamic thresholding to eliminate anomalous values (double the standard deviation). Finally, the data of each image was stored in a matrix to execute the statistical analysis. The processing of the images was done with the software MATLAB 8.0 (The Mathworks, USA).

Statistical analysis

The similarities between images data of Colombia variety and Castillo variety were determined using a Student's *t*-test. An analysis of variance (One-way ANOVA) was conducted in the first part of the study to determine whether there were significant differences. Each vector (color coordinates and morphology) was considered as a dependent variable and the variety was the factor. The least significant procedure (LSD) was used to test for the differences between means at a significance level of 5%. Additionally, in the second part of the study, a multifactor ANOVA was carried out for each vector to evaluate the effect of variety and roasting time and their interactions. In order to assess the feasibility of the artificial vision techniques to discriminate between coffee varieties and roasting times and to compress and select the information with the highest variance, a Principal Component Analysis (PCA) method was performed with a matrix of 44 variables obtained from the sensor, 30 for color (15 RGB and 15 HSV) and 14 for morphological features. All variance and multivariate analysis were carried out using Statgraphics centurion XVI program. (Manugistics Inc., Rockville, MD, USA).

3 RESULTS AND DISCUSSION

The similarity of the images obtained from the varieties studied presented a mean of 94.28%; the color coordinates showed a similarity of 93.33% for the RGB and 92.67 % for the HSV; a greater similarity was evidenced with the components of the shape vector 96.86%. The F-ratio and

P-values of the RGB coordinates obtained from the Colombia and Castillo varieties are shown in Table1, the values of the RGB coordinates only presented significant difference ($p < 0.05$) for the maximum green variable. The constant variables in all the images were eliminated. Therefore, information of the minimum blue channel was not included in the analysis; ANOVA data for HVS and morphology information are not shown.

Figure 1 shows the interaction between the factors time and variety for a variable analyzed in this study (Hue mean) of the HVS coordinates. Differences were observed in the first minutes of roasting, and the effect of temperature decreased differences caused by the mucilage residues, similar results were reported by other authors (LEE et al., 2017); the response was not significant ($p > 0.05$) for the varieties, while the roasting time values presented significant differences ($p < 0.05$). This analysis showed similar results for all the parameters studied which justifies the application of the algorithm in different coffee varieties.

At each time, changes in color and shape of the beans were observed. In the roasting stage, these changes relate to the development of properties such as flavor, aroma and color (HERNÁNDEZ et al., 2008).

Figure 2 shows the scores plot in the plane of the first and second principal components (PCs)

for the roasting stage, which together account for 94.22% of the total variability. Additionally, the value for each of the components established by the analysis is presented. From the third component, there are no significant increases for the variance, but 95.97% of the variability of the samples is noticed.

The analysis showed a good grouping of the samples in the study. The first component differentiated the coffee beans up to three minutes of roasting. In this time interval the beans still exhibited green colors and do not presented variation in volume. The second component separated the samples into four well-defined groups in which different sizes and color levels of roast were differed. At four minutes of roasting, when the internal temperature of the bean was 170 °C (Figure 3), the samples generated a group between the green color and the brown color and as the roasting process continued they got darker until reached minute seven when the temperature was approximately 220 °C (Figure 3).

At this time, the samples exhibited more homogeneous characteristics in both color RGB and HVS coordinates and shape morphology analyzed by textures of Haralick. Based on the results obtained, a robust classification algorithm capable of discriminating the roasting times can be performed.

TABLE 1 - F-ratio values and P-value obtained in a one-way ANOVA of the RGB color coordinates.

Variable	F-ratio	P-value
Mean Red	0.280	0.598
Mean Green	0.100	0.748
Mean Blue	0.050	0.816
Maximun Red	0.410	0.524
Maximun Green	3.950	0.048
Maximun Blue	1.340	0.248
Minimun Red	1.390	0.239
Minimun Green	0.060	0.806
Median Red	0.330	0.565
Median Green	0.150	0.698
Median Blue	0.060	0.814
SD Red	0.060	0.800
SD Green	0.180	0.674
SD Blue	0.260	0.612

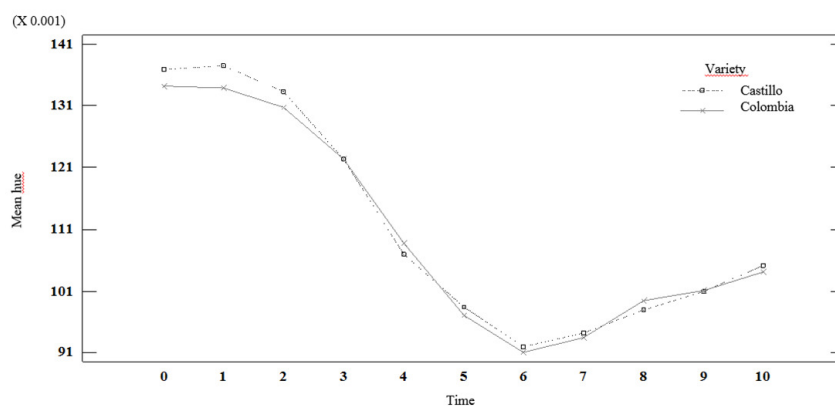


FIGURE 1 - Interactions between factors coffee variety and roasting time for one of the evaluated variables, mean hue.

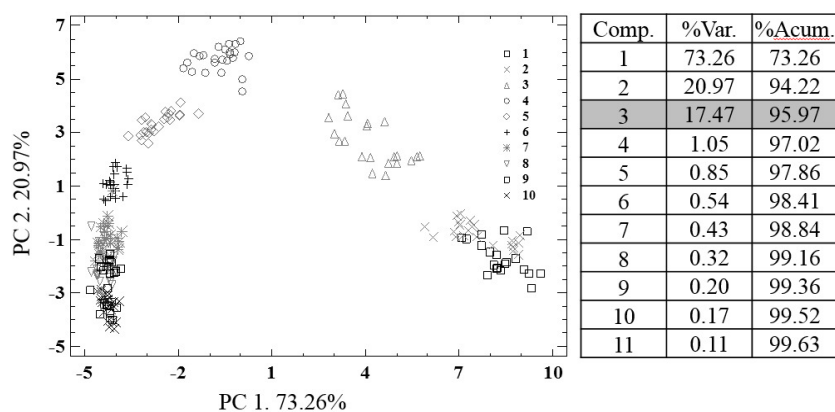


FIGURE 2 - Principal component analysis (PCA) performed on the image measurements for the roasting stage. With different times (1 (\square). 2 (\times). 3 (\triangle). 4 (\diamond). 5 (\diamond). 6 ($+$). 7 (\ast). 7.4 (∇). 8 (\square) and 9 (\times) minutes).

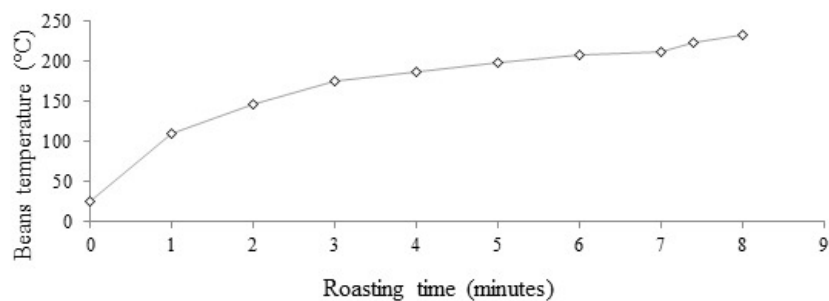


FIGURE 3 - Roasting times and the temperature at which the beans came out of the roaster.

4 CONCLUSION

The application of artificial vision techniques allowed the automatic and objective differentiation of coffee samples according to the roasting time. The coffee varieties used in the trial were not a factor of discrimination. Future studies with other varieties are necessary in order to generate a classification tool applicable to any variety of coffee for the control of the roasting stage.

5 REFERENCES

- Craig, A.; Botelho, B.; Oliveira, L.; Franca, A. Mid infrared spectroscopy and chemometrics as tools for the classification of roasted coffees by cup quality. **Food Chemistry**. V. 245. p 1052-1061. 2017.
- GARCÍA-LUNA, F.; MORALES-DÍAZ, A. Towards an artificial vision-robotic system for tomato identification. **IFAC-PapersOnLine**, San Diego, v. 49, n. 16, p. 365-370, Oct. 2016.
- GELADI, P.; GRAHN, H. Multivariate Image Analysis. **Encyclopedia of Analytical Chemistry**, New York, v. 1, n. 1, p.142-168, Sept. 2006.
- GIRÓN, J.; BARAT, J.; SÁNCHEZ, A.; GRAU, R. Aplicación de la espectrofotometría de infrarrojo (SW-NIR) para la clasificación de grasa de cerdos ibéricos. **Agromía Colombiana**, Bogotá, v. 34, n. 2, p. 473-476, Nov. 2016.
- HERNÁNDEZ, J.; HEYD, B.; TRYSTRAM, G. Prediction of brightness and surface area kinetics during coffee roasting. **Journal of Food Engineering**, San Diego, v. 89, n. 2, p. 156-163, Apr. 2008.
- IVORRA, E.; GIRÓN, J.; SÁNCHEZ, A.; VERDÚ, S.; BARAT, J.; GRAU, R. Detection of expired vacuum-packed smoked salmon based on PLS-DA method using hyperspectral images. **Journal of Food Engineering**, San Diego, v. 117, n. 3, p. 342-349, Apr. 2013.
- IVORRA, E.; SÁNCHEZ, A.; VERDÚ, S.; BARAT, J.; GRAU, R. Shelf life prediction of expired vacuum-packed chilled smoked salmon based on a KNN tissue segmentation method using hyperspectral images. **Journal of Food Engineering**, San Diego, v. 178, n. 4, p. 110-116, June 2016.
- LEE, L.; TAY, G.; CHEONG, M.; BIN, P.; LIU, S. Modulation of the volatile and non-volatile profiles of coffee fermented with *Yarrowia lipolytica*: II. Roasted coffee. **LWT-Food Science and Technology**. v. 80, n. 1 p. 32-42, July 2017.
- PICCARDI, M. "Background subtraction techniques: a review." IEEE International Conference on Systems, Man and Cybernetics (IEEE Cat. No.04CH37583), p. 3099-3104, Oct. 2004
- PRATS-MONTALBÁN, J.; DE JUAN, A.; FERRER, A. Multivariate image analysis: A review with applications. **Chemometrics and Intelligent Laboratory Systems**, San Diego, v. 107, n. 1, pp 1-23, May. 2011.
- Specialty coffee association SCAA. Grading Green Coffee. **Specialty Coffee Association of America**. USA: Santa Ana, Oct. 2009
- SUN, X.; YOUNG, J.; LIU, J.; NEWMAN, D. Prediction of pork loin quality using online computer vision system and artificial intelligence model. **Meat Science**, San Diego, v. 140, n. 2, p. 72-77, June 2018.
- VALA, H.; ASTHA, B. A review on Otsu image segmentation algorithm. **International Journal of Advanced Research in Computer Engineering and Technology**, Jawahar, v. 2, n. 2, p. 387-389, Feb. 2013.
- VERDÚ, S.; VÁSQUEZ, F.; IVORRA, E.; SÁNCHEZ, A.; BARAT, J.; GRAU, R. Hyperspectral image control of the heat-treatment process of oat flour to model composite bread properties. **Journal of Food Engineering**, San Diego, v. 192, n. 1, p. 45-52, Jan. 2017.

METEOROLOGICAL VARIABLES AND SENSORIAL QUALITY OF COFFEE IN THE MANTIQUEIRA REGION OF MINAS GERAIS

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ABSTRACT: The objective in this study was to identify meteorological variables related to the sensorial quality of the coffees from Mantiqueira region in Minas Gerais. Meteorological conditions are strongly related to the coffee's sensorial characteristics, however, there aren't many studies quantifying this relation. Air temperature and rainfall data were collected and spatialized for regional analysis. These were associated to the 2007 through 2011 coffees' beverage scores. The region was stratified according to relief characteristics. The bigger frequency of high scores occurred on the region's central-south, where coffee cultivation is performed above 900 m altitude. For the *in loco* study, meteorological data and coffee samples were collected in selected pilot areas. Coffee crops were selected in three altitude ranges: below 1000 m, between 1000 and 1200 m, and over 1200 m. Above 1000 m the meteorological variable that presented the biggest variation was the air temperature. Above 1000 m the smallest thermal amplitude occurred, which provided superior quality coffees. The study demonstrates the importance of the meteorological variable characterization aiming to identify locations with greater vocation to the specialty coffees production.

Index terms: Coffee production, agrometeorology, thermal amplitude, altitude.

VARIÁVEIS METEOROLÓGICAS E QUALIDADE SENSORIAL DO CAFÉ NA REGIÃO DA MANTIQUEIRA DE MINAS GERAIS

RESUMO: Objetivou-se com este estudo identificar variáveis meteorológicas que estão relacionadas à qualidade sensorial dos cafés da região da Mantiqueira de Minas Gerais. Condições meteorológicas são fortemente associadas às características sensoriais dos cafés, porém estudos que quantificam essa relação ainda são escassos. Para análise regional foram coletados e espacializados dados de temperatura do ar e precipitação pluvial, que foram relacionados com as notas de bebida dos cafés nos anos de 2007 a 2011. A região foi estratificada em função das características do relevo. A maior frequência de melhores notas da bebida ocorreu nos municípios localizados na área centro-sul da região, mais frios e chuvosos, e onde a cafeicultura localiza-se acima de 900 m de altitude. Para o estudo *in loco*, foram selecionadas áreas piloto para coleta de dados meteorológicos e de amostras de café. Foram selecionadas lavouras de café localizadas em três faixas de altitude: abaixo de 1000 m, entre 1000 e 1200 m e superior a 1200 m. Acima de 1000 m a temperatura do ar foi a variável meteorológica para a qual se verificou maior diferença. Acima de 1000 m ocorreu menor amplitude térmica do ar, o que proporcionou cafés com qualidade superior. O estudo demonstra a importância da caracterização de variáveis meteorológicas visando a identificação de locais com maior vocação para produção de cafés especiais.

Termos para indexação: Cafeicultura, agrometeorologia, amplitude térmica, altitude.

1 INTRODUCTION

The coffees produced at Mantiqueira region, located Southern Minas Gerais, have been gaining increasing recognition from the market, for they provide beverage with distinct sensorial characteristics. The crops are located in different environmental conditions and many climatic variables may be related to the coffees' sensorial characteristics.

Among the environmental variables related to the coffee's sensory quality, the altitude has been the most studied (Figueiredo et al., 2013; Barbosa et al., 2014; Silva et al., 2015). However, altitude's effects are considered to be indirect, for its variation results in different climatic conditions for the environment. Climate and soil characteristics are among the factors that most affect the coffee trees development (Guyot et al., 1996; Joët et al., 2010).

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In Central America, studies demonstrate that the average temperature during seed development has influenced the sensorial profile. Positive quality attributes, such as acidity, fruity character and flavor quality have been correlated to the coffee production in cooler microclimates (Bertrand et al., 2012). Workuad et al. (2018) observed that the sucrose content and acidity increase with altitude.

Studies performed at Mantiqueira region showed that coffee quality depends on the interaction between genotype, processing and altitude (Ribeiro et al., 2016; Ramos et al., 2016).

This study's purpose was to identify meteorological variables related to the sensorial quality of the coffees from Mantiqueira region in Minas Gerais and demonstrate the importance of the meteorological characterization to identify locations with greater vocation for specialty coffees production.

2 MATERIALS AND METHODS

Mantiqueira region is located Southern in Minas Gerais state, Brazil (Figure 1) with altitudes varying from 800 m (minimum) at Santa Rita do Sapucaí, to 2250 m (maximum) at Baependi (Figure 2). According to Alves et al. (2016), the coffee crops are distributed mainly on the region's central area (Santa Rita do Sapucaí, São Gonçalo do Sapucaí, Carmo de Minas, Campanha, Pedralva, Conceição do Rio Verde, Cambuquira and Lambari). Figure 3 shows the crops distribution by altitude. About 40% of the

crops are located between 900 and 1000 m and 50% between 1000 and 1400 m.

Data provided by AGRITEMPO (MAPA, 2014) were used to spatialize the meteorological variables. The location of the weather stations is shown in Table 1. Spatial distribution maps of annual precipitation and maximum and minimum temperature were obtained using the Inverse Distance Weighting interpolation, which has presented the best results for the region.

To describe the region's coffee sensory quality, beverage scores from the 2007/2008 through 2010/2011 harvests were handed over by the sectors companies that directly work in the region. Highest scores maps were elaborated for each harvest. Spatial distribution of annual precipitation and maximum and minimum temperature data were associated to the coffee's beverage scores.

Pilot areas were selected at Carmo de Minas for collection of meteorological data and coffee samples. Automatic weather stations were installed in coffee crops located at three altitude ranges (below 1000 m, between 1000 and 1200 m, and over 1200 m). Air temperature, relative humidity, solar radiation and precipitation data referring to the 2011/12 and 2012/13 harvests were collected.

The description of the coffee beverage sensory quality was carried out using field collected samples, by altitude range. The harvest was performed manually and selectively, collecting only mature fruits, which were processed according to Borém (2008) recommendations.

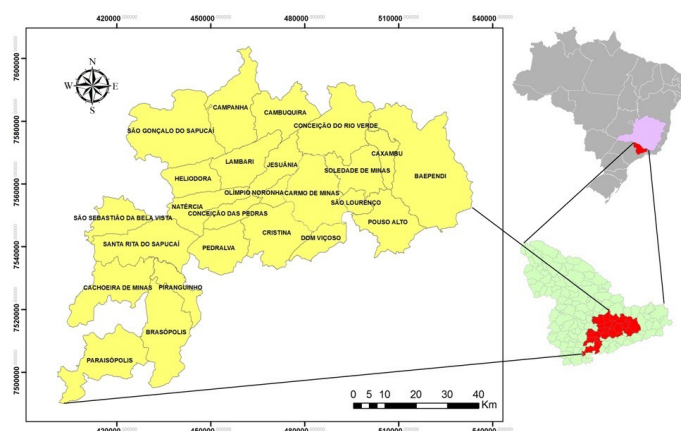


FIGURE 1 - Location of the Mantiqueira region, southern Minas Gerais state, Brazil.

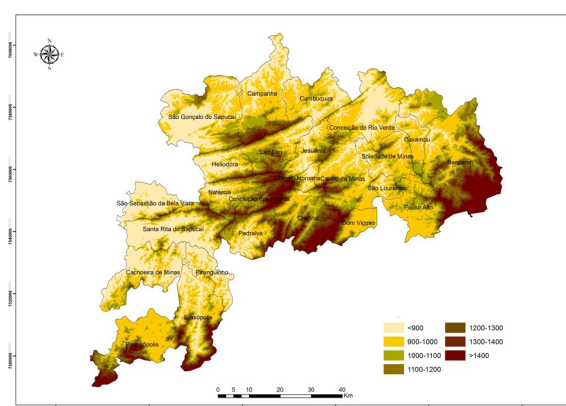


FIGURE 2 - Altitude class map of Mantiqueira region in Minas Gerais, in meters.

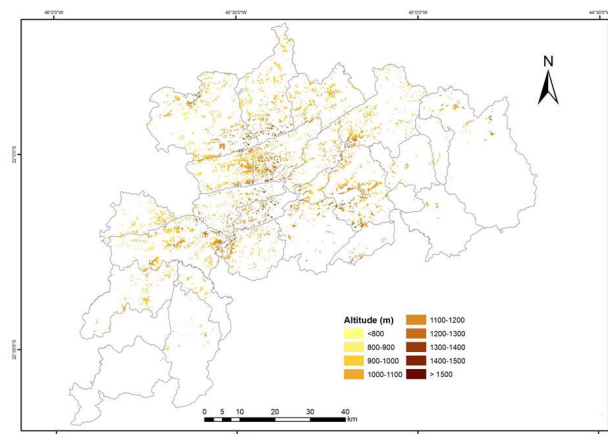


FIGURE 3 - Spatial distribution map of coffee occupied areas, by altitude class, in meters.

TABLE 1 - Locations used to provide air temperature and precipitation data.

Location	Station	Latitude	Longitude	Altitude (m)
Aiuruoca/MG	Agri tempo	21°45'	44°45'	1021
Camanducaia/MG	Agri tempo	22°45'	46°00'	645
Cambuquira/MG	Agri tempo	21°45'	45°15'	920
Campos do Jordão/SP	INMET	22°43'	45°34'	1580
Lavras/MG	Agri tempo	21°15'	45°00'	918
Machado/MG	INMET	21°40'	45°55'	873
Maria da Fé/MG	INMET	22°18'	45°22'	1276
São Lourenço/MG	INMET	22°08'	45°02'	953
Soledade de Minas/MG	CEMIG	22°01'	45°05'	1148
Varginha/MG	Agri tempo	21°30'	45°30'	832

Agri tempo: Agrometeorological Monitoring System; CEMIG: Minas Gerais Energy Company; INMET: National Meteorology Institute.

The sensorial analysis of the samples was carried out by trained tasters, using the methodology proposed by the Specialty Coffee Association of America (SCAA, 2009). The pilot areas' meteorological data were associated to the sensorial analysis results from the field collected samples.

Time series of meteorological variables (minimum, average and maximum temperature, thermal amplitude, precipitation, solar radiation and relative humidity) were used to perform the statistical analysis of the pilot area data, using the GRETl program. The data was checked for series periodicity. Then, seasonality patterns were graphically evaluated, with autocorrelogram construction. This method allowed the identification of seasonal dependence on the series.

Aiming a comparison, the subtraction between the time series of the meteorological variables on the different altitude ranges (below 1000 m, between 1000 and 1200 m, and over 1200 m) was performed. These subtractions were submitted to frequency-domain analysis to check for periodicity in the new series. The stationarity conditions were checked by the autocorrelation function.

3 RESULTS AND DISCUSSION

The spatial distribution of meteorological data from Mantiqueira region in Minas Gerais, for the 2007 to 2011 period is presented in Figures 4 through 6.

The region's annual precipitation ranged from 1000 to 2000 mm and the greatest quantities occurred at the central-south area. The rainier years were 2008, 2009 and 2011. The lower maximum and minimum temperatures occurred at the central-south area. The annual average temperatures ranged from 19 to 22°C, 18 to 20°C and 16 to 18°C at north-northeast, central and south areas respectively. According to Vianello and Alves (2012), for every 100 m of rising altitude, the air temperature drops 0.65°C. Therefore, the air temperature variation can be explained by latitude difference and by the altitude range of 800 to 2250 m, approximately.

Figures 7 to 10 present the distribution of the highest sensorial scores for the 2007/2008 to 2010/2011 harvests. It's noted values above 80 points for the Carmo de Minas, Dom Viçoso, Cristina, Pedralva, Pouso Alto, Jesuânia, Conceição do Rio Verde, Conceição das Pedras and Olímpio Noronha, at the region's central area. However, excessive rainfall in 2009's harvest period resulted in lower graded coffees.

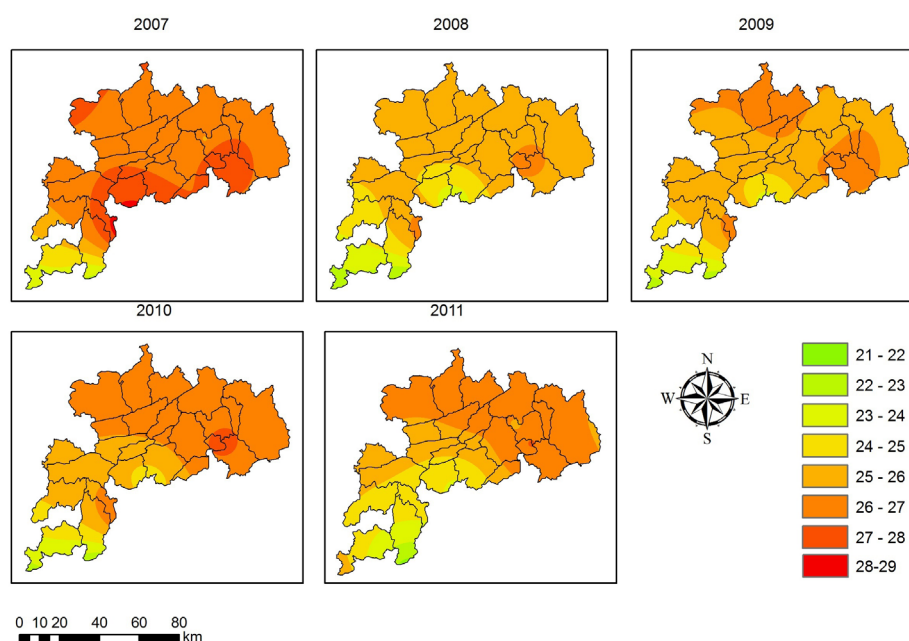


FIGURE 4 - Average maximum temperature maps for the 2007 to 2011 period, in °C.

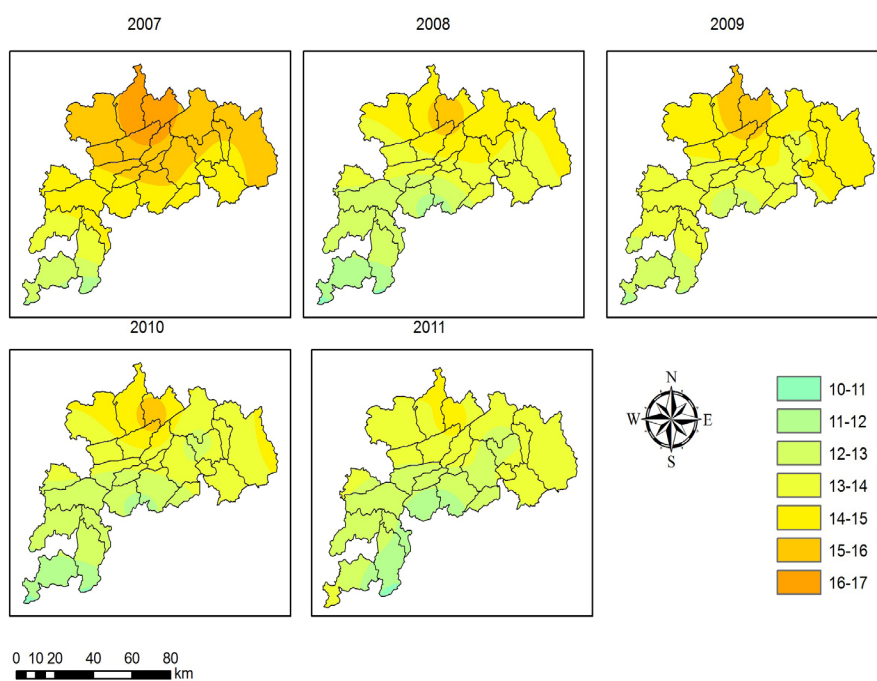


FIGURE 5 - Average minimum temperature maps for the 2007 to 2011 period, in °C.

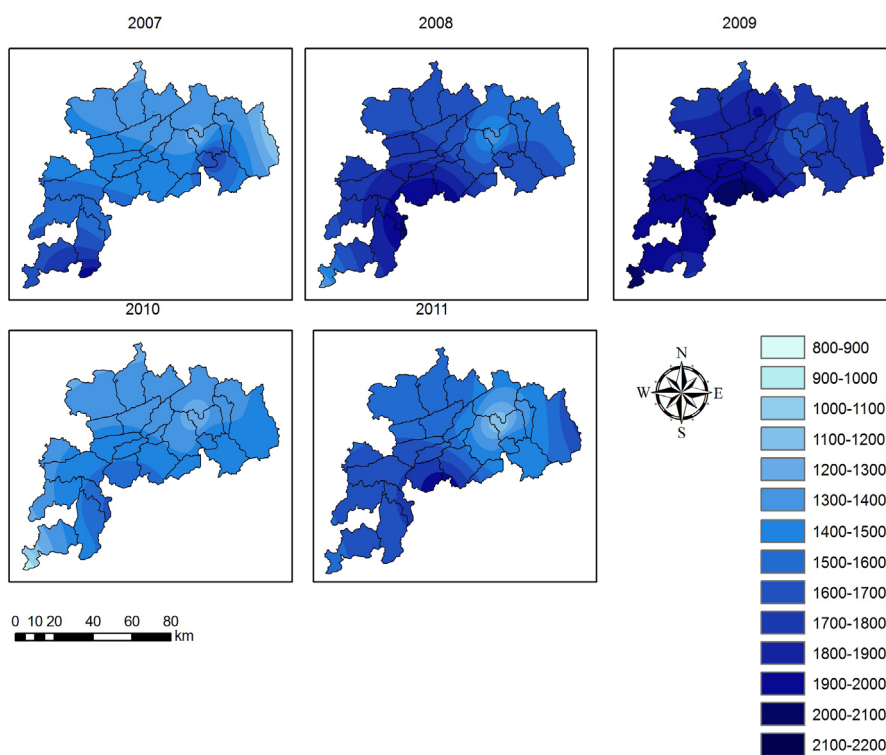


FIGURE 6 - Annual precipitation maps for the 2007 to 2011 period, in mm.

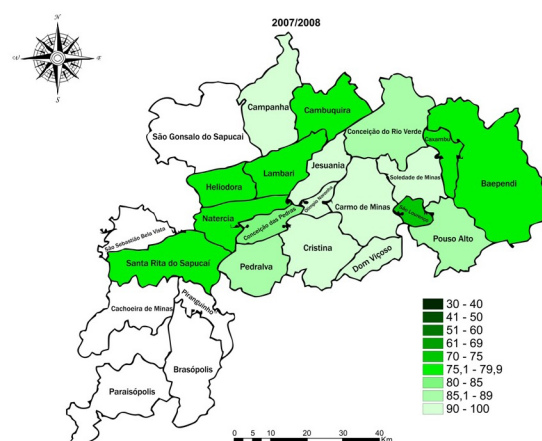


FIGURE 7 - Quality distribution map. Higher scores at 2007/2008 harvest.

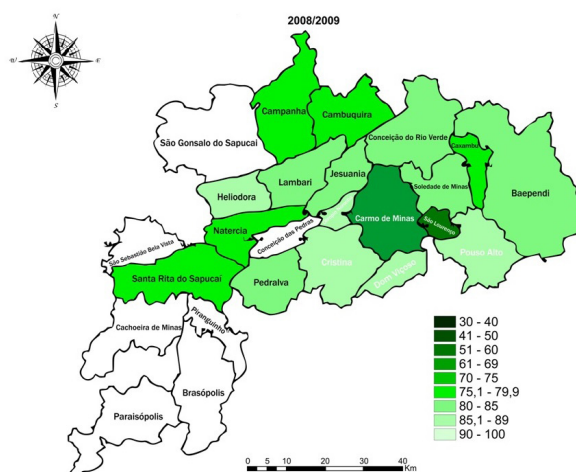


FIGURE 8 - Quality distribution map. Higher scores at 2008/2009 harvest.

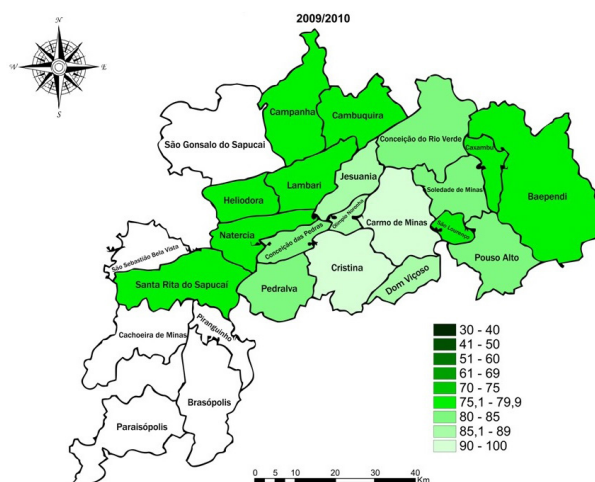


FIGURE 9 - Quality distribution map. Higher scores at 2009/2010 harvest.

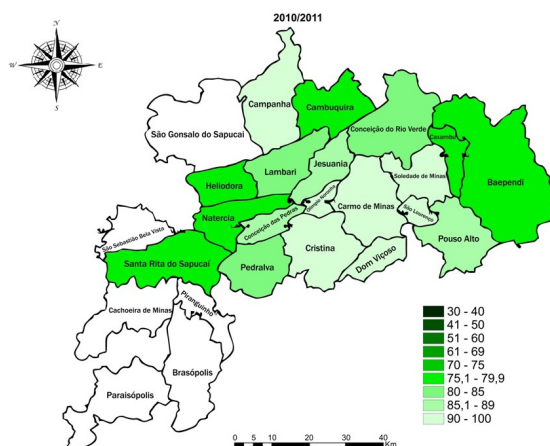


FIGURE 10 - Quality distribution map. Higher scores at 2010/2011 harvest.

In the rest of the region, highest scores values did not exceed 80 points, except for Campanha, which presented scores above 90 points in the 2007/2008 and 2010/2011 harvests.

Scores above 85 points are concentrated at the central-south region, which present the lowest maximum and minimum temperatures. Ribeiro et al. (2016) and Barbosa et al. (2014), while studying the genotype-environment interaction at Carmo de Minas, observed altitude effect on the coffee quality. The Yellow Bourbon genotype, when cultivated above 1200 m, presented a 90 point average grade at sensory quality scores.

These results reveal that the region is stratified according to meteorological characteristics and the coffees at higher and cooler regions present higher scores. Considering the region's great extension, pilot areas were selected to collect meteorological data, according to the altitude.

From the meteorological variables collected at the pilot areas, from July/2011 to June/2013, the air temperature and thermal range were the only ones to present difference between the altitude ranges. The average temperature was 21.6°C below 1000 m, and 19.7°C above 1000 m. The greater differences occurred in the hotter months (November to March), with values ranging from 2.4 to 2.7°C (Figure 11a). The thermal amplitude variation (Figure 11b) was bigger on the altitudes below 1000 m. The greater differences happened in the cooler months (May to September).

The time series subtraction graphs (Figure 12) and correlogram graphs (Figure 13) present

the differences, two by two, between the air temperature and thermal amplitude series, at the three altitude ranges. Figure 12 shows growth on the initial part of the series on A, B, C, D and F graphs, which means there's an upward trend.

Figure 12 presents the subtracted series of temperature (minimum, average and maximum) and thermal amplitude for different altitudes. Every situation presented non-zero values, which indicates that there is a difference between the temperature series for distinct altitudes. It is observed in graph (A), for example, that the first observations present an air temperature inversion for the below 1000 m and between 1000 and 1200 m environments comparison. The difference between the series remained around 2°C until the end of observations. Even though there wasn't temperature inversion in the tested environments from graph (F), the difference between the air temperatures presented a significant increase through the observations, stabilizing around 3°C. Graph (E) on the other hand presented decreasing values for the subtracted series, which shows a downward trend. These trends are confirmed by the correlograms presented on Figure 13. They show the series' non-stationarity, which is an indicative of the existence of a trend.

The presented results confirm classical literature reports regarding lower average temperatures and coffee's sensorial quality improvement (Avelino et al., 2005; Joët et al., 2010; Bertrand et al., 2012; Worku et al., 2018). However, a new fact was observed: higher thermal amplitudes are related to the occurrence of inferior quality coffees.

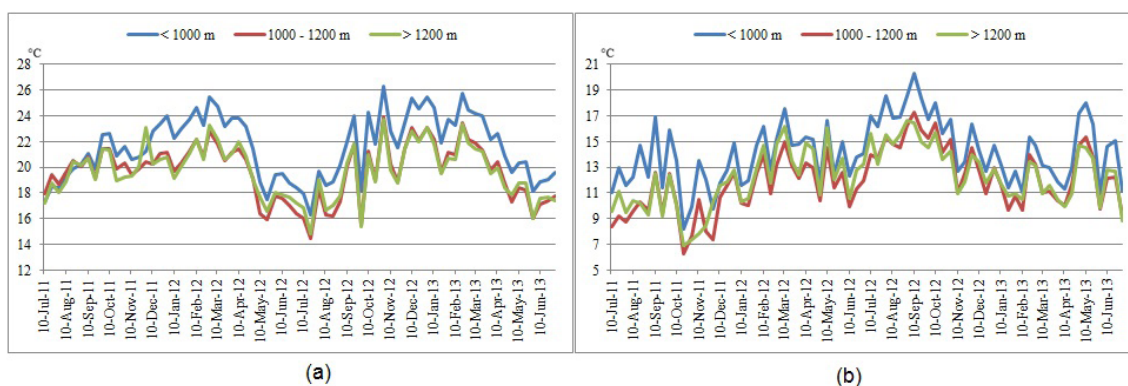


FIGURE 11 - Average temperature (a) and thermal amplitude (b) from July/2011 to June/2013.

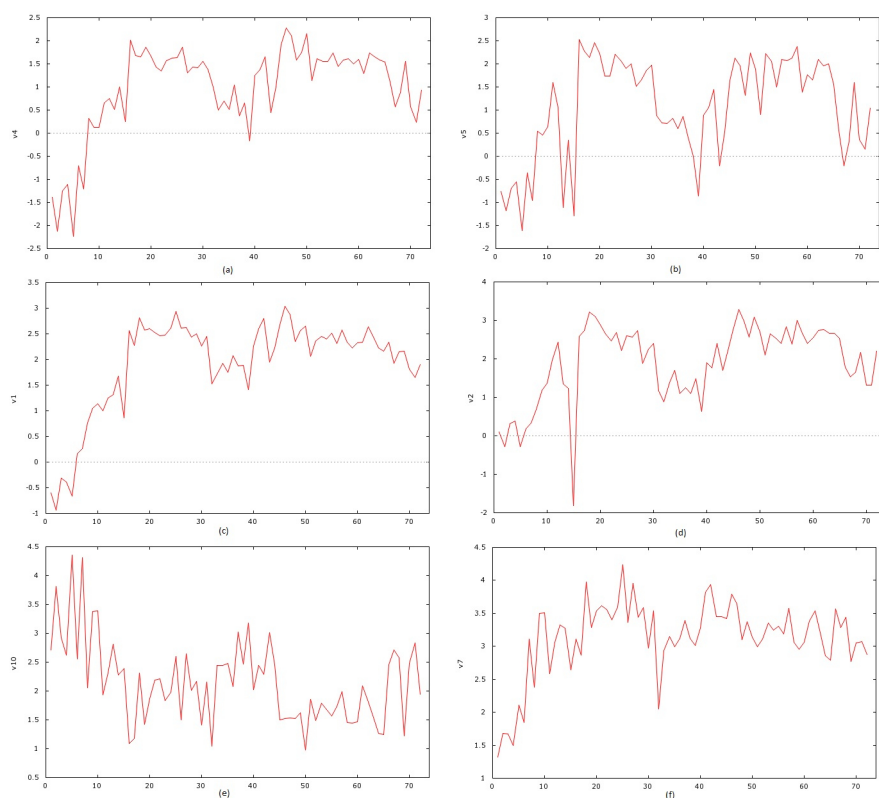


FIGURE 12 - Subtracted series graphs for (A) Minimum Temperature (below 1000 m – between 1000 and 1200 m), (B) Minimum Temperature (below 1000 m – above 1200 m), (C) Average Temperature (below 1000 m – between 1000 and 1200 m), (D) Average Temperature (below 1000 m – above 1200 m), (E) Thermal Amplitude (below 1000 m – between 1000 and 1200 m) e (F) Maximum Temperature (below 1000 m – between 1000 and 1200 m).

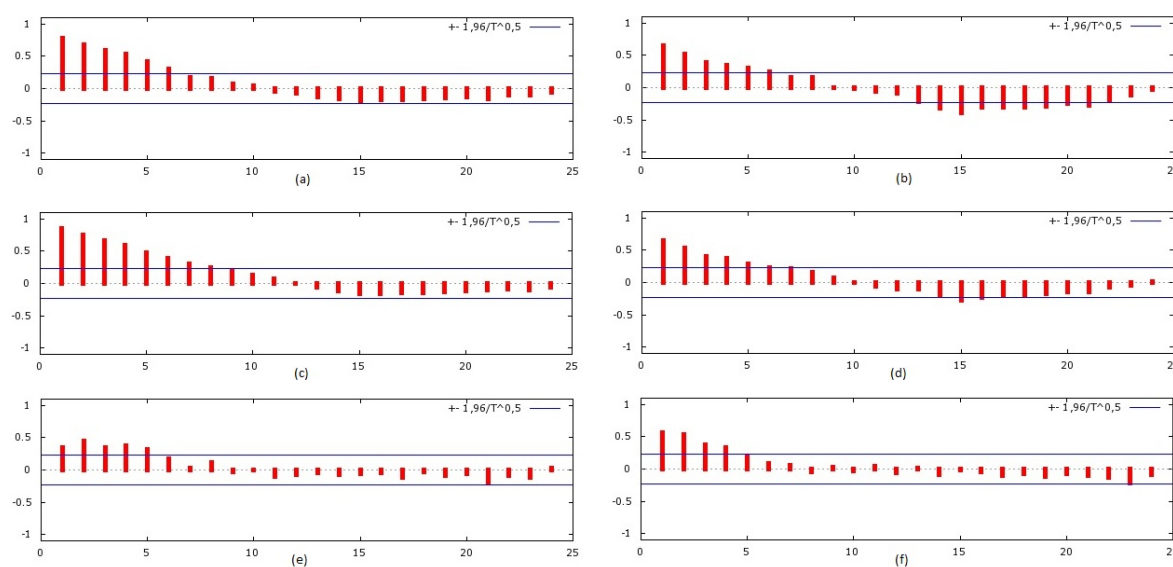


FIGURE 13 - Correlograms for (A) Minimum Temperature (below 1000 m – between 1000 and 1200 m), (B) Minimum Temperature (below 1000 m – above 1200 m), (C) Average Temperature (below 1000 m – between 1000 and 1200 m), (D) Average Temperature (below 1000 m – above 1200 m), (E) Thermal Amplitude (below 1000 m – between 1000 and 1200 m) e (F) Maximum Temperature (below 1000 m – between 1000 and 1200 m).

Many explanations could be related to this fact. Amongst the possibilities, the results obtained by Santos et al. (2018) noted that researches conducted at the same pilot areas, with the objective of studying the ecophysiological seasonal variation of arabica coffee of and metabolic attributes, verified that the altitude was determinant for physiological differences. Also, it was noted that plants located in lower altitudes suffered from a bigger oxidative stress, which decreases the beverage quality.)

4 CONCLUSIONS

Air temperature was the meteorological variable to present the biggest difference between the altitude ranges above and below the 1000 m. The difference was around 2.5°C on the hotter months.

Above 1000 m, the smallest thermal amplitude for the hotter months occurred, which provided superior quality coffees.

The study demonstrates the importance of the meteorological characterization aiming to identify locations with greater vocation to the specialty coffees production.

5 ACKNOWLEDGMENTS

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6 REFERENCES

- ALVES, H. M. R. et al. Geotechnologies for the characterization of specialty coffee environments of Mantiqueira de Minas in Brazil. **The International Archives of the Photogrammetry, Remote Sensing and Spatial Information Sciences**, V. 41-B8, p.797-799. June 2016. XXIII ISPRS Congress, Commission VIII, Prague, Czech Republic, 2016.
- AVELINO, J. et al. Effects of slope exposure, altitude and yield on coffee quality in two altitude terroirs of Costa Rica, Orosi and Santa Maria de Dota. **Journal of the Science of Food and Agriculture**, London, v. 85, n. 11, p. 1869-1876. May. 2005.
- BARBOSA, J. N. et al. Discrimination of production environments of specialty coffees by means of stable isotopes and discriminant model. **Journal of Agricultural Science**, Ottawa, v. 6, p. 55-64. Apr. 2014.
- BERTRAND, B. et al. Climatic factors directly impact the volatile organic compound fingerprint in green Arabica coffee bean as well as coffee beverage quality. **Food Chemistry**, London, v. 135, p. 2575–2583. July 2012.
- BORÉM, F. M. Pós-colheita do café. Lavras: UFLA, 2008. v. 1, p. 631.

- FIGUEIREDO, L. P. et al. The potential for high quality bourbon coffees from different environments. **Journal of Agricultural Science**, Ottawa, v. 5, n. 10, p. 87–98, Sept. 2013.
- GUYOT, B. et al. Influence de l'altitude et de l'ombrage sur la qualité des cafés Arabica. **Plantations Recherche Développement**, Paris, v.3, n. 4, p. 272–280, July – Aug. 1996.
- JOËT T. et al. Influence of environmental factors, wet processing and their interactions on the biochemical composition of green Arabica coffee beans. **Food Chemistry**, v. 118, n. 3, p. 693–701, Feb. 2010.
- MAPA - Ministério da Agricultura Pecuária e Abastecimento, **Sistema de Monitoramento Agrometeorológico**. Available in: <<http://www.agritempo.gov.br/agritempo/jsp/Sumario/index.jsp?siglaUF=MG>>. Access in: Jan. 28. 2014.
- RAMOS M. F. et al. Discrimination of the sensory quality of the Coffea arabica L. (cv. Yellow Bourbon) produced in different altitudes using decision trees obtained by the CHAID method. **Journal of the Science of Food and Agriculture**, London, v. 96, n.10, p 3543-3551, Aug. 2016.
- RIBEIRO, D. E. et al. Interaction of genotype, environment and processing in the chemical composition expression and sensorial quality of Arabica coffee. **African Journal of Agricultural Research**, Nairobi, v. 11, n. 27, p. 2412-2422, July 2016.
- SCAA - Specialty Coffee Association of America. 2009. SCAA Protocols - Cupping
- Specialty Coffee. Long Beach: SCAA. 2009. 7p.
- SANTOS, M. O. et al. Antioxidant system differential regulation is involved in coffee ripening time at different altitudes. **Tropical Plant Biology**, Switzerland, v. 11, n. 3, p. 131–140, June. 2018.
- SILVA, S. de A. et al. Mapping the potential beverage quality of coffee produced in the Zona da Mata, Minas Gerais, Brazil. **Journal of the Science of Food and Agriculture**, London, v. 96, n. 9, p. 3098–3108, Oct. 2015.
- VIANELLO, R.L.; ALVES, A.R. **Meteorologia básica e aplicações**. 2.ed. Viçosa: Ed. da UFV, 2012. 460p.
- WORKUAD, M. et al. Effect of altitude on biochemical composition and quality of green arabica coffee beans can be affected by shade and postharvest processing method. **Food Research International**, London, v. 105, p. 278-285, Mar. 2018.

