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Quality of clonal plantlets of *Coffea canephora* Pierre ex A. Froehner produced using coffee husk in the substrate

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Coffee husk is a residue produced during the fruit processing and it is an excellent source of organic matter. It is an interesting alternative that can be used in the formation of the substrate to plantlet production, but the proportion to be recommended is still unknown. In this context, this experiment was conducted with the objective to study the growth, quality and gas exchange rates of clonal plantlets of Conilon coffee produced using plastic tubes, filled with substrate composed of different proportions of coffee husk to partially replace the commercial substrate. The experiment was conducted in a nursery, following a 3×6 factorial scheme in a completely randomized design; studying three genotypes of Conilon coffee and six proportions of coffee husk in the composition of the substrate for plantlets production from 0% to 100%. Overall, the results showed gains in growth and quality of the plantlets when coffee husk was added in the substrate but decrease in gas exchanges, especially over the net carbon assimilation. Considering the growth and quality, most detrimental effects started being observed with proportions above 38%. Different patterns of response were observed among genotypes, which must be taken into consideration for further researches to help define safety levels and a possible recommendation to use coffee husk in the substrate.

Key words: Asexual reproduction, Conilon coffee, plant nursery, biomass, Robusta coffee.

INTRODUCTION

Coffee crops have an undeniable importance for several countries worldwide and, among those, Brazil stands out as the world's largest producer of this commodity. Brazil grows plantations of both species of

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cultivated coffee: Arabica coffee (*Coffea arabica* L.) and Conilon coffee (*Coffea canephora* Pierre ex A. Froehner) (Conab, 2018); and this primary product has large historical and socio-economic value for the country, being an important economic source and creating several jobs along its productive chain.

The species *C. canephora* originated from tropical rainforests and has its reproduction majorly done by outcrossing, due to its mechanism of gametophytic self-incompatibility, which prevents self-pollination and limits breeding between plants of similar genetic heritage (Devreux et al., 1959). This characteristic causes natural populations of this species to present high heterozygosity and genetic variability for several agronomic traits (Van Der Vossen, 1985; Carvalho et al., 1991). To help create plantations with more standardized plants to enhance the cultivation practices, the breeding programs developed clonal cultivars, exploring the advantages of the asexual propagation of a set amount of matrix genotypes (compatible among themselves for pollination). This is to create more homogeneous plantations regarding some agronomic aspects, such as higher uniformity of canopy architecture, ripening time, grain size, among others desirable characteristics (Bragança et al., 2001; Fonseca et al., 2008).

Among the forms of asexual propagation suitable for use in plants of *C. canephora*, the most commonly adopted in commercial nurseries is using cuttings of orthotropic stems to produce new clonal plantlets, this technique is viable in large scale and presents rooting percentage between 95 and 100% (Paulino et al., 1985). The most recent alternative for producing plantlets by cuttings is the use of plastic tubes in nurseries; however, it noticeably increases the production costs per plantlet, due to, among other factors, the requirement of using a more expensive substrate. Therefore, alternatives to decrease the production costs or to increase the quality of coffee plantlets produced in plastic tubes are important research goals.

Coffee husk is an interesting alternative among the materials that could be used to decrease the total amount of required substrate to produce plantlets. This material is a residue produced during the processing of coffee fruits (therefore already available in the coffee producing regions) and it is an excellent source of organic matter, being especially rich in nitrogen and potassium (Dzung et al., 2013; Zoca et al., 2014). However, the proportion in which coffee husk could be use in the mixture to partially replace the commercial substrate is still an incognita, as well as the possible effects that the use of this residue could have over the growth and quality of the plantlets.

In this context, the objective of this experiment was to investigate the influence of using coffee husk as partial component of the substrate to produce plantlets of *C.canephora*; verifying its possible effects over the plant growth, plantlet quality and gas exchange rates.

MATERIALS AND METHODS

Experimental design

The experiment was conducted under controlled conditions, in multiplication nursery specialized in producing clonal plantlets of *C. canephora* Pierre ex Froehner (Conilon coffee), located in the northern state of Espírito Santo, in Southeast Region of Brazil. The experiment followed a 3×6 factorial scheme, in a completely randomized design, studying three genotypes of Conilon coffee and six proportions of coffee husk in the composition of the substrate for plantlets production. Four repetitions were used and the experimental plots were composed by 16 plantlets grown in a 4×4 grid, with evaluations of the four central seedlings protected by borders in all adjacent spaces of the tray.