SUCROSE IN DETOXIFICATION OF COFFEE PLANTS WITH GLYPHOSATE DRIFT

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ABSTRACT: The weed control in coffee plants has great importance, as they compete for light, water and nutrients. The chemical control is the most used, emphasizing the glyphosate, however, when applied, drift can occur and consequently cause injuries to coffee. Many farmers use the sucrose application with the objective to reverse the damage caused by the herbicide drift, even without scientific basis to justify such action. The objective of this study was to evaluate the effect of the sucrose application on the detoxification of coffee plants in the implantation phase with glyphosate drift. The experiment was carried out in a greenhouse, using a randomized block design, arranged in a 3 x 3 factorial scheme with 2 additional treatments, using 3 sucrose doses (2, 4 and 8%) with 3 application times (1, 24 and 168 hours after intoxication with 10% of the commercial glyphosate dose) with an additional one in which the plants were not intoxicated and not treated with sucrose and another only with plants intoxicated by glyphosate. After 75 days performing the experiment, growth, physiological and anatomical characteristics were evaluated. The application of sucrose in the reversal of intoxication of growth variables (height, leaf area number of leaves, shoot dry weight and dry weight of the root system) was not efficient. The objective of this study was to evaluate the effect of the sucrose application on the detoxification of coffee plants in the implantation phase with glyphosate drift. For the physiological variables the application of 2% sucrose, one hour after glyphosate intoxication was the most efficient treatment.

Index terms: *Coffea arabica*, anatomy; physiology, intoxication, herbicide.

SACAROSE NA DESINTOXICAÇÃO DE PLANTAS DE CAFEEIRO COM DERIVA DE GLYPHOSATE

RESUMO: O manejo de plantas daninhas em cafeeiros é de grande importância, pois elas competem por luz, água e nutrientes. O controle químico é o mais utilizado, destacando-se o uso do glyphosate, porém, quando aplicado, pode ocorrer deriva e consequentemente provocar injúrias ao cafeeiro. Muitos cafeicultores utilizam a aplicação de sacarose com o objetivo de reverter os danos causados pela deriva do herbicida, mesmo sem embasamento científico que justifique tal ação. Objetivou-se avaliar o efeito da aplicação de sacarose na desintoxicação de cafeeiros em fase de implantação com deriva de glyphosate. O experimento foi conduzido em casa de vegetação, utilizando-se o delineamento em blocos casualizados, disposto em esquema fatorial 3 x 3 com 2 tratamentos adicionais, em que utilizaram-se 3 doses de sacarose (2, 4 e 8%) com 3 tempos de aplicação (1, 24 e 168 horas após a intoxicação com 10% da dose comercial de glyphosate) com um adicional em que as plantas não foram intoxicadas e não tratadas com sacarose e o outro adicional apenas com plantas intoxicadas por glyphosate. Após 75 dias de condução, avaliaram-se características de crescimento, fisiológicas e anatômicas. A aplicação de sacarose na reversão da intoxicação de variáveis de crescimento (altura, área foliar, Número de folhas, peso seco da parte aérea e peso seco do sistema radicular) não apresentou eficiência. Para as variáveis fisiológicas a aplicação de 2% de sacarose, uma hora após a intoxicação com glyphosate foi o tratamento mais eficiente.

Termos para indexação: *Coffea arabica*, anatomia, fisiologia, intoxicação, herbicida.

1 INTRODUCTION

Coffee cultivation has great importance to Brazil, which is the world's largest producer and exporter of coffee (CONAB, 2018).

The weeds' management in coffee crop is a very important practice, as these can compete for light, water and nutrients and negatively affect vegetative growth (RONCHI; SILVA, 2006).

The first year after planting of the seedlings in the field is characterized as the most critical phase of weed control in the coffee crop (SILVA et al., 2008), for even young plants leave

large areas of soil exposed to light, favoring the growth of weeds. In addition, young coffee plants present slower growth when compared to weeds (SILVA; RONCHI, 2008), favoring competition, being the control of fundamental importance for the good development of the crop.

Among the various weed control methods used in the coffee plant, chemical control with the use of herbicides stands out. This method is one of the most efficient and, in many cases, more economical (YAMASHITA et al., 2008), besides the application practicality, quickness of action and not soil revolving.

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Among the herbicides used to control weeds in coffee plants, glyphosate stands out, which has a systemic action, post-emergence and does not have coffee selectivity (AMARANTE JUNIOR; SANTOS, 2002). However, the glyphosate use, without observing the technology principles of agrochemicals application can cause "drift" and consequently injuries to the coffee. In order to avoid such injuries some coffee growers adopt some management techniques to avoid (directed jet, special nozzles, etc.) or to reverse (use of sugar spraying) the drift effect which, in the case of sugar application, does not scientific basis. The sugar application without scientific basis (doses, forms and times of application) increase the cost of production, and no guarantee of problem resolution.

Carbon is fundamental in the photosynthesis process, more active this process, less prone to intoxication will be the plants, or the sooner they will recover if intoxication has already occurred (TAIZ; ZEIGER, 2013).

Leaf spraying with dilute sugar solution (sucrose), as a source of carbon for plants, makes it possible to acquire carbon from plants through the photosynthetic pathway (SILVA et al., 2003). As this process is easily affected by adverse edaphoclimatic conditions, many technicians and coffee growers believe that the exogenous supply of carbon, via sucrose application, molasses or amino acids, can efficiently supply this element to the plant (Silva et al., 2003).

The work with sucrose application was done in other crops such as the study of the sucrose application role in the tolerance of *Arabidopsis thaliana* seedlings to the herbicide atrazine, in which Ramel et al. (2007) found that sucrose can induce tolerance to various xenobiotics types. In coffee, to date, no conclusive studies have been found indicating induction of tolerance to glyphosate drift, or even greater recovery of the plants with the use of sucrose.

The objective in this study was to evaluate the effect of the sucrose application on the detoxification of coffee plants in the implantation phase with glyphosate drift.

2 MATERIAL AND METHODS

The experiment was carried out in 2015, in a greenhouse, in the municipality of Lavras-MG. *Coffea arabica* plants of the cultivar Catuaí IAC 99 were used. At the six pairs of leaves stage, completely expanded, it was realized

the transplanting of coffee seedlings into pots containing 11 Liters of sieved and previously corrected soil was carried out in order to raise the base saturation to 60% (RIBEIRO et al., 1999). The soil used in the experiment was classified as a clayey, dystrophic dark red Latosol (EMBRAPA, 2006).

Fertilization planting and coverage were carried out according to the recommendations by Ribeiro et al. (1999). During conducting the experiment weed control was performed manually and the soil was maintained at field capacity.

The experiment consisted of 11 treatments arranged in a $3 \times 3 + 2$ factorial scheme, in a randomized block design, with four replicates. The treatments were consisted of the combinations between the sucrose levels (2, 4 and 8%, equivalent to 20, 40 and 80 g L⁻¹) and sucrose application time (1, 24 and 168 hours after a simulation of the glyphosate drift) and more two additional treatments, in which one was applied pure water (standard) and in the other was made the simulation of the glyphosate drift (intoxicated seedlings). Each plot consisted of one plant.

At 56 days after transplanting seedlings, the glyphosate drift was simulated using the original Roundup® commercial formulation, using a costal pressurized sprayer CO₂, calibrated at a constant pressure of 250 kPa, fitted with a bar and a fan-type nozzles (TT 11002), which allowed the application 200 L ha⁻¹ of syrup on the plants. To simulate drift, it was used 10% (144 ml) of the recommended glyphosate dose 1440 ml ha⁻¹.

For the sucrose application, the same sprayer was used in the glyphosate application, but adjusted to apply a syrup volume 400 L ha⁻¹.

All the evaluations were carried out 75 days after the treatments application and checking plant height (cm); number of leaves and leaf area (cm²) (CUNHA et al., 2010). Physiological evaluations were also carried out using a Portable Photosynthesis System (IRGA LICOR – 6400/XT), quantifying the photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), a stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) and the transpiration rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). For these evaluations, completely expanded leaves of plagiotropic branches of the middle third of the plants, located at the third node, were used from the branch apex.

For the anatomical studies, a completely expanded leaf was collected from the third node of plagiotropic branches, from the middle third of the plants. The leaves collected from each plant

were placed in a container containing 70% ethanol solution ($v\ v^{-1}$) to fix the plant material. After 72 hours the solution was renewed for preservation of the material. In the laboratory, two blades were assembled for each collected leaf, one blade with cross sections and another blade with paradermal sections.

The cross sections were obtained in a LPC-type tabletop microtome and the free-hand paradermal sections using a steel blade followed by the methodology proposed by Kraus; Arduin (1997) until the final preparation of the blade.

The blades were observed and photographed under an Olympus BX 60 model optical microscope coupled to the Canon A630 digital camera. The images were analyzed in UTHSCSA-Imagetool image analysis software. The diameter (μm) of the phloem was evaluated.

At the end of the experiment the plants were sectioned in the colon region, separating them in aerial part and root. Then, these were washed in running water and after in distilled water and placed in paper bags to be dried in an oven with forced air circulation at 65 °C until constant weight. After drying, each sample was weighed on a precision balance, determining the shoot dry weight and the root system (g).

For the statistical analysis, linear models were fitted to the data. After adjustment, the variance analysis was carried out, in which the effects of sucrose factors, application time and interaction were tested by the F test. The comparisons among the additional treatments with the treatments of the factorial part were made by means of orthogonal contrasts, which were also tested by the F test.

For the models and the contrasts adjustment, the function *aov* () of the *stats* library was used. The variance analysis was performed by the function *aov* (), also present in the *stats* library. In the response variables in which some of the factors had a significant effect ($p\text{-value} > 0.05$), the average study was done by comparing the confidence intervals at the 95% level for the adjusted averages by means of the *lsmeans* function () of the *lsmeans* library (LENTH; WEISBERG, 2016).

All the procedures to carry out analyzes were done by software R version 3.2.3 (R CORE TEAM, 2016).

3 RESULTS AND DISCUSSION

Plants that were intoxicated with glyphosate did not show visible and pronounced symptoms of intoxication 75 days after glyphosate

application. There was an interaction effect (Time x Sucrose) for the variables stem diameter, photosynthetic rate, stomatal conductance and transpiration rate, in function of time and sucrose dose (%) applied.

For plant height, number of leaves, leaf area, dry shoot mass, dry root mass and phloem thickness were significant effect ($p > 0.05$) only for sucrose factor. However, for the variables number of plagiotropic branches, stomatal density, abaxial and adaxial epidermal thickness, mesophyll, number of xylem vessels, root system volume and root surface area, there was no significant effect ($p < 0.05$) of the time and sucrose factors.

Coffee plants that were not intoxicated and received only water (pattern) were superior in height, number of leaves, leaf area and shoot dry mass compared to coffee plants that were intoxicated with no sucrose treatment (intoxicated) (Tables 1 and 2).

This result evidences the negative effects caused by glyphosate intoxication in coffee plants (FRANÇA et al., 2010a; FRANÇA et al., 2010b; FRANÇA et al., 2013).

Also in relation to the variables mentioned above, coffee plants with standard treatment were superior to the intoxicated coffee plants that received the application of sucrose at the dose 2%. It was also observed that coffee plants intoxicated and with no sucrose treatment did not present differences of the intoxicated coffee plants that received sucrose spraying, regardless of the applied dose. On the other hand, the supply of 4 and 8% of sucrose in coffee plants intoxicated with glyphosate resulted in an equivalent growth of plants that were not intoxicated in height, number of leaves and leaf area (Table 1).

Thirty-five percent of the dry matters of the plants on the planet are represented by derivatives of the shikimate pathway (FRANZ, MAO; SIKORSKI, 1997). As glyphosate-intoxicated plants have the shikimate pathway interrupted by the action of glyphosate (VELINE et al., 2008), it is explained the lower value of dry mass of coffee plants under glyphosate effect observed in the present study.

For the dry mass of the root system (Table 2), it was observed that there was no difference among the coffee plants from the standard treatment and the coffee plants intoxicated and not treated with sucrose, demonstrating that the intoxication with glyphosate at 10% dose did not cause negative effects to the coffee root system.

TABLE 1 - Height (cm), number of leaves, leaf area (cm²) of coffee plants intoxicated with glyphosate in function of sucrose dose (%) applied

Treatments	Height	Number of leaves	Leaf area
2	44.90 (43.30-46.50) b	40.67 (35.51-45.82) b	465.80 (393.00-538.60) b
4	45.40 (43.8-47.0) ab	46.17 (41.01-51.32) ab	547.90 (475.10-620.80) ab
8	46.51 (44.91-48.11) ab	47.67 (42.51-52.82) ab	543.70 (470.00-616.60) ab
Intoxicated	44.30 (41.53-47.07) b	36.75 (27.83-45.67) b	411.70 (285.50-537.80) b
Pattern	49.60 (46.83-52.37) a	56.50 (47.58-65.42) a	745.80 (619.60-872.00) a
VC (%)	5.88	17.59	23.38

*For each variable, average followed by the same lowercase letter, and by the same capital letter in the line, do not differ from each other by the overlapping confidence intervals system ($p < 0.05$).

TABLE 2 - Shoot dry weight (g) and root system dry weight (g) of coffee plants intoxicated with glyphosate in function of sucrose dose (%) applied.

Treatments	Shoot dry weight	Root system dry weight
2	17.4 (15.7-19.09) b	6.28 (5.42-7.14) b
4	19.12 (17.43-20.82) b	5.83 (4.97-6.69) b
8	18.85 (17.15-20.54) b	6.46 (5.60-7.32) ab
Intoxicated	16.18 (13.24-19.11) b	6.98 (5.49-8.48) ab
Pattern	25.28 (22.43-28.21) a	8.71 (7.21-10.2) a
VC (%)	14.88	21.81

*For each variable, average followed by the same lowercase letter, and by the same capital letter in the line, do not differ from each other by the overlapping confidence intervals system ($p < 0.05$).

Also Velini et al. (2008) observed no negative effects on the corn root system, conventional soybeans, *Eucalyptos grandis*, *Commelina benghalensis* and *Pinus caribaea*, when glyphosate was applied, possibly because of the slower effects on this part of the plants. Wagner Júnior et al. (2008) also reported that doses up to 345.6 g ha⁻¹ of glyphosate applied on yellow passion fruit did not promote root length reduction at 28 days after application.

Intoxicated coffee plants that received 2% sucrose one hour after glyphosate intoxication had higher photosynthetic rates and stomatal conductance when compared to coffee plants that were intoxicated with no sucrose treatment (Tables 3, 4 and 5).

Also, Martin (2003) concluded that the coffee plants spraying with 2% sugar, a week after glyphosate drift was efficient in the reversion process of intoxication by this herbicide. In the present work the higher photosynthetic efficiency observed in plants treated with 2% sucrose after one hour of glyphosate intoxication may have been caused by adaptive defense mechanisms.

It is known that stress caused by xenobiotics, such as glyphosate, causes oxidative stress in plants (RAMEL et al., 2009a). The sucrose application in plants of *Arabidopsis thaliana* intoxicated with atrazine was efficient to trigger defense mechanisms, activating antioxidant systems and altering the levels of reactive oxygen species (RAMEL et al., 2009a, b).

For the phloem thickness (Table 6) there was no sucrose effect, and the standard coffee plants showed no difference in relation to the intoxicated coffee plants and without sucrose. The leaves phloem thickness of intoxicated coffee plants that received application with 2% and 4% of sucrose was higher than those that received 8%.

These results confirm the anatomical modification for this characteristic among the plants intoxicated and treated with different sucrose dosages. It is through the phloem vessels that photo-assimilates translocation occurs, such as sucrose, produced during the photosynthetic process (GRIFFITHS et al., 2016).

TABLE 3 - Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) of coffee plants intoxicated with glyphosate in function of time and sucrose dose (%) applied.

Treatments	Application time (hours)		
	1	24	168
2	7.34 (6.01-8.68) aA	4.96 (3.63-6.30) aAB	4.26 (2.93-5.6) aB
4	5.94 (4.61-7.28) abA	4.94 (3.29-5.96) aA	4.63 (3.60-6.27) aA
8	4.24 (2.91-5.58) abA	6.28 (4.94-7.61) aA	4.98 (3.65-6.32) aA
Intoxicated	4.41 (3.08-5.75) b	4.41 (3.08-5.75) a	4.41 (3.08-5.75) a
Pattern	4.73 (3.40-6.07) ab	4.73 (3.40-6.07) a	4.73 (3.40-6.07) a
VC (%)	25.23		

*For each variable, average followed by the same lowercase letter, and by the same capital letter in the line, do not differ from each other by the overlapping confidence intervals system ($p < 0.05$).

TABLE 4 - Stomatal conductance ($\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of coffee plants intoxicated with glyphosate in function of time and sucrose dose (%) applied.

Treatments	Application time (hours)		
	1	24	168
2	0.087 (0.067-0.107) aA	0.045 (0.024-0.065) aB	0.040 (0.019-0.060) aB
4	0.055 (0.034-0.075) abA	0.040 (0.019-0.060) aA	0.052 (0.032-0.072) aA
8	0.040 (0.019-0.060) bA	0.070 (0.049-0.090) aA	0.052 (0.032-0.072) aA
Intoxicated	0.037 (0.017-0.057) b	0.037 (0.017-0.057) a	0.037 (0.017-0.057) a
Pattern	0.037 (0.017-0.057) b	0.037(0.017-0.057) a	0.037 (0.017-0.057) a
VC (%)	38.1		

*For each variable, average followed by the same lowercase letter, and by the same capital letter in the line, do not differ from each other by the overlapping confidence intervals system ($p < 0.05$).

TABLE 5 - Transpiratory rate ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) of coffee plants intoxicated with glyphosate in function of time and sucrose dose (%) applied.

Treatments	Application time (hours)		
	1	24	168
2	1.370 (1.123-1.617) aA	0.767 (0.502-1.015) abB	0.902 (0.655-1.150) aAB
4	0.920 (0.767-1.157) abA	0.820 (0.572-1.067) abA	0.925 (0.677-1.172) aA
8	0.897 (0.6501-1.145) bA	1.207 (0.9601-1.455) aA	1.047 (0.800-1.295) aA
Intoxicated	0.637 (0.390-0.8849) ab	0.637 (0.390-0.8849) b	0.637 (0.390-0.8849) a
Pattern	0.777 (0.5003-1.2025) b	0.777 (0.5003-1.2025) ab	0.777 (0.5003-1.2025) a
VC (%)	26.45		

*For each variable, average followed by the same lowercase letter, and by the same capital letter in the line, do not differ from each other by the overlapping confidence intervals system ($p < 0.05$).

TABLE 6 - Diameter (μm) of phloem vessels of coffee leaves intoxicated with glyphosate in function of sucrose dose (%) applied.

Treatments	Diameter (μm) of phloem vessels
2	39.43 (37.93-40.94) a
4	38.90 (37.39-40.40) a
8	35.37 (33.87-36.88) b
Intoxicated	38.11 (35.5-40.72) ab
Pattern	36.3 (33.69-38.91) ab
VC (%)	7.37

*For each variable, average followed by the same lowercase letter, and by the same capital letter in the line, do not differ from each other by the overlapping confidence intervals system ($p < 0.05$).

In the present work it is noticed that application of 2% sucrose in coffee plants intoxicated with glyphosate presented positive results for photosynthetic and transpiration rate and also for leaf phloem thickness. It is important to emphasize that the evaluations were carried out 75 days after treatments application, but the effects can be even more severe, harming the plants growth over time.

4 CONCLUSIONS

The application of sucrose in the reversal of intoxication of growth variables (height, leaf area, number of leaves, shoot dry weight and dry weight of the root system) was not efficient.

For the physiological variables the application of 2% sucrose, one hour after glyphosate intoxication was the most efficient treatment.

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6 REFERENCES

AMARANTE JUNIOR, O. P. et al. Glifosato: propriedades, toxicidade, usos e legislação. **Química Nova**. São Paulo-SP, vol.25, n.4, pp.589-593, 2002.

CASTRO, E. M., PEREIRA, F. J.; PAIVA, R. **Histologia vegetal: estrutura e função de órgãos vegetativos**. Lavras: Ed. UFLA, 2009.

COMPANHIA NACIONAL DE ABASTECIMENTO - CONAB. **Acompanhamento da safra brasileira**. 2016. Disponível em: <<http://www.conab.gov.br>>. Acesso em: 02 out. 2018.

CUNHA, J. L. X. L. et al. Comparação de métodos de área foliar em *Chrysobalanus icaco* L. **Agropecuária Científica no Semiárido**, Campina Grande, PB, v. 6, p. 22-27, 2010.

EMBRAPA. **Sistema brasileiro de classificação de solos**. 2. ed. Rio de Janeiro: Embrapa Solos, 2006.

FRANÇA, A. C. et al. Crescimento de cultivares de café arábica submetidos a doses do glyphosate. **Planta Daninha**, Viçosa, MG, v. 28, p. 599-607, 2010b.

FRANÇA, A. C. et al. Teores de nutrientes em cultivares de café arábica submetidos à deriva de glyphosate. **Planta Daninha**, Viçosa, MG, v. 24, p. 877-885, 2010b.

FRANÇA, A.C. et al. Deriva simulada do glyphosate em cultivares de café Acaiá e Catucaí. **Planta Daninha**, Viçosa, MG, v. 31, p. 443-451, 2013.

FRANZ, J. E., MAO, M. K.; SIKORSKI, J. A. **Glyphosate: a unique global herbicide**. Washington: American Chemical Society, 1997.

GRIFFITHS, C. A., MATTHEW, P. J.; CHRISTINE, F. H. Metabolite transport and associated sugar signalling systems underpinning source/sink interactions. **Biochimica e Biophysica Acta**, v. 1857, p. 1715-1725, 2016.

JORGE, L. A. C.; RODRIGUES, A. F. O. (2008). **Safira: sistema de análise de fibras e raízes**. **Boletim de Pesquisa e Desenvolvimento da Embrapa Instrumentação Agropecuária**, São Carlos, 24, 21.

RAMEL, F. et al. Natural variation reveals relationships between pre-stress carbohydrate nutritional status and subsequent responses to xenobiotic and oxidative stress in *Arabidopsis thaliana*. **Annals of botany**, v. 104, p. 1323-1337, 2009a.

- RAMEL, F. et al. Differential patterns of reactive oxygen species and antioxidative mechanisms during atrazine injury and sucrose-induced tolerance in *Arabidopsis thaliana* plantlets. **BMC Plant Biology**, v. 9, p. 1, 2009b
- RAMEL, F. et al. Genome-wide interacting effects of sucrose and herbicide-mediated stress in *Arabidopsis thaliana*: novel insights into atrazine toxicity and sucrose-induced tolerance. **BMC genomics**, v. 8, p. 1, 2007.
- RIBEIRO, A. C., GUIMARÃES, P. T. G.; ALVAREZ VIEGAS, V. H. (Ed.). **Recomendações para o uso de corretivos e fertilizantes em Minas Gerais: 5ª aproximação**. Viçosa, MG: Comissão de Fertilidade do Solo do Estado de Minas Gerais, p. 25-32, 1999.
- KRAUS, J. E.; ARDUIN, M. **Manual básico de métodos em morfologia vegetal**. Rio de Janeiro: EDUR, 1997.
- LENTH, J.; WEISBERG, S. **An R companion to applied regression**. 2nd ed. Washington: Sage, 2016.
- MARTIN, S. A. (2003). **Pulverização do cafeeiro com açúcar: potencial de uso em mudas submetidas à deficiência hídrica e na recuperação de plantas atingidas por glyphosate**. 67, Dissertação (Mestrado em Fisiologia Vegetal) -Universidade Federal de Lavras, 2003.
- R DEVELOPMENT CORE TEAM. **R: a language and environment for statistical computing**. Vienna: R Foundation for Statistical Computing, Disponível em: <<http://www.R-project.org>>. Acesso em: 10 Agosto, 2018.
- RONCHI, C. P.; SILVA, A. A. Effects of weed species competition on the growth of young coffee plants. **Planta Daninha**, Viçosa, MG, v. 24, p. 415-423, 2006.
- SILVA, A. A.; RONCHI, C. P. **Manejo e controle de plantas daninhas em café**. In: Vargas, L & Roman, E. S. (Org.). Manual de manejo e controle de plantas daninhas. Passo Fundo: Embrapa Trigo, p. 417-475, 2008.
- SILVA, A. C. et al. Produtividade e potencial hídrico foliar do cafeeiro 'Catuai' em função da época de irrigação. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande PB. v. 12, p. 21-25, 2008.
- SILVA, J. C. et al. D. Invertase and sucrose synthase activities in coffee plants sprayed with sucrose solution. **Sci. agric. Piracicaba, Braz**, Piracicaba, SP, v. 60, p. 239-244, 2003.
- TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 5. ed. Porto Alegre: Artmed, 2013. 954 p.
- VELINE, E. D. et al. Glyphosate. Botucatu: FEPAF, 2009.
- VELINI, E. D. et al. applied at low doses can stimulate plant growth. *Pest Management Science*, London. v. 64, 489-496, 2008.
- WAGNER JÚNIOR, A. et al. Deriva simulada de formulações comerciais de glyphosate sobre maracujazeiro-amarelo. **Planta Daninha**, Viçosa, MG, v. 26, p. 677-683, 2008.
- YAMASHITA, O. M. et al. Efeito de doses reduzidas de oxyfluorfen em cultivares de algodoeiro. **Planta Daninha**, Viçosa, MG, v.26, p. 917-921, 2008.

NITROGEN FERTILIZERS TECHNOLOGIES FOR COFFEE PLANTS

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ABSTRACT: The application of urea in coffee crop has caused high losses of nitrogen (N) by volatilization, causing low use and recovery of this nutrient. This low use of N may interfere in the growth and nutrition of the coffee crop and thus, influence the efficiency of the nitrogen fertilization. The aim in this work was to assess the growth, nutritional and physiological characteristics and the agronomic efficiency of the nitrogen fertilization with different nitrogen fertilizer technologies. The experiment was performed in a greenhouse in pots with a volume of 14 L. In each pot, it was performed the transplanting of two plants of coffee. The experimental design was entirely randomized, with four replicates. The following nitrogen fertilizers were applied in the dose of 10g pot⁻¹, divided into three applications with interval of 50 days: Conventional urea; ammonium nitrate; urea + formaldehyde; Polyblen Extend[®]; Polyblen Montanha[®]; Urea + polyurethane; urea + plastic resin; ammonium sulfate + CaCO₃ and the control, without N application. After cropping, it was evaluated the growth, nutritional and physiological parameters. Afterward, it was calculated two agronomic efficiency index of the nitrogen fertilization. The highest values of plants height, the total dry mass of plants and leaf area in coffee seedlings were found with the application of Polyblen Extend[®]. This blend also provided higher accumulation of N in the leaf and the whole plant. The application of Polyblen Extend[®] provided higher agronomic efficiency and also enhanced the photosynthetic rate in the coffee plants.

Index terms: *Coffea arabica*, agronomic efficiency, nitrogen fertilization.

TECNOLOGIAS DE FERTILIZANTES NITROGENADOS PARA PLANTAS DE CAFÉ

RESUMO: O baixo aproveitamento do N pode interferir no crescimento e na nutrição do cafeeiro e com isso influenciar a eficiência da adubação nitrogenada. Neste sentido, objetivou-se com este trabalho avaliar as características de crescimento, nutricionais, fisiológicas e a eficiência agrônômica da adubação nitrogenada com diferentes tecnologias de fertilizantes nitrogenados. O experimento foi realizado em casa de vegetação em vasos com volume de 14 L. Em cada vaso foi realizado o transplante de duas mudas de café. O delineamento foi inteiramente ao acaso, com quatro repetições. Os seguintes fertilizantes nitrogenados foram aplicados na dose de 10 g vaso⁻¹ de N, parceladas em três aplicações com intervalo de 50 dias. As fontes de N foram: Ureia convencional; Nitrato de Amônio; Ureia + formaldeído; Polyblen Extend[®]; Polyblen Montanha[®]; Ureia + Poliuretano; Ureia + resina plástica; Sulfato de Amônio + CaCO₃ e o controle, sem aplicação de N. Após o cultivo, foram avaliados os parâmetros de crescimento, nutricionais e fisiológicos nas plantas de café. Posteriormente foram calculados dois índices de eficiência agrônômica da adubação nitrogenada. Os maiores valores de altura de plantas, massa seca total de plantas e área foliar foram encontrados com a aplicação do Polyblen Extend[®]. Este Blend também proporcionou maior acúmulo de N na folha e na planta inteira. A aplicação do Polyblen Extend[®] promoveu maior eficiência agrônômica e aumentou a taxa fotossintética no cafeeiro.

Termos para indexação: *Coffea arabica*, eficiência agrônômica, adubação nitrogenada.

1 INTRODUCTION

Among the nitrogen fertilizers most used in coffee cultivation, urea is the most prominent. The use of this fertilizer has advantages such as lower cost per unit of N, high N concentration, low production cost, low corrosivity and lower acidifying power compared to other N sources (MARCHESAN et al., 2011).

However, the reaction of urea's hydrolysis in the soil causes an increase of the pH in the region of the granule (DOMINGHETTI et al., 2016). This alkaline pH environment hinders the passage of ammonia (NH₃) to ammonium (NH₄⁺) by the absence of protons (H⁺ ions), which

concentrate ammonia in this region and increase the loss by volatilization of N-NH₃ (CHAGAS et al., 2016). These losses contribute to a decrease in nitrogen fertilizer efficiency and can reach values up to 40% of the total N that is applied (FARIA et al., 2014).

Currently, one of the most studied practices to improve the efficiency of nitrogen fertilizers is the use of increased efficiency's fertilizers, classified as stabilized, slow release and controlled release (AZEEM et al., 2014; CHIEN et al., 2009).

Stabilized fertilizers are those having the addition of urease inhibitors and/or nitrification. Among the stabilized group, NBPT [N-(n-butyl)] thiophosphorictriamide is the most widely used

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urease inhibitor worldwide (SANZ-COBENA et al., 2012). However, there is still a need for more studies, especially in regions with a tropical climate, such as Brazil. This is justified because the NBPT urease inhibitory activity may decrease with increasing soil temperature (ABALOS et al., 2014).

Slow-release nitrogen fertilizers are products of the condensation of urea with aldehydes. Among the most commonly used is formaldehyde urea (GUELF, 2017). These fertilizers promote the reduction of the solubility of the N fractions present in the composition of the urea molecule (GUELF, 2017).

On the other hand, adding compounds that cover the fertilizer granule produce the controlled release fertilizers which reduce direct exposure to water and air, and slowly release the nutrient into the soil solution (TRENKEL, 2010). In this group, are highlighted those coated with sulfur, sulfur and polymers or only by the polymer (DOMINGHETTI et al., 2016).

Several authors studied these fertilizers, such as Pereira et al. (2009) and Frazão et al. (2014) in the maize cultivation and Garcia et al. (2011) in coffee. Pereira et al. (2009) and Frazão et al. (2014) found higher yields of maize with the application of Urea+NBPT compared to conventional urea application. Garcia et al. (2011), when evaluating the dry mass of coffee after 285 days of conventional urea and NBPT application, found a higher dry mass with Urea+NBPT application.

However, the factor that most limits the use of these fertilizers is the cost, which is higher when compared to conventional nitrogen fertilizers (GUELF, 2017). An alternative to reduce the costs of these fertilizers is the use of "Blends" (a physical mixture of fertilizers). With the use of "Blends", it is sought to use these technologies in an integrated way with conventional fertilizers and with this, to reduce costs compared to the exclusive use of fertilizers of increased efficiency (TRENKEL, 2010). In addition, can be created an adjustment of the N-release curve for each cultivation. According to Noellsch et al. (2009), the conventional fertilizer (with high solubility in water) can provide the initial demand for N by the cultivation and the controlled fertilizer release over a longer period.

In this context, the aim of this work was to assess the growth, nutritional and physiological characteristics and the agronomic efficiency of the nitrogen fertilization with different nitrogen fertilizer technologies.

2 MATERIAL AND METHODS

The experiment was carried out in a greenhouse of the Department of Soil Science of the Federal University of Lavras (UFLA), in Lavras city, Minas Gerais, from June 2015 to January 2016. It was used a soil of clay texture, classified as Red Latosol (RL) (SANTOS et al., 2013) collected in the B horizon, in Lavras. The collected soil was air-dried, passed in a sieve with a 4 mm opening, homogenized and placed in the pots. Concomitantly, soil samples were collected and used for chemical and physical characterization (Table 1), according to Comissão de Fertilidade do Solo do Estado de Minas Gerais - CFSEMG (1999).

Soil pH was determined in water, in the soil: water ratio of 1: 2,5; H+Al (Method Ca (OAc)₂ at concentration 0,5 mol L⁻¹, pH 7,0; Ca²⁺, Mg²⁺ and Al³⁺ exchangeable, KCl were extracted with 1 mol L⁻¹ and determined by titulometry; P and K were extracted by Mehlich-1 and analyzed by colorimetry (P) and flame photometry (K); the organic carbon was determined by oxidation with potassium dichromate; Zn, Mn, and Cu were extracted by Mehlich-1 and determined by atomic absorption spectrophotometry. The values of effective CTC (t); CTC at pH 7.0 (T), the sum of bases (SB) and percentages of saturation by bases (V%) and by aluminum (m%), were obtained indirectly, using the values of potential acidity, exchangeable bases, and exchangeable aluminum (CFSEMG, 1999).

It was used a completely randomized design with treatments composed of nitrogen fertilizers: Conventional urea, Ammonium Nitrate, Urea + Formaldehyde, Polyblen Extend®, PolyblenMontanha®, Urea + Polyurethane, Urea + Plastic Resin, Ammonium Sulfate + CaCO₃, and the Treatment control (without the addition of N) with four replicates.

Fertilizers' characteristics are:

- 1) Conventional urea – Granular with 45% of N;
- 2) Ammonium Nitrate – Granular with 30% of N;
- 3) Urea + formaldehyde – It has 26% of N and is obtained by reaction between formaldehyde (H₂CO) molecules with large amounts of urea, under controlled manufacturing conditions, resulting in a mixture of methylated urea chains of different sizes, gradually released to the soil by the action of microorganisms that decompose the chain.

TABLE 1 - Chemical and granulometric characteristics of the B horizon of the Red Latosol.

Chemical analysis																	
pH	P	K	Zn	Cu	Mn	B	Fe	Ca ²⁺	Mg ²⁺	Al ³⁺	(H+Al)	t	T	m	SB	MO	Prem
mg dm ⁻³								cmol _c dm ⁻³				—%—		g kg ⁻¹		mg L ⁻¹	
5,0	0,8	10	0,5	2,2	4,0	0,0	25,1	0,9	0,1	0,1	4,0	1,1	5,1	9,0	20,2	1,6	2,6
Physical Analysis																	
Silt						Clay						Sand					
g kg ⁻¹																	
140						670						190					

pH_{H2O} (ratio 1:2.5); P, K, Zn, Mn e Cu: Mehlich-1 Extractor; P-remaining: Alvarez et al. (1999); Ca²⁺, Mg²⁺, Al³⁺: KCl 1 mol L⁻¹ Extractor; B: hot water (H+Al): SMP Extractor; M.O.: oxidation Na₂Cr₂O₇ 0.67 mol L⁻¹ + H₂SO₄ 5 mol L⁻¹; Sand, silt, clay: pipette method.

4) Polyblen Extend®–Sulfur-coated (S) urea Blends + polymers (70%) and conventional urea + NBPT (30%): Urea coated with an elemental sulfur layer plus an organic polymer and granular urea + NBPT (uncoated). The total nitrogen content is 39%, with 12% elemental sulfur.

5) Polyblen Montanha®–Sulfur-coated (S) urea Blends + polymers (70%) and conventional urea + NBPT (30%). Urea coated with elemental sulfur plus an organic polymer and granular urea + NBPT (uncoated). The total of N content is 39%, with 12% elemental sulfur. Polyblen Montanha® differs from Polyblen Extend® because it has a greater thickness of the coating layer (CHAGAS et al., 2016).

6) Urea + Polyurethane – It has 40% of N and 2% of K₂O. Polyurethane coats the urea.

7) Urea + plastic resin – It has 39% of N. The urea is coated by plastic resin, which allows the contact of water with urea according to its degradation by the action of temperature and soil moisture.

8) Ammonium Sulfate + CaCO₃ – Contains 29% of N, 7% of S, 5% of Ca and 2% of Mg. Contains N in the amide and ammoniacal forms associated with marine calcium carbonate (CaCO₃).

The experimental unit was formed by pot filled with 14kg of soil and two coffee seedlings (*Coffea arabica* L., Cultivar Acaia IAC 474-19), produced from sowing in the washed sand and sieved with four months of age (after present the second pair of true leaves).

Before the transplanting of the seedlings, liming was performed to raise the saturation by bases to 70% (CFSEMG, 1999). The limestone

used was formed by the mixture of calcium carbonate and magnesium carbonate (P.A), passing through a period of incubation of 30 days. 4,56g pot⁻¹ of calcium and 1,11g pot⁻¹ of magnesium were applied.

Subsequently, fertilization with 20g of P₂O₅ + 6,72g of K₂O pot⁻¹ according to Chagas et al (2016), using triple superphosphate and potassium chloride as sources, was carried out which were homogenized to the soil on 06/22/2015. After that, the coffee plants were transplanted (two plants per pot), with pruning of the apical region of the root system.

The dose of N applied for each fertilizer was 10 g pot⁻¹ of N, according to Chagas et al (2016), divided into three applications with an interval of 50 days. The first application was performed after 40 days of transplanting.

During the whole period of the experiment, the soil moisture was maintained at 60% of the total pore volume (TPV), by weighing the pot and adding deionized water. At 60 days after transplanting, foliar fertilization with 0,3% of B and 0,3% of Zn applied in the form of boric acid and zinc sulfate, as recommended by Garcia (2011).

At the end of the experiment (six months after transplanting), were evaluated height (PH) and plant diameter (PD). The photosynthetic rate was measured on the day of the experiment, in the morning between 9:00 am and 11:00 am, in fully expanded leaves of the middle third of the plants, with the aid of Infra-Red Gas Analyzer (IRGA) model 266 LI6400-XT.

The relative chlorophyll index (RCI) was also calculated on the day of the experiment,

by reading with the chlorophyll meter SPAD-502 (Soil and Plant Analysis Development). The reading was performed in the middle third of the leaf, sampled in the middle part of the plant. Four readings per plant were carried out in the two experimental plot plants. Before carrying out the readings, the reading tester calibrated the instrument, according to the recommendations in the manual.

After these evaluations, the leaves were removed for determination of the leaf area (LA) with the aid of the Li-color leaf area integrator, model LI 3100.

Afterward, the plants were removed from the pots and, with the help of tap water, the shoot and roots were washed in sieves and then packed in paper bags and dried at 75°C in a forced circulation oven until constant weight to determine the weight of the dry mass. Then, the dry mass was ground, and samples equivalent to two grams were removed, which were submitted to nitroperchloric digestion followed by determination of the N content, according to Tedesco et al. (1995). The product between the dry weight and the N content in each part of the coffee plants determined the N accumulation.

After obtaining these data, the following efficiency indices of the nitrogen fertilization were calculated:

a) Relative agronomic efficiency index (RAEI) = $[(\text{total dry mass of the source tested, g pot}^{-1}) - (\text{total dry mass of control, g pot}^{-1})] / (\text{total dry mass of urea, g pot}^{-1}) - (\text{total dry mass of control, g pot}^{-1})] \times 100$.

b) Agronomic Efficiency of Applied N (AE) = $(\text{total dry mass of the source tested, g pot}^{-1}) - (\text{total dry mass of control, g pot}^{-1}) / \text{Dose of N applied, in g pot}^{-1}$ (FAGERIA; SANTOS; MORAES, 2010).

All data were submitted to analysis of variance (ANOVA) and means were compared by the Skott Knott test ($\alpha = 0,05$). ANOVA was performed after checking for normality (Shapiro-Wilk's test) and homogeneity of variance (Bartlett's Test) of the data. Statistical analyzes were performed using the SISVAR 5.3® statistical analysis program (FERREIRA, 2011).

3 RESULTS AND DISCUSSION

The Plant height, stem diameter, leaf dry mass, dry mass of roots, dry root mass and total dry mass of plants were significantly influenced ($p \leq 0,05$) by treatments with nitrogen fertilizers.

For all the N sources applied, there was an increase in plant height compared to the control treatment (Figure 1a).

The highest value of plant height was 55 cm with the application of Polyblen Extend®, it was an increase of 4 cm in comparison to the treatment with ammonium nitrate, and of 12 cm in comparison to the application of urea. The other fertilizers presented no differences in comparison to urea. The lowest height value was 23 cm in the control treatment (without application of N).

The lowest stem diameter value was 4 mm also in the control treatment. With the application of nitrogen fertilizers there was an increase in comparison to the control treatment, but no difference was found between the fertilizers (Figure 1b).

The minimum value of stem height and diameter found in the control treatment corroborate with the results found by Clemente et al. (2008). These authors studied critical bands of N-content in the coffee tree in post-planting and found lower height (50 cm) and stem diameter (3 mm) in the control treatment after 270 days of transplanting the seedlings. According to Fenilli et al. (2008), N is the main responsible for the vegetative growth in the coffee tree.

The increased height of 8% and 23% with the application of Polyblen Extend®, when compared to the application of ammonium nitrate and to the average of the other nitrogen fertilizers, respectively, may be associated to lower N losses due to volatilization of ammonia and also to the supply time of N.

Polyblen Extend® has lower loss of N-NH_3 compared to urea and can also provide N for a longer period than ammonium nitrate (soluble fertilizer) (CHAGAS et al., 2016). The part of urea treated with NBPT is soluble, less susceptible to losses by volatilization and meets the immediate demand of N by the culture (CANCELLIER et al., 2016). And the urea fraction coated with S^0 + polymers releases the N gradually in the medium and long term (NOELLSCH et al., 2009).

The dry leaf mass had a maximum value of 66,1 g pot^{-1} with the application of Polyblen Extend®, while the minimum value was 27,3 g pot^{-1} in the control treatment (Figure 2a).

For the dry mass of branches (Figure 2b) and roots (Figure 2c), the minimum values were 20,2 and 3,4 g pot^{-1} , respectively, found in the control treatment. Among the nitrogen fertilizers, there were no significant differences.

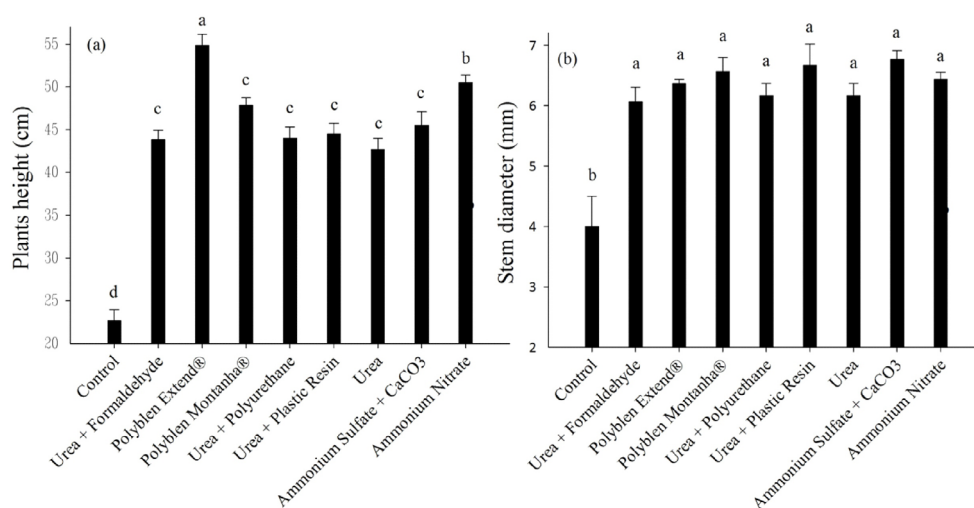


FIGURE 1 - Plants height (1a) and stem diameter (1b) of the coffee plants after application of nitrogen fertilizers. Values followed by the same letter belong to the same cluster by the Skott-Knott test at 5% probability. The vertical bars represent the standard error of the averages (n = 3).