Selection and multiplication of genotypes

The three genotypes selected to be used in the experiment are components of the clonal cultivar "Vitória Incaper 8142" (National Cultivar Register: #20471) (Brasil, 2006). The grouping of 13 highly productive genotypes composes this cultivar, and, among those, the genotypes referred as 2V, 5V and 12V were selected for this study due to their known contrasting characteristics. Mature stems were obtained from adult matrix plants, from each genotype, grown in clonal garden conduced with bending of orthotropic stems to stimulate sprouting. The plants were standardized on the subject of age, nutritional and phytosanitary aspects. The branches were cut from the middle section of the stems, discarding both ends (apex and base). Cuttings were made from the collected stems, sectioning them in parts of nearly 4 cm of length, using straight cutting on the base and bevel cutting on the apex, and leaving a pair of leaves per cutting with nearly one third of their original area. The cuttings were made following the current recommendation for asexual propagation for plants of Conilon coffee (Ferrão et al., 2012; Verdin Filho, 2014).

Substrate preparation

The prepared cuttings were inserted in plastic tubes of 280 mL of volume, filled with prepared substrate. The substrate was prepared with the standard material used commercially for multiplication of coffee plantlets. To compose the treatments, different proportions of the commercial substrate were replaced by coffee husk to fill the plastic tubes, at the levels of 0, 20, 40, 60, 80 and 100% of replacement, respectively. The plantlets were cultivated in nursery (Figure 1) and their nutrition, irrigation and pest management were made in accordance with current recommendations for plantlets production of Conilon coffee (Ferrão et al., 2012; Prezotti et al., 2007).

Evaluations

The plantlets were evaluated after 120 days of growth in nursery. The plant height (PH) was determined with graduated ruler (precision: 0.1 cm), from the substrate level to the apex of the stem. The total leaf area (LA) per plantlet was obtained using the non-destructive method of linear dimensions (Barros et al., 1973; Brinate et al., 2015). A portable infrared gas analyzer (IRGA, Licor, 6400XT) was used to evaluate the gas exchanges (from

Figure 1. Asexual multiplication using cuttings from matrix plants of each genotype in nursery.
Source: Marilândia, Espírito Santo, Brazil, 2018.

9:00 AM to 11:00 AM in a sunny day). The analyses were performed with irradiance of 1,000 PAR and concentration of CO_2 of 400 ppm. Among other gas exchange parameters, the net carbon assimilation (A), stomatal conductance (gs) and transpiration rate (E) were determined, and the intrinsic water use efficiency (iWUE) was estimated as the between net photosynthetic assimilation of CO_2 and the stomatal conductance.

After these evaluations, the plantlets were cut and each plant compartment (roots, stems and leaves) was separated and placed in paper bags, which were taken to laboratory oven with forced air circulation at 60°C, until constant weight. After drying, the biomass of each organ was stablished in electronic scale (0.001 g of precision). The sum of biomass of all organs was used to calculate the total dry matter (DM). The ratios between biomass accumulated in each organ and the total biomass per plantlet were used to calculate the leaf (LMR), root (RMR) and stem (SMR) mass ratios. The mass ratio between above ground (leaves + stems) and underground (roots) organs were used to calculate the proportion of biomass between shoot and roots (S:R). The ratio between the leaf area and the total biomass of the plantlet were used to calculate the leaf area ratio (LAR).

In addition to the plant height and biomass, the stem diameter was measured with digital caliper (precision: 0.01 mm) in order to estimate the quality of plantlets, which was calculated by the method proposed by Dickson et al. (1960), using the Equation 1:

$$DQ = \frac{TDM}{\frac{PH}{SD} + \frac{DM_{AP}}{DM_{RS}}}$$

Where: DQ: Dickson's quality index; TDM: total dry matter (g); PH: plant height (cm); SD: stem diameter (mm); DMAP: dry matter of aerial part (g); DMRS: dry matter of root system (g).

Data analyses

The data were subjected to analysis of variance and, according to the results for each variable, the interactions were unfolded or the factors studied separately, using the Scott-Knott test for the study the means of the three genotypes and regression analysis for the proportions of coffee husk in the substrate. All tests were performed at 5% probability, using the SISVAR software (Ferreira, 2011).