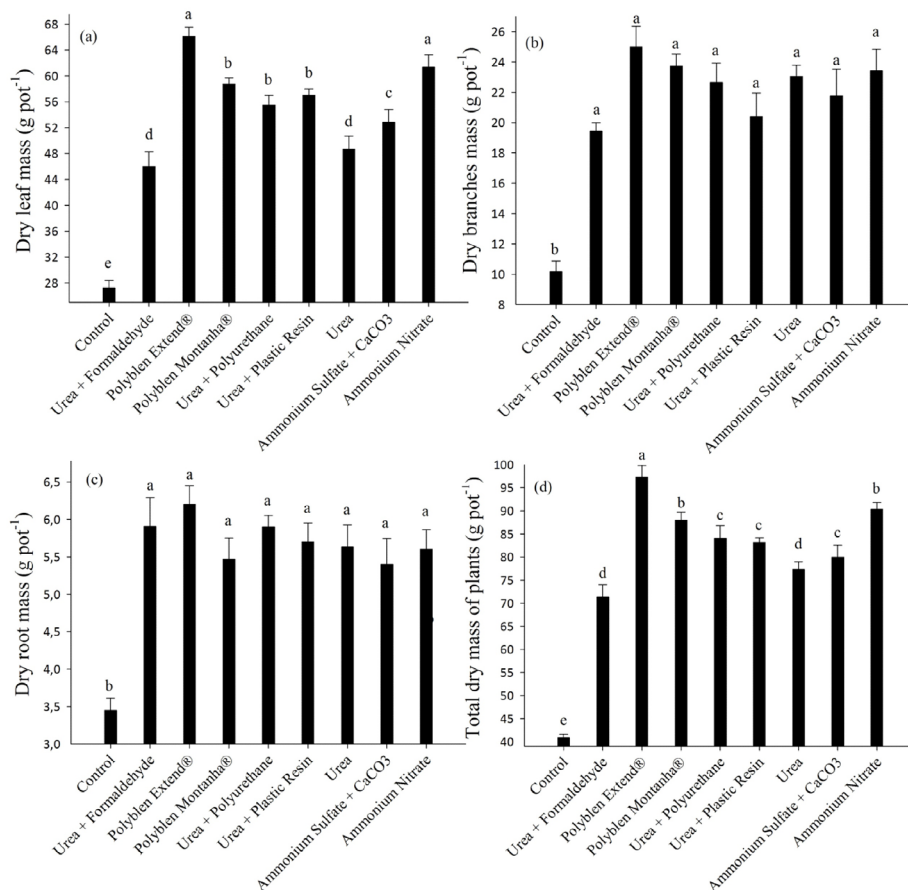


FIGURE 2 - Dry leaf mass, dry mass of branches, dry root mass and total dry mass of coffee plants after the application of nitrogen fertilizers. Values followed by the same letter belong to the same cluster by the Skott-Knott test at 5% probability. The vertical bars represent the standard error of the averages (n = 3).

The maximum value of the total dry mass of plants occurred with the application of Polyblen Extend® and was equal to 97,3 g pot⁻¹, while the minimum value was 40,9 g pot⁻¹ in the control treatment (Figure 2d).

With the application of urea, the dry mass was 77,3 g pot⁻¹. The increase in total dry mass of plants with the application of Polyblen Extend® was 20,6% compared to the treatment with urea application. These results confirm those found by Garcia et al. (2011), who, when studying the effect of urea with urease inhibitor on the growth of coffee tree seedlings, concluded that the use of NBPT associated with urea promotes an 18% gain in dry mass production of plants. The authors evaluated the dry mass of coffee after 285 days of conventional urea and Urea+NBPT application.

Possibly, this gain is due to the reduction of N losses by volatilization, since the coating of the fertilizer with S and the urease inhibitor (NBPT) decreases the rate of hydrolysis of urea, which allows better utilization of nitrogen. The coating of polymer urea causes less amount of urea to be hydrolyzed at one time, and this prevents the accumulation of N-NH₄⁺ in the soil, thereby reducing the risk of N-NH₃ formation (PENG et al., 2015).

According to Carelli et al. (2006), an adequate supply of N promotes a quick development of the

coffee tree, specifically through the increase in the number of pairs of leaves per plant. This increase, associated with other factors such as the number of nodes per branch, number of fruiting nodes and flowers per node, is responsible for the higher yields of the coffee tree (CARELLI et al., 2006; NAZARENO et al., 2003).

Martins et al. (2014), evaluating the dry mass of the aerial part of the maize after the application of conventional urea and polymer coated urea, did not find differences between the sources when they were applied during the rainy season. However, in another crop, where the fertilizers were applied under a drying condition after fertilization, the authors found a higher dry shoot mass with the application of polymer coated urea when compared to conventional urea.

The leaf area was significantly influenced ($p \leq 0,05$) by the treatments with nitrogen fertilizers and the control treatment (Figure 3).

Leaf area pot⁻¹ followed the descending order for nitrogen fertilizer treatments: Polyblen Extend® (2195,8 cm²) > Ammonium Nitrate (2010,6 cm²) = Polyblen Montanha® (1906,8 cm²) > Urea + Polyurethane (1771,1 cm²) = Ammonium Sulfate + CaCO₃ (1758,9 cm²) > Urea + Plastic Resin (1626,9 cm²) = Urea + Formaldehyde (1590,0 cm²) = Urea (1585,9 cm²) > Control (538,5 cm²).

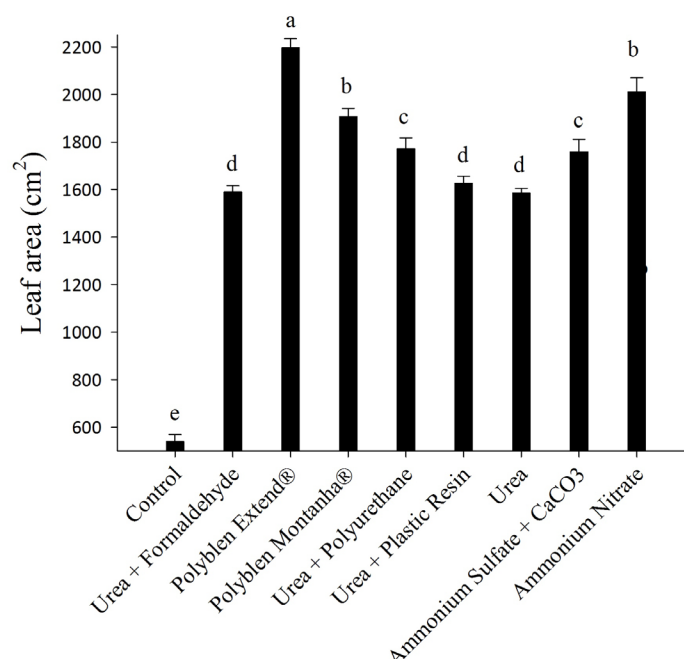


FIGURE 3 - Leaf area pot⁻¹ of coffee seedlings after nitrogen fertilizers application. Values followed by the same letter belong to the same cluster by the Skott-Knott test at 5% probability. The vertical bars represent the standard error of the averages (n = 3).

According to Taiz and Zieger (2013), nitrogen affects root formation, photosynthesis, photoassimilate production and translocation, and leaf growth rate, with leaf growth primarily affected.

The increase in leaf area with the application of Polyblen Extend® was 27,8% in comparison to the treatment with urea application and 75,5% in relation to the control treatment. According to Dominghetti et al. (2016), nitrogen fertilization and, consequently, the supply of N, promotes the quick growth of the new leaves of the coffee tree.

N is constituent of many components of the plant cell, such as amino acids and nucleic acids, when lacking causes chlorosis in the older leaves, which later senescence and with this can decrease the leaf area (TAIZ; ZIEGER, 2013).

The accumulation of N in the dry root mass, in the branches and leaves, together with the total accumulation in the coffee plant, were significantly ($p \leq 0,05$) influenced by the nitrogen fertilizers and the control treatment (without N application) (Table 2).

The highest values of N accumulated in the root and the branches occurred with the application of nitrogen fertilizers, with averages values of 89,8 and 370,2 mg pot⁻¹, respectively. In the control treatment, the accumulation of N in the root (38,1 mg pot⁻¹) and in the branches (142,9 mg pot⁻¹) were smaller in comparison to the other treatments.

Polyblen Extend® promoted greater N accumulation in the leaves (1608,6 mg pot⁻¹) and total in the plant (2168,0 mg pot⁻¹). The highest total accumulation value of N (2168,0 mg pot⁻¹) occurred with the application of Polyblen Extend®. Values followed by the same letter belong to the same cluster revealed a difference of 63% compared to Urea.

The lowest accumulation of N in the leaves (382,9 mg pot⁻¹) and in the plant (563,9 mg pot⁻¹) also occurred in the control treatment. The urea showed values of accumulation in the leaves (912,3 mg pot⁻¹) and in the plant (1364,4 mg pot⁻¹) higher than the control, similar to the Ammonium Sulfate + CaCO₃ and lower than the other treatments.

Silva et al. (2012) evaluating the accumulation of N in the dry mass of maize plants after the application of controlled release urea and conventional urea, did not find differences between the nitrogen sources. Valderrama et al. (2014) also did not find differences for N accumulation in maize leaf after the application of conventional urea and polymer-coated urea in two maize crops.

The photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and the relative index of chlorophyll (RIC) of coffee tree seedlings were influenced ($p \leq 0,05$) by nitrogen fertilizer application (Figure 4).

The highest value of photosynthetic rate in coffee plants was 2,8 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ with the application of Polyblen Extend®. The lowest value of photosynthetic rate was 1,4 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ found in the control treatment.

TABLE 2 - N accumulation in root, branches, leaves and total accumulation of N in the coffee tree after application of nitrogen fertilizer treatments.

Treatments	N accumulation in the root	N accumulation in the branches	N accumulation in leaves	Total N accumulation in the plant
----- mg pot ⁻¹ -----				
Control	38,1 b	142,9 b	382,9 e	563,9 e
Urea + Formaldehyde	90,2 a	343,8 a	997,9 d	1431,9 d
PolyblenExtend®	107,8 a	451,6 a	1608,6 a	2168,0 a
Polyblen Montanha®	82,3 a	370,4 a	1231,4 c	1684,1 c
Urea + Polyurethane	92,1 a	339,8 a	1110,3 c	1542,23 c
Urea + Plastic Resin	86,8 a	329,3 a	1235,0 c	1651,1 c
Urea	84,4 a	367,8 a	912,3 d	1364,4 d
Ammonium Sulfate + CaCO ₃	81,6 a	359,8 a	1018,1 d	1459,5 d
Ammonium Nitrate	93,3 a	399,4 a	1405,1 b	1897,8 b
Average	84,1	344,9	1100,2	1529,2
Variation coefficient (%)	12,9	13,8	8,8	7,7

Values followed by the same letter belong to the same cluster by the Skott-Knott test at 5% probability.

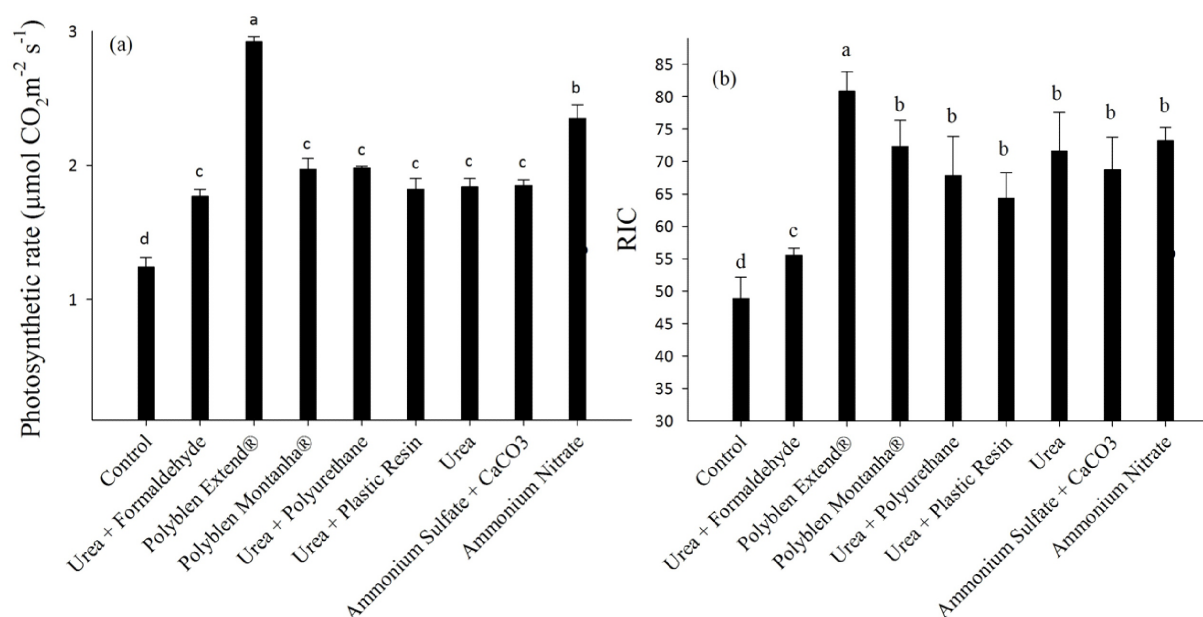


FIGURE 4 - Photosynthetic rate and relative index of chlorophyll (RIC) of coffee plants after application of nitrogen fertilizers. Values followed by the same letter belong to the same cluster by the Skott-Knott test at 5% probability. The vertical bars represent the standard error of the averages ($n = 3$).

The higher photosynthetic rate with application of Polyblen Extend®, together with the lower values in the control treatment, may be associated to the higher and lower accumulation of N in the leaves of the coffee tree by these treatments (Table 2). According to Andrade et al. (2015), the photosynthetic rate and the amount of N in the leaf show a significant positive correlation. According to the authors, N is a fundamental component for photosynthesis. According to Khamis et al. (1990), N levels in the leaf below the recommended critical for the plant may reduce the photosynthetic capacity.

The maximum RIC index value was 80,8 with the application of Polyblen Extend®. The minimum value was 48,8 in the control treatment. Guimarães et al. (1999), evaluating the RIC in tomato culture, with different doses of N, observed a linear increase of the RIC associated to increase of the doses of N. According to Godoy et al. (2008), the RIC reduces linearly with the reduction of N doses in the coffee tree. The authors evaluated in the period between flowering and coffee harvest.

Valderrama et al. (2014) evaluating the RIC in maize leaf after nitrogen fertilization with polymer-coated urea and conventional urea, did not find differences between the two sources. The authors evaluated the ICR after maize cultivations.

According to Malavolta et al. (2004), the RIC is based on the correlation between the N content in the leaf and the chlorophyll content. Torres Netto et al. (2005) and Reis et al. (2006) found positive correlations between the chlorophyll content and the N content in the coffee leaf in production.

The highest photosynthetic rate and RIC, associated to the highest accumulation of N in the leaves, and in the whole plant promoted by Polyblen Extend® is associated to the N supply by this Blend.

The N from the 30% urea + NBPT of this Blend may have supplied N uptake by the coffee tree in the first few days after fertilization, and 70% urea + S^o + polymers provided an adequate supply of N during the remainder of the experiment.

Ammonium nitrate (soluble), although promoting less loss of N-NH₃ compared to Polyblen Extend® (CHAGAS et al., 2016), may have supplied N in a large quantity in the first days after each fertilization and provided a smaller amount of N afterward. Thereby, the residual N of the fraction with urea + S^o + polymers may have been responsible for increasing the accumulation of N in the leaves, in the whole plant and consequently the photosynthetic rate and the RIC.

According to Dominghetti et al. (2016), ammonium nitrate promotes the reduction in volatilization losses compared to several fertilizer technologies of increased efficiency found in the Brazilian market. In a study carried out evaluating the losses due to volatilization by nitrogen fertilizers, after fertilization at the dose of 450 kg ha of N, divided in three applications in the coffee tree in production, the losses were 0,3% of the N applied with ammonium nitrate. Whereas, with conventional urea, Urea + Formaldehyde, Urea + Plastic Resin, losses by volatilization were 31,2; 1,1 and 8,6% of the N applied. However, the authors stress that ammonium nitrate does not have the slow or controlled release effect.

In addition, the urea coating with plastic resin, polyurethane, together with urea formaldehyde and ammonium sulfate + CaCO_3 may have the N-release time higher than the time used to partition the N application in this study (50 days). Thereby, the Polyblen Extend® may have promoted greater N supply during the period of the three fertilizations (150 days), in comparison to the other fertilizers.

Trenkel (2010) reports that the release time of the controlled release fertilizers (Urea + Plastic Resin and Urea + Polyurethane) depends on several factors, including the thickness of the coating and the quality of the coating process by the fertilizer industry. And the release time of the slow release fertilizers such as urea+formaldehyde

depends on the degree of polymerization to which the urea has been subjected (YAMAMOTO et al., 2016).

The application of Polyblen Extend® may have offered a better adjustment of the N released by the fertilizer and the absorption curve of the N by the coffee tree in the period of conduction of the experiment. According to Noellsch et al. (2009), conventional fertilizer (high solubility in water) can supply the initial demand for N by the crop and the controlled release fraction over a longer period of time.

PolyblenMontanha®, as well as Polyblen Extend®, has a soluble fraction (urea + NBPT) however, the controlled release fraction of PolyblenMontanha® (urea + S^0 + polymers) is able to provide N for a longer period of time compared to Polyblen Extend® (CHAGAS et al., 2016). This longer period may have promoted lower N supply during the conduction time of the experiment when compared to the Polyblen Extend®.

According to Chagas et al. (2016), the greater thickness of Polyblen Montanha® coating (coating thickness = 87-99 μm) compared to Polyblen Extend® (coating thickness = 67-68 μm) allows a longer delay in the release of N.

The relative agronomic efficiency index (RAEI) and Agronomic Efficiency (AE) were significantly ($p \leq 0,05$) influenced by nitrogen fertilizers (Figures 5a and 5b).

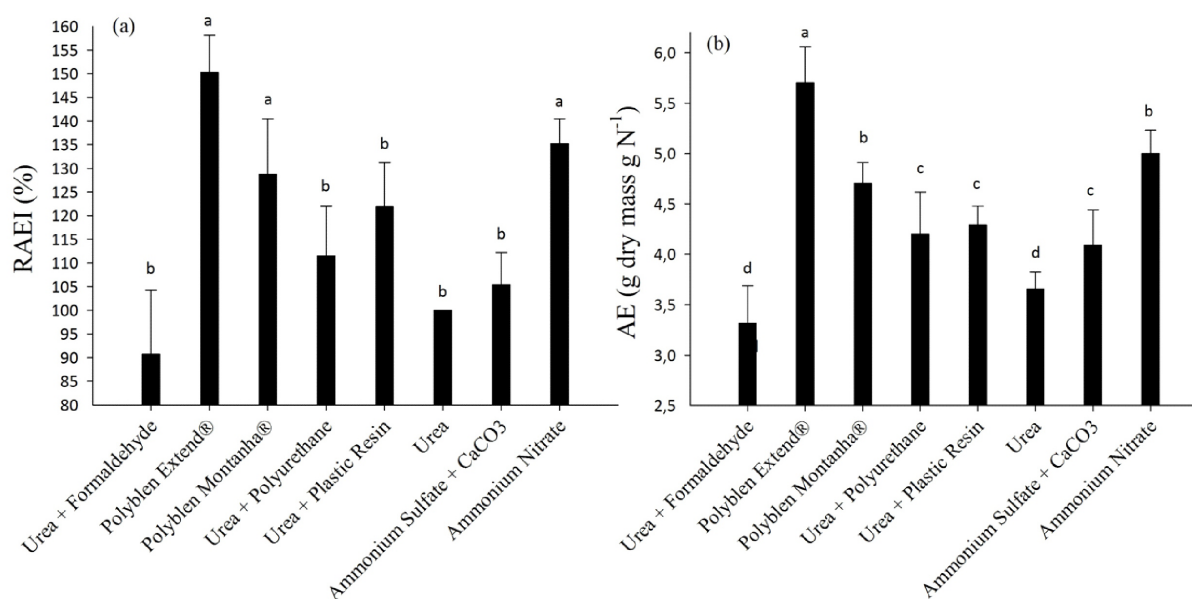


FIGURE 5 - Relative agronomic efficiency index (RAEI) and Agronomic Efficiency (AE) of nitrogen fertilizers after application in the coffee tree. Values followed by the same letter belong to the same cluster by the Skott-Knott test at 5% probability. The vertical bars represent the standard error of the averages (n = 3).

Polyblen Extend® (155%) and Ammonium Nitrate (136%) presented the highest values of RAEI. All other fertilizers did not present significant differences ($p \leq 0,05$) in comparison to urea. The average value of RAEI found for these two sources of N was 145%, which means that the dry mass production was on average 45% higher than urea (100%).

There are still few studies that evaluate the RAEI after the application of nitrogen fertilizers in the coffee tree. Fontoura and Bayer (2009) evaluated the RAEI for nitrogen sources applied to maize cultivated no-tillage system and found values equal to 108, 113, 104 and 122% for the incorporated urea, ammonium sulfate, ammonium nitrate and urea + NBPT. Also in maize, Zavaschi et al. (2014) found values of 92% for polymer-coated urea, applied at 90kg ha⁻¹ of N.

For AE, Urea + Formaldehyde and Urea gave the lowest values, which were 3,6 and 3,3 grams of dry mass per g of N applied, respectively. The highest AE value was found with Polyblen Extend® application and was 5,7 grams dry mass per gram of N applied.

The lowest AE value with Urea + Formaldehyde and conventional urea can be attributed to N-NH₃ losses by volatilization. In a study conducted by Viero et al. (2015), the authors found no reduction in N-NH₃ losses by volatilization with the application of slow release urea when compared to conventional urea.

Polyblen Extend® increased AE by 65% compared to the average values of Urea + formaldehyde and conventional urea. This reveals that Polyblen Extend® was 65% more efficient in producing the dry mass of coffee seedlings per unit of N compared to the application of urea and urea + formaldehyde. In relation to Polyblen Montanha® and Ammonium Nitrate, this increase was 39%.

In a study carried out on maize cultivation, Silva et al. (2012) did not find significant differences in grain yield per unit of N when using conventional or coated urea, although they observed increased productivity with increasing doses of N. Noellsch et al. (2009), when evaluating the efficiency of N recovery by maize plants, after the application of Blend (Urea + NBPT and polymer-coated urea), in the ratio 1:1 and conventional urea, found the highest values with the application of Blend. N recovery efficiency was 41% higher when compared to conventional urea. Both blends and conventional urea have been applied in the coating.

4 CONCLUSIONS

The highest values of plant height, total dry mass of plants and leaf area in coffee seedlings were obtained with the application of Polyblen Extend®. This Blend also provided greater accumulation of N in the leaf and whole plant.

The application of Polyblen Extend® promoted greater agronomic efficiency and increased the photosynthetic rate and relative index of chlorophyll in the coffee seedlings at the end of the period of conduction of the experiment.

5 REFERENCES

- ABALOS, D. et al. Meta-analysis of the effect of urease and nitrification inhibitors on crop productivity and nitrogen use efficiency. **Agriculture, Ecosystems & Environment**, Amsterdam, v. 189, p. 136-144, May 2014.
- ANDRADE, M. A. F.; RAMOS-CAIRO, P. A.; SANTOS, J. L.. Water relations and photosynthesis of young coffee plants under two water regimes and different n and k doses. **Agrociencia**, San Luis Huexotla, V. 49, n. 2, p. 153-161, Feb. 2015.
- AZEEM, B. et al. Review on materials & methods to produce controlled release coated urea fertilizer. **Journal of Controlled Release**, Amsterdam, v. 181, p. 11-21, May 2014.
- CANCELLIER, E.L. et al. Ammonia volatilization from enhanced-efficiency urea on no-till corn in Brazilian cerrado with improved soil fertility. **Ciência e Agrotecnologia**, Lavras, v. 40, n. 2, p. 133-144, Mar./Apr 2016.
- CARELLI, M. L. C.; FAHL, J. I.; RAMALHO, J. D. C. Aspects of nitrogen metabolism in coffee plants. **Brazilian Journal of Plant Physiology**, Londrina, v. 18, n. 1, p. 9-21, Jan./Feb. 2006.
- CHAGAS, W. F. T. et al. Ammonia volatilization from blends with stabilized and controlled-released urea in the coffee system. **Ciência e Agrotecnologia**, Lavras, v. 40, n. 5, p. 497-509, Sept./Oct. 2016.
- CHIEN, S.H.; PROCHNOW, L. I.; CANTARELLA, H. Recent developments of fertilizer production and use to increase nutrient efficiency and minimize environmental impacts. In: Bertsch, P. M.; Scow, K. M.; Phillips, R. L.; Wilding, L. P. (Ed.). **Advances in Agronomy**. Cambridge: Academic press, 2009. Chap. 8, p. 261-316.

CLEMENTE, F.M.V.T., et al. Faixas críticas de teores foliares de macronutrientes no café em pós-plantio-primeiro ano. **Coffee Science**, Lavras, v. 3, n. 1, p. 47-57, Jan./June 2008.

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: UFV, 1999. 310p.

DOMINGHETTI, A.W. et al. Nitrogen loss by volatilization of nitrogen fertilizers applied to coffee orchard. **Ciência & Agrotecnologia**, Lavras, v. 40, n. 2, p. 173-183, Mar./Apr. 2016.

FAGERIA, N.K.; SANTOS, A.B.; MORAES, M.F. Yield, Potassium Uptake, and Use Efficiency in Upland Rice Genotypes. **Communications in Soil Science and Plant Analysis**, New York, v. 41, n. 22, p. 2676-2684, Dec. 2010.

FARIA, L. A. et al. Hygroscopicity and ammonia volatilization losses from nitrogen sources in coated urea. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 38, n. 3, p. 942-948, May/June. 2014.

FENILLI, T. A. B. et al. Fertilizer ¹⁵N balance in a coffee cropping system: a case study in Brazil. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 32, n. 4, p. 1459-1469, July/Aug. 2008.

FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, Lavras, v. 35, n. 6, p. 1039-1042, Nov./Dec. 2011.

FONTOURA, S.M.V.; BAYER, C. Adubação nitrogenada para alto rendimento de milho em plantio direto na região centro-sul do Paraná. **Revista Brasileira de Ciência do Solo**, Viçosa v. 33, n. 6, p. 1721-1732, Nov./Dec. 2009.

FRAZÃO, J. J. et al. Fertilizantes nitrogenados de eficiência aumentada e ureia na cultura do milho. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v. 18, n. 12, p. 1262-1267, Dec. 2014.

GARCIA et al. Efeito da ureia com inibidor de urease no crescimento de mudas de café (*coffea arabica*). **Coffee Science**, Lavras, v. 6, n. 1, p. 1-7, Jan./Apr. 2011.

GODOY, L. J. G. et al. Relative chlorophyll index and nitrogen status of fertigated coffee plants during the crop season. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 32, n. 1, p. 217-226, Jan./Feb. 2008.

GUELFÍ, D. Fertilizantes Nitrogenados Estabilizados, de Liberação Lenta ou Controlada. **Informações Agrônomicas**, Piracicaba, n. 157, p. 1 – 14, Mar. 2017.

GUIMARÃES, T.G. et al. Teores de clorofila determinados por medidor portátil e sua relação com as formas de nitrogênio em folhas de tomateiro cultivado em dois tipos de solo. **Bragantia**, Campinas, v. 58, n. 1, p. 209-216, 1999.

MALAVOLTA, E. et al. Evaluation of nutritional status of the cotton plant with respect to nitrogen. **Communications in Soil Science and Plant Analysis**, New York, v. 35, n. 7-8, p. 1007-1019, 2004.

MALAVOLTA, E.; YAMADA, T.; AROALDO, J. **Nutrição e adubação do café**. Piracicaba: Instituto da Potassa e Fosfato, p. 1981. 226.

MARCHESAN, E. et al. Fontes alternativas à ureia no fornecimento de nitrogênio para o arroz irrigado. **Ciência Rural**, Santa Maria, v. 41, n. 12, p. 2053-2059, Dec. 2011.

MARTINS, I. S.; CAZETTA, J. O.; FUKUDA, A. J. F. Condições, modos de aplicação e doses de ureia revestida por polímeros na cultura do milho. **Pesquisa Agropecuária Tropical**, Goiânia, v. 44, n. 3, p. 271-279, Jul./Sep. 2014.

NAZARENO R.B. et al. Crescimento inicial do café Rubi em resposta a doses de nitrogênio, fósforo e potássio e a regimes hídricos. **Pesquisa Agropecuária Brasileira**, Brasília, v. 38, n. 8, p. 903-910, Aug. 2003.

NOELLSCH, A. J. et al. Corn response to conventional and slow-release nitrogen fertilizers across a claypan landscape. **Agronomy Journal**, Madison, v. 101, n. 3, p. 607-614, 2009.

PENG, X. et al. A Laboratory Evaluation of Ammonia Volatilization and Nitrate Leaching following Nitrogen Fertilizer Application on a Coarse-Textured Soil. **Agronomy Journal**, Madison, v. 107, n. 3, p. 871-879, Feb. 2015.

PEREIRA, H. S. et al. Ammonia volatilization of urea in the out-of-season corn. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 33, n. 6, p. 1685-1694, Nov./Dec. 2009.

- REIS, A.R. et al. Diagnosis of n requirements for coffee plant using a portable chlorophyllmeter. **Bragantia**, Campinas, v. 65, n. 1, p. 163-171, 2006.
- SANTOS H.G, et al. **Sistema brasileiro de classificação de solos**. 3.ed. Brasília: Embrapa, 2013. 353 p.
- SANZ-COBENA, A. et al. Gaseous emissions of N_2O and NO and NO_3^- – leaching from urea applied with urease and nitrification inhibitors to a maize (*Zea mays*) crop. **Agriculture, Ecosystems and Environment**, Amsterdam, v. 149, p. 64-73, Mar. 2012.
- SILVA, A. de A., et al. Aplicação de diferentes fontes de ureia de liberação gradual na cultura do milho. **Bioscience journal**. Uberlândia, v. 28, n. 1, p.104-111, 2012.
- TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 5.ed. Porto Alegre: Artmed, 2013. 918p.
- 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. (Boletim Técnico, 5).
- TORRES NETO, A. et al. Photosynthetic pigments nitrogen, chlorophyll fluorescence and SPAD-502 readings in coffee leaves. **Scientia Horticulturae**, Amsterdam, v. 104, n. 2, p. 199-209, Mar. 2005.
- TRENKEL, M. E. Slow and controlled-release and stabilized fertilizers: An option for enhancing nutrient use efficiency in agriculture. **International Fertilizer Industry Association**. Paris, 2010, 167p.
- VALDERRAMA, M. et al. Adubação nitrogenada na cultura do milho com ureia revestida por diferentes fontes de polímeros. **Semina: Ciências Agrárias**, Londrina, v. 35, n. 2, p. 659-670, Mar./April 2014.
- VIERO, F. et al. Management of Irrigation and Nitrogen Fertilizers To Reduce Ammonia Volatilization. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 39, n. 6, p. 1737-1743, Nov./Dec. 2015.
- YAMAMOTO, C.F. et al. Slow release fertilizar based on urea/urea-formaldehyde polymer nanocomposites. **Chemical Engineering Journal**, Amsterdam v. 287, n. 10, p. 390-397, Mar. 2016.
- ZAVASCHI, E. et al. Ammonia volatilization and yield components after application of polymer-coated urea to maize. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 38, n. 4, p. 1200-1206, Jul./Aug. 2014.

SELECTION OF COFFEE PROGENIES WITH LARGE BEANS RESISTANT TO RUST AND CERCOSPORA LEAF SPOT

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ABSTRACT: Rust is the main disease of coffee. Recently, cercospora leaf spot has grown in importance, intensifying defoliation and decreasing grain yield in *Coffea arabica*. The Big Coffee VL is a variety of *C. arabica*, with large beans. There is still little information on this variety. Because of this, the aim of this study was to use a mixed model approach to select the best progenies of “Big Coffee VL” for resistance to rust and cercospora leaf spot. There were evaluated 12 progenies with high bean yields. Based on bean size, leaves and plant size, plants within each progeny were classified as “small” (P), “medium” (M) and “large” (G). The experimental design was in blocks completely randomized, with six replicates and one plant per plot. Six measurements were carried out every 15 days for each plant by selecting 20 fully exposed leaves with higher and lower sun exposures. The measurements summarized disease severity using two diagrammatic scales to obtain the area of the leaf under rust or Cercospora leaf spot. A mixed model approach was used to calculate genotypic value (GV) and heritability estimates. The Mulamba and Mock index was used to select the most resistant progenies, in which the sum of rankings was weighted by a heritability value. Five progenies were selected; three progenies were G (G17, G9, and G12), one progeny was M (M5) and another progeny P (P23). Among these, M5 and P23 progenies are the most productive and may have potential use in future studies. This work presents the potential in investigating the new *C. arabica* variety.

Index terms: *Coffea arabica*, *Hemileia vastatrix*, *Cercospora coffeicola*, simultaneous selection.

SELEÇÃO DE PROGÊNIES DE CAFEEIROS DE GRÃOS GRAÚDOS RESISTENTES A FERRUGEM E CERCOSPORIOSE

RESUMO: A ferrugem é a principal doença do cafeeiro. Recentemente a cercosporiose tem crescido em importância, intensificando a desfolha e diminuição na produtividade de grãos da espécie *Coffea arabica*. O Big Coffee VL é uma variedade de *C. arabica*, com característica de grão graúdo. Ainda existe pouca informação sobre essa variedade, diante disso, objetivou-se selecionar progênies de cafeeiro “Big Coffee VL”, para resistência à ferrugem e cercosporiose simultaneamente, com a abordagem de modelos mistos. Foram consideradas as 12 progênies mais produtivas e classificadas em “pequenas” (P), “médias” (M) e “grandes” (G), de acordo com o tamanho dos frutos, folhas e o porte da planta. O delineamento utilizado foi em blocos casualizado (DBC), com seis repetições e uma planta por parcela. Em cada planta foram realizadas seis avaliações, com intervalos de 15 dias, em 20 folhas do terço médio da planta da face da planta com maior e menor exposição solar. As avaliações foram realizadas com o auxílio de duas escalas diagramáticas a fim de obter a área abaixo da curva de progresso da doença, que resume a severidade da doença. Foi utilizada a abordagem de modelos mistos para o cálculo do valor genotípico (VG) e as estimativas de herdabilidade. Para selecionar as progênies mais resistentes foi considerado o índice de Mulamba e Mock, no qual a soma de postos foi ponderada pelo valor da herdabilidade. Na seleção de cinco progênies, três delas são do grupo G (G17, G9 e G12), uma M (M5) e uma P (P23). Dentre essas, as progênies M5 e P23 são as mais produtivas e tem potencial, em termos de resistência às doenças, para serem utilizadas em trabalhos futuros. Esse trabalho mostra o potencial em investigar a nova variedade de *C. arabica*.

Termos para indexação: *Coffea arabica*, *Hemileia vastatrix*, *Cercospora coffeicola*, seleção simultânea.

1 INTRODUCTION

Development of coffee cultivars that are resistant and/or tolerant to diseases is important for increasing grain yield and decreasing production costs. The lack of information on how new cultivars resist attacks from major diseases is one of the limiting factors in the selection of the

best cultivars (CARVALHO et al., 2010). Coffee rust caused by the biotrophic fungus *Hemileia vastatrix* Berk. et Br is the most economically important coffee disease in the world. It has been found in all coffee plantations in Brazil and has caused damages and losses to coffee cultivation (MAFFIA; HADDAD; MIZUBUTI, 2009, ZAMBOLIM, 2016). The disease can reduce

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yields by 30 to 50% depending on the level of resistance of the genotype, the inoculum potential, and the environment conditions (CAPUCHO et al., 2013).

Favorable conditions for fungal infection and development include temperatures between 21 °C to 25 °C, and a high relative humidity (BEDENDO, 2011). The symptoms appear on the upper surfaces of the leaves, showing translucent chlorotic spots 1–3 mm in diameter. As these spots gradually increase in diameter masses of orange urediniospores appear on the bottom of the leaves. The centers of the spots eventually dry and turn brown, while the margins of the lesions continue to expand and produce urediniospores. With leaf defoliation occurring before flowering, both flowers and fruits are affected, causing the formation of abnormal grains (MATIELLO et al., 2015).

In recent years, another important disease that has increased in importance is cercospora leaf spot. This increase is attributed to technologies used in managing coffee plantations, high-density planting of crops, and irrigation practices that have altered the microclimate, favoring an increase in the incidence of diseases (PAIVA et al., 2013). *Cercospora coffeicola* Berk & Cook (SOUZA et al., 2011) is the causal agent of Cercospora leaf spot. *C. coffeicola* is more prevalent with air temperatures between 17 and 22 °C and high relative humidity (ZAMBOLIM et al., 2005). The infection occurs on the bottom of the leaf, where there is penetration of the fungus through stomata, causing colonization into the parenchyma cells. The main symptoms appear as leaf and fruit lesions. The lesions are characterized by necrotic spots with light colors in the center surrounded by brown rings with yellowish halos. Early leaf defoliation caused by the necrotic spots may reduce bean yield and fruit attack can reduce bean quality (ZAMBOLIM et al., 2005). However, it is difficult to control this disease (PATRICIO et al., 2010).

Bernardo (2010) noted that information on statistical variability is fundamental to understanding population genetics. A recent prediction methodology to characterize populations of perennial species is the restricted maximum likelihood/best linear unbiased prediction (REML/BLUP) (BALZARINI, 2001; PEREIRA et al., 2013). Data analysis in coffee experiments has benefited from using REML/BLUP because coffee is a perennial crop with

variable yields and unbalanced data in the field experiments. However, there remains a need for more accurate estimates (RODRIGUES et al., 2013; ANDRADE et al., 2001).

The objective of this study was to provide information on resistance to rust and cercospora leaf spot using the 12 most productive Big Coffee VL progenies.

2 MATERIAL AND METHODS

Origin of the variety

The “Big Coffee VL”, known for its large beans, originated from an Acaia plantation in southeast Brazil, municipality of Capitólio-MG. The parent plants present vigorous canopies and large fruits and leaves. In 2011, seeds from the parent plants were collected and planted on the farm in Piumhi-MG. Based upon fruit, leaves and plant size, these Big Coffee VL seedlings were classified as “small” (P), “medium” (M) and “large” (G).

In February 2012, 100 of these Big Coffee VL seedlings were used in an experiment carried out at the Department of Agriculture of the Federal University of Lavras. This experiment was designed to expand the genetic base of Arabica coffee and to promote the research on genetic breeding of this variety.

Experimental trials

The experiment was carried out at Department of Agriculture of the Federal University of Lavras, (21°14'43"S and 44°59'59"W, altitude of 919 m). All 100 progenies were distributed in a square lattice scheme. Twelve progenies of Big Coffee VL considered the most productive (SILVA, 2016) were selected (P5, P14, P32, M4, M4, M5, M11, M11, M20, G9, G12, G16, and G17) for resistance evaluation. A randomized complete block design was used with six replicates and one plant per plot. Each plant was evaluated every 15 days. Mean climatic data (precipitation, solar incidence, temperature and relative air humidity) were calculated for the 15 days prior to each measurement (Table 1).

During each evaluation, 40 leaves from each plant were selected from the middle-third of the plant. Twenty leaves were located on the side of the plant with the greatest sun exposure (North) and the other ones on the side with least sun exposure (South). For each leaf, the disease diagrammatic scales for coffee rust (CUNHA et al., 2001) and cercospora leaf spot (CUSTODIO et al., 2011) were used to calculate the severity of the disease.

TABLE 1 - Mean precipitation, solar incidence, temperature and relativity humidity data* 15 days prior to the day of plant evaluation.

Evaluations	Period	Precipitation (mm)	Solar incidence ** (hours)	Temperature (°C)	Relativity Humidity (%)
1	Feb 25 to Mar 10	4.3	5.0	22.9	78.1
2	Mar 11 to Mar 24	8.8	8.6	22.4	67.2
3	Mar 25 to Apr 07	0.2	8.1	21.4	68.3
4	Apr 08 to Apr 21	5.5	7.5	21.7	73.5
5	Apr 22 to May 05	2.2	5.6	19.8	76.6
6	May 06 to May 19	0.8	6.2	18.7	76.4

*Data supplied by INMET <http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep>

**Solar brightness length

For cercospora leaf spot, severity was calculated according to the formula:

$$x_i = \left[\frac{(n0 * 0) + (n1 * 2.2) + (n2 * 4.7) + (n3 * 8.3) + (n4 * 15.1) + (n5 * 20.1) + (n6 * 46.2)}{(nT * 100)} \right] * 100$$

where x_i is the severity at evaluation i , where $i = 1 \dots 6$, $n0$ is the number of leaves with a scale value = 0 up to $n6$ (scale value = 6); nT is the total number of leaves evaluated.

For coffee rust, the severity of the disease was calculated according to the formula:

$$x_i = \left[\frac{(n0 * 0) + (n1 * 1.5) + (n2 * 4.5) + (n3 * 9) + (n4 * 18.5) + (n5 * 37.5)}{(nT * 100)} \right] * 100$$

where x_i is the severity at evaluation i , where $i = 1 \dots 5$, $n0$ is the number of leaves with a scale value = 0 up to $n5$ (scale value = 5); nT is the total number of leaves evaluated.

Using the disease severity results, a curve was plotted to assess the progression of disease severity based upon the estimate of the area under the disease progress curve (AUDPC) (SHANER; FINNEY, 1977).

$$AUDPC = \sum_i^{n-1} \left[\frac{(x_i + x_{i+1})}{2} \right] * t$$

where n is the number of evaluations, x is the disease severity, i is the number of evaluations and t is the interval between two consecutive evaluations.

For the mixed model approach, the following matrix model was followed:

$$Y = X_r + Z_g + \varepsilon \quad \text{and} \quad \begin{cases} y|r, V \sim N(Xr, V) \\ g \sim N(0, I\sigma_g^2) \\ \varepsilon \sim N(0, R) \end{cases}$$

where y is the data vector; r is the vector of the main effects for repetitions and g is the vector of progeny effects. X and Z are the design matrices related to fixed and random effects.

The significance of random effects was verified by the likelihood test ratio (LTR) using the lmerTest library (KUZNETSOVA; BROCKHOFF; CHRISTENSEN, 2016). Because it is a mixed model, the means of the progenies cannot be evaluated with a multiple comparison test. Therefore, parameter estimation and ordering of the progenies was performed by the predicted genotypic values obtained by R program (R CORE TEAM, 2014), using lme4 (BATES et al., 2004) and lattice libraries (SARKAR, 2008).

Broad-sense heritability was also estimated following Ramalho (2012):

$$h^2 = \frac{\sigma_g^2}{\sigma_f^2}$$

where σ_g^2 is the variance of the genotypes; and

σ_f^2 is the phenotypic variance.

Progenies were classified in favorable order from the lowest to the highest disease severity value. To select promising progenies, the methodology of Mulamba and Mock (1978) as detailed by Cruz et al. (1997) was followed. After classification of each characteristic, the summation of the rankings was performed. Lower summation values indicated more disease-resistant progeny.

3 RESULTS AND DISCUSSION

The time required to fungus germination vary according to temperature, light regimes and exposure to light (SILVA et al., 2016). And the disease severity can be different according to the plant environment.

Coffee rust

The rust disease severity was obtained for each evaluation (Figure 1).

The greatest severity was at evaluation 6 (beginning of May) for plants under the lowest. During the time interval of 15 days before evaluation, means of relative humidity, solar incidence time, and temperature were 76.4%, 5.6 h, and 19.8 °C, respectively (Table 1). Although the ideal temperature for infection of the fungus in plant is between 21 °C and 25 °C, the pathogen was able to colonize the leaf tissue. Montes, Paulo e Fischer (2012) reported similar results, where the incidence of the disease correlated negatively with temperature.

In evaluation 1, the plants under higher had the highest severity of rust. However, the level of severity was comparatively lower compared to plants under lower sun exposure. During the time interval of 15 days before evaluation, means for average relative humidity, solar incidence and temperature were 78.1%, 5 h, and 22.9 °C, respectively (Table 1). In evaluation 1, the combination of relative humidity and temperature favored disease development. Similar to Moller and Stukenbrock (2017), we observed that *H. vastatrix* has the ability to rapidly adapt to different environmental conditions. The increase in solar incidence and decrease in relative humidity in the subsequent evaluation intervals supported a reduction in disease severity, primarily in plants under higher sun exposure.

Estimates of progeny variance were 229.7 (lower solar incidence) and 59.2 (higher solar incidence). There was a significant difference in both cases (p-value: $8,642 \times 10^{-7}$ and 0.009364, respectively) by the likelihood test ratio. Broad-sense heritability was 49.7% (lower solar incidence) and 36.2% (higher solar incidence).

According to Farias Neto et al. (2009), BLUP maximizes selective accuracy. In this work, the genotypic values are ordered from lower to higher values of rust severity (Table 2). The first position in both evaluations is associated with the G17 progeny. Considering the region of the plant with less solar incidence, two G progenies stand out (G17 and G12). Silvia (2016) observed that G progenies generally showed a lower average productivity, suggesting that resistance can incur a cost for grain yield as discussed by Bergelson and Purrington (1996). This is due to plants attempting to balance resource allocation between growth and defense, since a stress response can be costly and reduce growth and yield (TIAN et al., 2003).

Progenies P5 and P32 had the highest genotypic severity values in both evaluations. These are among the most productive progenies, confirming the negative correlation between grain yield and resistance. In general, the M progenies presented greatest mean values of severity as well as for yield (Silva et al., 2016).