RESULTS AND DISCUSSION

The total accumulation of biomass (in the form of dry matter), the leaf area ratio, the net carbon assimilation and the quality index of the plantlets were influenced by both factors. Independent effects from the variation originated from the differences among genotypes and among proportions of coffee husk in the substrate, without the occurrence of interaction between these factors was observed.

The comparison among the response of the genotypes is presented in Figure 2, where a faster growth of the plants from the genotype 12V is observed, which resulted in plantlets with higher accumulation of biomass (Figure 2A) and higher quality index (Figure 2D). The genotype 2V seems to present slower initial growth, showing plantlets of lower biomass production (Figure 2A) and lower quality index (Figure 2D) in the end of the same period of time than the other genotypes. The genotype 5V presented intermediate growth between the other two genotypes. The leaf area ratio (Figure 2B) and the photosynthetic rates (Figure 2C) were homogeneous among the three genotypes.

The isolated effect of the proportions of coffee husk in the substrate is presented in Figure 3. The total accumulation of biomass (Figure 3A) presented fit to a 2nd degree linear regression model with maximum point at 38% of replacement of substrate with coffee husk. Similarly, the leaf area ratio (Figure 3B) increased with the use of coffee husk up to the level of 49% of replacement, decreasing after this point. The overall quality of the plantlets (Figure 3D) followed a similar behavior, presenting fit to a 2nd degree linear model with maximum point at 38%. The photosynthetic rate (Figure 3C), however, decreased linearly with the use of coffee husk in the substrate, which caused limitations up to 24% over the net carbon assimilation achieved by the plantlets grown without use of coffee husk. For the