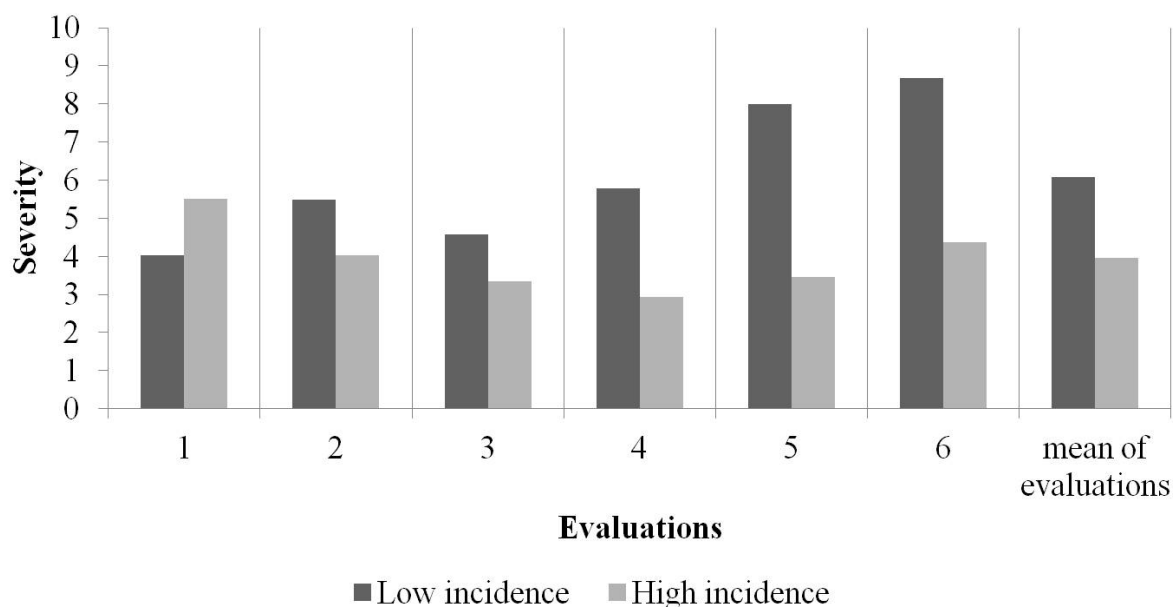


FIGURE 1 - Rust disease severity during the different evaluations in plants under low and high solar incidence.

TABLE 2 – Genotypic values for mean rust severity for 12 progenies of Big Coffee VL, arranged by genotypic values and solar exposure.

Low		High	
Progenies	Genotypic values	Progenies	Genotypic values
G17	1061.94	G17	396.04
G12	1090.2	P23	398.76
M4	1103.56	G16	404.03
P23	1108.92	G9	412.43
M5	1148.98	M5	419.29
G9	1162.69	M11	431.03
G16	1173.6	P14	437.31
M11	1211.4	G12	444.62
P14	1212.52	M20	450.39
P32	1244.71	M4	451.99
M20	1247.35	P32	501.65
P5	1864.07	P5	563.29

Cercospora leaf spot

The *Cercospora* leaf spot severity was obtained for each evaluation (Figure 2).

Few studies have investigated *C. coffeicola*; however, there are recent reports on new symptoms in coffee plantations caused by *Cercospora* leaf spot. Vale (2016) highlighted the importance of understanding the species identity and its relationship with a host. The likelihood test ratio was the only test that obtained a significant (p-value <0.05) difference for severity in plants under the highest sun exposure. The broad-sense heritability was 33.4%, considered low estimate. Unlike what was observed for rust, two progenies (P32 and P23) stood out for potential resistance to *C. coffeicola* (Table 3). However, there appears to be no exceptional progeny that is resistant to attacks from rust and *Cercospora* leaf spot. This raises the possibility that there may be different plant resistance mechanisms for each pathogen (NELSON et al., 2017).

Selection of Disease Resistant Progenies

To select disease resistant coffee trees, it is important to consider the genetic characteristics of a population (CARVALHO et al., 2017; PAIVA et al., 2013). A mixed model approach was originally proposed for estimating heritability values in animal breeding. Later, perennial crops breeders tried this approach to remove problems related to selecting individual genotypes in unbalanced

experiments. Recently the genetic resistance of coffee plants to *C. coffeicola* (BOTELHO et al., 2017) and *H. vastatrix* (PETEK et al., 2008) has been studied using the mixed model approach. When associated with statistical procedures, the mix model approach allows greater reliability in breeding programs (OLIVEIRA et al., 2015).

For simultaneous selection (Table 4) for resistance to rust and *Cercospora* leaf spot, the summation was weighted by heritability value in each case. Only genotypic values were considered due to the low solar incidence values for the rust and the high solar incidence values for the *Cercospora* leaf spots, at the time of the significance and value of the heritability, respectively.

Five progenies were selected; three of them are from group G (G17, G9 and G12), one from progeny M (M5) and one from progeny P (P23). There appears to be a positive correlation between vegetative development and fungus resistance, except for P progenies. The G progeny plants have large vegetative canopies, reducing possible photosynthetic losses due to reduction of the leaf area by fungus colonization (KUSHALAPPA; CHAVES, 1980).

A higher harvest index represents a greater investment in productive organs and consequently less investment in leaves and stem. The crops become less competitive and protected against losses of leaf area by pathogens (SADRAS; CALDERINI, 2015; ZAMBOLIM et al., 2005).

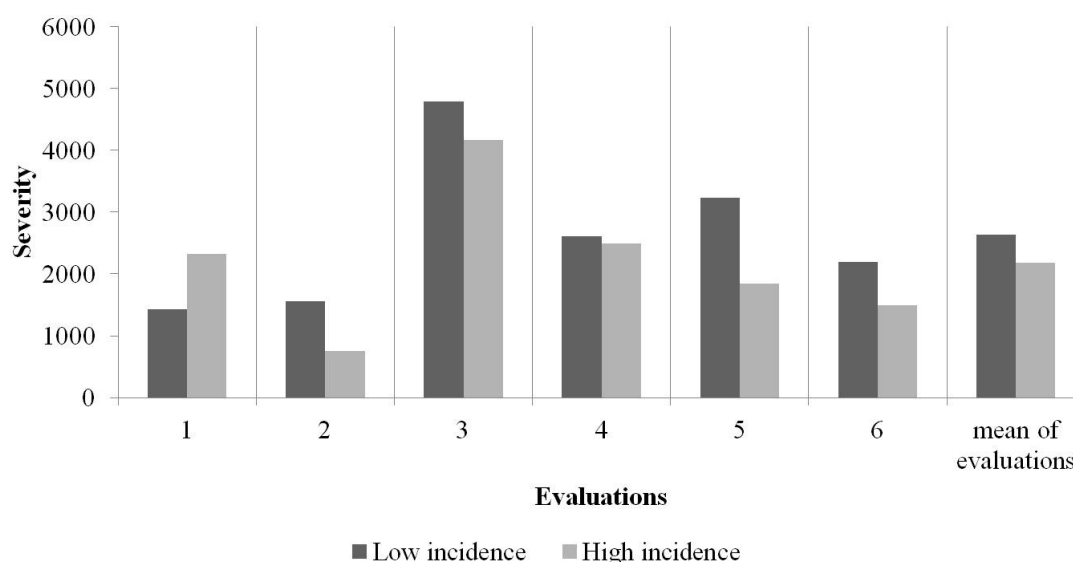


FIGURE 2 - Cercospora leaf spot severity in the different evaluations in plants under low and high solar incidence.

TABLE 3 - Genotypic values ordered for the Cercospora leaf spot severity in 12 progenies of Big Coffee VL.

High	
Progenies	Genotypic values
P32	236338
M5	248559
P23	254462
G9	262545
M20	271562
M4	280942
G17	285647
G12	287472
M11	298454
G16	299028
P14	302508
P5	353888

This is opposite to what we observed for the P23 and M5 progenies, which are among the most productive progenies (SILVA, 2016). Moreover, a lower disease severity may indicate horizontal resistance. Campbell and Madden (1990) found that under natural epidemic conditions, severity is the component that best discriminates between levels of horizontal resistance. The heritability estimates that were found below 50% indicate a possible presence of resistance genes that could be strongly influenced by the environment (BERNARDO,

2010). Partial and non-specific polygenic resistances have been found in *C. canephora*, in some *C. arabica* genotypes and in interspecific hybrids (SILVA et al., 2006). There is great genetic diversity within *H. vastatrix* (CABRAL et al., 2015), *C. coffeicola* (DELL'ACQUA et al., 2011) because of this durable long-term resistance in *C. arabica* has not been achieved so far. Therefore, breeding programs need to develop varieties with sustainable resistance for a highly fit and variable pathogen.

TABLE 4 - Classification by the summation of rankings by Mulamba and Mock (1978) considering rust and cercospora leaf spot severity in Big Coffee progenies.

Progenies	Summation of rankings
G17	3.197
P23	3.714
M5	4.963
G9	5.766
G12	6.562
M4	7.115
G16	7.905
M11	9.154
P32	9.286
M20	10.395
P14	10.681
P5	14.316

4 CONCLUSIONS

From the evaluated progenies it is possible to select superior plants more resistant to rust and Cercospora leaf spot simultaneously. The selection index associated with an advanced statistical approach related to genotype value prediction facilitates the selection of the genetically superior individuals, maximizing the success on selection.

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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.
- BALZARINI, M. Applications of mixed models in plant breeding. In: KANG, M. S. (Ed.) **Quantitative genetics, genomics and plant breeding**. New York: CABI Publishing, 2001. p. 353-363.
- BATES, D. et al. Fitting linear mixed-effects models using lme4. **Journal of Statistical Software**, Linz, v. 67, n. 1, Dec. 2015.
- BERGELSON, J.; PURRINGTON, C. B. Surveying patterns in the cost of resistance in plants. **American Naturalist**, Chicago, v. 148, n. 3, p. 536-558, Sept. 1996.
- BEDENDO, I. Ferrugens. In: AMORIM, L.; REZENDE, J. A. M.; BERGAMIN FILHO, A. (Ed.) **Manual de fitopatologia: princípios e conceitos**. 4. ed. Piracicaba: Agronômica Ceres, 2011, p.479-484.
- BOTELHO, D. M. S. et al. Cercosporiosis resistance in coffee germplasm collection. **Euphytica**, Wageningen, v. 213, n. 1, p. 117, May. 2017.
- CAPUCHO, A. S. et al. Climate favorability to leaf rust in Conilon coffee. **Australasian Plant Pathology**, v. 42, n. 5, p. 511-514, Sept. 2013.
- CARVALHO, V. L.; CHALFOUN, S. M.; CUNHA, R. L. Manejo de doenças do cafeeiro. In: REIS, P. R.; CUNHA, R. L. (Ed.) **Café Arábica do plantio a colheita**. Lavras: URESM, 2010. v. 1, p. 689-756.
- 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, Mar. 2017.
- CUNHA, R. L. et al. Desenvolvimento e validação de uma escala diagramática para avaliar a severidade da ferrugem (*Hemileia vastatrix*) do cafeeiro. In: SIMPÓSIO DE PESQUISA DOS CAFÉS DO BRASIL, 2., 2001, Vitória. **Resumos Expandidos...** Vitória, ES: EMBRAPA Café, 2001. p.77-78.
- CUSTODIO, A. A. et al. Comparison and validation of diagrammatic scales for brown eye spots in coffee tree leaves. **Ciência e Agrotecnologia**, Lavras, v. 35, n. 6, p. 1067-1076, Nov. 2011.

- DELL' ACQUA R. et al. Variabilidade in vitro, in vivo e molecular de isolados de *Cercospora coffeicola*. **Tropical Plant Pathology**, Brasília, v. 36, n. 5, p. 313-326, Sept. 2011.
- FAZUOLI, L. C. et al. Identification and use of sources of durable resistance to coffee leaf rust at the IAC. In: ZAMBOLIM, L.; ZAMBOLIM, E.M.; VÁRZEA, V.M. **Durable resistance to coffee leaf rust**. Viçosa: Universidade Federal de Viçosa, Departamento de Fitopatologia, 2005, p. 137-185.
- KUSHALAPPA, A. C.; CHAVES, G. M. An analysis of the development of coffee rust in the field. **Fitopatologia Brasileira**, Brasília, v. 5, p. 95-183, Dec. 1980.
- KUZNETSOVA, A., BROCKHOFF, P. B.; CHRISTENSEN, R. H. B. **lmerTest: Tests in Linear Mixed Effects Models**. R package version 2.0-33, 2016. Available: <<https://cran.r-project.org/web/packages/lmerTest/lmerTest.pdf>>. Access in: Jan. 17. 2019.
- MAFFIA, L. A.; HADDAD, F.; MIZUBUTI, E. S. G. Controle biológico da ferrugem do cafeeiro. In: BETTIOL, W.; MORANDI, M. A. B. **Biocontrole de doenças de plantas: uso e perspectivas**. 1 ed. Jaguariúna: Embrapa Meio Ambiente, 2009, p. 267-276.
- MATIELLO, J. B. et al. **Cultura de café no Brasil: manual de recomendações**. Rio de Janeiro: PROCAFÉ, 2015, p. 387.
- MOLLER, M.; STUKENBROCK, E. H. Evolution and genome architecture in fungal plant pathogens. **Nature Reviews | Microbiology**, p.756-771, Nov. 2017.
- MONTES, S. M. N. M.; PAULO, E. M.; FISCHER, I. H. Incidência da ferrugem alaranjada em cafeeiros nas condições climáticas de Presidente Prudente, SP. **Coffee Science**, Lavras, v. 7, n. 3, p. 208-214, Sept. 2012.
- MULAMBA, N. N. AND MOCK, J. J. Improvement of yield potential of the Eto Blanco maize (*Zea mays* L.) population by breeding for plant traits. **Egyptian Journal of Genetics and Cytology**, Cairo, v. 7, p. 740-51, 1978.
- NELSON, R. et al. Navigating complexity to breed disease-resistant crops. **Nature Reviews | Genetics**. Nov. 2017.
- OLIVEIRA, A. C. B. et al. Prediction of genetic gains from selection in Arabica coffee progenies. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 2, n. 2, p. 106-113, Feb 2011.
- PAIVA, B. R. L. et al. Progresso da cercosporiose do cafeeiro sob diferentes manejos de irrigação e densidades de plantio. **Coffee Science**, Lavras, v. 8, n. 2, p. 166-175, June 2013.
- PATRICIO, F. R. A.; BRAGHINI, M. T.; FAZUOLI, L. C. Resistência de plantas de *Coffea arabica*, *Coffea canephora* e híbridos interespecíficos à cercosporiose. **Bragantia**, Campinas, v. 69, n. 4, p. 883-890, Dec. 2010.
- 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, Dec. 2013.
- PETEK, M. R.; SERA, T.; FONSECA, I. C. B. Prediction of genetic additive values for development of a coffee cultivar with increased rust resistance. **Bragantia**, Campinas, v. 67, p. 133-140, Feb. 2008.
- RAMALHO, M. A. P. et al. **Aplicação da genética quantitativa ao melhoramento de plantas autógamas**. Lavras: Editora UFLA, 2012. p. 522.
- R CORE TEAM. **R: A language and environment for statistical computing**. Viena, 2014. Available: <<http://www.r-project.org>>. Access in: July 5 2015.
- RODRIGUES, W. P. et al. Adaptability and genotypic stability of *Coffea arabica* genotypes based on REML/BLUP analysis in Rio de Janeiro State, Brazil. **Genetics and Molecular Research**, Ribeirão Preto, v. 12, n. 3, p. 2391-2399, Aug.2013.
- SADRAS, V. O.; CALDERINI, D. F. **Crop physiology applications for genetic improvement and agronomy**. Second edition. Elsevier, Amsterdam, The Netherlands, 2015, p. 580.
- SARKAR, D. **Lattice: multivariate data visualization with R**. New York: Springer-Verlag, 2008, 280 p.
- SCOTT, A. J.; KNOTT, M. Cluster analysis method for grouping means in the analysis of variance. **Biometrics**, Arlington, v. 30, n. 3, p. 507-512, Sept. 1974.
- SHANER, G.; FINNEY, R. E. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. **Phytopathology**, Lancaster, v. 67, p. 1051-1056, Nov. 1977.

- SILVA, M. C. et al. Coffee resistance to the main diseases: leaf rust and coffee berry disease. **Brazilian Journal of Plant Physiology**, v. 18, n. 1, p. 119–147, Mar. 2006.
- SILVA, M. G. da. Temperature and light intensity interaction on *Cercospora coffeicola* sporulation and conidia germination. **Ciência e Agrotecnologia**, v. 40, n. 2, 2016.
- SILVA, J. A. da. **Diversidade genética e seleção de progênies de cafeeiros do grupo “Big Coffee VL”**. 2016. 66 p. Tese (Doutorado em Fitotecnia) - Universidade Federal de Lavras, Lavras, 2016.
- SOUZA, A. G. C. et al. Infection Process of *Cercospora coffeicola* on Coffee Leaf. **Journal of Phytopathology**, San Diego, v. 159, n. 1, p. 6-11, June 2011.
- SOUZA, A. G. C. et al. A time series analysis of brown eye spot progress in conventional and organic coffee production systems. **Plant Pathology**, New Jersey, v. 64, n. 1, p. 157-166, May. 2014.
- TIAN, D. et al. Fitness costs of R-gene-mediated resistance in *Arabidopsis thaliana*, **Nature**, London, v. 423, p. 74-77, June 2003.
- VALE, P. A. S. **Caracterização de *Cercospora coffeicola* por filogenia molecular multigênica**. 2016. 64 p. Dissertação (Mestrado em Biotecnologia Vegetal) – Universidade Federal de Lavras, Lavras, 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.
- ZAMBOLIM, L. Current status and management of coffee leaf rust in Brazil. **Tropical Plant Pathology**, Brasília, v. 41, n.1, Jan. 2016.

GROWTH, ANATOMY AND PHYSIOLOGY OF COFFEE PLANTS INTOXICATED BY THE HERBICIDE GLYPHOSATE

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ABSTRACT: Weed control is fundamental in coffee cultivation due to their high interference, competing with the crop for water, light and nutrients. Among the control methods used, chemical control is highlighted, due to its high efficiency and low cost. However, due to application failures, herbicide drift phytotoxicity is common. Aiming at the search for selective active ingredients in coffee, the objective of this study was to growth, anatomy and physiology of coffee plants intoxicated by the herbicide Glyphosate. The experiment was carried out in a greenhouse with 'Topázio MG1190' coffee plants (*Coffea arabica* L.), cultivated in pots with an 11 L-substrate capacity. The statistical design was randomized block design (RBD), with four replicates and four doses of the herbicide, making up 16 experimental plots. Each plot consisted of three plants. The treatments were: (i) 0%; (ii) 10%; (iii) 25% and (iv) 50% of the commercial dose of the herbicide Glyphosate. The evaluations were performed at 104 days after application of the treatments. Growth, morphological, and physiological characteristics were evaluated. The effect of glyphosate drift impairs plant growth. However, after 104 days of intoxication, there is no longer any effect on the physiology and leaf anatomy of coffee plants.

Index Terms: Selectivity; chemical control; *Coffea arabica* L.

CRESCIMENTO, ANATOMIA E FISILOGIA DE CAFEEIROS INTOXICADOS PELO HERBICIDA GLYPHOSATE

RESUMO: O controle de plantas daninhas é fundamental na cafeicultura devido à elevada interferência que as mesmas implicam, competindo com a cultura por água, luz e nutrientes. Dentre os métodos de controle utilizados destaca-se o controle químico, por sua alta eficiência e baixo custo. Contudo, devido a falhas na aplicação, são frequentes casos de fitotoxicidade pela deriva dos herbicidas. Visando a busca por ingredientes ativos seletivos ao café, objetivou-se com este trabalho, avaliar o crescimento, anatomia e fisiologia de plantas de café intoxicadas pelo herbicida Glyphosate. O experimento foi realizado em casa de vegetação, com plantas de café (*Coffea arabica* L.) da cultivar Topázio MG1190, cultivadas em vasos com capacidade de 11 litros de substrato. O delineamento estatístico utilizado foi o Delineamento em Blocos Casualizados (DBC), com quatro repetições e quatro doses do herbicida, perfazendo, 16 parcelas experimentais. Cada parcela foi composta por três plantas. Os tratamentos foram: (i) 0%; (ii) 10%; (iii) 25% e (iv) 50% da dose comercial do herbicida Glyphosate. As avaliações foram realizadas aos 104 dias após a aplicação dos tratamentos. Foram avaliadas características de crescimento, fisiológicas e anatômicas. O efeito da deriva de glyphosate prejudica o crescimento das plantas, porém após 104 dias da ocorrência da intoxicação já não se observa consequências na fisiologia e na anatomia foliar dos cafeeiros.

Termos indexados: Fitotoxicidade, seletividade, controle químico, café.

1 INTRODUCTION

The coffee agribusiness is of great importance in the Brazilian economy, and it is responsible for the generation of income and jobs (CAIXETA, 2008). According to CONAB (2018), the Brazilian production expected for 2018 is between 54.4 and 58.5 million 60 kg-processed bags, which is higher than the previous year. However, coffee crop yield is directly related to several factors, among them, soil fertility, plant nutrition, phytosanitary control, application technologies, climatic conditions and weed management can be highlighted (OLIVEIRA, 2013).

In this context, weed incidence stands out as one of the most important, mainly due to the competition with coffee for water, light and nutrients (FIALHO et al, 2011; LORENZI, 2014). In addition, it may also hamper crop cultivation, such as fertilization, harvesting and pest and disease control (RONCHI et al., 2003b).

The period of greatest crop sensitivity to weed incidence occurs in the first year of implantation, mainly in the planting line, where it is frequently necessary to adopt manual control, which is more difficult and costly due to the increasing scarcity of manpower in the field and

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the high rainfall that coincides with the time of greatest weed interference in the crop. In this context, a viable alternative for an efficient weed control in the coffee crop is by herbicides (RONCHI et al., 2001).

However, even though there are several herbicides registered for weed control in coffee cultivation, few are those that show total selectivity for direct application on coffee (ALCÂNTARA, 2000). It is emphasized that these non-selective herbicides should be used with caution, avoiding them to reach the crop, causing phytotoxicity symptoms (SILVA et al., 2017).

Glyphosate is one of the main non-selective herbicides used in coffee plants. However, due to the occurrence of winds and the lack of use of recommended application technologies, product drift can occur, reaching the crop and, consequently, causing phytotoxicity symptoms (RONCHI et al., 2003a).

Several studies have demonstrated the characterization of these symptoms by the appearance of chlorotic, small and brittle leaves, similar to those of nutritional deficiencies of nitrogen (N), boron (B) and zinc (Zn) (FRANÇA, et al., 2010b). However, there is a lack of studies that associate the appearance of these visual characteristics with the internal modifications in plants, as well as with the physiological changes caused by these alterations. The objective of this study was to evaluate the changes in the anatomical and physiological characteristics of coffee plants intoxicated by the herbicide Glyphosate.

2 MATERIAL AND METHODS

The trial was carried out in a greenhouse in the municipality of Lavras-MG, located in the southern region of the state of Minas Gerais, at an altitude of 918 m, 21°14' S latitude and 45°00' W longitude, from December 2016 to March 2017. The annual average air temperature is 19.4 °C, with a maximum of 26.1 °C and the minimum, 14.4 °C.

For the simulation of intoxication, 'Topázio MG1190' coffee (*Coffea arabica* L.) seedlings were planted in 11-L pots, with 4-5 pairs of leaves. A randomized block design with four replicates and four doses of the herbicide Glyphosate was used: (i) 0%; (ii) 10%; (iii) 25% and (iv) 50% of the recommended commercial dose (3.0 L ha⁻¹) applied directly to coffee plants. Each plot consisted of three plants.

The application of the herbicide at different doses was carried out using a CO₂ pressurized costal sprayer, with pressure of 45 pounds, and the spray bar directed close to the top of the plants, with a spray volume of 300 L.ha⁻¹. After the application, the plants remained in the greenhouse for 104 days, daily irrigated, aiming at the maintenance of field capacity (100%). The management and cultural treatment were carried out as recommended by Matiello (2010).

Visual observation and imaging of the symptoms caused by the action of the herbicide were performed daily. The anatomical and physiological evaluations of the plants were performed at the end of the experiment. The following growth characteristics were evaluated: plant height, measured in centimeters from the ground level to the apical bud of the orthotropic branch; number of leaves; stem diameter, measured in millimeters at the collar of the plants; number of plagiotropic branches; leaf area, in cm², quantified by leaf discs (Cunha et al., 2010); shoot, root, plagiotropic branches, ortotropic branche and leaf dry matter, in grams. Root length analysis, in centimeters, was also determined by the sum of the linear extension of each fragment of the root system and the mean root diameter, in centimeters, both using the imaging software Safira (JORGE; SILVA, 2010).

For the evaluation of leaf anatomy, at 104 days, leaves located between the second and third node of the plagiotropic branch of the plants were collected. Subsequently, in the laboratory, the paradermic and transverse sections were performed. Those paradermic were obtained before of collection, using the universal instant adhesive (cyanoacrylate ester) method (SEGATTO et al., 2004). In order to obtain the cross sections, the plant material underwent dehydration in an ethylic series, and then immersed in methacrylate (methodology according to the manufacturer) and sectioned with 0.8 µm thickness using a rotary microtome. Subsequently, these were stained with toluidine blue (O'Brien; Feder; Mccully, 1964) and the blades were assembled using Entelan® as a medium. The slides of both sections (paradermic and transverse) were observed and photographed under an optical microscope coupled to a digital camera. The images obtained were analyzed in the UTHSCSA-Imagetool program and then the stomatal characteristics, leaf tissues and vascular bundles were evaluated. For the stomatal characterization, the following characteristics

were evaluated: stomatal number; polar diameter of the stomata and equatorial diameter of the stomata. Stomatal density (number of stomata per mm^2) and the polar diameter/equatorial diameters stomatal ratio, which is highly correlated with stomatal functionality (Silva, et al., 2014, Souza et al., 2013), was calculated using these data. To evaluate leaf tissues, the following were measured: epidermal thickness of the adaxial face; thickness of the palisade parenchyma; thickness of the spongy parenchyma; and thickness of the mesophyll. In the evaluation of vascular bundles, the following parameters were measured: thickness of the phloem region; diameter of xylem vessels; and number of xylem vessels.

For the physiological analyses, a portable infrared gas analysis system (LICOR - 6400XT) was used to evaluate the net photosynthetic rate ($A - \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$), stomatal conductance ($g_s - \text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$), and transpiration rate ($E - \text{mmol m}^{-2} \text{ s}^{-1}$). The water use efficiency ($EiUA - \mu\text{mol CO}_2 \text{ mmol}^{-1} \text{ H}_2\text{O}$), calculated by the A/E ratio (Yan et al., 2015), was also evaluated. The evaluations were performed between 9 and 11 o'clock in the morning under artificial light ($1000 \mu\text{mol m}^{-2} \text{ s}^{-1}$), using leaves located between the second and third node of the plagiotropic branch of the plants.

For data analysis, the SISVAR Statistical Software (Ferreira, 2011) was used. For the response variables that showed differences ($p < 0.05$), the regression models that were able to explain the variation maximum of the response variable as a function of herbicide doses were adjusted.

3 RESULTS AND DISCUSSION

By the observation of the symptoms and verification of the obtained images, the appearance of the phytotoxicity symptoms occurred from the 10th day after the application of the herbicide Glyphosate. Initially, in the 10th day leaves with mild chlorosis were observed in the meristematic regions; subsequently, after 20 days, leaf limb tightening and excessive shooting were observed in axillary bud regions. (Figures 1, 2 and 3).

Leaf chlorosis is related to the reduction in chlorophyll synthesis by the plant, since Glyphosate directly blocks its formation (TAN et al., 2006). However, leaf limb tightening maybe associated with dysfunction in the cell caused by the herbicide, causing that three essential amino acids are not produced for plant development, tyrosine, phenylalanine and tryptophan, from the

inhibition of the site of action of the target enzyme, 5-enolpyruvyl-chiquimate-3-phosphate synthase (EPSPs) (ZABLOTOWICZ; REDDY, 2004).

The analysis of variance showed that the variables stem diameter, leaf number, number of plagiotropic branches, shoot and ortotropic dry matter, all physiological, anatomical and root system characteristics of the plants did not present significant differences ($p < 0.05$) among the doses. However, for the variables height, leaf dry matter, leaf area and dry matter of plagiotropic branches, there was a significant difference.

Despite the initial symptoms after the application of the herbicide, the plants apparently presented recovery, without damage to the physiology and foliar anatomy, after 104 days of application. Tuffi Santos et al. (2008) did not observe variations in epidermal thickness in plants exposed to different doses of Glyphosate in the eucalyptus crop. However, Tuffi Santos et al. (2009) observed an increased thickness of the palisade parenchyma at 7 days after application of Glyphosate in doses from 345.6 g ha^{-1} in three different eucalyptus clones. This variability in the occurrence of symptoms may have occurred due to the different clones used in the studies.

On the other hand, Reis (2013) observed that, with the increase in Glyphosate doses, there was a decrease in total leaf thickness in 'Catuaí' plants, due to the reduction in the palisade parenchyma, 30 days after application of herbicide.

There was no significant effect on the plants at 104 days after the simulated drift for the physiological characteristics. This may have occurred due to the fact that coffee plants have the ability to recover from the injuries inherent to the herbicide Glyphosate after a certain time after application. In this context, França et al. (2010a) observed a recovery of coffee plants at 120 days after application of Glyphosate, with a decrease in the intoxication level of the plants that received the herbicide. Likewise, Tuffi Santos et al. (2008) chose eucalyptus plants with symptoms of Glyphosate intoxication ranging from 0 to 50% of the dose in the field and verified that, after 180 days of application, the plants were recovered.

No significant differences were observed in the coffee seedlings for the characteristics related to the root system, and these results differ from those found by França et al. (2013), in which the authors found differences in root dry matter for cultivars Acaia and Catuaí, using with up to 32% of the recommended herbicide dose.



FIGURE 1 - Foliar chlorosis in the coffee growing regions submitted to the herbicide Glyphosate.



FIGURE 2 - (A) and (B) Leaf limb tightening in coffee plants submitted to the herbicide Glyphosate.



FIGURE 3 - Leaf limb tightening and excessive shooting in axillary bud regions in coffee plants submitted to the herbicide Glyphosate.

However, Wagner Jr. et al. (2008) also reported that doses of Glyphosate applied on yellow passion fruit seedlings did not lead to reductions in root length at 28 DAA, up to 24 % of the recommended herbicide dose, corroborating the results of this study.

In relation to the characteristics that presented significant differences, as the doses of the herbicide Glyphosate were increased, there was a linear decrease in values, in height, leaf area, leaf dry matter and in the dry matter of the plagiotropic branches of the plants (Figure 4).

A negative influence of Glyphosate drift was observed under the growth of the coffee plant, so that, by the replacement the dose of herbicide in the adjusted equation linear regression, at every 1% of the recommended dose, the height was reduced by 0.096 cm, the leaf area in 35.31 cm², leaf dry matter in 0.233 g and the dry matter of plagiotropic branches in 0.046 g.

The decrease observed in the values of the studied characteristics may be associated with the mode of action of the herbicide, which acts on the secondary metabolism of the plant (VIDAL, 1997). The smaller leaf area caused a lower leaf dry matter, maybe this happened due to the possibly reduction in chlorophyll synthesis, evidenced by chlorosis, and the absence of aromatic amino acid formation, the leaf increment of the plant was impaired (VIDAL, 1997). The smallest leaf area is directly related to leaf blade tightening, a phytotoxicity symptom observed at the highest doses of the herbicide (Figure 2).

However, the lower plant height and the lower dry matter of the plagiotropic branches in plants that received higher doses of Glyphosate can be explained by the possible reduction in syntheses of indole-3-acetic acid (IAA), since it is synthesized from tryptophan which, due to the inhibition of the chiquimate pathway by this herbicide, is not produced.

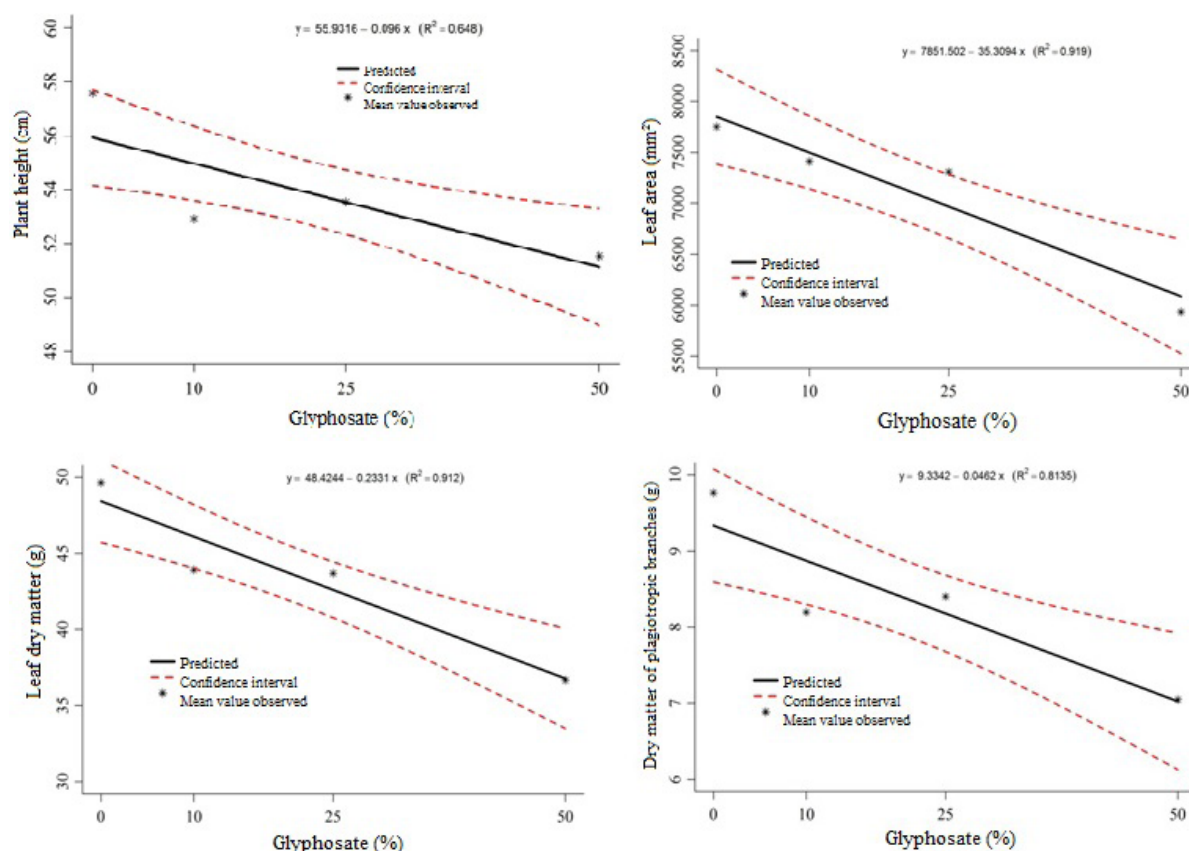


FIGURE 4 - Plant height, leaf area, leaf dry matter and dry matter of plagiotropic branches of coffee plants as a function of the effects of Glyphosate doses after 104 days at evaluation.

Above all, the hormone indole-3-acetic acid is of great importance in the plant, responsible for cell expansion and maintenance of apical dominance, which is directly related to plant growth (GRUYS; SIKORSKI, 1999). França et al. (2010b) also report losses in the growth of coffee seedlings when submitted to the simulated drift of the herbicide Glyphosate.

4 CONCLUSION

The effect of glyphosate drift impairs coffee plant growth.

Leaf anatomy and physiology were not affected at 104 days after application of the herbicide.

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6 REFERENCES

- ALCÂNTARA, E. N.; FERREIRA, M. M. Efeitos de métodos de controle de plantas daninhas na cultura do cafeeiro (*Coffea arabica* L.) sobre a qualidade física do solo. **Revista Brasileira de Ciência do Solo**, Viçosa, v. 24, n.4, p. 711-721, Oct./Dec. 2000.
- CAIXETA, G. Z. T. et al. Gerenciamento como forma de garantir a competitividade da cafeicultura. **Informe Agropecuário**, Belo Horizonte v. 29, n. 247, p. 14-23, Nov./Dec. 2008.
- CONAB. **2º levantamento da safra de café 2017/2018**. Brasília, 2018. Available in: <<https://www.conab.gov.br/info-agro/safras/cafe>> Access in: June 2018.
- CUNHA, J. L. X. L. et al. Comparação de métodos de área foliar em *Chrysobalanus icaco* L. **Agropecuária Científica no Semiárido**, Patos, v. 6, n. 3, p. 22-27, July/Sept. 2010.
- FERREIRA, D. F. Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia**, Lavras, v. 35, n. 6, p. 1039-1042, Nov./Dec. 2011.
- FIALHO, C. M. T., et al. Interferência de plantas daninhas sobre o crescimento inicial de *Coffea arabica* L. **Planta Daninha**, Viçosa, v.29, n.1, p. 137-147, Mar. 2011.
- FRANÇA, A. C. et al. Teores de nutrientes em cultivares de café arábica submetidos à deriva de glyphosate. **Planta Daninha**, Viçosa, MG, v. 28, n. 4, p. 877-885, Dec. 2010.
- FRANÇA, A.C. et al. Crescimento de cultivares de café arábica submetidos a doses do glyphosate. **Planta daninha**, Viçosa, v. 28, n. 3, p. 599-607, Sept. 2010.
- FRANÇA, A.C. et al. Deriva simulada do glyphosate em cultivares de café Acaiá e Catucaí. **Planta daninha**, Viçosa, v. 31, n. 2, p. 443-451, June 2013.
- GRUYS, K. J.; SIKORSKI, J. A. Inhibitors of tryptophan, phenylalanine and tyrosine biosynthesis as herbicides. In: SINGH, B. K. **Plant amino acids: biochemistry and biotechnology**. New York: Marcel Dekker, 1999, p. 357-384.
- JORGE, L. A. C.; SILVA, D. J. C. B. SAFIRA: Manual de utilização. São Carlos: **Embrapa CPDIA**, 2010. 29 p.
- LORENZI, H. **Manual de Identificação e controle de plantas daninhas**. 7ª Ed. Nova Odessa – SP. Instituto Plantarum, 2014. 384 p.
- MATIELLO, J. B. et al. **Cultura de Café no Brasil: manual de recomendações**. Rio de Janeiro: MAPA/ PROCAFE, 2010. 542 p.
- O'BRIEN, T. P.; FEDER, N.; MCCULLY, M. E. Polychromatic staining of plant cell walls by toluidine blue O. **Protoplasma**, Leipzig, v. 59, n. 2, p. 368-373, June 1964.
- OLIVEIRA, C. M., et al. Economic impact of exotic insect pests in Brazilian agriculture. **Journal of Applied Entomology**, Berlin, v. 137, n. 1-2, p. 1-15, Nov 2012.
- REIS, L. A. C. **Influência do glyphosate na anatomia e fisiologia de cultivares de café arábica**. 2013. 61 p. Dissertação (Mestrado em Produção Vegetal)-Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, 2013.
- RONCHI, C. P. et al. Acúmulo de nutrientes pelo cafeeiro sob interferência de plantas daninhas. **Planta Daninha**, Viçosa, v. 21, n. 2, p. 219-227, Aug. 2003.
- RONCHI, C. P. et al. Efeito do 2, 4-D na produtividade do cafeeiro. In: SIMPOSIO DE PESQUISA DOS CAFÉS DO BRASIL, 2. 2001, Vitória. **Resumos Expandidos...**, Brasília: Embrapa Café; MINASPLAN, 2001. V. 1, p. 24-27.
- RONCHI, C.P.; SILVA, A.A. Tolerância de mudas de café a herbicidas aplicados em pós-emergência. **Planta Daninha**, Viçosa, v. 21, n. 3, p.421-426, Sept./Dec. 2003.

- SEGATTO, F. B.; et al. Técnica para o estudo da anatomia da epiderme foliar de batata. **Ciência Rural**, Santa Maria, v.34, n.5, p.1597-1601, Oct. 2004.
- SILVA, H., et al. Relationships between leaf anatomy, morphology, and water use efficiency in *Aloe vera* (L) Burm f. as a function of water availability. **Revista chilena de história natural**, Santiago, v. 87, n. 1, p. 13, July 2014.
- SILVA, L. G., et al. Sintomas de fitotoxicidade e crescimento de mudas de café submetidas aos herbicidas inibidores da PROTOX. **Coffee Science**, Lavras, v.12, n.3, Sept 2017
- TAN, S. et al. Herbicidal inhibitors of amino acid biosynthesis and herbicide-tolerant crops. **Amino Acids**, Vienna, v. 30, p. 195– 204, Mar. 2006.
- TUFFI SANTOS, L. D. et al. Anatomia e morfometria foliar em clones de eucalipto tratados com glyphosate. **Brazilian Journal of Biology**, London, v. 69, n. 1, p. 129-136, Mar. 2009.
- TUFFI SANTOS, L. D. et al. Danos visuais e anatômicos causados pelo glyphosate em folhas de *Eucalyptus grandis*. **Planta Daninha**, Viçosa, MG, v. 26, n. 1, p. 9-16, Jan./Mar. 2008.
- VIDAL, R. A. **Herbicidas: Mecanismos de ação e resistência de plantas**. Porto Alegre, RS. 1997. 165 p.
- WAGNER JR, A. et al. Deriva simulada de formulações comerciais de glyphosate sobre maracujazeiro amarelo. **Planta Daninha**, Viçosa, v. 26, n. 3, p. 677-683, Sept. 2008.
- YAN, J., et al. High photosynthetic rate and water use efficiency of *Miscanthus lutarioriparius* characterize an energy crop in the semiarid temperate region. **Gcb Bioenergy**, San Diego, v. 7, n. 2, p. 207-218, Mar. 2014.
- ZABLOTOWICZ, R. M.; REDDY, K. N. Impact of glyphosate and *Bradyrhizobium japonicum* symbiosis; with glyphosate-resistant transgenic soybean: a minireview. **Journal of Environmental Quality**, Madison, v. 33, p. 825-831, May. 2004.

SIMULTANEOUS SELECTION IN COFFEE PROGENIES OF MUNDO NOVO BY SELECTION INDICES

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ABSTRACT: Coffee cultivars that are better adapted and more productive can help reduce production costs and make coffee cultivation more profitable. The Mundo Novo cultivar has stood out as one of the most productive cultivars of *Coffea arabica* L. Therefore, the aim of this work was to select Mundo Novo progenies with high bean yield capacity and with other agronomic characteristics of interest. We evaluated the bean yield, seed size, plant height, upper and lower canopy diameter, stem diameter, and plant vigor of 24 F₄ progenies and one check cultivar. The experimental design consisted of a randomized complete block design with three replicates and experiments were carried out in two sites: São Sebastião do Paraíso (SSP) and Três Pontas (TP), MG, Brazil. The mixed model approach was used to calculate the genotypic value for each progeny, after which the selective accuracy and genetic gain of the two best progenies in each trait were calculated, the simultaneous selection index of Mulamba and Mock was used to select the two most promising progenies at each site. In general, the genetic gain was higher at SSP than at TP. In SSP, the most promising progenies were 1 [IAC376-2 (M. Novo) x IAC386-17 (M. Novo)] and 20 [IAC382-10 (M. Novo) x IAC388-20 (M. Novo)]. In TP, the most promising progeny was 22 [H1535/181 (M. Novo) x (S795) 1344/10/5], together with the check (Mundo Novo IAC379/19). The highest selection gain was expressed for bean size (the percentage of beans retained in sieve 17).

Index terms: *Coffea arabica*, ranking, mixed model.

SELEÇÃO SIMULTÂNEA DE PROGÊNIES DE CAFEIROS MUNDO NOVO POR ÍNDICE DE SELEÇÃO

RESUMO: Cultivares de café mais adaptadas e produtivas podem auxiliar na redução dos custos de produção e tornar a cafeicultura mais rentável. A cultivar Mundo Novo tem se destacado como uma das cultivares mais produtivas de *Coffea arabica* L. Diante disso, objetivou-se com este trabalho selecionar progênies de cafeeiro Mundo Novo com elevada capacidade produtiva e portadoras de outras características agrônomicas de interesse. Foram conduzidos experimentos em blocos casualizados em São Sebastião do Paraíso e Três Pontas-MG para a avaliação de 24 progênies F₄ e uma testemunha quanto às características de produtividade, peneira 17 e acima, altura, diâmetro de copa do terço inferior e do terço médio, diâmetro do caule e vigor de plantas. Foi utilizada a abordagem de modelos mistos para o cálculo do valor genotípico para cada progênie, posteriormente foi calculada a acurácia seletiva e o ganho genético da seleção das duas melhores plantas em cada característica. Para resumir, foi considerado um índice de seleção simultâneo de Mulamba e Mock para selecionar as duas progênies mais promissoras em cada local. De um modo geral, o ganho genético foi maior para São Sebastião do Paraíso quando comparado com Três Pontas. Em São Sebastião do Paraíso as progênies mais promissoras foram a 1 [IAC376-2 (M. Novo) x IAC386-17 (M. Novo)] e 20 [IAC382-10 (M. Novo) x IAC388-20 (M. Novo)]. E em Três Pontas, foi a progênie 22 [H1535/181 (M. Novo) x (S795)1344/10/5], juntamente com a testemunha (Mundo Novo IAC379/19). O ganho de seleção foi expressivo, principalmente para a característica peneira 17 e acima.

Termos para indexação: *Coffea arabica*, ranqueamento, modelos mistos.

1 INTRODUCTION

The Mundo Novo cultivar was discovered by researchers of the Instituto Agrônomo de Campinas (IAC) in the city of Mundo Novo (Aparecida Farm), now Urupês, in the region near Araraquara City, state of São Paulo, in mid-1943. The tillage was composed of plants from a Sumatra cultivar that showed phenotypical variations

but exceptional growth and productivity. The seeds for this crop came from another farm that originally collected the seeds from the city of Jaú. Researchers who visited the farm were provided with the seeds from Jaú that was subsequently cultivated at the Aparecida Farm to form a crop similar to that previously established. The owner reported that the seeds provided for the formation of this crop came from another crop in a property

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at Mineiro do Tietê, whose plants would have been produced from seeds of a plant that had been grown on the edge of a dirt road at Santa Terra farm. From this plant, seeds were collected to form other crops in the region (CARVALHO et al., 1952).

After research efforts by the IAC, there are currently several cultivars of the “Mundo Novo” group available in the market. In the breeding programs, the “Mundo Novo” cultivar has been used mainly as a parent, with the purpose of exploring its high bean yield, vegetative vigor, and rusticity (PEREIRA et al., 2010). In addition to these characteristics, this cultivar has been considered important because of market demand and other traits such as quality, size, stability, adaptability, and others.

The breeding programs have developed varieties of coffee to increase productivity and other important agronomic traits and adapted to local climate and soil conditions (PETEK et al., 2006). However, the cultivar responses differ in different environments because of genotype-environment interactions (PINTO et al., 2012).

The use of more refined genetic-statistical procedures, such as the mixed model approach, is a trend in plant breeding. These procedures provide additional parameters relevant to the identification of superior genotypes and to obtain more precise estimates (RAMALHO; ARAÚJO, 2011).

Selecting plants to improve several characteristics simultaneously, to obtain more advantageous cultivars than the preceding cultivar is a great challenge (RAMALHO et al., 2012), mainly for perennial crops (OLIVEIRA et al., 2011; CORRÊA et al., 2017). An alternative is the use of selection indexes that have frequently been used for selection in various plant species (VIEIRA et al., 2017; SILVA et al., 2017; GHOSH et al., 2018). One of the simplest methods is that of Mulamba and Mock (1978), which consists of an ordering of the genotypes for each one of the traits in the order most favorable to improvement, and subsequently, the orders of each genotype referring to each trait are summed, resulting in an additional measurement taken as a selection index (CRUZ et al., 2014).

An important point to increasing the selection efficiency is the use of statistical methods that have the advantage of obtaining the most information about genotypes, resulting in accurate estimations or predictions of breeding values (PEREIRA et al., 2013; BERNARDO, 2010).

Thus, the objective in this work was to select “Mundo Novo” coffee progenies with a high productive capacity and other agronomic characteristics of interest.

2 MATERIAL AND METHODS

The hybridizations were carried out between “Mundo Novo” and “Mundo Novo” progenies and between “Mundo Novo” and “Bourbon” progenies. Twenty-four progenies from the F_4 generation were obtained at Machado-MG.

Two experiments were carried out in a randomized complete block design, both with three replicates and 24 treatments with one common check (Mundo Novo IAC379/19). Each plot consisted of a line with eight plants spaced $3.2 \text{ m} \times 0.8 \text{ m}$ apart. Descriptions of the treatments with their respective crosses are shown in Table 1.

The first experiment was established in January 2008 at São Sebastião do Paraíso-MG, on the Experimental Field of São Sebastião do Paraíso (EFSSP: $21^{\circ}20'45.7'' \text{ S}$ and $45^{\circ}28'45.7'' \text{ W}$; 939 m altitude), in Distroferic Red Latosol soil with clay texture and undulating relief. The average annual rainfall is 1470.4 mm and the average temperature is 20.8°C . The second experiment was established in March 2008 at Três Pontas-MG, on the Experimental Field of Três Pontas (EFTP $20^{\circ}54'72.8'' \text{ S}$ and $47^{\circ}07'33.2'' \text{ W}$, 890 m altitude) in a Distroferic Red Latosol soil with medium texture and undulating relief. The average annual rainfall is 1670 mm and the average temperature is 20.1°C .

Plant sowing and care were performed in accordance with the technical recommendations for the crop.

Evaluated traits

Yield: The harvests were performed manually in both sites during four crop years. The beans of each plot were weighed, and the yield in processed coffee as bags/hectare (sc ha^{-1}) was determined (BARTHOLO et al., 1989). In 2015, the plants that were eight years old was evaluated by the following traits: Seed size (%S17), measured on 300 g samples of processed beans that were classified on a set of sieves specific for the classification of coffee based on size of the raw beans, which were classified into only two groups, i.e., those that passed through sieve 17 and those above, representing the coarse flat type beans; Plant height, measured using a graduated

rule, from the base to the apical meristem; Upper and lower canopy diameter, measured using a graduated rule considering the reach of the largest branch (cm) in a transverse direction to the planting line; Stem diameter, measured with a caliper 10 cm from the soil surface; Plant vigor, scored from 1 to 10 based on attributes of plants in the field, in which 10 corresponds to plants with the best development and greatest plant vigor. The scores were based on the general aspect of plant as suggested by Carvalho et al. (1979).

The analyses of all traits were conducted based on a mixed model with random effect for the progeny factor and fixed effect for the blocks, expressed in the following equation:

$$y = X_m + Z_p + e$$

where y is the data vector; m is the vector representing the constant and blocks fixed effect; p is the vector representing the progenies effects, where; $p \sim N(0, I\sigma_p^2)$ e is the error vector, and;

$e \sim (0, I\sigma_e^2)$ X and Z are the incidence matrices of the fixed and random effects, respectively. We used SAS (SAS Institute, 2009) with PROC MIXED procedure.

The E-BLUP (empirical best linear unbiased prediction) for the genotypic values of the progenies was carried out subsequently.

We estimated heritability associated with the individual means by the following expression:

$$\hat{h}_i^2 = \frac{\hat{\sigma}_p}{\hat{\sigma}_p + \sigma_e}$$

where $\hat{\sigma}_p$ is the variance of progenies; and σ_e is the variance of error. The heritability associated with the plot mean was estimated by the following expression:

$$\hat{h}_m^2 = \frac{\hat{\sigma}_p}{\hat{\sigma}_p + \frac{\sigma_e}{j}}$$

where $\hat{\sigma}_p$ is the variance of progenies; σ_e is the variance of error; and j is the number of replicates. The square root of heritability provides the selective accuracy (SA) estimate.

For selection, the progenies were ranked in the order of favorability for each trait. Thus, the methodology of Mulamba and Mock (1978) detailed by Cruz et al. (2012) was used. In this methodology, after the ranking of each trait, the

sum of the positions in the ranking is then carried out. The two progenies selected in each site were those that showed the lowest value in terms of the sum of the positions.

The genetic gain was quantified by comparing the general average of the population with the check in each trait. The method used was presented by Vencovsky (1992) by the following formula:

$$SG = ds \cdot \hat{h}_m^2$$

where ds is the selection differential (sd), which is the difference between the mean of the selected genotypes (X_s) and the general population mean (X_0) or check (X_c); \hat{h}_m^2 and is the heritability associated with the plot mean.

In practical terms, the percentage of selection gain was obtained as follows:

$$SG (\%) = \frac{SG}{\bar{X}_0 \text{ or } \bar{X}_c} * 100$$

3 RESULTS AND DISCUSSION

Resende and Duarte (2007) has proposed a scale of accuracy, ordered in levels of experimental precision that are considered high at values higher than 70%, average at values between 40% and 70%, and low at values between 10% and 40%. These parameters indicate whether the experiment has been carried out appropriately and the predicted genetic values are fairly accurate.

In EFSSP, the mean SA level of the progeny ranged from 31% to 92%. The values were considered low for plant height and average for plant vigor and yield, and for other traits, the SA values were classified as high. The SA values associated with the individual means ranged from 40% to 64% and were considered low for plant vigor and height, high for lower canopy diameter, and low for other traits (Table 2).

In EFTP, the experiment generally provided good precision, with mean SA levels in the progeny ranging from 60% to 83%, close to the values found by Silva et al (2017) (74.89% to 91.28%) in conilon clones, suggesting reliable genetic progress in response to selection (Resende, 2007). The average accuracy values for productivity and for the other characteristics are high (Table 2).

In both sites, the SA values at the individual progeny level were lower than those at the mean progeny level for all traits, which shows that the selection will be much more efficient when considering all the progeny plants.

TABLE 1 - Genealogy of F₄ progenies of *Coffea arabica* evaluated in the Experimental Field of São Sebastião do Paraíso (EFSSP) and Experimental Field of Três Pontas (EFTP), MG.

ID	Progeny	Genealogy
1	H 6664	IAC 376-2 (M. Novo) x IAC386-17 (M. Novo)
2	H 6667	IAC 376-10 (M. Novo) x IAC 379-13 (M. Novo)
3	H 6669	IAC 376-10 (M. Novo) x IAC 382-10 (M. Novo)
4	H 6669	IAC 376-10 (M. Novo) x IAC 382-10 (M. Novo)
5	H 6683	IAC 382-14 (M. Novo) x IAC 382-12 (M. Novo)
6	H 6683	IAC 382-14 (M. Novo) x IAC 382-12 (M. Novo)
7	H 6683	IAC 382-14 (M. Novo) x IAC 382-12 (M. Novo)
8	H 6684	IAC 382-14 (M. Novo) x IAC 386-06 (M. Novo)
9	H 6672	IAC 379-13 (M. Novo) x IAC 379-19 (M. Novo)
10	H 6674	IAC 379-13 (M. Novo) x IAC 382-12 (M. Novo)
11	H 6684	IAC 382-14 (M. Novo) x IAC 386-06 (M. Novo)
12	H 6677	IAC 379-19 (M. Novo) x IAC 382-10 (M. Novo)
13	H 6682	IAC 382-12 (M. Novo) x IAC 386-6 (M. Novo)
14	H 6679	IAC 379-19 (M. Novo) x IAC 386-6 (M. Novo)
15	H 6680	IAC 382-10 (M. Novo) x IAC 382-12 (M. Novo)
16	H 6705	IAC 386-6 (M. Novo) x IAC 387-17 (M. Novo)
17	H 6698	IAC 386-5 (M. Novo) x IAC 387-15 (M. Novo)
18	H 6672	IAC 379-13 (M. Novo) x IAC 379-19 (M. Novo)
19	H 3223	IAC 379-19 (M. Novo) x [393-20-4(Bourbon) x 370(Bourbon)]5 (F ₁)
20	H 2917	IAC 382-10 (M. Novo) x IAC 388-20 (M. Novo)
21	H 2931	IAC 388-20 (M. Novo) x IAC 382-14 (M. Novo)
22	H 1596	H 1535/181 (M. Novo) x (S795) 1344/10/5
23	H 6669	IAC 376-10 (M. Novo) x IAC 382-10 (M. Novo)
24	H 2917	IAC 382-10 (M. Novo) x IAC 388-20 (M. Novo)
25	Check	Mundo Novo IAC 379/19

ID: Identification of progeny

TABLE 2 - Selective accuracy at individual and progeny levels for São Sebastião do Paraíso and Três Pontas, MG.

Traits	São Sebastião do Paraíso		Três Pontas	
	SA_m	SA_i	SA_m	SA_i
Yield	0.62	0.40	0.60	0.41
%S17	0.82	0.55	0.75	0.64
Height	0.31	0.52	0.73	0.18
Upper canopy diameter	0.92	0.64	0.83	0.81
Lower canopy diameter	0.72	0.54	0.75	0.51
Stem diameter	0.79	0.59	0.78	0.59
Vigor	0.40	0.57	0.77	0.25

Experimental Field of São Sebastião do Paraíso

The rankings and genotypic values of each variable are presented in Table 3.

The mean yield ranged from 22.4 sc ha⁻¹ (progeny 18) to 28 sc ha⁻¹ (progeny 22). Because of drought and high temperatures (CONAB, 2014, 2015), the mean yields of the experiments were low in 2014 and 2015.

The classification by sieve size has been an important criterion in selections for superior plants; it is a characteristic related to bean quality, in which the larger seeds present superior physiological qualities (GIOMO et al., 2008). Batches with larger quantities of large flat beans are more valued (BARTHOLO; GUIMARÃES, 1997). In this case, the trait %S17 ranged from 22.4% (progeny 22) to 42.9% (progeny 17).

TABLE 3 - Ranking of 25 progenies and their genotypic values (GV) for yield (sc ha⁻¹), %S17 (%), height (m), upper canopy diameter (m), lower canopy diameter (meters), stem diameter (mm), and vigor (score) in São Sebastião do Paraíso.

Rank	Yield		%S17		Height		Upper canopy diameter		Lower canopy diameter		Stem diameter		Vigor	
	ID	GV	ID	GV	ID	GV	ID	GV	ID	GV	ID	GV	ID	GV
1	22	28.0	17	42.9	9	3.24	14	2.03	17	1.75	14	80.4	14	7.9
2	21	27.3	16	39.9	16	3.24	17	2.03	8	1.75	23	78.9	6	7.8
3	6	26.9	4	38.1	17	3.25	11	2.05	22	1.77	15	78.9	10	7.8
4	20	26.1	1	38.0	2	3.25	8	2.05	19	1.79	20	78.9	12	7.8
5	5	25.8	3	35.1	8	3.25	16	2.07	11	1.80	5	78.8	23	7.8
6	15	25.5	24	34.1	1	3.25	25*	2.12	20	1.80	4	78.7	1	7.8
7	7	25.2	23	33.6	19	3.25	1	2.12	25*	1.81	25*	78.7	5	7.8
8	13	25.0	19	32.8	22	3.25	22	2.13	24	1.82	18	78.3	11	7.8
9	23	24.7	15	32.1	20	3.25	7	2.15	14	1.82	12	78.2	15	7.8
10	11	24.3	2	31.7	13	3.26	13	2.15	16	1.82	1	78.1	7	7.7
11	1	24.2	7	29.6	6	3.26	10	2.18	1	1.85	3	77.8	8	7.7
12	14	24.1	18	29.0	25*	3.26	18	2.20	10	1.85	9	77.7	9	7.7
13	24	24.1	25*	28.8	21	3.26	19	2.21	21	1.86	10	77.5	13	7.7
14	4	23.8	5	28.6	24	3.26	20	2.21	18	1.86	13	77.2	18	7.7
15	17	23.7	10	27.6	3	3.27	2	2.23	2	1.87	11	76.9	20	7.7
16	25*	23.4	6	27.6	12	3.27	15	2.24	13	1.87	6	76.8	21	7.7
17	8	23.3	12	27.5	18	3.27	21	2.29	12	1.88	8	76.8	24	7.7
18	12	23.2	21	27.3	5	3.27	12	2.30	7	1.90	22	76.1	25*	7.7
19	3	23.2	14	27.0	4	3.27	6	2.31	3	1.91	24	76.1	4	7.6
20	16	23.2	8	25.5	11	3.27	24	2.31	6	1.92	16	75.4	19	7.6
21	19	23.2	20	24.6	10	3.27	5	2.34	5	1.92	7	75.3	22	7.6
22	9	23.1	11	23.9	7	3.27	23	2.36	4	1.92	17	74.9	2	7.6
23	2	23.0	13	23.8	14	3.28	3	2.43	23	1.97	19	73.9	3	7.5
24	10	23.0	9	23.4	15	3.28	4	2.46	9	1.97	2	73.8	16	7.5
25	18	22.4	22	22.4	23	3.28	9	2.53	15	2.01	21	73.6	17	7.5
Média		24.4		30.2		3.3		2.2		1.9		77.1		7.7

* Check: Mundo Novo IAC 379/19

The selection of shorter plants has been a priority for coffee breeding programs, especially for mechanized regions; non-significant correlations between height and yield have been found (CARVALHO et al., 2010; SEVERINO et al., 2002). Among the evaluated progenies, mean plant height values ranged from 3.24 m (progeny 9) to 3.28 m (progeny 23). This is the characteristic that showed the lowest variation among the progenies, confirming the low selective accuracy (31%) in the mean progeny level at this site.

The capacity of high-density planting progenies is useful information to the breeder, since the larger the number of plants in the stand, the greater the average productivity of the field over the years. Moreover, taking the average over a number of years (PEREIRA et al., 2007) tends to attenuate adverse environmental effects. Estimating the plant density involves measuring the canopy structure and dimension of the plants. The lower canopy diameter ranged from 2.03 m (progeny 14) to 2.53 m (progeny 9). The upper canopy diameter of the progenies ranged from 1.75 m (progeny 17) to 2.01 m (progeny 15).

Among the evaluated progenies, the stem diameters ranged from 73.6 mm (progeny 21) to 80.3 mm (progeny 14). According to Ferreira et al. (2014) the stem of the coffee plant contains reserve tissues that represent an important source of carbohydrates to supply the vegetative and reproductive demands of plants. A larger stem diameter also contributes to reduce the tendency of the plants to tip, thus improving the management of coffee trees.

Plant vigor scores sum up, in the view of a breeder, the overall performance of the plant. There was low variation in plant vigor, which ranged from 7.5 (progeny 17) to 7.9 (progeny 14); the mean value is much higher than that found by Fazuoli et al. (2005) in “Mundo Novo” progenies in Campinas, SP. According to Severino et al. (2002), high plant vigor correlates positively with the adaptation of the cultivar to the environment. Therefore, a selection process that considers plant vigor may favor the selection of plants with a lower possibility of depletion and greater longevity.

In general, the average stem diameter of 56% of progenies was higher than the general average; for other traits (yield, %S17, and vigor), only 36% of progenies had averages that were above the general average. For plant height, upper canopy diameter and lower canopy diameter, the aim of the selection was to decrease the average, and in this case, 56%, 48%, and 36% of the progenies, respectively, had averages below the general average.

Experimental Field of Três Pontas

Mean yields ranged from 23.7 sc ha⁻¹ (progeny 8) to 29.7 sc ha⁻¹ (progeny 22). Progeny 22 was the most productive at both sites. Similar results were found by Raso et al. (2015), who observed average yields of 29.7 sc ha⁻¹ in cultivars of “Mundo Novo” in Três Pontas-MG (Table 4). There is no strong correlation between rankings considering the both sites, this shows the presence of genotypes by environments interaction (GEI) also observed by Carvalho et al. (2010), and Beksisa et al. (2018) that effectively determined the existence of GEI among *Coffea arabica* genotypes.

The %S17 values ranged from 19.2% (progeny 20) to 30.4% (progeny 3). In comparison to the other site, the average %S17 was lower by 4.5%.

Plant heights ranged from 2.89 m (progeny 20) to 3.08 m (progeny 15). The level of variation observed among the progenies is slightly higher than in São Sebastião do Paraíso. This level of variation favored a higher level of selective accuracy at the progeny level (73%).

The lower canopy diameters ranged from 2.09 m (progeny 11) to 2.40 m (progeny 9) while the upper canopy diameter ranged from 1.69 m (progeny 8) to 1.91 m (progeny 9). In the São Sebastião do Paraíso, progeny 9 also had a larger lower canopy diameter.

The stem diameters varied from 64.8 mm (progeny 8) to 74.7 mm (progeny 18). The general mean was 6.6 mm lower than in São Sebastião do Paraíso, which can be explained by the different management and cultivation practices in each site.

Plant vigor scores varied between 5.9 (progeny 17) and 7.5 (progeny 13), which was a higher level of variation compared to the previous site and reflects a higher mean score at the progeny level. A similar trend was observed for plant height, which shows how much these two characteristics are heavily influenced by the environment.

The percentage of progenies that had averages above the general average for yield, %S17, stem diameter and vigor were 64%, 48%, 56% and 60%, respectively. These percentages, except for stem diameter, were higher at this location. There were no differences between sites in the percentages of progenies that had averages above the general average for case upper and lower canopy diameter and plant height.

TABLE 4 - Ranking of 25 progenies and their genotypic values for yield (sc ha⁻¹), %S17 (%), height (m), upper canopy diameter (m), lower canopy diameter (m), stem diameter (mm), and vigor (score) in Três Pontas, MG.

Rank	Yield		%S17		Height		Upper canopy diameter		Lower canopy diameter		Stem diameter		Vigor	
	ID	GV	ID	GV	ID	GV	ID	GV	ID	GV	ID	GV	ID	GV
1	22	29.7	3	30.4	20	2.89	11	2.09	8	1.69	18	74.7	13	7.5
2	14	29.6	1	30.0	8	2.94	8	2.14	20	1.70	23	73.3	18	7.3
3	17	29.6	17	28.9	5	2.95	14	2.14	22	1.71	2	72.7	1	7.1
4	12	29.2	19	28.7	11	2.96	10	2.16	1	1.72	16	72.4	6	7.1
5	10	29.0	24	28.5	6	2.97	22	2.17	24	1.72	10	71.7	10	7.1
6	20	29.0	16	28.5	22	2.98	25*	2.17	2	1.73	9	71.7	11	7.1
7	2	28.9	15	28.3	17	2.99	2	2.18	16	1.74	25*	71.7	25*	7.1
8	25*	28.9	22	27.8	13	2.99	20	2.19	11	1.75	19	71.1	5	6.9
9	9	28.7	14	27.7	21	2.99	16	2.19	25*	1.76	12	71.0	7	6.9
10	21	28.5	25*	27.2	9	3.01	17	2.20	5	1.77	4	70.8	8	6.9
11	4	28.4	2	25.8	24	3.01	19	2.21	10	1.77	17	70.8	12	6.9
12	6	28.1	18	25.8	14	3.01	1	2.22	15	1.77	24	70.7	14	6.9
13	19	28.1	21	25.7	7	3.02	21	2.22	6	1.79	13	70.7	21	6.9
14	13	28.1	13	25.5	19	3.02	5	2.25	14	1.79	3	70.6	22	6.9
15	16	28.1	8	25.1	1	3.02	12	2.26	17	1.80	7	70.4	23	6.9
16	15	27.9	7	24.7	3	3.03	18	2.27	18	1.81	1	70.4	9	6.7
17	7	27.6	4	24.6	25*	3.04	23	2.28	19	1.82	21	69.9	15	6.7
18	5	27.6	6	24.3	10	3.04	24	2.29	13	1.82	15	69.8	19	6.7
19	24	27.2	12	24.1	12	3.05	7	2.29	7	1.83	22	69.6	24	6.7
20	11	26.8	11	24.1	16	3.05	6	2.31	21	1.83	6	69.4	2	6.5
21	23	26.5	9	23.2	4	3.05	15	2.31	12	1.86	14	69.2	3	6.5
22	18	25.5	10	23.0	18	3.05	4	2.32	4	1.87	20	69.1	16	6.3
23	1	25.3	23	21.9	23	3.06	13	2.33	23	1.90	5	68.7	20	6.3
24	3	24.9	5	19.2	2	3.07	3	2.39	3	1.90	11	67.4	4	5.9
25	8	23.7	20	19.2	15	3.08	9	2.40	9	1.91	8	64.9	17	5.9
Média		27.8		25.7		3.0		2.2		1.8		70.5		6.8

* Check

ID: identification of progeny

Selection

We simulated the selection of two progenies for each trait and the selection gain was quantified considering the general mean of the progenies and the check (Table 5).

For both sites, the highest gains were observed for the %S17, thus, the population subjected to the selection process has a high level of genetic variability for grain size and the possibility exists to obtain greater success in the selection for

this trait. Negative gains refer to the traits that are selected based on their reduction compared to the average; among them, plant height presented the lowest gain of selection in São Sebastião do Paraíso. In contrast to plant height, in Três Pontas, the selection gain for productivity was not as significant as that in São Sebastião do Paraíso. These results show how much the environment can interfere with genotypic characteristics and selection as found by Ferreira et al. (2005).

Falconer (1996) reported that it is possible to achieve faster progress through a correlated response rather than through the direct selection

of the desired trait. In order to summarize the characteristics and to carry out the simultaneous selection, the sum of ranking at each site is presented in Table 6.

TABLE 5 - Selection gain (%) for all traits evaluated in São Sebastião do Paraíso and Três Pontas in relation to the general population mean and the check (Mundo Novo IAC 379/19).

Traits	São Sebastião do Paraíso		Três Pontas	
	SG mean	SG check	SG mean	SG check
Yield	5.12	6.98	2.40	0.93
%S17	24.93	29.41	9.85	6.20
Height	-0.06	-0.06	-1.68	-2.19
Upper canopy diameter	-7.24	-3.59	-3.84	-1.75
Lower canopy diameter	-3.06	-1.71	-2.99	-2.08
Stem diameter	2.08	0.77	3.05	2.03
Vigor	0.31	0.31	5.23	2.51

TABLE 6 - Ranking by the sum of individual ranks of 24 progenies and the cultivar Mundo Novo IAC 379/19, according to the Mulamba and Mock (1978) index, in São Sebastião do Paraíso and Três Pontas, MG.

São Sebastião do Paraíso		Três Pontas	
ID	Sum of ranks	ID	Sum of ranks
1	47	22	50
20	60	25*	58
14	65	14	65
17	66	10	66
25*	69	17	72
8	71	1	73
11	72	16	77
16	77	2	78
22	77	8	78
5	82	11	80
15	84	19	81
24	84	20	84
6	85	24	84
19	88	13	87
23	88	21	88
10	90	18	89
7	91	6	91
18	95	12	95
21	95	5	99
12	96	7	106
13	99	9	112
4	101	15	112
2	109	23	117
3	114	3	122
9	117	4	127

* Check

ID: identification of progeny

After ranking in the ascending order, we observed that in São Sebastião do Paraíso, the best progenies were 1 [IAC376-2 (M. Novo) x IAC386-17 (M. Novo)] and 20 [IAC382-10 (M. Novo) x IAC388-20 (M. Novo)]. In addition, progeny 14 [IAC379/19 (M. Novo) x IAC386-6 (M. Novo)] and 17 [IAC386-5 (M. Novo) x IAC387-15 (M. Novo)] ranked higher than the control, which ranked fifth overall.

In Três Pontas, progeny 22 [H1535/181 (M. Novo) x (S795) 1344/10/5] and the check (Mundo Novo IAC379/19) ranked highest. The differentiation of progeny 22 at this site reflects its genealogy, which includes a genetic factor for resistance to rust, the *SH3* gene. Van-der-Vossen (2005) observed that coffee plants carrying the *SH3* gene are likely to express durable resistance. Furthermore, the yield of progeny 22 was higher, which was also observed in São Sebastião do Paraíso.

4 CONCLUSIONS

The genetic breeding program will benefit from the information and selection data from this study, especially since the genetic material used originated from “Mundo Novo,” the most planted coffee cultivar. The selection gain was expressed mainly for the trait bean size (percentage of beans retained in sieve 17). In general, the genetic gain was higher in São Sebastião do Paraíso than in Três Pontas.

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6 REFERENCES

BARTHOLO, G. F. et al. Cuidados na colheita, no preparo e no armazenamento do café. **Informe Agropecuário**, Campinas, v. 14, p. 33-44, 1989.

BEKSISA, L. et al. Genotype × environment interaction and yield stability of Arabica coffee (*Coffea arabica* L.) genotypes. **African Journal of Agricultural Research**, v. 13, n.4, p. 210-219, 2018.

BERNARDO, R. **Breeding for quantitative traits in plants**. Stemma Press, Woodbury, 2010.

CARVALHO, A.; ANTUNES FILHO, H. Melhoramento do cafeeiro: X - Seleção visando eliminar o defeito “Lojas vazias do fruto” no cafeeiro Mundo Novo. **Bragantia**, Campinas, v. 14, p. 51-62, 1955.

CARVALHO, A.; MÔNACO, L. C.; FAZUOLI, L. C. Melhoramento do café. XL - Estudos de progênies e híbridos de café Catuaí. **Bragantia**, Campinas, v. 38, p. 202-2016, 1979.

CARVALHO, A. M. de et al. Correlação entre crescimento e produtividade de cultivares de café em diferentes regiões de Minas Gerais, Brasil. **Pesquisa Agropecuária Brasileira**, Brasília, v. 45, p. 269-275, 2010.

CARVALHO, A. et al. Melhoramento do cafeeiro. IV-Café Mundo Novo. **Bragantia**, Campinas, v. 12, p. 97-129, 1952.

CONAB - COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira: café, safra 2014, quarto levantamento**. Brasília, 2014, 51 p.

CONAB - COMPANHIA NACIONAL DE ABASTECIMENTO. **Acompanhamento da safra brasileira: café, safra 2015, quarto levantamento**. Brasília, 2015, 60 p.

CORRÊA, T. R. et al. Phenotypic markers in early selection for tolerance to dieback in Eucalyptus. **Industrial Crops and Products**, v. 107, p. 130–138, 2017.

CRUZ, C. D.; REGAZZI, A. J.; CARNEIRO, P. C. S. **Modelos biométricos aplicados ao melhoramento genético**. Viçosa: Editora da UFV. v. 2. 2014, 514p.

FALCONER, D. S.; MACKAY, T. F. C. **Introduction to Quantitative Genetics**. Longman Pearson. 4 edition. 1996, 480p.

FAZUOLI, L. C. et al. Avaliação das cultivares Mundo Novo, Bourbon Amarelo e Bourbon Vermelho de *Coffea arabica* L. em Campinas. **Bragantia**, Campinas, v. 64, p. 533-546, 2005.

FERREIRA, A. et al. Seleção simultânea de *Coffea canephora* por meio da combinação de análise de fatores e índices de seleção. **Pesquisa Agropecuária Brasileira**, Brasília, v. 40, n.12, p. 1189-1195, 2005.

FERREIRA, I. N. M.; SANTOS, C. S.; CHAVES FILHO, J. T. Caracterização anatômica dos órgãos vegetativos do cafeeiro arábica (*Coffea arabica* L.). **Fragmentos de Cultura**, Goiânia, v. 24, p. 153-161, 2014.

GIOMO, G. S.; NAKAGAWA, J.; GALLO, P. B. Beneficiamento de sementes de café e efeitos na qualidade física. **Bragantia**, Campinas, v. 67, p. 997-1010, 2008.

- GHOSH, T. K. et al. Genetic variability and selection index evaluation of some selected tomato lines for their yield and yield components. **Bangladesh journal of environmental science**, Dhaka, v. 34, p. 73-78, 2018.
- LITTELL, R. C. et al. **SAS for mixed models**. SAS Institute, Cary, 2006.
- MULAMBA, N. N.; MOCK, J. J. Improvement of yield potential of the Eto Blanco maize (*Zea mays* L.) population by breeding for plant traits. **Egyptian Journal of Genetics and Cytology**, Cairo, v. 7, p. 40-51, 1978.
- OLIVEIRA, A. C. B. et al. Prediction of genetic gains from selection in Arabica coffee progênies. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 2, p. 106-113, 2011.
- PEREIRA, T. B. et al. Eficiência da seleção de progênies de café F₄ pela metodologia de modelos mistos (REML/BLUP). **Bragantia**, Campinas, v. 72, p. 230-236, 2013.
- PETEK, M.R. et al. Seleção de progênies de *Coffea arabica* com resistência simultânea à mancha aureolada e à ferrugem alaranjada. **Bragantia**, Campinas, v. 65, p. 65-73, 2006.
- PIEPHO, H. P.; BUCHSE, A.; RICHTER, C. A mixed modeling approach for randomized experiments with repeated measures. **Journal of Agronomy and Crop Science**, v. 190, p. 230-247, 2004.
- PINTO, M. F. et al. Eficiência na seleção de progênies de cafeeiro avaliadas em Minas Gerais. **Bragantia**, Campinas, v. 71, p. 1-7, 2012.
- PEREIRA, S. P. et al. Crescimento vegetativo e produção de cafeeiros (*Coffea arabica* L.) recepados em duas épocas, conduzidos em espaçamentos crescentes. **Ciência e Agrotecnologia**, Lavras, v. 31, p. 643-649, 2007.
- PEREIRA, A. A. et al. Cultivares: origem e suas características In: REIS, P. R. AND CUNHA, R. L. (Eds). **Café Arábica do plantio a colheita**. Lavras: EPAMIG, 2010, p. 163-222.
- RAMALHO, M. A. P.; ARAUJO, L. C. A. de. Breeding self-pollinated plants. **Crop Breeding and Applied Biotechnology**, Viçosa, v. 11, p. 1-7, 2011.
- RAMALHO, M. A. P.; SANTOS, J. B. dos; ZIMMERMANN, M. J. de O. **Aplicações da genética quantitativa no melhoramento de plantas autógamas**. Lavras: UFLA, 2012.
- RESENDE, M.D.V. de; DUARTE, J.B. Precisão e controle de qualidade em experimentos de avaliação de cultivares. *Pesquisa Agropecuária Tropical*, v.37, p.182-194, 2007.
- SAS - STATISTICAL ANALYSIS SYSTEM INSTITUTE. **SAS/STAT 9.0: user' guide**. Software, 2009.
- SEVERINO, L. S. et al. Associações da produtividade com outras características agrônômicas de café (*Coffea arabica* L. "Catimor"). **Acta Scientiarum**, v. 24, p. 1467-1471, 2002.
- SILVA, L. A. et al. Selection strategy for indication of crosses between potential sugarcane genotypes aiming at the production of bioenergy. **Industrial Crops and Products**, Amsterdam, v. 104, p. 62-67, 2017.
- VAN DER VOSSEN, H. A. M. The cup quality of disease-resistant cultivars of Arabica Coffee (*Coffea arabica*). **Experimental Agriculture**, v. 45, p. 323-332, 2009.
- VENCOVSKY, R.; BARRIGA, P. **Genética biométrica no fitomelhoramento**. Ribeirão Preto: Sociedade Brasileira de Genética, 1992.
- VIEIRA, R. A. et al. Selection index based on the relative importance of traits and possibilities in breeding popcorn. **Genetics and Molecular Research**, Brasília, v. 15, n. 2, p. 1-10, 2016.

MODELING DYNAMIC ADSORPTION ISOTHERMS AND THERMODYNAMIC PROPERTIES OF SPECIALTY GROUND ROASTED-COFFEE (*Coffea arabica* L.)

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ABSTRACT: Specialty coffee is highly differentiated product because of its sensorial attributes: aroma, body and brand reputation. In specialized markets, these products are highly valued, and sometimes up to six times their commercial value is paid. Thus, it is essential to preserve their freshness. Sorption isotherms are necessary for determining and studying water sorption changes in specialty coffee during storage. This study aimed to determine the adsorption isotherms of specialty ground roasted-coffee at temperatures of 25 °C, 30 °C and 40 °C and water activities between 0.1 and 0.8 using the dynamic dewpoint method (DDI). The experiment sorption data were modeled using 12 different equations with non-linear regression to represent the dependence of the equilibrium moisture content with both water activity and temperature. In addition, the thermodynamic properties were determined with the experiment adsorption data. The results showed that type III isotherms were obtained according to the Brunauer classification, and the Weibull equation satisfactorily modeled the effect of the temperature on the hygroscopic equilibrium in the specialty ground roasted-coffee. The results of thermodynamic analysis showed that the net isosteric heat of adsorption and Gibbs free energy decreased as the equilibrium moisture content increased, indicating the amount of energy released, a strong bond energy between water molecules in the product components and spontaneity in the adsorption process. The entropy of the adsorption increased with the moisture content, leading to product stability conditions during storage; it was possible to conclude that to guarantee the stability of high quality, ground, roasted coffee should store in environments where the water activity does not exceed 0.5 at temperatures between 25 °C and 40 °C. The results were similar to those reported for the roasted and ground coffee of others cultivars.

Index terms: Water activity, equilibrium moisture content, hygroscopicity.

MODELAGEM DE ISOTERMAS DE ADSORÇÃO DINÂMICA E PROPRIEDADES TERMODINÂMICAS DE CAFÉ ESPECIAL TORRADO E MOÍDO (*Coffea arabica* L.)

RESUMO: O café especial é um produto altamente diferenciado por seus atributos sensoriais: aroma, corpo e reputação da marca. Nos mercados especializados, estes produtos são altamente valorizados e as vezes podem ser pagados em até seis vezes seu valor comercial. Deste modo, é essencial preservar seu frescor. As isotermas de sorção são necessárias para a determinação e o estudo das mudanças da sorção de água no café especial durante o armazenamento. O objetivo deste trabalho foi determinar as isotermas de adsorção de café especial torrado e moído para temperaturas de 25 °C, 30 °C e 40 °C e atividades de água entre 0.1 e 0.8 usando o método dinâmico de ponto de orvalho (DDI). Os dados de sorção experimentais foram modelados usando 12 equações diferentes através de regressão não linear, para representar a dependência do teor de água de equilíbrio com a atividade de água e a temperatura. Adicionalmente, foram determinadas as propriedades termodinâmicas a partir dos dados experimentais de adsorção. Os resultados mostraram que foram obtidas isotermas tipo III, de acordo com a classificação Brunauer e a equação Weibull modelou satisfatoriamente o efeito da temperatura sobre o equilíbrio higroscópico em café especial torrado e moído. Os resultados da análise termodinâmica mostraram que o calor isostérico líquido de adsorção e a energia livre de Gibbs diminuíram com o aumento do teor de água de equilíbrio, indicando a quantidade de energia liberada, uma forte energia de ligação entre as moléculas de água nos componentes do produto e a espontaneidade no processo de adsorção. A entropia de adsorção aumentou com o teor de água, levando a condições de estabilidade do produto durante o armazenamento; foi possível concluir que para garantir a estabilidade do café torrado e moído de alta qualidade deve-se armazenar em ambientes onde a atividade de água não exceda de 0.5 para temperaturas entre 25 °C e 40 °C. Os resultados foram semelhantes aos relatados para café torrado e moído de outras cultivares.

Termos para indexação: Atividade de água, teor de água de equilíbrio, higroscopicidade.

1 INTRODUCTION

“Coffee is an ancient beverage that has a relationship with the development of human civilization” (TSAI et al., 2016), and is widely consumed throughout the world because of its unique sensory properties and physiological effects (KREUML et al., 2013). It is considered one of

the most important products in the world because of income from exportation and industrialization (CORRÊA et al., 2010). The specialty coffee is generally defined by an excellent and unique flavor (PICCINO et al., 2014), it has become a highly differentiated product, in terms of sensorial attributes (aroma, body) and brand reputation as Colombian coffee (ÖZDESTAN et al., 2013),

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this product is generally destined to specialized markets where the prices reached can be up to six times the value of a traditional coffee (TAPIERO et al., 2017).

Coffee is a highly hygroscopic material and can readily take up moisture when exposed to the environment during storage (IACCHERI et al., 2015); this condition may affect coffee freshness. For preserving desirable sensory quality, it is necessary to guarantee the storage conditions (BORÉM et al., 2013). For the proper handling of ground roasted-coffee it is necessary to know, among other properties, the water sorption isotherms, which describe the relationship between the equilibrium moisture content and the water activity at constant temperature and pressure (BASTIOĞLU et al., 2017). There is a large numbers of sorption equations for the characterization of the water sorption behavior of foods (MOUSA et al., 2012), such models can be theoretical, empirical or semi-empirical equations. Because of the complex nature of food matrices, there is not a unique model to entirely describe agri-food products (BON et al., 2012); therefore, is necessary to validate the best model for both the material and the environment conditions using experiment data.

Traditionally, sorption isotherms in coffee have been generated using the static method. This method has disadvantages such as long periods to reach product equilibrium, difficulty in obtaining conclusive and precise measurements, large samples and great effort on the part of researchers to obtain weight measurements when seeking balance (SCHMIDT & LEE, 2012). These drawbacks have been overcome with the development of the dynamic in the sorption of water vapor, in particular the dynamic dewpoint isotherm method (IACCHERI et al., 2015). This method offers highly available experiment points, broad working conditions and high precision, thus eliminating the disturbances associated with the manipulation of samples and the possible mass transfer phenomenon with the environment where the experiment is carried out (SCHMIDT & LEE, 2012). IACCHERI et al. (2015) have employed the DDI method to obtain sorption isotherms of green coffee and roasted coffee, however, they have not included the temperature effect in the sorption isotherms, moreover, in the literature has not been reported sorption isotherms specialty coffee.

Knowledge of the temperature effect on sorption isotherms is of great importance since foods can be exposed to a wide range of temperatures during storage and processing (EIM

et al., 2011). By observing the effect of temperature on sorption isotherms, it is possible to determine the thermodynamic properties of agricultural products and obtain helpful information with respect to energy changes in the system (VILLA-VELEZ et al., 2012); thus, thermodynamic properties are a key resource that can reveal the effect of water activity on storage (OLIVEIRA et al., 2016).

The net isosteric heat of sorption represents the total energy available to do work (MARTÍNEZ-LAS HERAS et al., 2014) and provides important information on the energy requirement for different processes and the state of water molecules in food (TADAPANENI et al., 2017). Gibbs free energy represents the spontaneity of water gain and loss processes between a product and the environment (TEIXEIRA et al., 2018), and sorption entropy (differential entropy) indicates the number of available sorption sites at a specific energy level and can be related to the attraction-repulsive forces in a system (YOGENDRARAJAH et al., 2015); it also indicates the degree of order of the water molecules in a product (PROKOPIUK et al., 2010).

Because of the importance of understanding the hygroscopicity of Colombian specialty ground roasted-coffee from Huila region and its dependence on water activity and temperature, the objective of the present study was to determine the adsorption isotherms of specialty ground roasted-coffee at 25 °C, 30 °C and 40 °C, and water activities between 0.1 and 0.8 using the dynamic dew point method, to evaluate the ability of different models in describing the adsorption isotherms and to determine the net isosteric heat of adsorption, Gibbs free energy and adsorption entropy with the purpose of defining the best storage conditions for this product.

2 MATERIALS AND METHODS

2.1 Coffee samples

Nine specialty coffee samples of variety Colombia, Castillo and Caturra (*Coffea arabica* L.), from different farmers of Huila region of Colombia, that were processed by the wet method, underwent sensory analysis with the Specialty Coffee Association of America (SCAA) methodology (SCAA, 2015) in the South Colombian Coffee Research Center (CESURCAFÉ) by four expert panelists (DI DONFRANCESCO et al., 2014), cup scores were between 84 to 86.5 the samples were considered as high-quality specialty coffee.

The coffee beans were roasted at 180 ± 2 °C during 8 ± 2 min, in a laboratory with a TC-150R (Quantik, Colombia), at medium roast ($21.0 < L^* < 24.2$) (WEI & TANOKURA, 2015). After roasting, the coffee samples were ground separately to medium size particle (particles retained in a #20 standard sieve; hole diameter of 0.85 mm), using a Bunn G3HD milling device (Bunn Coffee Mill, Springfield, IL, USA).

2.2. Experiment determination of the adsorption isotherms

For the development of the dynamic dewpoint method (DDI), 2 to 3 g of specialty ground roasted-coffee were placed inside a vapor sorption analyzer (VSA Aqualab Decagon Device, Inc. Pullman, WA, USA). The a_w range for the measurements was defined between 0.1 and 0.8 at temperatures of 25 °C, 30 °C and 40 °C, and the a_w interval was 0.01 for the adsorption with an air flow rate of $100 \text{ ml} \cdot \text{min}^{-1}$. The initial moisture content of the samples was made with a moisture tester (Kett PM-450, Science of Sensing, Japan). All tests were performed in triplicate for each temperature.

2.3 Modeling the adsorption isotherms

The adsorption isotherms of the ground, roasted specialty coffee was represented mathematically using the twelve models shown in Table 1, where X_e is the equilibrium moisture content (% dry basis), a_w is the water activity, A , B and C are the GAB model parameters (CORRÊA et al., 2010), T is the absolute temperature (K) and b_i is the empirical model parameters.

2.3.1 Parameter estimation and statistical analysis

The non-linear regression analysis Matlab® R2016b (The MathWorks Inc., Natick, MA, USA) to identify the model parameters and calculate the 95% confidence intervals of the parameters. The adjusted determination coefficient (R^2_{adj}) and the root mean square error ($RMSE$) were used as goodness-of-fit statistics. High values of $R^2_{adj} \geq 0.9$ and low values of $RMSE$ (lower than 10 % d.b.) are considered to be a reasonable fit (BASTIOĞLU et al., 2017).