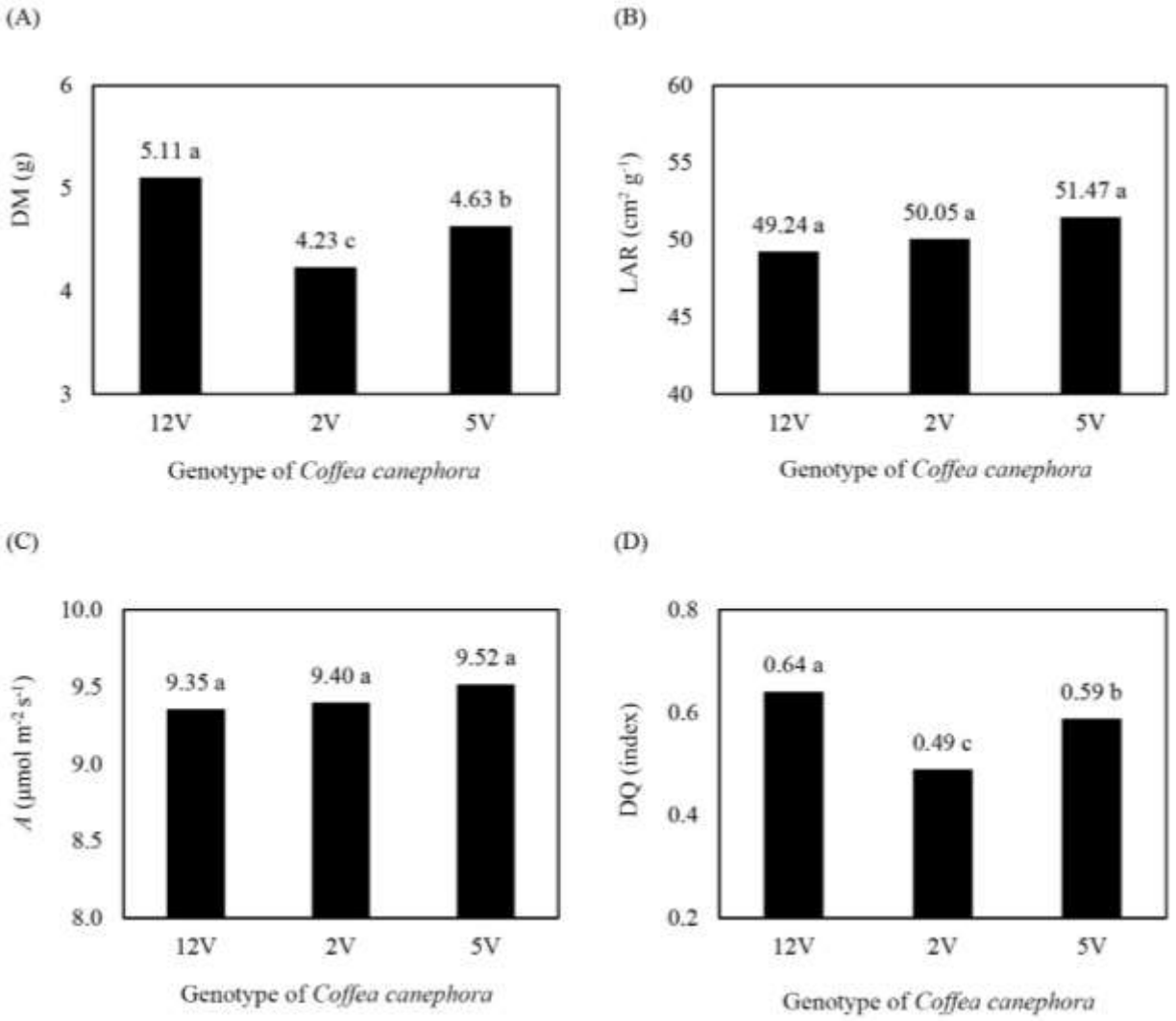

Figure 2. Means of total dry matter (DM), leaf area ratio (LAR), net carbon assimilation (A) and Dickson's quality index (DQ) of plantlets of three genotypes of *C. canephora* (Means followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability).

remaining variables, there was significant interaction between the effects of genotypes and levels of coffee husk in the substrate; therefore, the unfolding of the interactions was performed. The comparison among genotypes in each level of coffee husk used to replace the commercial substrate is presented on Table 1. For height, the genotype 2V presented the taller plantlets without the use of coffee husk in the substrate, showing that this genotype maybe metabolically invested in the early vertical gain, resulting in taller and thinner stems. For the levels of 20% and 40% of coffee husk in the substrate, the genotype 2V also presented taller plantlets, but the means did not differ from the genotype 12V. Above this level of use, the effects of the coffee husk seem to limit the differentiation among genotypes, resulting in similar means (Table 1).

The leaf area only differed among genotypes for the levels of coffee husk between 40 and 80%. The genotypes 12V and 5V presented larger leaf area for the levels of 40 and 60%, respectively, while the genotype 12V alone presented a higher mean of leaf area for the level of 80%; showing that this genotype invests early in the development of leaves (Table 1). The ratio of biomass between roots and aerial organs seems to be homogeneous among genotypes regardless of the use of coffee husk. The only exception happened with the proportion of 80% of the husk in the substrate. For this isolated condition, the genotype 12V allocated higher amount of its biomass towards the roots than the others did (Table 1). There were significant differences among genotypes for the mass ratio allocate towards each organ (leaves, roots