2.4 Thermodynamic properties

The thermodynamic properties were calculated from the experiment data of moisture adsorption isotherms. The net isosteric heat of adsorption can be computed using the Clausius–Clapeyron equation (Eq. 13), from the slope of the plot of $\ln(a_w)$ versus $1/T$ (DOMIAN et al., 2018; TADAPANENI et al., 2017). The net isosteric heat of sorption (q_{sn}) can be calculated by integrating Eq. 13 between two temperatures (Eq. 14). In the literature also has been reported the Riedel equation (Eq. 15) to describe the influence of temperature on water activity. Thus, another expression to estimate net isosteric heat of sorption may be considered (Eq. 16) by combining Eqs. (14) and (15) (GARCÍA-PÉREZ et al., 2008).

$$q_{sn} = -R \left[\frac{\partial(\ln a_w)}{\partial \left(\frac{1}{T} \right)} \right]_{X_e} \quad (13)$$

$$q_{sn} = R \left[\frac{T_1 T_2}{T_2 - T_1} \ln \frac{a_{w2}}{a_{w1}} \right] \quad (14)$$

$$\ln \left(\frac{a_{w2}}{a_{w1}} \right) = A_r \exp(-B_r X_e) \left(\frac{1}{T_1} - \frac{1}{T_2} \right) \quad (15)$$

$$q_{sn} = C_r \exp(-B_r X_e) \quad (16)$$

Where R is the universal gas constant for water vapor ($8.314 \times 10^{-3} \text{ kJ} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$), A_r , B_r are constants of the Riedel equation and C_r being $A_r \cdot R$. Gibbs free energy (ΔG) was defined according to Eq. (17); where the changes of free energy were positive for desorption and negative for the adsorption (BAPTESTINI et al., 2017). The entropy of adsorption (ΔS) was calculated with Eq. (18).

$$\Delta G = \pm RT \ln(a_w) \quad (17)$$

$$\Delta S = \frac{q_{sn} - \Delta G}{T} \quad (18)$$

TABLE 1 - Mathematical models used to represent the ground roasted-coffee isotherms*

Model	Mathematical expression	Reference
Modified GAB (Eq. 1)	$X_e = \frac{AB(C/T)(a_w)}{[1 - B(a_w)][1 - B(a_w) + B(C/T)(a_w)]}$	(CORRÊA et al., 2010)
Oswin (Eq. 2)	$X_e = b_1 \left(\frac{a_w}{1 - a_w} \right)^{b_2}$	(MARTÍNEZ-LAS HERAS et al., 2014)
Halsey (Eq. 3)	$X_e = \left[\frac{\exp(b_1 - b_2)}{-\ln a_w} \right]^{\frac{1}{b_3}}$	(OLIVEIRA et al., 2016)
Smith (Eq. 4)	$X_e = b_1 + b_2 \ln(1 - a_w)$	(SHIGEHISA et al., 2015)
Chung-Pfost (Eq. 5)	$X_e = b_1 + b_2 \ln(-\ln a_w)$	(MOUSA et al., 2012)
Caurie (Eq. 6)	$X_e = \exp(b_1 + b_2 a_w)$	(CABALLERO-CERÓN et al., 2017)
Iglesias and Chirife (Eq. 7)	$X_e = b_1 + b_2 \left(\frac{a_w}{1 - a_w} \right)$	(KHAWAS & CHANDRA, 2016)
White and Eiring (Eq. 8)	$X_e = \frac{1}{(b_1 + b_2 a_w)}$	(SHITTU et al., 2015)
Peleg (Eq. 9)	$X_e = b_0 a_w^{b_1} + b_2 a_w^{b_3}$	(BONNER & KENNEY, 2013)
DLP (Eq. 10)	$X_e = b_0 + b_1 x + b_2 x^2 + b_3 x^3$ $x = \ln(-\ln a_w)$	(SHITTU et al., 2015)
Kuhn (Eq. 11)	$X_e = \left(\frac{b_1}{\ln a_w} + b_2 \right)$	(DOMIAN et al., 2018)
Weibull (Eq. 12)	$X_e = b_1 + \exp^{b_2(1-a_w)^{b_3}}$	(URIBE et al., 2011)

*In all cases, a linear dependence of temperature on the bi parameter was considered ($b_i T + b_{i,1}$)

3 RESULTS AND DISCUSSION

Table 2 shows the ten mathematical models with the best fit results and others models, considering the effect of temperature on the isotherms of adsorption.

The Weibull model may be the best equation to represent all of the DDI experiment data because it reached the highest R^2_{adj} (0.975) and the lowest $RMSE$ (0.389 % d.b.) values. In addition, the confidence intervals showed that all parameters of the model were statistically significant at a confidence level of 95%, indicating a good fit for practical purposes (BAPTESTINI et al., 2017). The others nine mathematical models (GAB, Halsey, Iglesias and Chirife, White and Eiring, Peleg, DLP, Kuhn, Oswin, and Caurie) resulted in values of the R^2_{adj} coefficient below 0.975 and of the $RMSE$ higher than 0.389 % d.b., with respect to those obtained by the Weibull model however, the models showed a well goodness of

fit and parameters statistically significant (95%), these models have been used in others food product and they have obtained an excellent goodness of fit: YOGENDRARAJAH et al. (2015) in adsorption isotherms of whole black peppercorns (Peleg, Oswin, and DLP), OLIVEIRA et al. (2016) in roasted coffee.

The Smith and Chung-Pfost models did not showed a good fit better than other models due to the lower $R^2_{adj} < 0.9$ and the highest $RMSE$ values Table 2, and should not be considered for representing the adsorption process of specialty ground roasted-coffee.

Our results were different from those mentioned above, because the number of experiment data with DDI method, with often > 75 (SCHMIDT & LEE, 2012). Thus, the Weibull model represented better the hygroscopic behavior with great ability for describing the adsorption process and it was the best option for the prediction of the equilibrium moisture content of specialty ground roasted-coffee under the studied conditions.

TABLE 2 - Estimated model parameters and statistical results.

Models	Parameters	Confidence Intervals 95%	R^2_{adj}	RMSE (% d.b.)
Modified GAB	$C = 1050 \text{ K}^{-1}$ $B = 1.00$ $A = 2.213 \text{ \% d.b.}$	[905.9, 1194] [Fixed at bound] [2.163, 2.263]	0.916	0.724
Halsey	$b_3 = 0.939$ $b_2 = 8.980 \times 10^{-3} \text{ K}^{-1}$ $b_1 = 3.336 \times 10^{-3} \text{ K}^{-1}$	[0.922, 0.955] [8.945 $\times 10^{-3}$, 9.014 $\times 10^{-3}$] [3.323 $\times 10^{-3}$, 3.348 $\times 10^{-3}$]	0.956	0.521
Iglesias and Chirife	$b_{2,1} = -0.086$ $b_2 = 3.620 \times 10^{-4} \text{ K}^{-1}$ $b_1 = 2.936 \times 10^{-5} \text{ K}^{-1}$	[-0.098, -0.074] [3.239 $\times 10^{-4}$, 4.014 $\times 10^{-4}$] [2.739 $\times 10^{-5}$, 3.133 $\times 10^{-5}$]	0.958	0.509
White and Eiring	$b_{2,1} = -480.8$ $b_2 = 1.320 \text{ K}^{-1}$ $b_{1,1} = 405.6$ $b_1 = -1.097 \text{ K}^{-1}$	[-546.0, -415.6] [1.108, 1.532] [356.2, 455] [-1.259, -0.937]	0.969	0.437
Peleg	$b_3 = 0.279$ $b_2 = 9.475 \times 10^{-5} \text{ K}^{-1}$ $b_1 = 5.701$ $b_{0,1} = -0.953$ $b_0 = 4.118 \times 10^{-3} \text{ K}^{-1}$	[0.209, 0.348] [8.587 $\times 10^{-5}$, 1.036 $\times 10^{-4}$] [5.401, 6.002] [-1.144, -0.763] [3.467 $\times 10^{-3}$, 4.77 $\times 10^{-3}$]	0.970	0.427
DLP	$b_3 = -0.011 \text{ K}^{-1}$ $b_{2,1} = -0.145$ $b_2 = 5.247 \times 10^{-4} \text{ K}^{-1}$ $b_1 = -0.013$ $b_0 = 7.414 \times 10^{-5} \text{ K}^{-1}$	[-0.013, -0.010] [-0.167, -0.122] [4.506 $\times 10^{-4}$, 5.987 $\times 10^{-4}$] [-0.014, -0.012] [7.235 $\times 10^{-5}$, 7.593 $\times 10^{-5}$]	0.966	0.46
Kunh	$b_{1,1} = 0.053$ $b_1 = -2.523 \times 10^{-4} \text{ K}^{-1}$	[0.043, 0.063] [-2.856 $\times 10^{-4}$, -2.191 $\times 10^{-4}$]	0.953	0.54
Weibull	$b_{3,1} = 3.354$ $b_3 = -8.843 \times 10^{-3} \text{ K}^{-1}$ $b_{2,1} = -42.90$ $b_2 = 0.118 \text{ K}^{-1}$ $b_{1,1} = 0.115$ $b_1 = -3.308 \times 10^{-4} \text{ K}^{-1}$	[2.699, 4.008] [-10.980 $\times 10^{-3}$, -6.706 $\times 10^{-3}$] [-49.66, -36.15] [0.096, 0.140] [0.075, 0.155] [-4.629 $\times 10^{-4}$, -1.987 $\times 10^{-4}$]	0.975	0.389
Oswin	$b_2 = 0.823$ $b_{1,1} = -0.077$ $b_1 = 3.645 \times 10^{-4} \text{ K}^{-1}$	[0.804, 0.841] [-0.094, -0.06] [3.078 $\times 10^{-4}$, 4.213 $\times 10^{-4}$]	0.934	0.641
Smith	$b_{2,1} = 0.1346$ $b_2 = -6.274 \times 10^{-4} \text{ K}^{-1}$ $b_1 = -4.944 \times 10^{-6} \text{ K}^{-1}$	[0.095, 0.173] [-7.552 $\times 10^{-4}$, -4.996 $\times 10^{-4}$] [-9.188 $\times 10^{-6}$, -6.997 $\times 10^{-6}$]	0.878	0.873
Chung-Pfost	$b_{2,1} = 0.161$ $b_2 = -6.499 \times 10^{-4} \text{ K}^{-1}$ $b_1 = 8.914 \times 10^{-5} \text{ K}^{-1}$	[0.103, 0.22] [-8.427 $\times 10^{-4}$, -4.572 $\times 10^{-4}$] [8.61 $\times 10^{-5}$, 9.219 $\times 10^{-5}$]	0.804	1.11
Caurie	$b_2 = 3.664$ $b_{1,1} = -8.632$ $b_1 = 0.011 \text{ K}^{-1}$	[3.573, 3.755] [-9.172, -8.093] [9.361 $\times 10^{-3}$, 0.013]	0.926	0.68

The figure 1 shows the experiment values and the estimations obtained with the Weibull model at 25 °C, 30 °C and 40 °C.

The resulting DDI isotherms were type III according to the Brunauer classification (BRUNAUER et al., 1940). Similar isotherm forms were reported in roasted coffee by BAPTISTINI et al. (2017); OLIVEIRA et al. (2016) and IACCHERI et al. (2015). According to LABUZA & ALTUNAKAR (2007), type III isotherms represent food systems composed mainly of crystalline components, such as sugars and salt. Thus, the type III isotherms obtained may have been due to reactions in the coffee roasting process, where the water-soluble polysaccharides were formed after roasting (WEI & TANOKURA, 2015). The values of equilibrium moisture content as function of water activity at 25 °C were similar to those reported by IACCHERI et al. (2015) in DDI isotherms of roasted coffee beans and HERMAN et al. (2018) in fermented cocoa beans. As can be seen in figure 1, there were not statistically significant differences (95%) from adsorption isotherms at 25, 30 and 40 °C in the range of water activity between 0.1–0.6 as confirmed by analysis of variance, however, in $a_w > 0.6$ the effect of the temperature on the adsorption isotherm at 40 °C presented statistically significant differences in respect to 25 and 30 °C and did not differ in the last water activity value (0.8). As can be observed, at the constant temperature, the equilibrium moisture content of the specialty, ground roasted-coffee increased with a_w ; however, at a constant

a_w , the equilibrium moisture content increased with temperature, indicating that the specialty, ground roasted-coffee is more hygroscopic. This trend was reported by EIM et al. (2011) in carrots; the authors explained that this behavior was due to the dissolution of sugar, which results in complete leaching of sugar in solution, a change of the crystalline structure of sugar to the amorphous state and an increase in the number of sorption sites upon swelling of biopolymers. The similar effect has been reported by ČERVENKA et al. (2015) in roasted yerba mate.

For thermodynamic properties, VILLA-VÉLEZ et al. (2012) stated that, during adsorption, ΔS will be negative because the adsorbate-sorbate system becomes ordered upon adsorption and loses degrees of freedom. If ΔG is less than zero, q_{sn} will have to be negative and, thus, adsorption will be exothermic. Otherwise, desorption will be endothermic.

Figure 2 shows the variation of the net isosteric heat of adsorption with the equilibrium moisture content.

The net isosteric heat of adsorption (Figure 2) decreased as the water content increased. This could be related to the amount of energy released ($-q_{sn}$) by the adsorption of water in the product. The negative values of differential enthalpy of adsorption have been reported by OLIVEIRA et al. (2016) in roasted coffee and PROKOPIUK et al. (2010) in algarroba pods. The results of the net isosteric heat of adsorption calculated using the Riedel equation are shown in Table 3.

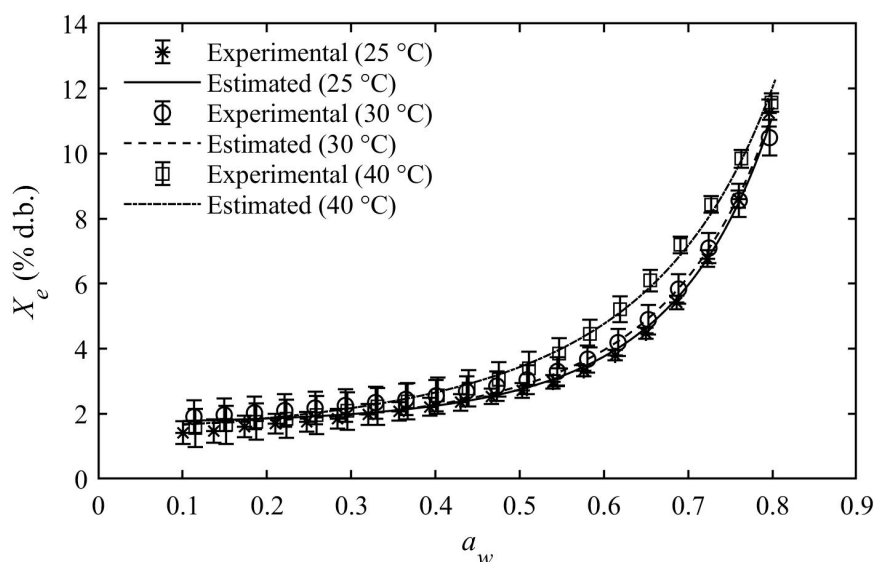


FIGURE 1 - Experiment adsorption isotherms and estimations using the Weibull model for the specialty, ground roasted-coffee at 25 °C, 30 °C and 40 °C.

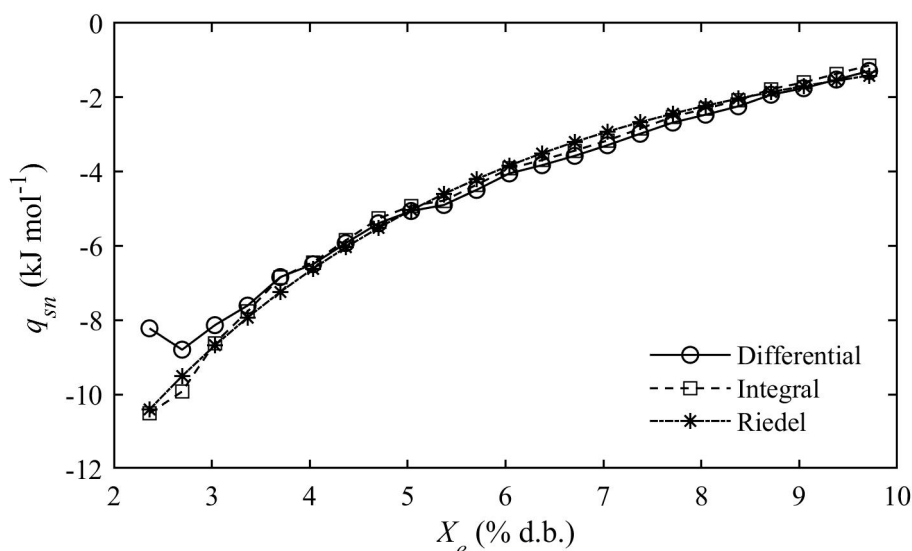


FIGURE 2 - Net isosteric heat of adsorption of the ground, roasted-coffee at 25 °C, 30 °C and 40°C as a function of the equilibrium moisture content

TABLE 3 - Estimation of the net isosteric heat of adsorption with the Riedel equation.

Parameters	95% Confidence Intervals	R^2_{adj}	RMSE (% d.b.)
$A_r = -2376$	$[-2484, -2268]$	0.994	0.4
$B_r = 27.13$	$[26.05, 28.21]$		

The Riedel equation satisfactorily represented the net isosteric heat of adsorption because it reached a high value for the R^2_{adj} coefficient, 0.994, and RMSE was the same at 0.4 % d.b. In addition, the confidence intervals showed that the parameters of the model were statistically significant at a confidence level of 95%. This equation can be used to predict the net isosteric heat of adsorption as a function of the equilibrium moisture content.

The Gibbs free energy of the ground roasted-coffee is presented in Figure 3.

Gibbs free energy is known as an indicator of the affinity between the food sorbent and water (BASTIOĞLU et al., 2017). Changes in Gibbs free energy during water sorption between the product and the environment can be defined as the energy required to transfer water molecules from the vapor state to a solid surface, or vice versa (TEIXEIRA et al., 2018). This is the amount of work generated by the system during the adsorption process (BAPTESTINI et al., 2017).

Figure 3 presents the negative change in the ΔG with the water content, indicating the typical

behavior of an exergonic reaction, which does not require the addition of energy from the environment surrounding the product for the reaction to occur, that is, the reaction is spontaneous (OLIVEIRA et al., 2016). Similar results have been reported by OLIVEIRA et al. (2016) for roasted coffee grain and other agricultural products, such as algarroba pods (PROKOPIUK et al. 2010), whole black peppercorns (YOGENDRARAJAH et al. 2015), passion fruit seeds (FERREIRA DE SOUZA et al. 2014), and pineapple pulp powder (VIGANÓ et al. 2012). Since ΔG was close to 0 as the moisture content increased, it is possible to conclude that there will be fewer sites available for sorption at higher water content levels (CANO-HIGUITA et al., 2015).

The figure 4 shows the variation of the entropy of adsorption, which represents the change in the order or disorder after water molecules are adsorbed by the system at the same hydration level (FERREIRA DE SOUZA et al., 2014; BASTIOĞLU et al., 2017). It can be observed that the entropy of adsorption increased with the increase in the equilibrium moisture content.

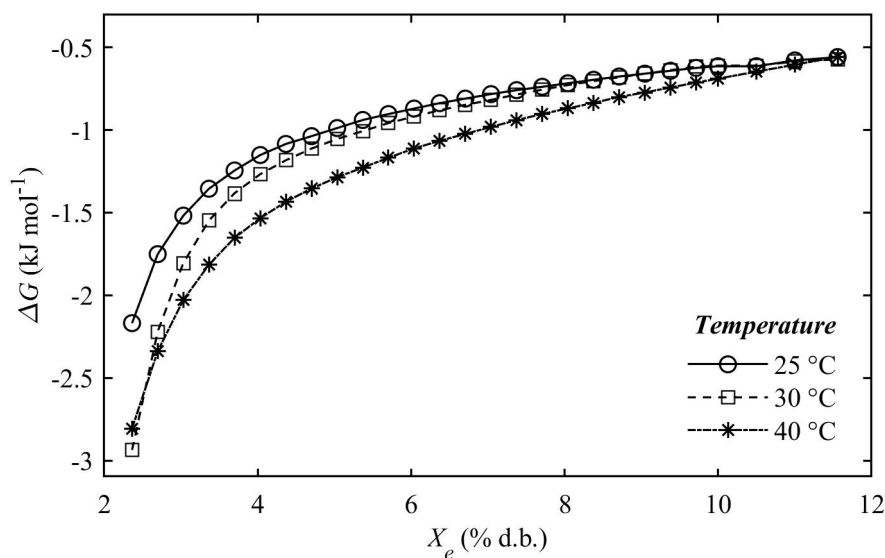


FIGURE 3 - Gibbs free energy of the ground roasted-coffee at temperatures of 25 °C, 30 °C and 40 °C as a function of the equilibrium moisture content.

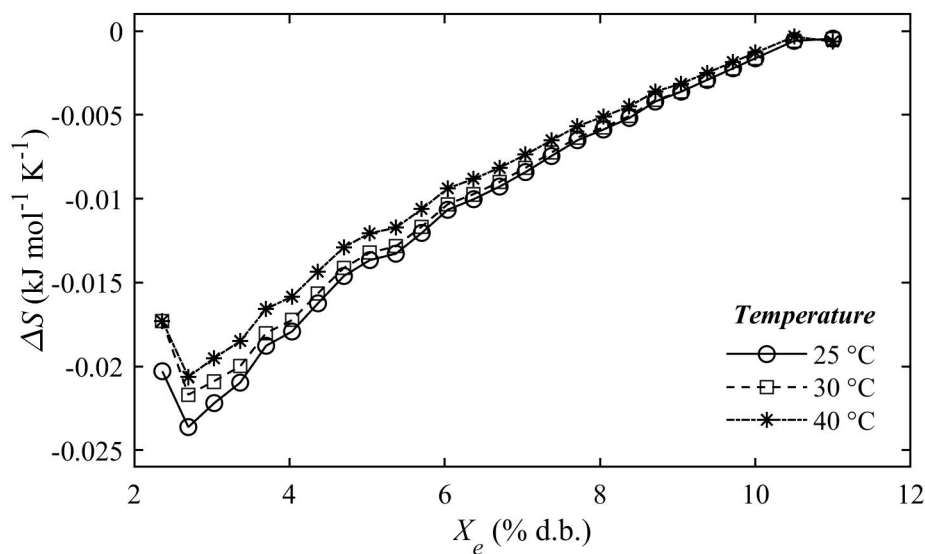


FIGURE 4 - Entropy of adsorption of ground roasted-coffee at temperatures of 25 °C, 30 °C and 40 °C as a function of the equilibrium moisture content.

The increase of the adsorption entropy indicates the increase in the degrees of freedom of the water in the product, which increased with the equilibrium moisture content; therefore, a less structured system is described. Similar negative values of entropy of adsorption have been reported by OLIVEIRA et al. (2016) in roasted coffee and PROKOPIUK et al. (2010) in algarroba pods.

According to MOREIRA et al. (2016), the minimum values of entropy can be interpreted as the condition at which a product has the best stability. Thus, with the results seen in Figure 4, it is possible to conclude that the equilibrium moisture content corresponded to a higher order degree of the water molecules in the specialty, ground roasted-coffee ($\Delta_s < 0$) was $X_e = 2.6$ % d.b.

The Weibull model offers a tool for the prediction of water activity intervals that correspond to the equilibrium moisture content, which evidenced the lowest entropy of adsorption of the system; therefore, it is possible to guarantee the stability of high quality, ground, roasted coffee if stored in environments where the water activity does not exceed 0.5 at temperatures between 25 °C and 40 °C.

4 CONCLUSIONS

The moisture adsorption behavior of the specialty, ground roasted-coffee exhibited the Type III common sigmoid pattern. The adsorption isotherms were modeled with the Weibull model, which proved to be satisfactory for the prediction of the experiment data and can be used to describe the behavior of specialty, ground roasted-coffee (medium roast and medium particle size) during storage conditions with a water activity interval between 0.1 and 0.8 and temperatures between 25 °C and 40 °C. The adsorption isotherms obtained with the dynamic dewpoint isotherms method (DDI) offered high availability for the experiment points, a broad range of work and great precision, thus eliminating the disturbances associated with the handling and manipulation of samples and the possible phenomenon of mass transfer.

Likewise, the amount of energy released by the water adsorption in the coffee increased as the water content decreased, which was expressed by the net isosteric heat of adsorption, and the Gibbs free energy values showed that the water adsorption was a spontaneous process. The entropy of adsorption increased with the moisture content and put in showed at to guarantee the stability of high-quality ground roasted coffee, the storage should be in environments where the water activity does not exceed 0.5 at temperatures between 25 °C and 40 °C.

5 ACKNOWLEDGMENT

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6 REFERENCES

- BAPTESTINI, M. F.; CORRÊA, P. C.; HORTA DE OLIVEIRA, G. H.; CECON, P. R.; SOARES, F. N. D. Kinetic modeling of water sorption by roasted and ground coffee. *Acta Scientiarum*, Maringá, v. 39, n. 3, p. 273-281, July-Sept., 2017. DOI: 10.4025/actasciagron.v39i3.32576.
- BASTIOĞLU, A. Z.; KOÇ, M.; ERTEKIN, F. G. Moisture sorption isotherm of microencapsulated extra virgin olive oil by spray drying. *Food Measure*, 2017. DOI: 10.1007/s11694-017-9507-4
- BON, J.; VAQUIRO, H. A.; MULET, A. Modeling sorption isotherms and isosteric heat of sorption of Mango pulp cv. tommy atkins. *Biotecnología en el Sector Agropecuario y Agroindustrial*. Vol 10 No. 2 (34 - 43) 2012. Available on: <http://www.scielo.org.co/scielo.php?script=sci_arttext&pid=S1692-35612012000200005>. Access in: 21 oct. 2018.
- BONNER, I. J.; KENNEY, K. L. Moisture sorption characteristics and modeling of energy sorghum (*Sorghum bicolor* L.) Moench. *Journal of Stored Products Research*, 2013. DOI: <http://dx.doi.org/10.1016/j.jspr.2012.11.002>
- BORÉM, F. M.; RIBEIRO, C. F.; FIGUEIREDO, P. L.; GIOMO, G. S.; FORTUNATO, V. A.; ISQUIERDO, E. P. Evaluation of the sensory and color quality of coffee beans stored in hermetic packaging. *Journal of Stored Products Research*, 2013. <http://dx.doi.org/10.1016/j.jspr.2012.08.004>
- BRUNAUER, S.; DEMING, S. L.; DEMING, E. W.; TELLER, E. On a theory of the Van der Waals Adsorption of Gases. *J. Am. Chem. Soc.*, 1940. DOI: 10.1021/ja01864a025
- CABALLERO-CERÓN, C.; SERMENT-MORENO, V.; VELAZQUEZ, G.; TORRES, J. A.; WELTI-CHANGES, J. Hygroscopic properties and glass transition of dehydrated Mango, apple and Banana. *J Food Sci Technol*, 2017. DOI: <https://doi.org/10.1007/s13197-017-2963-3>
- CANO-HIGUITA, D. M.; VILLA-VÉLEZ, H. A.; TELIS-ROMERO, J.; VÁQUIRO, H. A.; NICOLETTI, T. V. R. Influence of alternative drying aids on watersorption of spray dried mango mix powders: A thermodynamic approach. *Food and Bioprocess Processing* 93 (2015) 19–28, 2015. DOI: <http://dx.doi.org/10.1016/j.fbp.2013.10.005>
- ČERVENKA, L.; HLOUŠKOVÁ, L.; ŽABČÍKOVÁ, S. Moisture adsorption isotherms and thermodynamic properties of green and roasted Yerba mate (*Ilex paraguariensis*). *Food Bioscience*, 2015. <https://doi.org/10.1016/j.fbio.2015.10.001>
- CORRÊA, P. C.; GONELI, L. A.; JUNIOR, A.; OLIVEIRA, H. G.; VALENTE, D. Moisture sorption isotherms and isosteric heat of sorption of coffee in different processing levels. *International Journal of Food Science and Technology*, 2010. DOI: 10.1111/j.1365-2621.2010.02373.x

- DI DONFRANCESCO, B.; GUTIERREZ-GUZMAN, N.; CHAMBERS, E. Comparison of results from cupping and descriptive sensory analysis of colombian brewed coffee. **Journal of Sensory Studies**, 2014. DOI:10.1111/joss.12104
- DOMIAN, E.; BRYNDA-KOPYTOWSKA, A.; CIESLA, J.; GÓRSKA, A. Effect of carbohydrate type on the DVS isotherm-induced phase transitions in spray-dried fat-filled pea protein-based powders. **Journal of Food Engineering**, 2018. DOI: <https://doi.org/10.1016/j.jfoodeng.2017.11.012>
- EIM, V. S.; ROSSELLO, C.; FEMENIA, A.; SIMAL, S. Moisture Sorption Isotherms and Thermodynamic Properties of Carrot. **International Journal of Food Engineering**, 2011. DOI: 10.2202/1556-3758.1804
- FERREIRA DE SOUZA, J. S.; VAQUIRO, H. A.; VILLA-VÉLEZ, A. H.; POLACHINI, C. T.; TELIS-ROMERO, J. Physical, Thermal and Water-Sorption Properties of Passion Fruit Seeds. **International Journal of Food Engineering**, 2014. DOI: 10.1515/ijfe-2014-0138
- GARCÍA-PÉREZ, J. V.; CÁRCEL, J. A.; CLEMENTE, G.; MULET, A. Water sorption isotherms for lemon peel at different temperatures and isosteric heats. **Swiss Society of Food Science and Technology**, 2008. DOI: 10.1016/j.lwt.2007.02.010
- HERMAN, C.; SPREUTELS, L.; TUROMZSA, N.; KONAGANO, M. E.; HAUT, B. Convective drying of fermented Amazonian cocoa beans (*Theobroma cacao* var. Forasteiro). Experiments and mathematical modeling. **Food and Bioproducts Processing**, v 108, p.81-94, 2018. <https://doi.org/10.1016/j.fbp.2018.01.002>
- IACCHERI, E.; LAGHI, L.; CEVOLI, C.; BERARDINELLI, A.; RAGNI, L.; ROMANI, S.; ROCCULI, P. Different analytical approaches for the study of water features in green and roasted coffee beans. **Journal of Food Engineering**, 2015. DOI: <https://doi.org/10.1016/j.jfoodeng.2014.08.016>
- KHAWAS, P.; CHANDRA, D. S. Moisture sorption isotherm of underutilized culinary banana flour and its antioxidant stability during storage. **Journal of Food Processing and Preservation**, 2016. DOI:10.1111/jfpp.13087
- KREUML, M. T. L.; MAJCHRZAK, D.; PLOEDERL, B.; KOENIG, J. Changes in sensory quality characteristics of coffee during storage. **Food Science & Nutrition**, 2013. DOI: 10.1002/fsn3.35
- LABUZA, P. T.; ALTUNAKAR, B. Water Activity Prediction and Moisture Sorption Isotherms. **Water Activity in Foods: Fundamentals and Applications**, Chapter 5, 2007. DOI: 10.1002/9780470376454.ch5
- MARTÍNEZ-LAS HERAS, R. A.; HEREDIA, M. L.; CASTELLÓ, A. A. Moisture sorption isotherms and isosteric heat of sorption of dry persimmon leaves. **Food Bioscience-ScienceDirect**, 2014. DOI: <http://dx.doi.org/10.1016/j.fbio.2014.06.002>
- MOREIRA, R.; CHENLO, F.; SINEIRO, J.; ARUFE, S.; SEXTO, S. Water sorption isotherms and air drying kinetics of fucus vesiculosus brown seaweed. **Journal of Food Processing and Preservation**, 2016. DOI: 10.1111/jfpp.12997
- MOUSA, W.; MOHAMAD, F.; JINAP, S. G.; MOHD, H.; RADU, S. Sorption isotherms and isosteric heats of sorption of Malaysian paddy. **J Food Sci Technol**, 2012. DOI: 10.1007/s13197-012-0799-4
- OLIVEIRA, G. H.; CORRÊA, P. C.; RODRIGUES DE OLIVEIRA, A. P.; BAPTESTINI, F. M.; VARGAS-ELÍAS, G. A. Roasting, grinding, and storage impact on thermodynamic properties and adsorption isotherms of arabica coffee. **Journal of food processing and preservation**, 2016. DOI:10.1111/jfpp.12779
- ÖZDESTAN, Ö.; VAN RUTH, S. M.; ALEWIJN, M.; KOOT, A.; ROMANO, A.; CAPPELLIN, L.; BIASIOLI, F. Differentiation of specialty coffees by proton transfer reaction-mass spectrometry. **Food Research International**, 2013. DOI: <http://dx.doi.org/10.1016/j.foodres.2013.05.013>
- PICCINO, S.; BOULANGER, R.; DESCROIX, F.; SING, C. A. S. Aromatic composition and potent odorants of the “specialty coffee” brew “Bourbon Pointu” correlated to its three trade classifications. **Food Research International**, 2014. DOI: <http://dx.doi.org/10.1016/j.foodres.2013.07.034>
- PROKOPIUK, D.; MARTÍNEZ-NAVARRETE, N.; ANDRÉS, A.; CHIRALT, A.; CRUZ, G. Influence of Roasting on the Water Sorption Isotherms of Argentinean Algarroba (*Prosopis alba* Griseb) Pods. **International Journal of Food Properties**, 2010. DOI: 10.1080/10942910902742055
- SCHMIDT, S. J.; LEE, W. J. Comparison Between Water Vapor Sorption Isotherms Obtained Using The New Dynamic Dewpoint Isotherm Method and those Obtained Using The Standard Saturated Salt Slurry Method. **International Journal of Food Properties** 15(1-2):236-248, 2012. DOI: 10.1080/10942911003778014

- SHIGEHISA, T.; INOUE, T.; KUMAGAI, H. Mathematical model of water sorption isotherms of UBC. **Fuel Processing Technology**, 2015. DOI: <http://dx.doi.org/10.1016/j.fuproc.2014.11.023>
- SHITTU, T.A.; IDOWU-ADEBAYO, F.; ADEDOKUN, I. I.; ALADE, O. Water vapor adsorption characteristics of starch-albumen powder and rheological behavior of its paste. **Nigerian Food Journal**, 2015. DOI: <http://dx.doi.org/10.1016/j.nifoj.2015.04.014>
- 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.
- TADAPANENI, R. K.; YANG, R.; CARTER, B.; TANG J. A new method to determine the water activity and the net isosteric heats of sorption for low moisture foods at elevated temperatures. **Food Research International**, 2017. DOI: <http://dx.doi.org/10.1016/j.foodres.2017.09.070>
- TAPIERO, S. O.; TRUJILLO, B. D.; GUTIÉRREZ G. N. Aplicación del proceso analítico jerárquico ahp para definir la mejor taza en evaluación de cafés especiales, **Coffee Science**, 2017. DOI: <http://dx.doi.org/10.25186/cs.v12i3.1301>
- TEIXEIRA, P. L.; T. DE ANDRADE, E.; DEVILLA, A. I. Isosteric heat, entropy, and gibbs free energy of pumpkin seeds (*Cucurbita moschata*). **Engenharia Agrícola**, 2018. DOI: <http://dx.doi.org/10.1590/1809-4430-eng.agric.v38n1p97-102/2018>
- TSAI, S-Y.; HWANG, S-P.; LIN, C-P.; A kinetics study of coffee bean of roasting and storage conditions. **Journal of Food Processing and Preservation**, 2016. DOI: 10.1111/jfpp.13040
- URIBE, L.; VEGA-GÁLVEZ, A.; DI SCALA, K.; OYANADEL, R.; TORRICO, S. J.; MIRANDA, M. Characteristics of Convective Drying of Pepino Fruit (*Solanum muricatum* Ait.): Application of Weibull Distribution. **Food Bioprocess Technol**, 2011. DOI: 10.1007/s11947-009-0230-y
- VIGANÓ, J.; AZUARA, E.; TELIS, V. R. N.; BERISTAIN, C. I.; JIMÉNEZ, M. Role of enthalpy and entropy in moisture sorption behavior of pineapple pulp powder produced by different drying methods. **Thermochimica Acta**, 2012. DOI: 10.1016/j.tca.2011.11.011
- VILLA-VELEZ, H. A.; VÁQUIRO, H. A.; BON, J.; TELIS-ROMERO, J. Modelling thermodynamic properties of Banana waste by analytical derivation of desorption isotherms. **International Journal of Food Engineering**, 2012. DOI: <https://doi.org/10.1515/1556-3758.2191>
- WEI, F.; TANOKURA, M. Chemical Changes in the Components of Coffee Beans during Roasting-Chapter 10. **Coffee in Health and Disease Prevention**, 2015. DOI: <http://dx.doi.org/10.1016/B978-0-12-409517-5.00010-3>
- YOGENDRARAJAH, P.; SAMAPUNDO, S.; DEVLIEGHERE, F.; DE SAEGER, S.; DE MEULENAER, B. Moisture sorption isotherms and thermodynamic properties of whole black peppercorns (*Piper nigrum* L.). **LWT - Food Science and Technology**, 2015. DOI: <http://dx.doi.org/10.1016/j.lwt.2015.05.045>

AGRONOMIC TECHNIQUES FOR MITIGATING THE EFFECTS OF WATER RESTRICTION ON COFFEE CROPS

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ABSTRACT: Water restriction significantly affects coffee (*Coffea arabica* L.) production. The study of a few agronomic techniques that optimizes water use can generate technologies for mitigating the effects of climatic variations on coffee crops. The aim in this study was to indicate agronomic techniques that mitigate the effects of water restriction on coffee crops. For this end, we analyzed the morphophysiological changes in coffee plants cultivated in a greenhouse with different types of fertilizers and soil conditioners and under two levels of irrigation. The evaluations were performed 130 days after planting, assessing the morphological and physiological characteristics of the plants. We also quantified soil moisture in the different treatments. The water restriction expressively hindered plant growth. The use of controlled release fertilizers and soil conditioners, especially coffee husk, is indicated for mitigating water restriction in coffee crops.

Index Terms: *Coffea arabica* L., climatic variations, water use efficiency.

TÉCNICAS AGRONÔMICAS PARA A MITIGAÇÃO DOS EFEITOS DA RESTRIÇÃO HÍDRICA NO CAFEIEIRO

RESUMO: A restrição hídrica já afeta significativamente a produção do cafeeiro (*Coffea arabica* L.). O estudo de algumas técnicas agronômicas que proporcionem a otimização da água poderá gerar tecnologias eficazes na mitigação dos efeitos das variações climáticas na cultura do café. Objetivou-se neste trabalho indicar técnicas agronômicas que mitiguem os efeitos da restrição hídrica em cafeeiro. Para isso foram analisadas as alterações morfofisiológicas em cafeeiros cultivados em casa de vegetação com diferentes tipos de fertilizantes e condicionadores de solo sob dois níveis de irrigação. As avaliações foram realizadas aos 130 dias após o plantio. Foram avaliadas características morfológicas e fisiológicas das plantas. Também se quantificou a umidade do solo nos diferentes tratamentos. A restrição hídrica prejudicou de forma expressiva o crescimento do cafeeiro. Fertilizantes de liberação controlada e condicionadores de solo, em especial a casca de café, são técnicas agronômicas indicadas para mitigação da restrição hídrica em cafeeiros.

Termos para indexação: *Coffea arabica* L., Variações climáticas, Eficiência do uso da água.

1 INTRODUCTION

Water availability is one of the most limiting of the essential plants growth factors and contributes significantly to an increased agricultural production worldwide. Most coffee plant tissues are approximately 85% water, which is also the principle medium for chemical processes that support plants metabolism (TAIZ; ZEIGER, 2013).

Climate is continually varying on time scales ranging from days to seasons. However, considering temperature increase and reduction in water availability as consequences of climatic variations (IPCC, 2014), the study on agronomic techniques aiming to minimize such impacts is found to be an utmost important approach for agriculture. Regarding coffee plants, a significant

loss of areas considered suitable for the cultivation of *Coffea arabica* L. and *Coffea canephora* Pierre is likely to happen (BUNN, 2015).

The increased efficiency fertilizers are emphasized as a great alternative for plant nutrition under adverse cropping conditions. These fertilizers show greater efficiency in providing nutrients to plants and allow the reduction of nutrients losses through leaching and volatilization, making fertilization safer (TIMILSENA et al., 2015).

Few soil conditioners such as agricultural gypsum and organic residues enhance soil properties and root environment, then allowing better access to available water for plants (SANTOS et al., 2014).

A successfully study on water-retaining polymer able to retain and provide water to plants

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for extended periods of time was developed in coffee crops and shown as a potential tool for water optimization in crops production processes (SOUZA et al., 2016).

However, there is no information regarding the effects of the simultaneous use of technologies such as controlled release fertilizers and soil conditioners with higher water use efficiency in coffee crops. Thus, it highlights here the relevance of developing a sustainable coffee production process.

Anatomical and physiological aspects, as well as the aerial parts growth and root systems of plants, can help in choosing the technology that better mitigates the effects of water restriction (CASTANHEIRA et al., 2016). Therefore, this study was developed aiming to describe agronomic methods that better mitigate the effects of water restriction on coffee crops.

2 MATERIALS AND METHODS

Study site, growth conditions and experiment design

The experiment was carried out in a greenhouse, in the municipality of Lavras, State of Minas Gerais, Brazil, at latitude 21°15'S, longitude 45°00'W and 918 m elevation. An electronic gauge was used to measure the temperature, relative humidity and luminance during the experiment development and mean estimates were 21.36°, 64% and 8945.14 lx, respectively.

Coffee seedlings (*Coffea arabica* L.) of cultivar Mundo Novo IAC 379/19 were planted, when containing four pairs of leaves, in 14-liters pots arranged on a stand at 0.8 m height. The substrate used to fill up pots was Humic Rhodic Acrudox, clayey texture with 67% clay, 15% silt and 18% sand and the following chemical characteristics: pH 5.8, 22 mg dm⁻³ K, 0.28 mg dm⁻³ P, 0.3 cmol_c dm⁻³ Ca, 0.1 cmol_c dm⁻³ Mg, 0.0 cmol_c dm⁻³ Al, 0.84 cmol_c dm⁻³ potential acidity (H+Al), 0.46 cmol_c dm⁻³ sum of bases (SB), 0.46 cmol_c dm⁻³ effective cation exchange capacity (t), 1.3 cmol_c dm⁻³ cation exchange capacity at pH 7.0 (T), 35.11% base saturation (V), 0.0% aluminum saturation (m), 0.04 dag kg⁻¹ organic matter (OM), 1.52 mg L⁻¹ reminescent phosphorus (P-rem), 0.59 mg dm⁻³ Zn, 34.67 mg dm⁻³ Fe, 4.30 mg dm⁻³ Mn, 0.57 mg dm⁻³ Cu, 0.56 mg dm⁻³ B and 7.82 mg dm⁻³ S.

The correction of soil acidity was carried out by raising the base saturation to 70% using 570 kg ha⁻¹ of dolomitic limestone with 35%

CaO and 14% MgO and 80% effective calcium carbonate (ECC). The limestone was added to the soil substrate and incubated in pots for 60 days, keeping the moisture at 60% total pore volume (TPV) occupied by water. Phosphate fertilizer was applied during planting as outlined in Malavolta (1981), using Single Superphosphate (21% P₂O₅).

After planting seedlings into pots, the substrate moisture was kept at 100% field capacity for 30 days to ensure the full establishment of seedlings and growth uniformity. Then, the irrigation was performed as per each treatment.

The experiment was carried out in randomized block design with four replicates. Treatments were arranged in 2 x 2 x 5 factorial scheme, where two levels of irrigation (40% and 80% of field capacity), two types of fertilizers (conventional fertilizer and controlled release fertilizer) and five types of soil conditioners (coffee husk, agricultural gypsum, water-retaining polymer, organic compost and negative control or treatment without soil conditioners). A total of 80 plots were used, each plot consisting of one pot.

Irrigation, fertilizers and conditioners management

The irrigation water was replaced based on the gravimetric weight difference estimated by means of electronic scales. The weight of pots under field capacity conditions was measured. Then, the weight of pots for each treatment, at 40% and 80% field capacity was also measured (LANNA et al., 2016).

Seedlings were checked three times a week (Monday, Wednesday and Friday) to assess the need for irrigation, using two reference pots located at strategic points inside the greenhouse, one for each level of irrigation. The evapotranspiration of water from seedlings leaves led to weigh losses. Thus, seedlings were then irrigated to reestablish the corresponding weight for each treatment (LANNA et al., 2016).

Two types of fertilizers were used to supply nitrogen (N) and potassium (K) to coffee plants. The conventional fertilizer consisted of 20-00-20 (N-P-K) and urea, used in cover application on planting day and at 30 and 60 days after planting, as outlined in Guimarães et al. (1999). The controlled release fertilizer consisted of a commercial product containing 37% N and urea coated with elemental sulfur particles plus a layer of organic polymers and another commercial product containing 52% K₂O with potassium chloride, also coated with elemental sulfur particles plus a layer of organic

polymers. A single application of fertilizers was done at planting date via side holes, according to manufacturer's instructions.

Doses of fertilizer were estimated as outlined in Malavolta (1981), considering a supplement of 300 ppm N and 150 ppm K.

Coffee husk, organic compost and gypsum soil conditioners were applied as cover at planting date, as outlined in Guimarães et al. (1999). For treatments containing coffee husk and organic compost, 0.16 L dm⁻³ was added. The organic compost consisted of a commercial product containing farm and food industry residues. An amount of 0.7 g dm⁻³ estimated based on the type of soil texture was added to the treatment containing agricultural gypsum.

Regarding treatments containing water-retaining polymer, the solution was prepared adding 1.5 kg of polymer in 400 liters of water. Then 1.5 liters of the obtained solution was applied in the planting hole (SOUZA et al., 2016).

Measurements

Field data collect was performed for 130 days after planting due to the growth limitation of plants under water restriction. The following parameters were evaluated: plant height in cm, measured using graduated scale; stem diameter in mm, measured using digital pachymeter; number of leaves; number of plagiotropic branches; total root length in cm, obtained as the sum of the linear extension of each fragment of the root system and measured using the Safira program; dry weight of the aerial part in g; and dry weight of the root system in g. To evaluate the dry weight, plants were harvested, separated into aerial part and roots, washed in running water and dried in a forced air heating system (hothouse) at 65° until reaching a constant weight.

For anatomical studies, fully expanded leaves located between the second and third node from the tip of the orthotropic branch were collected and, then, placed on germitest paper previously identified according to treatments and moisturized with deionized water. Paradermic sections were obtained in the laboratory by the impression of the epidermis, using the leaf printing technique with universal instantaneous adhesive (cyanoacrylate ester). For obtaining transversal sections, leaves were fixed in 70% ethanol (v v⁻¹) and, after 72 hours, exchanged for the same reagent for good biomass conservation.

The dehydration process of biomass in series of ethyl was also performed. The plant

material was placed in methacrylate according to the manufacturer's instructions and divided into sections approximately 0.8 µm thickness using a rotary microtome. Sections were stained using toluidine blue dye (O'BRIEN; FEDER; MCCULLY, 1964) and slides were assembled using Entelan® as medium. Slides were observed with an optic microscope and photographs captured with a digital camera coupled to it. Images were analyzed using the UTHSCSA-Imagetool program.

The number of stomata, the polar diameter of the stomata and the equatorial diameter of the stomata were evaluated for stomatal characterization. The stomatal density (number of stomata per mm²) and the ratio polar diameter/equatorial diameter of the stomata, which is highly correlated to stomatal functioning were calculated (SOUZA et al., 2010, 2013). The adaxial epidermis thickness, palisade parenchyma thickness, spongy parenchyma thickness and mesophyll thickness were evaluated to determine the thickness of leaf tissues. The thickness of the phloem region, the diameter of xylem vessels and the number of xylem vessels were measured for the evaluation of the vascular beams.

A portable gauge was used for gas analysis in the infrared region (LI 6400-XT, LICOR), seeking to evaluate the gas exchanges. The net photosynthetic rate (µmol CO₂ m⁻² s⁻¹), stomatal conductance (mol H₂O m⁻² s⁻¹) and the transpiration rate (mmol H₂O m⁻² s⁻¹) were evaluated. The instantaneous water use efficiency (µmol CO₂ mmol⁻¹ H₂O) was calculated using the photosynthetic rate/transpiration rate ratio (YAN et al., 2015). Evaluations were conducted from 8:00 a.m. to 11:00 a.m, under artificial lighting (1000 µmol m⁻² s⁻¹), using fully expanded leaves located at the third node from the tip of the orthotropic branch.

The leaf water potential (MPa) was determined in the early morning using a Scholander pressure chamber (model 1000, PMS Instrument Company). Leaves fully expanded and without symptoms of pests and/or diseases were collected for evaluation in a chamber applying pressure until it could observe exudation runoff from the cut section of the leaf petiole.

A simple and deformed sample was collected at 15 cm depth from each plot to assess the soil moisture. The wet weight was measured using a precision scale and samples were placed in a hothouse at 105°C until reaching constant weight. Then, the dry weight and percentage of water in the soil were estimated.

Statistical analysis

The R statistical software (2016) was run for Analysis of Variance to verify the significance of variation sources using the F test at 5% probability. The aov package was used to adjust the linear model and the summery function was used for Analysis of Variance. The Scott-Knott test was used to compare means at 5% significance.

3 RESULTS AND DISCUSSION

Results on interactions within levels of irrigation, types of fertilizers and types of soil conditioners under study did not show differences at 5% probability ($p > 0.05$). However, there were differences for interactions between factors ($p < 0.05$), as follows: (i) levels of irrigation and types of soil conditioner; (ii) types of fertilizer and types of soil conditioner; and (iii) levels of irrigation and types of fertilizer. In addition, differences were also found for each of factor effects under study ($p < 0.05$).

Higher plants growth was recorded for most of types of soil conditioners when compared to levels of irrigation at 80% of field capacity and adequate water availability for the crop (TABLE 1). However, the same trend was found with regard to plants grown in soil conditioners containing coffee husk and organic compost, both used at 40% irrigation level, for the following variables: plants height, stem diameter, number of leaves, number of plagiotropic branches, dry weight of the aerial part and dry weight of the root system, as well as for stem diameter and dry weight of the root system when used water-retaining conditioner also associated with 40% irrigation. Lesser estimates were found for plant height, number of leaves, number of plagiotropic branches and dry weight of the aerial part, with regard to plants grown under water-retaining polymer, agricultural gypsum and negative control (treatment without soil conditioners), under water restricting conditions (TABLE 1).

The coffee husk allowed better plant growth, with higher values of plant height, number of leaves, dry weight of the aerial part and dry weight of the root system for plants under water-restricting at 40% field capacity. The organic compost showed lesser values when compared to coffee husk. However, such estimates were greater than those found for treatments consisting of the following plants growth factors: water-retaining polymer, agricultural gypsum and negative control. Regarding coffee husk and organic compost, both associated with water restriction,

plants showed stem diameter and number of plagiotropic branches superior to those found for other conditioners (TABLE 1).

Regarding interaction between types of soil conditioners and levels of irrigation, there was no differences at 80% field capacity ($p > 0.05$) for the following variables: palisade parenchyma thickness, spongy parenchyma thickness, mesophile thickness, xylem vessel diameter, number of xylem vessels and leaf water potential (TABLE 1).

Estimate of palisade parenchyma thickness was greater in plants grown under water-retaining polymer and the negative control. Estimates of xylem vessel diameter and number of xylem vessels were greater in plants grown under coffee husk and organic compost and lesser for treatments consisting of the following plants growth factors: water-retaining polymer, agricultural gypsum and the negative control. Besides, lesser estimates were obtained for spongy parenchyma thickness, mesophile thickness and number of xylem vessels with regard to plants grown in agricultural gypsum (TABLE 1).

Plants grown in coffee husk showed greater estimates of leaf water potential, followed by plants grown in organic compost, both at 40% irrigation. However, plants grown in water-retaining polymer and agricultural gypsum, showed not differences (TABLE 1). Plants of the negative control showed lesser estimates of leaf water potential. However, plants grown in coffee husk and organic compost at 40% irrigation showed estimates of leaf water potential with a trend similar to those found for plants grown at 80% irrigation, for either plant height, stem diameter, number of leaves, number of plagiotropic branches, dry weight of the aerial part, dry weight of the root system, xylem vessel diameter of number of xylem vessels (TABLE 1).

Irrigation at 80% field capacity showed greater contents of soil moisture than those found at 40% irrigation for all types of soil conditioners under study (TABLE 1). However, the use of coffee husk showed higher estimates of soil moisture for both irrigation levels than those found for other types of soil conditioners under study. Treatments consisting of agricultural gypsum and the negative control showed lesser content of soil moisture at 80% irrigation, with no differences from each other. The content of soil moisture obtained for treatments consisting of organic compost and water-retaining polymer did not show any difference. However, treatments consisting of water-retaining polymer, agricultural gypsum and the negative control showed lesser content of soil moisture under water restrictions.

TABLE 1 - Plant height, stem diameter, number of leaves, plagiotropic branches, dry weight of the aerial part, dry weight of the root, palisade parenchyma, spongy parenchyma, mesophyll, xylem vessel diameter, number of xylem vessels, leaf water potential and soil moisture of coffee plants of coffee plants cultivated under different levels of irrigation and soil conditioners.

Treatments	Plant height		Stem diameter		Number of leaves	
	80% ^a	40% ^b	80%	40%	80%	40%
Coffee husk	49.8 Aa	55.3 Aa	6.61 Aa	6.60 Aa	46.6 Aa	44.3 Aa
Organic compost	44.4 Ab	47.6 Ab	6.17 Aa	5.96 Aa	32.5 Ab	34.9 Ab
Water-retaining polymer	42.5 Ab	35.6 Bc	5.72 Aa	5.28 Ab	36.4 Ab	13.9 Bc
Agricultural gypsum	53.5 Aa	33.8 Bc	6.49 Aa	4.16 Bc	40.6 Aa	7.3 Bc
Witness	51.4 Aa	36.3 Bc	6.31 Aa	4.39 Bc	35.3 Ab	9.0 Bc
	Plagiotropic branches		Dry weight of the aerial part		Dry weight of the root	
	80%	40%	80%	40%	80%	40%
Coffee husk	7.1 Aa	6.8 Aa	17.1 Aa	16.9 Aa	3.3 Aa	3.8 Aa
Organic compost	5.9 Aa	5.8 Aa	12.3 Aa	11.9 Ab	2.3 Ab	2.7 Ab
Water-retaining polymer	6.0 Aa	2.6 Bb	7.5 Ac	3.3 Bc	1.8 Ab	1.2 Ac
Agricultural gypsum	6.3 Aa	2.3 Bb	16.6 Ab	3.1 Bc	3.4 Aa	1.2 Bc
Witness	6.1 Aa	1.8 Bb	12.7 Ab	3.1 Bc	2.9 Aa	1.2 Bc
	Palisade parenchyma		Spongy parenchyma		Mesophyll	
	80% ^a	40% ^b	80%	40%	80%	40%
Coffee husk	47.8 Aa	45.1 Ab	180.6 Aa	180.5 Aa	226.0 Aa	222.7 Aa
Organic compost	47.0 Aa	43.6 Ab	178.0 Aa	170.9 Aa	221.2 Aa	213.2 Aa
Water-retaining polymer	47.7 Aa	47.8 Aa	168.5 Ba	189.1 Aa	214.0 Ba	234.1 Aa
Agricultural gypsum	45.9 Aa	42.6 Ab	177.2 Aa	146.1 Bb	221.0 Aa	187.3 Bb
Witness	43.3 Ba	49.3 Aa	172.8 Aa	174.1 Aa	214.0 Aa	226.2 Aa
	Xylem vessel diameter		Number of xylem vessels		Leaf water potential	
	80%	40%	80%	40%	80%	40%
Coffee husk	14.4 Aa	14.6 Aa	129.8 Aa	136.4 Aa	-0.99 Aa	-1.06 Aa
Organic compost	14.4 Aa	13.1 Aa	127.1 Aa	129.3 Aa	-1.21 Aa	-1.51 Ab
Water-retaining polymer	14.2 Aa	10.9 Bb	131.4 Aa	116.7 Ab	-1.04 Aa	-2.56 Bc
Agricultural gypsum	14.8 Aa	12.4 Bb	136.7 Aa	94.17 Bc	-1.18 Aa	-2.84 Bc
Witness	14.9 Aa	12.2 Bb	136.9 Aa	108.4 Bb	-1.22 Aa	-3.73 Bd
	Soil moisture					
	80%			40%		
Coffee husk	38.08 Aa			34.18 Ba		
Organic compost	34.62 Ab			25.52 Bb		
Water-retaining polymer	34.02 Ab			18.21 Bc		
Agricultural gypsum	29.42 Ac			16.74 Bc		
Witness	27.19 Ac			15.89 Bc		

^a80%=levels of irrigation 80% of field capacity; ^b40%=levels of irrigation 40% of field capacity.

Means followed by the same letter, uppercase in the line and lowercase in the column belong to the same cluster by the Scott-Knott test ($p < 0.05$).

The organic compost showed an intermediate estimate of soil moisture when compared to other types of soil conditioners (TABLE 1).

The controlled release fertilizer associated with coffee husk promoted a significant increase in plant height, stem diameter and dry weight of the root system when compared to the conventional fertilizer (TABLE 2). Higher estimates of number of leaves and dry weight of the aerial part were found in controlled release fertilizer used for plants grown under coffee husk and organic compost. In addition, the use of controlled release fertilizer showed higher estimates of number of leaves for plants of the negative control. Regarding stem diameter, higher mean estimate was found for plants grown in conventional fertilizer associated with water-retaining polymer conditioner (TABLE 2).

Plants grown in conventional fertilizer, for different types of soil conditioners, did not differ from each other regarding plant height and stem diameter ($p > 0.05$) (TABLE 2). Plants grown in coffee husk showed higher estimates for both types of fertilizer, regarding number of leaves, dry weight of the aerial part and dry weight of the root system. Plants grown in controlled release fertilizer, under water-retaining polymer and with agricultural gypsum, as well as the negative control showed lesser estimates of stem diameter, number of leaves, dry weight of the aerial part and dry weight of the root system. In addition, lesser estimates were also found with regard to dry weight of the aerial part and dry weight of the root system for coffee plants under water-retaining polymer, when compared to other types of soil conditioners used in the study (TABLE 2).

TABLE 2 - Plant height, stem diameter, number of leaves, dry weight of the aerial part, dry weight of the root, stomatal density, adaxial epidermis thickness and leaf water potential of coffee plants cultivated using different fertilizers and soil conditioners.

Treatments	Plant height		Stem diameter		Number of leaves	
	CRF ^a	CF ^b	CRF	CF	CRF	CF
Coffee husk	57.56 Aa	47.56 Ba	7.18 Aa	6.04 Ba	50.25 Aa	40.67 Ba
Organic compost	48.88 Ab	43.06 Aa	6.52 Aa	5.61 Aa	40.38 Ab	27.00 Bb
Water-retaining polymer	37.06 Ac	41.06 Aa	4.97 Bb	6.02 Aa	22.25 Ac	28.00 Ab
Agricultural gypsum	43.94 Ab	43.31 Aa	5.41 Ab	5.25 Aa	28.00 Ac	21.75 Ac
Witness	45.75 Ab	41.88 Aa	5.51 Ab	5.19 Aa	26.88 Ac	17.38 Bc
	Dry weight of the aerial part		Dry weight of the root		Stomatal density	
	CRF	CF	CRF	CF	CRF	CF
Coffee husk	21.16 Aa	12.96 Ba	4.06 Aa	3.04 Ba	1617 Ab	1606 Ab
Organic compost	14.88 Ab	9.31 Bb	2.95 Ab	2.04 Ab	1436 Ab	1602 Ab
Water-retaining polymer	4.62 Ad	6.18 Ab	1.13 Ad	1.83 Ab	1785 Aa	1873 Ab
Agricultural gypsum	10.28 Ac	9.40 Ab	2.39 Ac	2.23 Ab	1894 Aa	1658 Ab
Witness	8.86 Ac	6.94 Ab	2.43 Ac	1.79 Ab	1973 Ba	2441 Aa
	Thickness adaxial epidermis		Leaf water potential			
	CRF	CF	CRF		CF	
Coffee husk	24.46 Aa	24.34 Aa	-1.09 Aa		-0.95 Aa	
Organic compost	22.90 Ba	24.95 Aa	-1.38 Aa		-1.35 Ab	
Water-retaining polymer	23.59 Ba	25.29 Aa	-1.91 Ab		-1.69 Ac	
Agricultural gypsum	23.00 Aa	22.12 Ab	-1.94 Ab		-2.07 Ad	
Witness	22.39 Aa	22.37 Ab	-2.25 Ab		-2.70 Be	

^aCRF= controlled release fertilizer; ^bCF= conventional fertilizer.

Means followed by the same letter, uppercase in the line and lowercase in the column belong to the same cluster by the Scott-Knott test ($p < 0.05$).

Beyond these findings, the stomatal density was greater in plants of the negative control, as well as those grown in conventional fertilizer. Treatments consisting of controlled release fertilizer showed lesser estimates of stomatal density in coffee plants grown in a soil without conditioners, when compared to treatment containing conventional fertilizer. Lesser estimates of stomatal density were also found for treatments containing controlled release fertilizer associated with coffee husk and organic compost (TABLE 2).

Plants of the negative control and with agricultural gypsum, both with conventional fertilizer added as type of fertilizer, showed lesser adaxial epidermis thickness (TABLE 2). A similar trend was found for the same treatments regarding leaf water potential, in which lesser estimates were obtained (TABLE 2).

Plants grown in soils without conditioners showed greater estimates of leaf water potential for treatments containing controlled release fertilizer than for treatments containing conventional fertilizer. However, regarding conventional fertilizer, different estimates of leaf water potential were found in plants grown in different types of soil conditioners, as follows: greater estimates for coffee husk, followed by organic compost, water-retaining polymer, agricultural gypsum and the control treatment. Coffee plants grown in coffee husk and organic compost, with controlled release fertilizer added, showed greater estimates of leaf water potential when compared to other types of soil conditioners (TABLE 2).

The controlled release fertilizer provided greater estimates of palisade parenchyma thickness and water use efficiency when compared to treatments with conventional fertilizer added, at 40% irrigation (TABLE 3). Regarding number of xylem vessels, there was no statistical difference between types of fertilizers for plants grown under the same conditions (TABLE 3).

Regarding palisade parenchyma thickness and number of xylem vessels, there was no difference between levels of irrigation in treatments with controlled release fertilizer added. The use of conventional fertilizer caused decreasing of estimates of palisade parenchyma thickness and number of xylem vessels at 40% irrigation. The highest water use efficiency at the same level of irrigation was found in coffee plants of treatments containing controlled release fertilizer (TABLE 3).

The irrigation at 80% of field capacity allowed the obtaining of greater estimates of total root length, adaxial epidermis thickness, photosynthetic rate, stomatal conductance

and transpiration rate. Treatments with water restriction at 40% field capacity showed greater estimates of stomatal density and phloem region (TABLE 4).

The controlled release fertilizers provided greater estimates of number of plagiotropic branches and photosynthetic rate, regardless of the level of irrigation and type of soil conditioner used (TABLE 5).

The coffee husk provided greater estimates of total root length, photosynthetic rate, stomatal conductance and transpiration rate when compared to other types of soil conditioners under study (the control treatment). Besides, treatments under water-retaining polymer showed lesser estimates of total root length (TABLE 6).

Treatments containing coffee husk, organic compost and water-retaining polymer showed greater estimates of the ratio polar diameter/equatorial diameter of the stomata when compared to treatments containing agricultural gypsum and the treatment without soil conditioners (TABLE 6).

The water deficit is found to be one of the most limiting of climatological factors in coffee growing, with direct effect in the decrease of coffee production (APARECIDO; ROLIM; SOUZA, 2015). In this work, the lesser water availability (40% field capacity) led to low plants growth and lesser amount of plants biomass (aerial part and root system), decrease in gas exchange and changes in the leaf tissues when compared to plants irrigated at 80% of field capacity.

Assis et al. (2014) demonstrate the importance of water on coffee growing and variations recorded on vegetative development in plants grown under water restrictions. In this study, plants irrigated at 80% field capacity and/or cultivated in soils containing conditioners such as coffee husk, agricultural gypsum, water-retaining polymer and organic compost showed greater vegetative growth when compared to plants cultivated under water restrictions.

The efficiency of plants in water and nutrients uptake varies significantly according to water restrictions adopted in the production system. Thus, plants grown at 40% field capacity showed lesser growth of the root system. Similar results were reported in Souza et al. (2016), where coffee plants grown at 25% irrigation level showed lesser ratio between dry weight of the root system and dry weight of shoots. These authors also found that 100% irrigation causes damages to plants root system due to the decrease of soil aeration.

TABLE 3 - Palisade parenchyma thickness, number of xylem vessels and water use efficiency of coffee plants submitted to different levels of irrigation and fertilizers.

Irrigation	Palisade parenchyma		Number of xylem vessels		Water use efficiency	
	CRF ^a	CF ^b	CRF	CF	CRF	CF
80% of field capacity	45.4 Aa	47.26 Aa	119.0 Ba	145.8 Aa	4.20 Ab	4.51 Aa
40% of field capacity	47.6 Aa	43.76 Bb	113.0 Aa	121.0 Ab	5.22 Aa	4.40 Ba

^aCRF= controlled release fertilizer; ^bCF= conventional fertilizer.

Means followed by the same letter, uppercase in the line and lowercase in the column belong to the same cluster by the Scott-Knott test ($p < 0.05$).

TABLE 4 - Means of the total root length, stomatal density, adaxial epidermis thickness, phloem thickness, photosynthetic rate, stomatal conductance and transpiration rate of coffee plants submitted to different levels of irrigation.

Irrigation	Total root length	Stomatal density	Adaxial epidermis	Phloem	Photosynthetic rate	Stomatal conductance	Transpiration rate
80% of field capacity	593.9 a	1607 b	24.60 a	47.16 b	6.28 a	0.07 a	1.40 a
40% of field capacity	384.8 b	1970 a	22.49 b	50.32 a	3.73 b	0.04 b	0.77 b

Means followed by the same letter in the column belong to the same cluster by the Scott-Knott test ($p < 0.05$).

TABLE 5 - Mean of the number of plagiotropic branches and photosynthetic rate of coffee plants cultivated using different fertilizers.

Fertilizer	Number of plagiotropic branches	Photosynthetic rate
Controlled release	5.4 a	5.43 a
Conventional	4.7 b	4.58 b

Means followed by the same letter in the column belong to the same cluster by the Scott-Knott test ($p < 0.05$).

TABLE 6 - Means of the total root length, polar diameter/equatorial diameter relation of the stomata, photosynthetic rate, stomatal conductance and transpiration rate of coffee plants with different soil conditioners.

Conditioner	Root length	Polar / equatorial diameter relation of the stomata	Photosynthetic rate	Stomatal conductance	Transpiration rate
Coffee husk	904.8 a	1.88 a	7.52 a	0.09 a	1.64 a
Organic compost	562.4 b	1.90 a	4.69 b	0.05 b	1.13 b
Water-retaining polymer	262.4 c	1.83 a	4.06 b	0.04 b	0.83 c
Agricultural gypsum	436.0 b	1.73 b	4.09 b	0.04 b	0.76 c
Witness	281.1 c	1.77 b	4.54 b	0.05 b	1.05 b

Means followed by the same letter in the column belong to the same cluster by the Scott-Knott test ($p < 0.05$).

Coffee plants show anatomical and physiological changes related to the existing limiting growing factors such as water stress and radiation exposure levels (MENEZES-SILVA et al., 2017). In this study, difference in the leaf water potential, tissue spacing, and vascular bundles were found in plants grown under water restrictions.

Changes in the diameter and number of xylem vessels indicate the adaptation level of plants grown under adverse environmental conditions (CASTANHEIRA et al., 2016; HACKE; SPICER; SCHREIBER, 2017). Smaller diameter and higher number of xylem vessels indicate high plants efficiency for sap conduction since they decrease chances for air embolism in plants grown, for example, under water stress (SEVANTO et al., 2018). However, the decrease in number of xylem vessels found in treatments containing agricultural gypsum and the control treatment in this study may be the result of damages caused by water restriction to plants grown at 40% irrigation. In addition, these treatments showed values of leaf water potential estimated in -2.84 Mpa for treatments containing agricultural gypsum and -3.73 MPa for the control treatment, which indicate an existing sharp water deficit in the production system. Thus, the reduction in xylem vessel diameter reflects the effect of water restrictions in plants grown in different types of soil conditioners, when compared to plants grown at 80% field capacity.

The higher spacing of the phloem observed in plants grown under water stress is also an indicating of plants adaptation form developed to address adverse growing conditions, to allow greater translocation of photoassimilates and favor plants growth (SEVANTO et al., 2018).

Higher estimates of palisade parenchyma thickness found in treatments with no soil conditioners indicate the existing anatomical adaptations of plants to develop under water deficit conditions. The palisade parenchyma is part of the leaf mesophyll and can have direct effect on plants gas exchanges. However, plants with greater tissue thickness tend to tolerate water deficiency even without a higher photosynthetic capacity (BINKS et al., 2016).

Water availability can alter stomatal density and the relationship between these variables may be described by means of an inverse proportionality. In addition, the decreased in size of epidermis cells results in greater number of stomata per area

(SILVA et al., 2014). These findings explain the trends obtained for some variables, for example, higher estimates of stomatal density and lesser estimates of adaxial epidermis thickness obtained in plants grown under different levels of water availability.

Anatomical changes found in this work do not indicate levels of tolerance of water stress since aspects related to plants physiology must also be taken into consideration. Thus, although there were anatomical changes, lesser estimates of gas exchanges in coffee plants grown under water restriction were found in this study, which reveals low plant growth.

Lesser estimates of leaf water potential (-3.73 Mpa) found in coffee plants grown with no soil conditioner and the low irrigation level, indicate an extreme water stress condition. Studies describe estimates of leaf water potential about -2.15 Mpa found in coffee plants, which is usually found in plants at the permanent wilting point (SANTOS; MAZZAFERA, 2012). In this work, plants grown at 40% field capacity showed lesser estimates of leaf water potential, which probably reflects lesser estimates of the following variables: plant height, number of leaves, number of plagiotropic branches, dry weight of the aerial part, xylem vessel diameter, total root length, photosynthetic rate, stomatal conductance and transpiration rate.

The low soil moisture has a direct effect in the decrease of leaf water potential in coffee plants irrigated at 40%. Beyond this effect, lesser contents of soil moisture were found in treatments irrigated at 40% field capacity, which may have affected the pattern of morphophysiological parameters under study. Studies state that the water availability affects significantly the plants growth and development, since the water participates in the transport and translocation of photoassimilates, cellular expansion and opening and closing of stomata (MCELDRONE et al., 2013).

The controlled release fertilizer was found to be an efficient type of fertilizer used to stimulate the coffee plants growth since it led to greater estimates of plant height, number of leaves, dry weight of the aerial part and dry weight of the root system than those found in conventional fertilizer. This soil effect occurred due to the significant supplementation of N and K existing in the controlled release fertilizer, as well as the lesser level of nutrient losses through volatilization and leaching. The controlled release fertilizer is usually

formulated seeking for optimization of plants nutrition, which depends on the following factors: coating thickness, plants nutrients requirements and level of potential losses that occur in the production process (TIMILSENA et al., 2015).

The controlled release fertilizer favors indirectly the level of water stress, since higher estimates of leaf water potential were found in plants of treatments containing controlled release fertilizer and the treatment with no soil conditioners. In the same context, the increase in estimates of stomatal density becomes expressive in plants impacted by water restrictions (SILVA et al., 2014). Besides, the increase in stomatal density is usually recorded in plants grown under increased water restriction. This is associated to the size of stomata, where smaller sized stomata can quickly open and close, then increasing stomatal conductance (XU; ZHOU, 2008).

In this study, it highlights the estimate of controlled release fertilizer found for coffee plants grown under water restrictions, which was higher and probably reduced the transpiration rate without affecting the photosynthetic rate of plants (FARES et al., 2016). Higher estimates of palisade parenchyma thickness can be associated to higher estimates of water use efficiency, since the palisade parenchyma is a chlorophyll-containing tissue specialized for photosynthesis (TERASHIMA et al., 2011). Thus, the thickness of this plant tissue may have contributed to the maintaining the photosynthetic efficiency in coffee plants grown in soils with controlled release fertilizer added. A higher photosynthetic rate was recorded in coffee plants for treatments with controlled release fertilizer added, regardless of the level of irrigation and type of soil conditioners. This finding had effect on estimates of number of plagiotropic branches due to higher production and translocation of photoassimilates to promote plants growth.

Coffee plants grown in soils with controlled release fertilizer added, associated with coffee husk, showed a greater vegetative growth than those found in coffee plants grown only in soils with conventional fertilizer added, indicating a beneficial interaction between these plants growth factors. This effect may be related to substantial supplementation of nutrients existing in both this type of fertilizer and the coffee husk, which is also a source of N and K (FERNANDES et al., 2013).

The importance of studying types of soil conditioners is highlighted, since the coffee husk

and organic compost provided higher growth rate and greater number of vascular bundles, with greater estimates of xylem vessel diameter in plants grown under water restriction conditions. This factors determining relationship has possibly occurred in function of types of soil covers provided by soil conditioners under study, which contributed to the decreased of water losses through evapotranspiration and the obtaining of higher contents of soil moisture. The coffee husk and organic compost are sources of organic matter, which allow greater water retention and improve soil properties. Fernandes et al. (2013) found greater productivity in coffee plants of treatments containing coffee husk, due to the improvement of the physical-chemical and biological soil properties, as well as gradual availability of nutrients provided by soil organic matter fractions found in soils containing coffee husk.

Despite the water-retaining polymer providing low plants growth as found in plants grown in soils containing coffee husk and organic compost, this soil conditioner allowed obtaining estimates of stem diameter, dry weight of the root system and number of xylem vessels with similar trend for either plants grown under water restriction or not. Regarding these variables, plants grown in soils containing water-retaining polymer were subjected to water stress conditioning treatments. Beyond these findings, coffee plants grown under water-retaining polymer showed greater estimates of plant height, dry weight of the aerial part, total dry weight and absolute growth when compared to plants of treatments grown without soil polymers (SOUZA et al., 2016).

Regarding the water-retaining polymer, estimates of spongy parenchyma thickness and mesophyll thickness at 40% irrigation level were greater those found in plants grown at 80% irrigation level. The higher thickness of spongy parenchyma and leaf mesophyll in plants grown under water restrictions can favor the gas exchanges due to high accumulation and storage of CO₂ required for photosynthesis (TERASHIMA et al., 2011).

Among types of soil conditioners under study, it highlights the effect of agricultural gypsum on the following anatomical variables: palisade parenchyma thickness, spongy parenchyma thickness, mesophyll thickness and number of xylem vessels, which was found to be lesser than estimates found in other treatments and the treatment with no soil conditioners.

Coffee plants grown in soils without conditioners at 40% irrigation level, showed lesser estimates of leaf water potential, i.e., this treatment causes greater water stress. This finding showed that soil conditioners are prominent determining factors in the management of water deficit in coffee growing.

Results obtained in this study show that the coffee husk was able to provide greater gas exchanges and total root length to coffee plants, regardless of the level of irrigation. This is one of soil conditioners that allowed obtaining higher estimate of the ratio polar diameter/equatorial diameter of the stomata. High estimates of soil moisture in plants grown in this type of soil conditioner indicates high photosynthetic performance, regardless of the level of irrigation, resulting in significant production and translocation of photoassimilates and greater estimates of total root length. Studies indicate that high estimates of the ratio polar diameter/equatorial diameter of the stomata favor photosynthetic capacity in coffee plants grown in soils with coffee husk. Besides, the increase in the polar diameter of stomata can favor the absorption of CO₂ required for photosynthesis, resulting in high production of energy and plant growth (SOUZA et al., 2010).

4 CONCLUSIONS

Controlled release fertilizers and soil conditioners were found to be potential agronomic techniques for the implementation of a water restriction attenuation plan in coffee plantations.

The coffee husk allowed greater plant growth under water restriction, thus, being the most appropriate type of soil conditioner recommended for mitigating the effects of water deficit.

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6 REFERENCES

- APARECIDO, L.E.O.; ROLIM, G.S.; SOUZA, P.S. Sensitivity of newly transplanted coffee plants to climatic conditions at altitudes of Minas Gerais, Brazil. **Australian Journal of Crop Science**, Queensland, v. 9, n. 2, p. 160-167, 2015.
- ASSIS, G.A. et al. Correlation between coffee plant growth and yield as function of water supply regime and planting density. **Bioscience Journal**, Uberlandia, v. 30, n. 3, p. 666-676, 2014.
- BINKS, O. et al. Limited acclimation in leaf anatomy to experimental drought in tropical rainforest trees. **Tree physiology**, Oxford, v. 36, n. 12, p. 1550-1561, 2016.
- BUNN, C. et al. A bitter cup: climate change profile of global production of Arabica and Robusta coffee. **Climatic Change**, v.129, n. 1-2, p.89-101, 2015.
- CASTANHEIRA, D.T. et al. 2016. Potential use of anatomical and physiological characteristics in the selection of coffee progenies. **Coffee Science**, Lavras, v. 11, n. 3, p. 375 – 386, 2016.
- FARES, A. et al. Irrigation water requirements for seed corn and coffee under potential climate change scenarios. **Journal of Water and Climate Change**, v.7, n.1, p. 39-51, 2016.
- FERNANDES, A.L.T. et al. Reduction of mineral fertilization of arabic coffee using coffee straw. **Coffee Science**, Lavras, v.8, n.3, p.324-336, 2013.
- GUIMARÃES, P. T. G. et al. Cafeeiro. In: RIBEIRO, A. C.; GUIMARÃES, P. T. G.; ALVARES, V. H. (Ed). **Recomendação para o uso de corretivos e fertilizantes em Minas Gerais: 5ª aproximação**. Viçosa, MG: Editora da UFV, 1999. p. 289-302.
- HACKE, U.G.; SPICER, R.; SCHREIBER, S.G. An ecophysiological and developmental perspective on variation in vessel diameter. **Plant, cell & environment**, v.40, n.6, p. 831-845, 2017.
- INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE - IPCC. **Climate Change 2014: Impacts, Adaptation and Vulnerability**. Summary for Policymakers. WG II. Geneva: IPCC, 2014.
- LANNA, A.C. et al. Physiological characterization of common bean (*Phaseolus vulgaris* L.) genotypes, water-stress induced with contrasting response towards drought. **Australian Journal of Crop Science**, Queensland, v.10, n.1, p.1-6, 2016.
- MALAVOLTA, E. **Manual de química agrícola: adubos e adubação**. 3ed. São Paulo: Agronômica Ceres, 1981. 584p.
- MCELTRONE, A.J. et al. Water uptake and transport in vascular plants. **Nature Education Knowledge**, Cambridge, v.4, p.1-6, 2013.
- MENEZES-SILVA, P.E. et al. Photosynthetic and metabolic acclimation to repeated drought events play key roles in drought tolerance in coffee. **Journal of Experimental Botany**, Oxford, v.68, p.4309-4322, 2017.

O'BRIEN, T.P.; FEDER, N.; MCCULLY, M.E. Polychromatic staining of plant cell walls by toluidine blue O. **Protoplasma**, v. 59, n. 2, p. 368-373, 1964.

R DEVELOPMENT CORE TEAM. **R**: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing, 2016. Disponível em: <<http://www.R-project.org>>. Acesso em: 10 Agosto, 2018.

SANTOS, W.J.R. et al. Soil moisture in the root zone and its relation to plant vigor assessed by remote sensing at management scale. **Geoderma**, v. 221-222, n. 1, p. 91-95, 2014.

SANTOS, A.B.; MAZZAFERA, P. Dehydrins are highly expressed in water stressed plants of two coffee species. **Tropical Plant Biology**, New York, v. 5, n. 3, p. 218-232, 2012

SEVANTO, S. et al. Is desiccation tolerance and avoidance reflected in xylem and phloem anatomy of two coexisting aridzone coniferous trees?. **Plant, Cell & environment**, v.41, p.1551-1564, 2018.

SILVA, H. et al. Relationships between leaf anatomy, morphology, and water use efficiency in *Aloe vera* (L) Burm f. as a function of water availability. **Revista Chilena de Historia Natural**, Santiago, v.87, p.1-10, 2014.

SOUZA, T.C. et al. Leaf plasticity in successive selection cycles of *Saracura maize* in response to periodic soil flooding. **Pesquisa Agropecuária Brasileira**, Brasília, v.45, n.1, p.16-24, 2010.

SOUZA, T.C. et al. Morphophysiology, morphoanatomy, and grain yield under field conditions for two maize hybrids with contrasting response to drought stress. **Acta Physiologiae Plantarum**, v.35, n.11, p.3201-3211, 2013.

SOUZA, A.J.J. et al. Quantitative analysis of growth in coffee plants cultivated with a water-retaining polymer in an irrigated system. **Revista Ciência Agronômica**, Fortaleza, v.47, n.1, p.162-171, 2016.

TAIZ, L.; ZEIGER, E. **Fisiologia vegetal**. 5ed. Porto Alegre: ArtMed, 2013. 918p.

TERASHIMA, I. et al. Leaf functional anatomy in relation to photosynthesis. **Plant Physiology**, v.155, n.1, p.108-116, 2011.

TIMILSENA, Y.P. et al. Enhanced efficiency fertilisers: a review of formulation and nutrient release patterns. **Journal of the Science and Food Agriculture**, v.95, n.6, p.1131-1142, 2015.

XU, Z.; ZHOU, G. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. **Journal of experimental botany**, Oxford, v.59, n.12, p.3317-3325, 2008.

YAN, J. et al. High photosynthetic rate and water use efficiency of *Miscanthus lutarioriparius* characterize an energy crop in the semiarid temperate region. **Gcb Bioenergy**, Illinois, v.7, n.2, p.207-218, 2015.

INFLUENCE OF THE USE OF COVERAGE PLANTS AND THE BIOACTIVATOR IN THE PHYSICAL-BIOLOGICAL CHARACTERISTICS OF SOIL CULTIVATED WITH COFFEE

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ABSTRACT: One of the alternatives to increase productivity and without harming the environment is the use of green cover associated with soil bioactivators. However, there are still incipient studies reporting the association influence of these two techniques on soil quality. In this sense, the aim in this work was to evaluate the Penergetic® bioactivator effect associated to different cover plants on the physical and biological characteristics of the soil cultivated with coffee tree. The experiment was carried out in a coffee field with *Catuai Vermelho* cultivar IAC 144, in a randomized block design in a factorial scheme 4 x 2, consisting of control (without plant cover); oats (*Avena strigosa*) + forage turnip (*Raphanus sativus*); oats (*Avena strigosa*) + forage turnip (*Raphanus sativus* L) + lupine (*Lupinus albus*) + rye (*Secale cereale*) + vetch (*Vicia sativa*) (OFLRV); *Brachiaria brizantha* (*Urochloa brizantha*), associated or not with the Penergetic® bioactivator use. The experiment was conducted for 6 months and after that period physical and biological soil characteristics were analyzed. The data were submitted to variance analysis and the means of treatment were grouped by the Scott-Knott test at 5% probability. The treatments containing cover plants had lower temperature and greater water retention. The bioactivator use reduced the nematodes population from the *Meloidogyne* genus and when associated to the cover plants showed increased the density of diazotrophic bacteria and solubilizers of phosphorus and potassium.

Index terms: Coffee cultivation, Penergetic®, Soil microbiota, Sustainable management.

INFLUENCIA DA UTILIZAÇÃO DE PLANTAS DE COBERTURA E BIOATIVADOR NAS CARACTERÍSTICAS FÍSICO-BIOLÓGICAS DO SOLO CULTIVADO COM CAFÉ

RESUMO: Uma das alternativas visando aumentar a produtividade e sem agredir o meio ambiente consiste no uso de cobertura verde associada aos bioativadores de solo. No entanto, ainda são incipientes os estudos relatando a influência da associação destas duas técnicas na qualidade do solo. Nesse sentido, o objetivo deste trabalho foi avaliar o efeito do bioativador Penergetic® associado a diferentes plantas de cobertura sobre as características físicas e biológicas do solo cultivado com cafeeiro. O experimento foi instalado em gleba de café contendo a cultivar Catuai Vermelho IAC 144, em delineamento de blocos casualizados em esquema fatorial 4 x 2, sendo constituído por: controle (sem planta de cobertura); aveia (*Avena strigosa*) + nabo forrageiro (*Raphanus sativus* L); aveia (*Avena strigosa*) + nabo forrageiro (*Raphanus sativus*) + tremoço (*Lupinus albus*) + centeio (*Secale cereale*) + ervilhaca (*Vicia sativa*) (OFLRV); *Brachiaria brizantha* (*Urochloa brizantha*), associados ou não ao uso do bioativador Penergetic®. O experimento foi conduzido por 6 meses e após esse período foram analisadas características físicas e biológicas do solo. Os dados foram submetidos à análise de variância e as médias dos tratamentos foram agrupadas pelo teste de Scott-Knott a 5% de probabilidade. Os tratamentos contendo plantas de cobertura apresentaram menor temperatura e maior retenção de água. O uso do bioativador reduziu a população de nematoides do gênero *Meloidogyne* e quando associado às plantas de cobertura apresentou aumento da densidade de bactérias diazotróficas e solubilizadoras de fósforo e potássio.

Termos para indexação: Cafeicultura, Penergetic®, Microbiota do solo, Manejo sustentável.

1 INTRODUCTION

The green manure consists of the rotating or consortium plants use with the economic interest crops, being considered a management technique that promotes improvement of the physical, chemical and biological characteristics of the soil (NUNES et al., 2006; CARVALHO et al., 2012), and is also responsible for reducing

environmental pollution promoted by erosion and leaching of fertilizers and pesticides to watercourses (AVANZI et al., 2013). In addition, it provides reduction of the thermal and moisture oscillations, thus ensuring better conditions for the development of plants and soil organisms.

The organic material provided by the practice of green manure provides an increase in the density and diversity of soil microorganisms

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(FILSER, 1995; DUDA et al., 2003), since its residues serve as energy source for metabolic activities. These, in turn, play a fundamental role in the decomposition of organic matter and increase the availability of nutrients to the plants (MOREIRA; SIQUEIRA, 2006). Among the groups of soil microorganisms, deserve to be highlighted, the N_2 fixers (FENG et al., 2018) and the phosphorus solubilizers (WEI et al., 2018) and potassium (ZHANG; KONG, 2014; MEENA et al., 2015), responsible for increasing the availability of this macronutrients in the soil.

However, in addition to the groups of beneficial organisms, there are pathogens, such as phytonematodes, responsible for large losses in productivity, widely disseminated and difficult to control (CASTRO et al., 2008). Mattei et al. (2017), report on the importance of maintaining the soil organisms population balance, thus favoring the occurrence of ecological interactions, thereby avoiding the population explosion occurrence of the phytonematodes and other pests.

In this way, the green manure use promotes better soil quality, controlling the ecosystem key functions (BATISTA et al., 2013). In addition to this, in the market are found the soil bioactivators, which are constituted by organic substances, humic and fulvic acids, amino acids, algae extract and vitamins that may or may not be associated with micronutrients. These products, according to Castro and Pereira (2008), act in the vegetal development by diverse mechanisms. Külen et al. (2011), declare that these products act in all development stages of the cultivated plants, being able to benefit different crops, such as soybean, corn, wheat and vegetables. According to Assis et al. (2012), the bioactivators reduce the aluminum toxicity and this is due to the formation of organic complexes with calcium and magnesium, which favors their soil movement, thus filling phosphorus adsorption sites and making it available to the plants, besides potentiating the use of fertilizers and contributing to the soil microbiological activity.

The Penergetic® bioactivator has been widely used in Brazil and, among its mechanisms of action, is responsible for favoring the microbial soil activity and, with this, increases the nutrient cycling and, consequently, reduction in the use of chemical fertilizers, providing a better life quality for rural producers and less aggression to the environment (BRITO; DEQUECH; BRITO, 2012). However, despite all these benefits, studies on the joint use the on cover plants of green manure and bioactivators are still incipient.

In this sense, the aim in this work was to evaluate the Penergetic® bioactivator effect associated to different cover plants on the physical and biological characteristics of the soil cultivated with coffee.

2 MATERIAL AND METHODS

The experiment was carried out at Farm Boa Esperança, located in the country of Serrania, South of Minas Gerais, during the months from May to December 2016. The experimental area has the following geographical coordinates: Latitude: 21°36'18.29"S, Longitude: 46°7'46.29"W and Altitude of 982m. The coffee grounds selected were those cultivated with *Catuaí Vermelho* IAC 144, implanted in 2011, spacing 3.5 m between rows and 0.7 m between plants, totaling 4081 pls/ha.

The experimental design was a randomized complete block design in a 4x2 factorial scheme, with 4 soil cover conditions associated to the use or not of Penergetic® bioactivator and 4 replications, totaling 32 experimental units. The four soil cover conditions were: 1) control, without cover plant; 2) oats (*Avena strigosa*) + forage turnip (*Raphanus sativus* L); 3) oats (*Avena strigosa*) + forage turnip (*Raphanus sativus* L) + lupine (*Lupinus albus*) + rye (*Secale cereale*) + vetch (*Vicia sativa*) (OFLRV); 4) *Brachiaria brizantha*.

Each experimental unit consisted of 10 coffee plants, being considered for the analysis only the 6 central plants, being the useful area of the parcel of approximately 74 m² and the experimental area of 0.24 ha⁻¹. The planting densities of the cover plants were: *Brachiaria brizantha* and forage turnip, 10 kg ha⁻¹, oats 40 kg ha⁻¹, lupine and rye 20 kg ha⁻¹ and vetch 15 kg ha⁻¹. The bioactivator was used in the dosage of 0.6 kg ha⁻¹. The sowing was done in shallow grooves, using 3 lines in the middle of the coffee streets, distant from each other by 50 cm. For both the seed quantity and the Penergetic® bioactivator to be applied, the manufacturer's recommendations were followed.

The cover plants were kept in the area from May to October, when the mowing was done and these were conserved in the coffee intercropped. The soil temperature was measured on its surface, using an infrared thermometer, at 1pm, weekly, for 32 weeks (may/december), resulting in an average. Two months later, soil samples were taken for moisture analysis, which was determined by the direct (gravimetric) method, which is the most

used, consisting of sampling the soil and, through weighing, determining its gravimetric moisture, correlating the water mass with the sample solids mass or the volumetric moisture, relating the water volume contained in the sample and its volume (EMBRAPA, 1997).

Soil and root samples, for nematode analysis, were collected under the canopy, where the highest root concentration was found according to the methodology recommended by Salgado, Carneiro and Pinho (2011) and sent to the nematology laboratory of the Federal University of Lavras - UFLA.

The soil samples collected for the microbiological analyzes were carried out with flambé auger to avoid contamination between the treatments. The population density of the bacterial groups was evaluated by the serial dilution technique using the following culture media: associative diazotrophs - FAM Medium (DÖBEREINER et al., 1995), phosphate solubilizing bacteria - NBRIP Medium (NAUTIYAL et al., 2000) and potassium - Aleksandrov Medium (PARMAR; SINDHU, 2013). In the soil samples collection for the nematode and microbiological analyzes, the auger was flambé after collection in each treatment to avoid contamination, being placed in sterilized containers, transported in a thermal box and taken to the Agricultural Microbiology Laboratory of the José do Rosário Vellano University (UNIFENAS).

The data were submitted to variance analysis and the averages were compared by the Scott-Knott test at 5% probability using the Sisvar® computer program (FERREIRA, 2014).

3 RESULTS AND DISCUSSION

Analyzing the values of the physical parameters, temperature and moisture, it was observed that there was no interaction between soil cover *versus* bioactivator, only soil cover influenced the values of these parameters (Table 1).

In relation to the temperature, it was verified that the different treatments containing cover plants were similar to each other, presenting smaller values when compared to the control treatment (without soil cover). For moisture, the positive effect of soil cover was also observed, and the treatments containing OFLRV and *B. brizantha* were the ones that presented higher water retention in the soil.

These results agree with those reported by Lima et al. (2017) and Ribeiro et al. (2018), who

mention the positive influence of cover plants on the reduction of soil water evaporation and mitigate the temperature, factors that favors the root development with greater absorption of water and nutrients and, consequently, ensuring greater agricultural productivity.

The OFLRV treatment, constituted by cruciferous, grass and leguminous plants, which have different morphological characteristics of the root system may have had a positive influence on the higher water retention. Studies show the importance of grasses, such as the *Brachiaria* genus in soil quality improvement, with emphasis on maintaining moisture (ANDRIOLI et al., 2008). Prando et al. (2010), relate to higher water retention in soils grown with *Brachiaria ruziziensis* due to the improvement in the size and numbers of biopores, which facilitates the penetration of water into the soil, in agreement with the results found in this study.