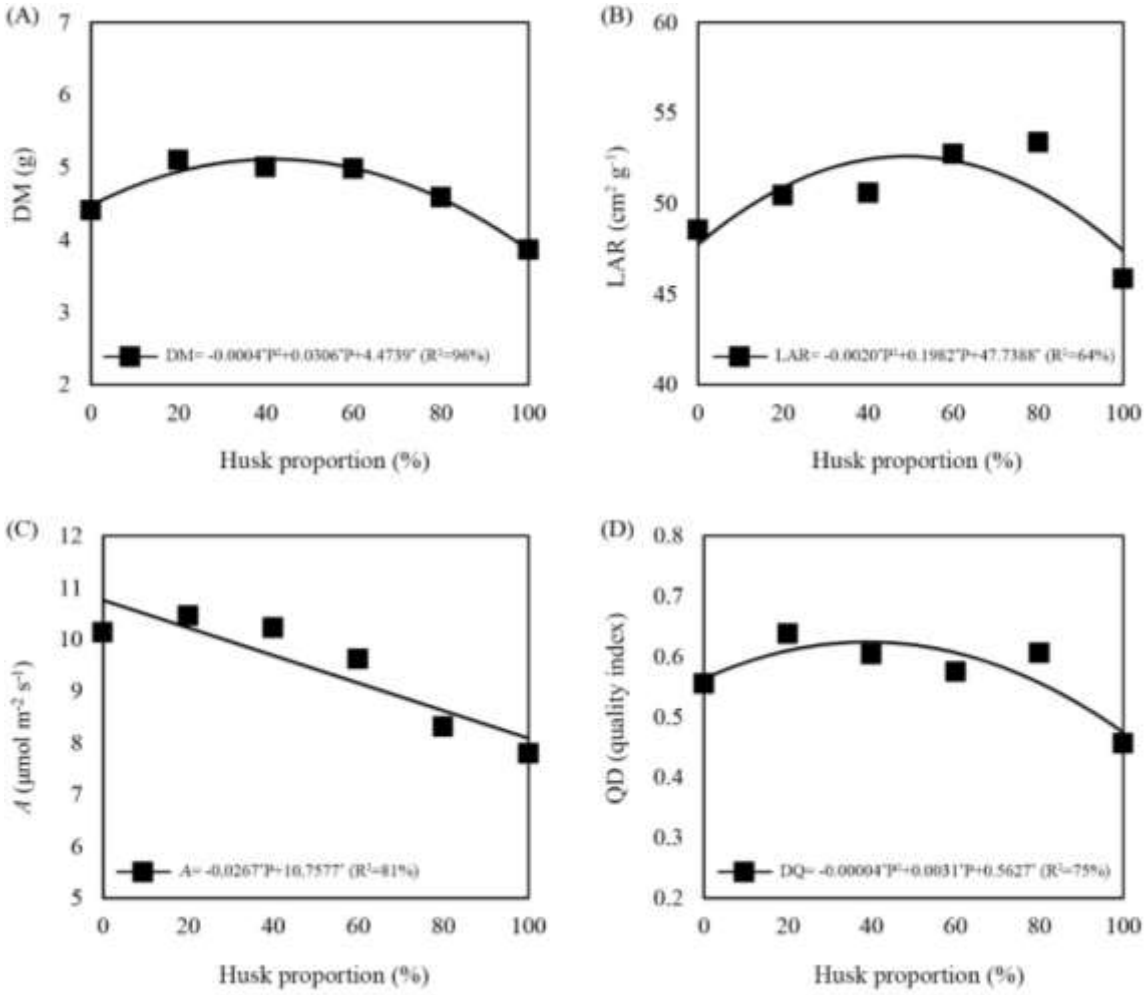

Figure 3. Regression analyzes for total dry matter (DM), leaf area ratio (LAR), net carbon assimilation (A) and Dickson's quality index (DQ) of plantlets of *C. canephora* grown with different proportions of coffee husk in the substrate (Coefficients followed by * are significant by the t-test at 5% of probability).

and stems); however, a similarity of patterns can be observed from the genotypes regardless of the use of coffee husk. Overall, the genotype 2V, compared to the others, seems to have an early allocation of biomass towards the leaves in detriment of stems (Table 1).

The unfolding of the effect of the use of coffee husk for each studied genotype is presented in the regression analyzes of Figure 4. Even if the magnitude of the coefficients is different, a similar pattern of response can be noticed among the genotypes. For the growth in height (Figure 4A) and leafiness (Figure 4B), the genotypes 12V and 5V present significant fit to linear regression models of 2nd degree, with maximum points at the levels of 46% (12V) and 43% (5V) of replacement of substrate with coffee husk for plant height, and at 46% (12V) and 49% (5V) for leaf area. The genotype 5V had both the plantlet height and leafiness decreasing linearly with the use of coffee husk (Figure 4A and B).

The mass ratios were not significantly influenced by the proportion of coffee husk in the substrate, each genotype keeping its own pattern of biomass allocation unchanged. Therefore, these variables did not present adjustment to linear regression models (Figure 4C, D, E and F). In relation to the other parameters of gas exchange, there was significant interaction between the factors for stomatal conductance, transpiration rate and for intrinsic water use efficiency. The factors did not cause significant effects over the intercellular CO_2 concentration. The comparison among genotypes in each level of coffee husk used to replace the commercial substrate is presented on Table 2.

The genotype 2V presented the higher means of stomatal conductance for the plantlets grown without use of coffee husk and up to 40% of replacement. However, its net assimilation of CO_2 was not enhanced by this behavior; indicating that this may be a specific response of this genotype to environmental factors,

Table 1. Means of plantlet height (PH), total leaf area (LA), mass ratio between roots and shoot (R:S), leaf mass ratio (LMR), root mass ratio (RMR) and stem mass ratio (SMR) of plantlets of three genotypes of *C. canephora* for each level of coffee husk used in the substrate.