Other studies confirm the importance of soil cover to increase infiltration and moisture maintenance, since they reduce the raindrops impact, disintegrating the soil and, as observed in this study, reduce soil temperature, besides increasing the organic matter contents (CARVALHO et al., 2012; MOREIRA; PAIVA; DINIZ, 2017).

In the soil biological characteristics, in relation to the population density of nematodes, no interaction between soil cover *versus* bioactivator was observed, and the population of the *Meloidogyne* genus was only influenced by the bioactivator use (Table 2), while the nematodes density of the *Xiphinema* genus was affected by the soil cover type (Table 3).

Analyzing Table 2, there is a significant reduction of the nematode population of the *Meloidogyne* genus by the bioactivator use. According to Assis et al. (2012), bioactivators increase soil microbiological activity, which, according to Elhady et al. (2017) can act in the phytonematodes control. Mattei et al. (2017), report on the bacteria efficiency of the *Pasteuria* genus, found in soil, in reducing the number of galls formed by *Meloidogyne* spp.

Considering the importance of the nematodes control for agriculture and studies are still incipient on the effects of the use of soil bioactivators, the results obtained are of great relevance, since the *Meloidogyne* genus occurs in all the coffee regions of Brazil, mainly in the states of São Paulo, Rio de Janeiro, Paraná, Minas Gerais, Espírito Santo and Bahia, causing damages to the national coffee industry (MATIELLO et al., 2010).

TABLE 1 - Temperature and soil after handling the cover plants, moisture and soil in the coffee plantation as a function of the cover plants in december 2016.

Soil cover	Temperature (°C)	Moisture (%)
Control	30.88 a	15.75 c
Oats and turnips	27.99 b	21.75 b
OFLRV*	27.78 b	27.00 a
<i>Brachiaria brizantha</i>	27.27 b	30.38 a

Averagets of treatment followed by the same letter, in the column, do not differ by Scott Knott's test at 5% probability. *Abbreviation of treatment: oat + forage turnip + lupine + rye + vetch.

TABLE 2- Nematodes density of the *Meloidogyne* genus in soil samples collected in the coffee intercropped grown under different cover plants, after handling the cover plants.

Penergetic®	<i>Meloidogyne</i> density (100 cm ³ soil)
With	7.12 b
Without	12.50 a

Averagets of treatment followed by the same letter, in the column, do not differ by Scott Knott's test at 5% probability.

TABLE 3 - Nematodes density of the *Xiphinema* genus in soil samples collected in the coffee intercropped grown under different cover plants, after handling the cover plants.

Soil cover	<i>Xiphinema</i> density (100 cm ³ soil)
Control	5.75 a
Oats and turnips	2.75 a
OFLRV*	0.00 b
<i>Brachiaria brizantha</i>	0.25 b

Averagets of treatment followed by the same letter, in the column, do not differ by Scott Knott's test at 5% probability. *Abbreviation of treatment: oat + forage turnip + lupine + rye + vetch.

Table 3 shows the results of the nematode density of the *Xiphinema* genus, which was influenced by the different types of soil cover.

It is observed that the OFLRV and *B. brizantha* treatment significantly reduced the nematode population, presenting lower results than the other treatments. Although this nematodes genus is not a problem in the coffee crop, this confirms the importance of cover plants, which, by promoting diversity in the population of predatory fungi, plays an important role in reducing population nematodes density, of different genera, that cause damage in many crops (INOMOTO; ASMUS, 2014).

In the microbiological parameters, it was observed that there was interaction between soil cover *versus* bioactivator, and, in general, the presence of cover plants associated to the bioactivator use increased the density of the bacteria groups, evidencing the synergistic effect between cover plants and bioactivators.

Averagets of treatment followed by the same letter, in the column, do not differ by Scott Knott's test at 5% probability. *Abbreviation of treatment: oat + forage turnip + lupine + rye + vetch.

In the bioactivator and cover plants presence a largest population of diazotrophic bacteria was obtained.

TABLE 4 - Average of log of UFC.mL⁻¹ for the diazotrophic bacteria groups, potassium and phosphorus solubilizers isolated from soil samples collected in the coffee intercropped grown with different cover plants with or without the Penegetic® bioactivator, after handling the cover plants.

Soil cover	Bioactivator					
	With	Without	With	Without	With	Without
	Diazotrophic		Potassium solubilizers		Phosphorus solubilizers	
Control	4.64 Ba	3.74 Aa	3.09 Ca	3.23 Ba	3.07 Ba	3.35 Ba
OFLRV*	5.74 Aa	4.09 Ab	7.30 Aa	5.37 Ab	6.44 Aa	3.39 Bb
Oats and Turnip	5.75 Aa	3.80 Ab	3.24 Ca	2.96 Ba	3.71 Ba	4.22 Ba
<i>Brachiaria</i> <i>Brizantha</i>	6.95 Aa	4.64 Ab	5.29 Ba	2.98 Bb	5.97 Aa	5.91 Aa

Averagets of treatment followed by the same letter, in the column, do not differ by Scott Knott's test at 5% probability. *Abbreviation of treatment: oat + forage turnip + lupine + rye + vetch.

On the other hand, when the bioactivator was not used, no statistical difference was observed in the density of these bacteria.

For the potassium solubilizers group, the OFLRV treatment associated to the bioactivator provided a higher population density, followed by the soil cover containing *B. brizantha*. In the phosphorus solubilizers, the OFLRV treatment with the bioactivator use was also highlighted, which was similar to that of *B. brizantha*.

The cover plants influence, as well as the rhizospheric effect on the soil microbial population is well reported by Moreira and Siqueira (2006), however, studies are needed aiming to identify possible soil bioactivators action mechanisms associated with cover plants on the soil micro-organisms. In this sense, the results obtained in this study are of great relevance for research and for coffee cultivation in general, since there is now a need to identify alternative sources of nutrients to reduce dependence on fertilizer imports by Brazil and that are sustainable, thus ensuring the products competitiveness on the external market.

4 CONCLUSIONS

The use of cover plants positively influenced soil temperature and moisture in the coffee intercropped. The use of the Penegetic® bioactivator reduced the nematode population of the *Meloidogyne* genus and when associated with the use of cover plants, increased the population density of diazotrophic and solubilizers bacteria of phosphorus and potassium.

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6 REFERENCES

- ANDRIOLI, Itamar et al. Produção de milho em plantio direto com adubação nitrogenada e cobertura do solo na pré-safra. **Revista Brasileira Ciência do Solo**. Viçosa, v. 32, n. 4, p. 1691-1698, 2008.
- ASSIS, R.T.; TOMAZ, H.V.Q. O cerrado hoje e o seu potencial de produção agrícola. In: SILVA, J.C.; SILVA, A.A.S. **Sustentabilidade Produtiva do Cerrado**. Uberlândia: Composer, 2012. p. 204-219.
- AVANZI, J. C. et al. Spatial distribution of water erosion risk in a watershed with eucalyptus and Atlantic Forest. **Ciência e Agrotecnologia**, v. 37, n. 5, p. 427-34, set-out. 2013.

- BATISTA, M.A.V.; BEZERRA NETO, F.; AMBRÓSIO, M.M.Q.; GUIMARÃES, L.M.S.; SARAIVA, J.P.B. E SILVA, M.L. Atributos microbiológicos do solo e produtividade de rabanete influenciados pelo uso de espécies espontâneas. **Revista Horticultura Brasileira**, vol. 31, n. 4, p.587-594, 2013.
- BRITO, O. R.; DEQUECH, F. K.; BRITO, R. M. Use of penergetic procutcs P and K in snap bean production. **Annual report of the bean improvement cooperative**, v. 55, p. 279- 280, 2012.
- CASTRO, J. M. da C. e; CAMPOS, V. P.; POZZA, E. A.; NAVES, R. de L.; ANDRADE JÚNIOR, W. C.; DUTRA, M. R.; COIMBRA, J. L.; MAXIMINIANO, C.; SILVA, J. R. C.. Levantamento de fitonematóides em cafezais do sul de Minas Gerais. **Nematologia Brasileira**, Piracicaba, v. 32, n. 1, p. 56- 64, mar. 2008.
- CASTRO, P.R.R.; PEREIRA, M. A. Bioativadores na agricultura. In: GAZZONI, D. L. (Ed.). **Tiametoxam: uma revolução na agricultura brasileira**. Petrópolis: Vozes, p. 115-122. 2008
- CARVALHO, M. A. R.; MIRANDA, J. H.; DUARTE, S. N; CARVALHO, L. C. C. Escoamento superficial na interação: cobertura vegetal e práticas de controle de erosão. **Engenharia Agrícola**, v. 32, n. 6, p. 1116-1125, 2012.
- DÖBEREINER, J.; BALDANI, V. L. D.: BALDANI, J. I. **Como isolar e identificar bactérias diazotróficas de plantas não-leguminosas**, Brasília: Embrapa-SPI, 1995 60 p.
- DUDA, G.P.; GUERRA, J.G.M.; MONTEIRO, M.T.; DE-POLLI, H.; TEIXEIRA, M.G. Perennial herbaceous legumes as live soil mulches and their effects on C, N and P of the microbial biomass. **Scientia Agricola**, v.60, p.139-147, 2003.
- ELHADY, A.; GINÉ, A.; TOPALOVIC, O.; JACQUIOD, S.; SØRENSEN, S. J.; SORRIBAS, F. J. and HEUER, H. Microbiomes associated with infective stages of root-knot and lesion nematodes in soil. **PloS one**, v.12, n.5, 2017.
- EMBRAPA. **Manual de métodos de análise de solo**. 2. ed. Rio de Janeiro, 1997. 212p. (Documentos, 1).
- FENG, M.; ADAMS, J. A.; FAN, K.; SHI, Y.; SUN, R.; WANG, D.; GUO, X.. CHU H. Long-term fertilization influences community assembly processes of soil diazotrophs. **Soil Biology and Biochemistry**, V.126, p. 151-158, 2018.
- FERREIRA, D.F. Sisvar: um guiado dos seus procedimentos de comparações múltiplas *Bootstrap*. **Ciência e Agrotecnologia**, v. 38, n. 2. p. 109-112, 2014.
- FILSER, J. The effect of green manure on the distribution of collembola in a permanent row crop. **Biology and Fertility of Soils**, Berlin, v.19, n.4, p.303-8, 1995.
- INOMOTO, M. M.; ASMUS, G. L. Adubos verdes das famílias fabaceae e mimosaceae para o controle de fitonematoides. In: LIMA FILHO, O.F. et al. **Adubação verde e plantas de cobertura no Brasil**. Brasília: Embrapa, 2014. cap.12, p.441-479.
- KÜLEN, O. et al. Gibberellic acid and ethephon alter potato minituber bud dormancy and improve seed tuber yield. **American Journal of Potato Research**, v. 88, n. 2, p. 167-174, abril 2011.
- LIMA, B. V.; SOUZA, G. G.; OLIVEIRA, T. O. P.; OLIVEIRA, J. A. G. EFEITOS DA ADUBAÇÃO VERDE NOS ATRIBUTOS DO SOLO. **Revista Conexão Eletrônica** – Três Lagoas, MS – V.14, n.1, p.515-523, 2017.
- MATEI, D.; HENKEMEIER, P.; HELING, A. L.; LORENZETTI, E.; KUHN, O. J.; STANGARLIN, J. R. **Produtos fitossanitários biológicos disponíveis para agricultura e perspectivas de novos produtos**. In: ZAMBOM, M. A.; KUHN, O. J.; SILVA, N. L. S.; STANGARLIN, J. R.; NUNES, R. V.; FULBER, V. M.; EYNG, C.(Org.) **Ciências agrárias: ética do cuidado, legislação e tecnologia na agropecuária / C569** . Marechal Cândido Rondon, 2017. 228 p.
- MATIELLO, J. B. et al. **Cultura de café no Brasil: novo manual de recomendações**. Rio de Janeiro e Varginha: MAPA/PROCAFÉ, 2010. 542 p.
- MEENA, V. S. et al. Potassium solubilizing rhizobacteria (KSR): Isolation, identification, and K-release dynamics from waste mica. **Ecological Engineering**, v. 81, p. 340-347, aug. 2015.
- MOREIRA, F.M.S. & SIQUEIRA, J.O. **Microbiologia e bioquímica do solo**. 2.ed. Lavras, Universidade Federal de Lavras, 2006. 729p.
- MOREIRA, C. F.; PAIVA, A. O.; DINIZ, C. V. C. **Clima e água para uma cafeicultura**. Machado: Associação de Cafeicultura Orgânica do Brasil, 2017. 44 p. il.

- NAUTIYAL, C. S. et al. Stress induced phosphate solubilization in bacteria isolated from alkaline soils. **FEMS Microbiol. Lett.**, v. 182, n. 2, p. 291-296, 2000.
- NUNES, U.R.; ANDRADE JÚNIOR, V.C.; SILVA, E.B. SANTOS, N.F.; COSTA, H.A.O.; FERREIRA, C.A. Produção de palhada de plantas de cobertura e produtividade do feijão em plantio direto. **Pesquisa Agropecuária Brasileira**, v.41, n.6, p.943-948, jun. 2006.
- PARMAR, P.; SINDHU, S.S. Potassium solubilization by rhizosphere bacteria: influence of nutritional and environmental conditions. **International of Journal Microbiology Research**, v.3, n. 1, p. 25–31, out. 2013.
- PRANDO, M. B.; OLIBONE, D.; OLIBONE, A. P. E.; ROSELEM, C. A. Infiltração de água no solo sob escarificação e rotação de culturas. *Revista Brasileira de Ciência do Solo*. vol.34 no.3 Viçosa May/June 2010. P. 693-700.
- RIBEIRO, H. A. B. R.; DINIZ, E. R.; SOUZA JÚNIOR, J. B.; MOURO, G. F.; MARTINKOSKI, L. Qualidade do solo sobre manejo com palha e adubação verde em cobertura. **Cadernos de Agroecologia** – ISSN 2236-7934 – Anais do VI CLAA, X CBA e V SEMDF – Vol. 13, Nº 1, Jul. 2018.
- SALGADO, S.M. de L. , CARNEIRO, R.M. D.G.: PINHO, R. S. C de: **Aspectos técnicos dos nematoides parasitas do cafeeiro**. - Belo Horizonte: EPAMIG, 2011. p 29 -33.
- WEI, Y.; ZHAO, Y.; SHI, M.; CAO, Z.; LU, Q.; YANG, T.; FAN, Y.; WEI, Z. Effect of organic acids production and bacterial community on the possible mechanism of phosphorus solubilization during composting with enriched phosphate-solubilizing bacteria inoculation. **Bioresource Technology**, Volume 247, January 2018, Pages 190-199.
- ZHANG, C.; KONG F. Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. **Applied Soil Ecology**, v. 82, p. 18–25, oct. 2014.

NOTA PRÉVIA

APPLICATIONS OF COPPER-BASED FUNGICIDES AND INFESTATIONS OF *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) IN COFFEE PLANTS

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ABSTRACT: The present study aimed to evaluate the effects of applying fungicides with different sources of copper and of the number of applications on the occurrence of *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) and on the wax content on leaves in a coffee plantation. Four applications of fungicides were carried out, and the effects on the number of leaves mined by the insect and on the wax content on the leaf surface were evaluated. The copper-based fungicides increased the number of leaves mined by the leaf-miner and reduced the wax content on the coffee leaf surfaces in both periods studied.

Index terms: *Coffea arabica*, pulverization, epicuticular wax, occurrence, leaf-miner.

APLICAÇÃO DE FUNGICIDAS CÚPRICOS E INFESTAÇÃO DE *Leucoptera coffeella* (Guérin-Mèneville & Perrottet) NO CAFEEIRO

RESUMO: O presente trabalho teve como objetivo avaliar os efeitos de aplicações de diferentes fontes de fungicidas cúpricos e de números de aplicações, na ocorrência de *Leucoptera coffeella* (Guérin-Mèneville & Perrottet, 1842) (Lepidoptera: Lyonetiidae) e no teor de cera das folhas em lavoura cafeeira. Foram realizadas quatro aplicações de fungicidas e foi avaliado os efeitos no número de folhas minadas pelo inseto e no teor de cera da superfície foliar. Os fungicidas cúpricos aumentaram o número de folhas minadas pelo bicho-mineiro e reduziram o teor de cera das superfícies das folhas do cafeeiro nas duas épocas estudadas.

Termo de indexação: *Coffea arabica*, pulverização, cera epicuticular, ocorrência, bicho-mineiro.

The coffee leaf-miner, *Leucoptera coffeella* (Guérin-Mèneville, 1842) (Lepidoptera: Lyonetiidae), is the main pest of coffee plants (*Coffea arabica* L.) (BARRERA, 2008). The occurrence of this insect pest in plantations is associated with various factors, and notable among these is the application of copper-based fungicides, based on copper hydroxide and copper oxychloride. These compounds are applied in coffee plantations throughout the crop cycle in order to carry out preventive control of the fungus *Hemileia vastatrix* (CARVALHO et al., 2008) and also of bacterial infections. Copper fungicides may act on the wax layer of leaves, which is an important defense structure against attack by herbivorous insects (LICHSTON; GODOY, 2006), and changes to this layer can increase *L. coffeella* egg-laying and population size.

In planning management programs for this insect, it is very important to confirm if the application of copper fungicides is correlated with an increase in the population of the coffee

leaf-miner. Therefore, the present study aimed to evaluate the effects of applying fungicides with different sources of copper and the effects of the number of applications on the occurrence of *L. coffeella* and on the wax layer of leaves in a coffee plantation.

The assays were carried out at Fazenda Vitória, a farm belonging to the José do Rosário Vellano University (UNIFENAS), in the county of Fama, MG, Brazil, between 21° 24' 23" S and 45° 49' 43" W, at 776 m altitude. The climate is Cwa according to the Köppen classification. Six-year-old coffee cv. Catuai plants, cultivated with spacing of 3.5 x 0.80 m, were used to conduct two assays in different periods of the year. The period from November 2016 to February 2017 was denominated period 1, the period from June 2017 to September 2017 was denominated period 2, and all assays were carried out on the same piece of land. Each assay received four applications of copper fungicides.

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The experimental design was randomized blocks in a 4x4 factorial scheme. It consisted of four applications of fungicide in a combination with three copper-based products plus the control treatment (water), with four replicates, totaling 64 experimental plots, each of which contained 10 plants. The useful area of each plot was composed of the six central plants, and between the blocks a line of coffee plants was left as a barrier. The fungicides and the doses used were: Cuprozeb® (copper oxychloride) – 3 kg of commercial product (c.p.).ha⁻¹, Supera® (copper hydroxide) – 3 L c.p. ha⁻¹ and Supa cobre (copper hydroxide – product used for plant nutrition) – 2 L c.p. ha⁻¹.

The applications of the products were carried out every 30 days in each of periods 1 and 2, using a manual back-sprayer (Jacto®, model PJH 20, with a cone-shaped nozzle) and with application volume of 200 L.ha⁻¹. The adjuvant Agral® was added at a concentration of 0.5% (v/v) to the chemical mixture and to the control water treatment. Crop treatment followed the agronomic practices recommended for coffee. No insecticides were applied in the experimental area.

For the evaluations of insect pest attack, four branches were marked in the middle third of the plant, by means of colored tape, with two on each side, making a total of 24 branches marked per plot. The applications of copper fungicides were carried out monthly. Twenty-four hours before the first application, the leaves that had already been mined were removed from the marked branches, in order not to count them during the first evaluation. The evaluations of the number of leaves with mines made by leaf-miner insects were carried out one day before each application, by counting the leaves with at least one intact mine; in other words, with no sign of predation by wasps. Immediately after each evaluation, the leaves with mines were removed from all the marked branches. In this way, it was possible to determine the infestation by leaf-miner throughout the evaluation periods.

In period 1 and in period 2, the wax content on leaf surfaces was analyzed at 10 days after the fourth fungicide application. This amount of time is sufficient for copper-based products to have been absorbed. Leaves from the third and fourth pair of branches in the middle third of the plants were collected, which were exactly those that had previously been marked with colored tape. Two leaves were taken from each side of the plant. These leaves were taken to the laboratory to quantify the wax content, following the methodology modified by Guimarães et al. (2009). For this, the leaves were separately placed on to a Petri dish with 100 mL of chloroform for 30 seconds, with gentle

shaking. This procedure was carried out with great care to avoid rupturing the leaf tissues, thereby releasing cell compounds. The extracts obtained were filtered through filter paper and transferred to a beaker, in which they were evaporated over a steam bath until the volume was reduced to approximately 15 mL. This solution (chloroform and wax) was transferred to 25 mL test tubes of known weight. The chloroform was evaporated in a steam bath to obtain the solid residue (wax). The quantity of wax was expressed in mass per leaf area unit (µg. cm⁻²).

The data on mined leaves and wax content were submitted to the Shapiro-Wilk test for analysis of normality. The data were transformed into $\sqrt{x} + 0.5$ and submitted to analysis of variance with the use of software R version 3.2.4 (R CORE TEAM, 2016). The means were grouped by the Scott-Knott test at 5% significance.

In relation to the percentage of leaves mined in the assay carried out in period 1, there was a significant effect on the interaction between the evaluated products and the number of applications (gl = 9; F = 2.53; p < 0.05) (Table 1). After the first two applications, no difference was observed between the evaluated products regarding the increase in leaf-miner infestation; however, from the third application onwards, the copper-based fungicides provoked an increase in the percentage of mines in relation to the control treatment. With three and four applications of the fungicides Supa cobre, Cuprozeb® and Supera®, an increase in the percentage of mined leaves was noted (Table 1).

There was a significant effect on the interaction between the evaluated fungicides and the number of applications with regard to the percentage of mined leaves in period 2 (gl = 9; F = 1.96; p < 0.05) (Table 1). There was no difference between the products in the first three applications; however, when the fourth application took place, the products Supa cobre and Cuprozeb® differed from Supera® and from the control treatment (Table 1). The number of applications of copper fungicides contributed to the increase in the percentage of mined leaves, and with four applications the number of mined leaves was higher.

The economic threshold of coffee leaf-miner in Brazil was previously determined to be 20% of mined leaves with no signs of predation, mainly in the third and fourth pair of leaves (REIS; SOUZA, 1996).

TABLE 1 – Percentage of leaves mined by the coffee leaf-miner (\pm standard error) after applications of copper fungicides in period 1 (November 2016 to February 2017) and period 2 (June 2017 to September 2017) in field conditions.

PERIOD 1				
Number of applications of copper fungicides				
Treatments	1	2	3	4
Supa cobre	2.27 \pm 0.66 a B*	2.49 \pm 0.7 a B	4.09 \pm 1.56 a A	4.19 \pm 0.58 a A
Cuprozeb	2.13 \pm 0.6 a B	2.59 \pm 0.68 a B	4.66 \pm 1.3 a A	5.12 \pm 0.77 a A
Supera	1.83 \pm 0.69 a B	1.99 \pm 0.8 a B	3.98 \pm 1.11 a A	4.32 \pm 0.57 a A
Control	1.83 \pm 0.58 a A	1.83 \pm 0.8 a A	1.92 \pm 0.63 b A	2.11 \pm 0.51 b A
PERIOD 2				
Number of applications of copper fungicides				
Treatments	1	2	3	4
Supa cobre	4.20 \pm 1.64 a C*	8.40 \pm 3.54 a B	10.75 \pm 3.04 a B	22.47 \pm 5.34 a A
Cuprozeb	2.31 \pm 1.37 a C	11.52 \pm 3.15 a B	13.41 \pm 3.27 a B	22.72 \pm 4.99 a A
Supera	5.47 \pm 2.43 a C	10.21 \pm 2.13 a B	11.09 \pm 1.95 a B	16.83 \pm 3.02 b A
Control	2.47 \pm 1.21 a C	5.97 \pm 3.59 a C	12.13 \pm 3.14 a B	17.06 \pm 3.6 b A

*Lower-case letters in the column and upper-case letters in the line do not differ among themselves by Scott-Knott test at a 5% level of significance.

In our study, the percentage of leaves mined in September was higher than 20% in plants treated with supa cobre (22.47%) and cuprozeb (22.72%) in older leaves. This information is important for improving management strategies for the coffee leaf-miner.

In period 1 (November 2016 to February 2017) the infestation of the coffee leaf-miner did not reach the economic threshold. These results were also observed by Custódio et al. (2009), in a study that identified low infestation of the leaf-miner during the same period in the south of Minas Gerais state. The South of Minas region presents high levels of rainfall, which leads to unsuitable conditions for the reproduction of the coffee leaf-miner (ASSIS et al., 2012).

The wax content in coffee leaves differed between the treatments from periods 1 and 2 (gl = 3; F = 4.91; p < 0.05 and gl = 3; F = 4.94; p < 0.05) (Table 2). The three copper-based fungicides reduced the wax content on leaves in both periods 1 and 2.

Studies have been carried out on the effects of copper fungicide application on the wax content of coffee leaves; however, little is known about its influence on infestation by *L. coffeella*. Linchoston and Godoy (2006) performed four

cumulative applications of fungicides based on copper oxychloride on coffee plants and affirmed that these applications diminished the wax content on the leaf surface. Therefore, they concluded that substances in contact with the leaf surface may damage the leaf morphology and wax content, jeopardizing the plant by reducing its capacity to retain water by means of the wax. This may cause hydric stress, which is favorable to leaf-miner attack, because the moth prefers to lay eggs on leaves with a drier surface (CUSTÓDIO et al., 2009).

According to Cunha, Mendes and Chalfoun (2004), applications of copper fungicides make the coffee leaves greener and shinier. The greener coloration of leaves with less wax may be considered an attractive characteristic for the leaf-miner moth, when it makes an egg-laying choice under field conditions. Another hypothesis that could explain the increase in mined leaves is that the fungicides may interact with the plant, affecting its metabolic routes, with the release of volatiles that attract the leaf-miner (COQUERET et al., 2017; VEROMANN et al., 2013). However, the real cause is not yet clear, and more research is needed to examine this event more deeply.

TABLE 2 - Mass of epicuticular wax ($\mu\text{g. cm}^{-2}$) extracted from coffee leaves (\pm standard error) after four applications of different copper fungicides in period 1 (November 2016 to February 2017) and in period 2 (June 2017 to September 2017) in field conditions.

Treatments	Period 1	Period 2
Cuprozeb	22.31 \pm 2.48 b*	51.53 \pm 5.57 b
Supera	32.24 \pm 3.89 b	57.06 \pm 3.26 b
Supa cobre	33.94 \pm 7.37 b	41.45 \pm 2.74 b
Control	46.50 \pm 1.71 a	76.01 \pm 4.69 a

*Means followed by the same lowercase letter in the column belong to the same cluster by Scott-Knott test at a 5% level of significance.

In coffee crops of the Minas Gerais savanna the occurrence of *L. coffeella* is higher than in the south of the same state, due to environmental conditions (REIS; SOUZA, 1996). It is therefore also important to carry out the present study in the savanna region, in order to see the effects of copper fungicides on pest occurrence. It was concluded that the application of fungicides based on copper hydroxide and copper oxychloride in coffee plantations increases the infestation of *L. coffeella* and reduces the wax layer on coffee leaves.

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REFERENCES

- 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, fev, 2012.
- BARRERA, J. F. **Encyclopedia of Entomology**. 2. ed. Netherlands: Capinera, J. L, 2008. 4346 p.
- CARVALHO, V. L. et al. Influência do zinco na incidência de doenças do café. **Ciência e agrotecnologia**, Lavras, v. 32, n. 3, p. 804-808, maio/jun, 2008.
- 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. 2017.
- CUNHA, R. L.; MENDES, A. N. G.; CHALFOUN, S. A. Controle químico da ferrugem do café (*Coffea arabica* L.) e seus efeitos na produção e preservação do enfolhamento. **Ciência e agrotecnologia**, Lavras, v. 28, n. 5, p. 990-996, Out. 2004.
- CUSTÓDIO, A. A. P. et al. Incidência do bicho-mineiro do café em lavoura irrigada sob pivô central. **Coffee Science**, Lavras, v. 4, n. 1, p. 16-26, Jan./Jun. 2009.
- GUIMARÃES, A. A. et al. Composição química da cera epicuticular de biótipos de azevém resistente e suscetível ao glyphosate. **Planta Daninha**, Viçosa, v. 27, n. 1, p. 149-154, Mar. 2009.
- LICHSTON, J. E.; GODOY, S.A.P. Morfologia e teor de cera de folhas de café após aplicação de fungicida. **Pesquisa Agropecuária Brasileira**, Brasília, v. 41, n. 6, p. 919-926, June. 2006.
- R CORE TEAM. **R: A language and environment for statistical computing**. Vienna: R Foundation for Statistical Computing, 2016.
- REIS, P. R.; SOUZA, J. C. Manejo integrado das pragas do café em Minas Gerais. **Informe Agropecuário**, Belo Horizonte, v. 19, n. 193, p. 17-25, Oct 1998.
- 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.

NOTA PRÉVIA

INFECTION PROCESS OF *Cercospora coffeicola* IN IMMATURE COFFEE FRUITS

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ABSTRACT: Cercosporiosis is caused by *Cercospora coffeicola* and represents a very important coffee plants phytosanitary problem. Catuaí IAC 144 and Topázio cultivars at the F2 stage were inoculated with a conidial suspension. Samples were collected at 4, 8, 12, 24, 36, 48, 72, 96, and 168 hours after inoculation (hai) for scanning electron microscope studies. Fungal germination in epidermal surface occurred four hai; penetration only through epidermal wounds, without appressoria, started at 12 hai while the mycelial colonization occurred at 72 hai. The infection in immature coffee fruits implies brown eyespot control management anticipation need, even before the first symptoms.

Index terms: Brown eyespot, *Coffea arabica*, scanning electron microscopy.

PROCESSO INFECCIOSO DE *Cercospora coffeicola* EM FRUTOS DE CAFÉ IMATUROS

RESUMO: Cercosporiose é causada por *Cercospora coffeicola* e representa grave problema fitossanitário do cafeeiro. Frutos de cv. Catuaí IAC 144 e Topázio no estágio F2 foram inoculados com suspensão conidial. Amostras foram coletadas 4, 8, 12, 24, 36, 48, 72, 96, e 168 horas após a inoculação (hai) para estudos de microscopia eletrônica de varredura. A germinação conidial na superfície epidérmica ocorreu quatro hai; a penetração na epiderme ocorreu através de ferimentos, sem formação de apressórios, 12 hai, já a colonização micelial interna ocorreu 72 hai. A infecção de frutos imaturos implica na necessidade de antecipação no manejo da mancha de olho pardo, mesmo antes dos primeiros sintomas.

Termos para indexação: Mancha de olho pardo, *Coffea arabica*, microscopia eletrônica de varredura.

One of the main phytosanitary problems of coffee is cercosporiosis, also known as brown eye spot disease, whose etiological agent is the fungus *Cercospora coffeicola* Berkeley & Cooke. The pathogen can infect leaves and fruits of coffee tree, causing losses of 15% to 30% (GUIMARÃES; MENDES; BALIZA, 2010), and can affect the final product quality: consumers' cup of coffee (POZZA *et al.*, 2010). This adverse effect on quality is due to change in staining and, as proportion of diseased grains increases, a reduction in grain size, total sugars, total solids, and pH (LIMA; POZZA; SANTOS, 2012).

In order to reduce this infection and to guarantee quality to final product, adequate management strategies for cercosporiosis control must be employed. Therefore, it is necessary to know how the pathogen germinates and penetrates the fruit, whether by stomata or wounds, or by appressoria formation. The infection process of *C. coffeicola* has been frequently studied and characterized in foliar tissues of coffee (SOUZA *et al.*, 2011) but little is known about how this

process occurs in fruits. Infections occurrence during the early flowering stages has been frequently reported in the field; however there is not scientific confirmation. Our results explain how this pathogen penetrates the coffee fruit and helps to determine when coffee grain disease monitoring and biological control agents and fungicides application should start. The objective in this study was to describe the infection process of *Cercospora coffeicola* in coffee fruit.

The isolate of *C. coffeicola* used was CML 2489 and was deposited in mycological collection of Federal University of Lavras (UFLA) in Lavras, Minas Gerais, Brazil. Its sporulation was induced as described by Souza *et al.* (2011), using a conidia suspension of 6×10^4 conidia.mL⁻¹ to inoculate the pathogen. The trials were carried out with fruit obtained from detached branches of the middle third of cv. Catuaí IAC 144 and cv. Topázio sampled from the UFLA Coffee Growing Field in November 2013. It was used two branches of each cultivar with 10 fruit rosettes in F2 stage ('pin head' stage, with green color and 3.1 to 4 mm). It was

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performed the fruits disinfestation by immersion in 1 mg L⁻¹ sodium hypochlorite (twice), followed by rinsing with distilled water and drying on filter paper in a laminar flow chamber for 2 hours; afterwards, the samples were placed in a plastic tray.

The fruit branch cut site was introduced in moist phenolic foam and wrapped in plastic film, with nutrient solution from Hoagland and Arnon (1950), to ensure nutrition for the fruit during the experiment period. The inoculation was performed using 100 µL of conidial suspension, applied to F2 stage fruits surface, just after petals fell. The samples were incubated at 25 ± 2 °C and 90 ± 5% relative humidity with continuous white fluorescent light. The samples were processed to electron microscope analysis as done by Silva et al. (2017). Ten fruit units from each cultivar were collected at 4, 8, 12, 24, 36, 48, 72, 96, and 168 hours after inoculation (hai); fragments of 25 mm² were fixed with Karnovsky solution (pH 7.2) and post-fixed with 1% osmium tetroxide solution and dehydrated with acetone series. The specimens were dried in a Critical Point Apparatus (Baltec CPD 030 Balzers) followed by gold coating on a Sputter Coater Evaporator (Baltec SCD 050 Balzers). The acquisition of the images was performed with at the Electron Microscopy Lab in Federal University of Lavras using the Zeiss LEO EVO 40 XVP MEV and Smart Sem software, at 20 Kv and 9 mm working distance. The images obtained were edited using Corel Draw software.

Our analysis showed the germination of some *C. coffeicola* conidia on the coffee fruit surface at four hai (Figure 1A) and this was verified for both cultivars; germination occasionally showed two germ tubes (Figure 1A, arrows) and most of the conidia germinated at eight hai. The germ tubes grew towards the stomata in an attempt to penetrate them, but as they not clearly penetrate directed the sub-stomatal cavity, demonstrating a possibly absence of chemotropism to the stomata (Figures 1 B and C). However it was observed that the germ tubes clearly grew towards the wounds (Figures 1 D to G), thus showing a probable wounds chemotropism. In fact, the penetration of the pathogen occurred only through lesions on the fruits epidermis (Figures 1 D to G), for both coffee cultivars. Germ tubes developed and moved on open stomata but did not penetrate these natural openings (Figures 1 B and C). The penetration of *C. coffeicola* into the fruit occurred at 12 hai (Figure 1 D) and in subsequent evaluations: 24 hai

(Figures 1 E and F), 36 hai (Figure 1 G), and 168 hai (Figure 1 H). It was not observed appressoria formation, reinforcing the passive penetration hypothesis, by wounding. Samples evaluated at 168 hai had internal mycelial tissue colonization (Figure 1 H). No symptoms were observed during the evaluation.

In our work, the presence of germinative tubes in fruits at four hai was verified on the fruit epidermal surface. Similarly, Souza et al. (2011) observed *C. coffeicola* conidial germination both on adaxial and abaxial in coffee leaves surfaces at four hai. These authors also observed the growth of the germinative tubes towards stomatal openings, as occurred in the fruit; however, they observed penetration by stomata. They also verified that penetration into coffee leaves occurred at 36 hai and was mainly through the stomata on the epidermal surface or, occasionally, due to wounds in the epidermis, also without the appressoria formation. Some *Cercospora* species penetrate leaf tissues in this way, such as *C. arachidicola* (SMITH; PAUER; SHOKES, 1992), and *C. moricola* (GUPTA et al., 1995); however, *C. henningsii* penetrates directly through the epidermis of leaf tissues, and also without appressoria formation (BABU et al., 2009; PAULA et al., 2015). In this way, the infection process of *Cercospora* spp. may differ depending on the organ of the plant or the studied pathosystem.

The importance of infection process of *C. coffeicola* studies in immature fruit at young stages, shortly after the dry petals fall, is evidenced with the present results because certain species of cercosporioides fungi can go through long periods of latency; as example, *Pseudocercospora musae* has a latency periods around 40 days (FREITAS et al., 2017). That is, penetration occurs and symptoms are observed days or months after this process. Therefore, it is recommended starting control measures in the early fruiting stages from F2 to F5. The germination of *C. coffeicola* on the surface of the fruit from coffee cv. Catuaí IAC 144 and Topázio in the F2 stage is observed at four hai and its penetration only occurs by wounds in the epidermis and after 12 hai. Additionally, its colonization in the fruit's internal tissues occurs after 72 hai.

In order to evaluate brown eye spot in yellow and red coffee fruits, diagrammatic scales were validated by Paula et al. (2016). These scales are important to assessing the severity progress in already infected fields and compare control treatments.

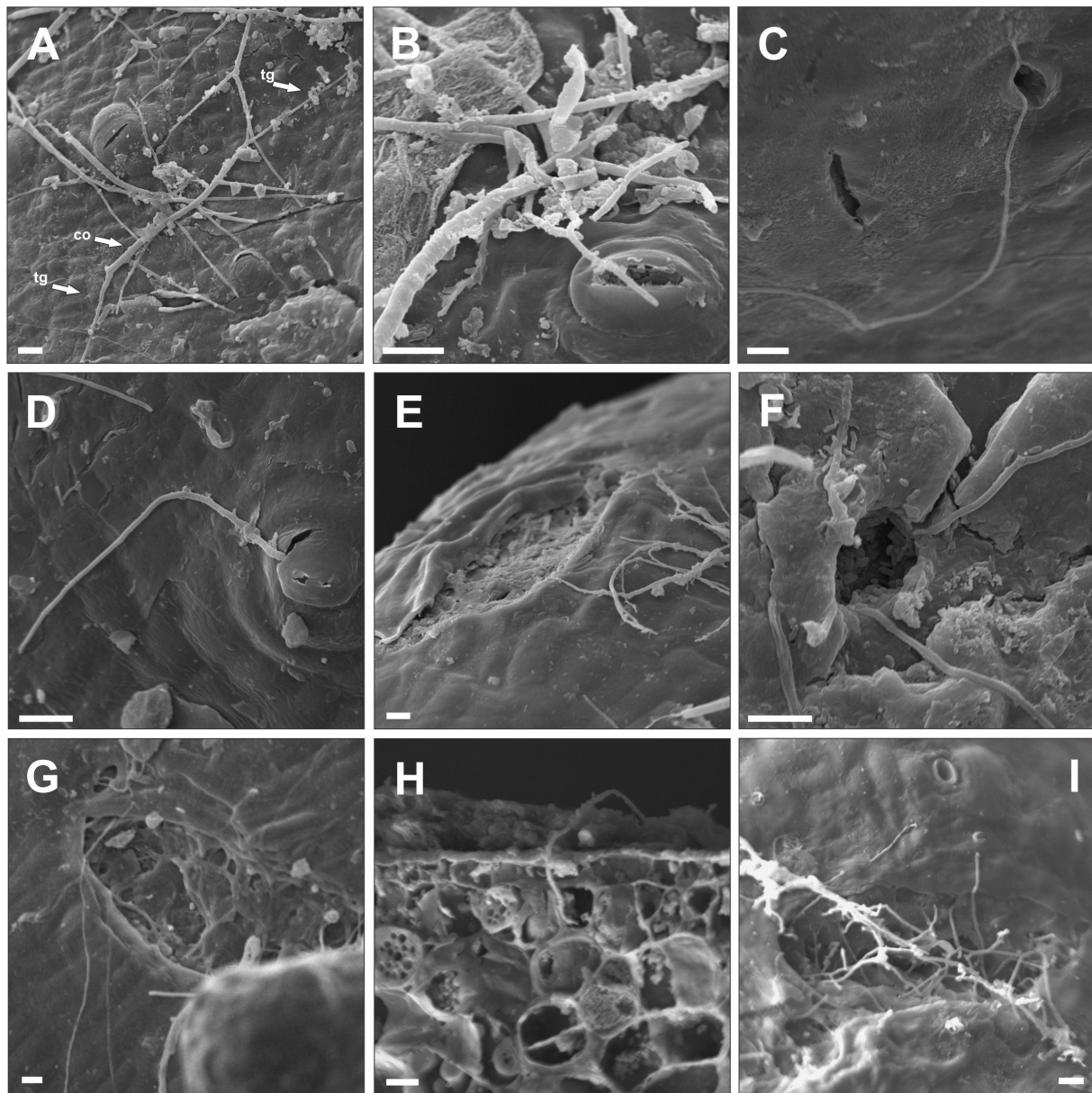


FIGURE 1 - Scanning Electron Micrographs. **A.** Germination of *Cercospora coffeicola* conidia four hours after inoculation (hai) in fruits of coffee at F2 stage. **B** and **C.** Growth of germinative tubes on stomata at eight hai. Penetration by wound at 12 (**D**), 24 (**E** and **F**) and 36 (**G**) hai. **H.** Cross-section of a penetration through injury at 168 hai. **I.** Internal mycelial colonization of tissues by *C. coffeicola* at 72 hai. Co: conidium, tg: germ tube. Scale bars = 10µm.

However, once the latent infection is possible in immature fruits, the preventive control is required, especially under optimal disease conditions, such as mild temperatures, high relative humidity, soaked soil, cold winds, nutritional deficiencies (GODOY; BERGAMIN FILHO; SALGADO, 2016) and intense sunlight, which may increase cercosporin production (SILVA et al., 2016). The control methods to reduce the initial

C. coffeicola inoculum include avoiding favorable conditions, cultural management (VASCO et al., 2015), chemical and or biological control (GODOY; BERGAMIN FILHO; SALGADO, 2016). In addition, it is possible that the use of resistant varieties contributes to the reduction of disease severity, and in consequence, fruit infection by the pathogen. (BOTELHO et al., 2017).

The *Cercospora coffeicola* infection in immature coffee fruits is very important, and implies in brown eyespot control management anticipation need, even before the first symptoms. Thus, the control actions should be preventive, since it is only possible to observe this infection through microscope.

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REFERENCES

- BABUA. M., et al. Scanning electron microscopy of the infection process of *Cercospora henningsii* on cassava leaves. **Journal of Phytopathology**, Göttingen, v. 157, n. 2, p. 57-62, Aug 2008.
- BOTELHO D. M. S., et al. Cercosporiosis resistance in coffee germplasm collection. **Euphytica**, Wageningen, v. 213, n. 2, p. 117-129, May 2017.
- FREITAS, A. S., et al. Infection process of *Pseudocercospora musae* on banana leaf. **Phytoparasitica**, Rotterdam, v. 45, n. 3, p. 317-324, June 2017.
- GODOY C. V.; BERGAMIN FILHO A.; SALGADO C. L. Doenças do cafeeiro (*Coffea arabica* L.). In: AMORIM L.; REZENDE J. A. M.; CAMARGO L. F. A. (Eds.) **Manual de Fitopatologia** v.2, 5ª Ed. Ouro Fino: Agronômica Ceres, 2016. p. 178-193.
- GUIMARÃES R. J.; MENDES A. N. G.; BALIZA D. P. **Semiologia do cafeeiro: Sintomas de desordens nutricionais, fitossanitárias e fisiológicas**. Lavras: Ed. UFLA, 2010.
- GUPTA V., et al. Observations on the surface ultrastructure of conidial stage of *Cercospora moricola* and its infection process in mulberry. **Sericologia**, Lyon, v. 35, n. 4, p. 123-131, Oct. 1995.
- HOAGLAND D. R.; ARNON D. I. The water-culture method for growing plants without soil. **Circular** 347. San Diego: Ed. University of California, 1950.
- LIMA L. M; POZZA E. A.; SANTOS F. S. Relationship between Incidence of Brown Eye Spot of Coffee Cherries and the Chemical Composition of Coffee Beans. **Journal of Phytopathology**, Göttingen, v. 160, p. 209-211, 2012.
- PAULA P. V. A. A., et al. Formas de Penetração do Gênero *Cercospora*. **Nucleus**, Ituverava, v. 12, p. 271-279, Oct. 2015.
- PAULA P. V. A. A., et al. Diagrammatic Scales for Assessing Brown Eye Spot (*Cercospora coffeicola*) in Red and Yellow Coffee Cherries. **Journal of Phytopathology**, Göttingen, v. 164, p. 791-800, Oct. 2015.
- SILVA, F. F., et al. Emergência e análise ultraestrutural de plântulas de soja inoculadas com *Sclerotinia sclerotiorum* sob efeito da aplicação de *Trichoderma harzianum*. **Summa Phytopathologica**, Botucatu, v. 43, n. 1, p. 41-45, Jan./Mar. 2017.
- SILVA M. G., et al. Effect of light and temperature on *Cercospora coffeicola* and *Coffea arabica* pathosystem. **Coffee Science**, Lavras, v. 11, n. 2, p. 148-160, June 2016.
- SMITH D. H.; PAUER G. D. C.; SHOKES F. M. Cercosporidium and Cercospora leaf spots of peanut. In: CHAUBE H.S., et al. (Eds.). **Plant diseases of international importance** v. 2. New Jersey: Prentice Hall Inc., 1992. p. 285-304.
- SOUZAA. G. C., et al. Infection process of *Cercospora coffeicola* on coffee leaf. **Journal of Phytopathology**, Göttingen, v.159, p. 6-11, 2011.
- VASCO G. B. et al. Incidência da cercosporiose em frutos de cafeeiro: diferentes densidades de plantio e manejos de irrigação. **Coffee Science**, Lavras, v. 10, n. 1, p. 38-45, Mar. 2015.