Husk proportion (%)	Genotype	PH (cm)	LA (cm^2)	R:S ($g\ g^{-1}$)	LMR ($g\ g^{-1}$)	RMR ($g\ g^{-1}$)	SMR ($g\ g^{-1}$)
0	12V	8.21[b]	227.99[a]	0.20[a]	0.23[b]	0.17[a]	0.60[a]
	2V	13.22[a]	223.08[a]	0.20[a]	0.26[a]	0.17[a]	0.57[b]
	5V	8.30[b]	189.20[a]	0.21[a]	0.21c	0.18[a]	0.61[a]
20	12V	11.70[a]	268.28[a]	0.21[a]	0.22[b]	0.17[a]	0.61[a]
	2V	11.50[a]	238.07[a]	0.20[a]	0.24[a]	0.16[a]	0.59[a]
	5V	9.25[b]	264.72[a]	0.21[a]	0.23[b]	0.18[a]	0.60[a]
40	12V	11.73[a]	275.73[a]	0.21[a]	0.22[b]	0.18[a]	0.61[a]
	2V	11.41[a]	220.00[b]	0.21[a]	0.25[a]	0.17[a]	0.58[b]
	5V	9.25[b]	259.49[a]	0.20[a]	0.23[b]	0.16[a]	0.61[a]
60	12V	12.21[a]	290.56[a]	0.20[a]	0.22[b]	0.16[a]	0.62[a]
	2V	10.69[a]	208.04[b]	0.18[a]	0.27[a]	0.15[a]	0.58[b]
	5V	10.14[a]	290.65[a]	0.19[a]	0.21[b]	0.16[a]	0.63[a]
80	12V	10.00[a]	286.19[a]	0.24[a]	0.22[b]	0.19[a]	0.58[a]
	2V	10.15[a]	211.45[b]	0.20[b]	0.27[a]	0.17[b]	0.56[b]
	5V	8.82[a]	234.70[b]	0.20[b]	0.23[b]	0.17[b]	0.60[a]
100	12V	7.21[a]	168.60[a]	0.17[a]	0.23[b]	0.14[a]	0.62[a]
	2V	9.02[a]	168.88[a]	0.16[a]	0.29[a]	0.14[a]	0.57[b]
	5V	7.44[a]	190.56[a]	0.17[a]	0.22[b]	0.14[a]	0.64[a]

Means followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability.

such as humidity, vapor pressure deficit differences and temperature, which may not be observed in the same intensity in the other genotypes grown in the same conditions. Above the proportion of 60%, there was not differentiation among the behavior of the genotypes for the stomatal conductance (Table 2). The transpiration rate was relatively homogenous among genotypes, only presenting differences for the plantlets grown with use of 80% of coffee husk in the substrate and above. Plantlets from the genotypes 4V and 2V presented higher transpiration rates at the levels of 80 and 100% of use of coffee husk in the substrate (Table 2).

The water use efficiency presented difference among genotypes for most levels of coffee husk used in the substrate, only not differing at the levels of 40 and 80%. The genotype 12V presented higher efficiency than the others when grown without use of coffee husk did. Overall, for the plantlets grown using coffee husk in the substrate, this genotype together with the genotype 5V presented the higher water use efficiencies (Table 2). This fact may be related to the investment in stem growth and leafiness, which the genotypes 12V and 5V presented in the same conditions, which may have promoted the development of a more robust transport system to sustain the carbon metabolism of the larger leaves (Table 1). The unfolding of the effect of the use of coffee husk over the remaining gas exchange parameters for each studied genotype is presented in the regression analyses of Figure 5. For stomatal conductance, it was observed that only the behavior of the genotype 12V presented adjustment to linear region model of 2nd degree, with maximum point at 50% of coffee husk use. The genotypes 2V and 5V presented linear decrease in the stomatal conductance with the use of coffee husk, with a sharper decrease being observed for the genotype 2V, as observed by the higher angular coefficient from its regression model (Figure 5A).

The transpiration rate was similarly affected by the proportion of coffee husk used in the substrate for all genotypes. The intensity of the effect and the regression coefficients presented differences, but all genotypes presented adjustment to linear regression models of 2nd degree, with minimum points at 44, 74 and 42% for the genotypes 2V, 12V and 5V respectively (Figure 5B). The genotypes 2V and 5V

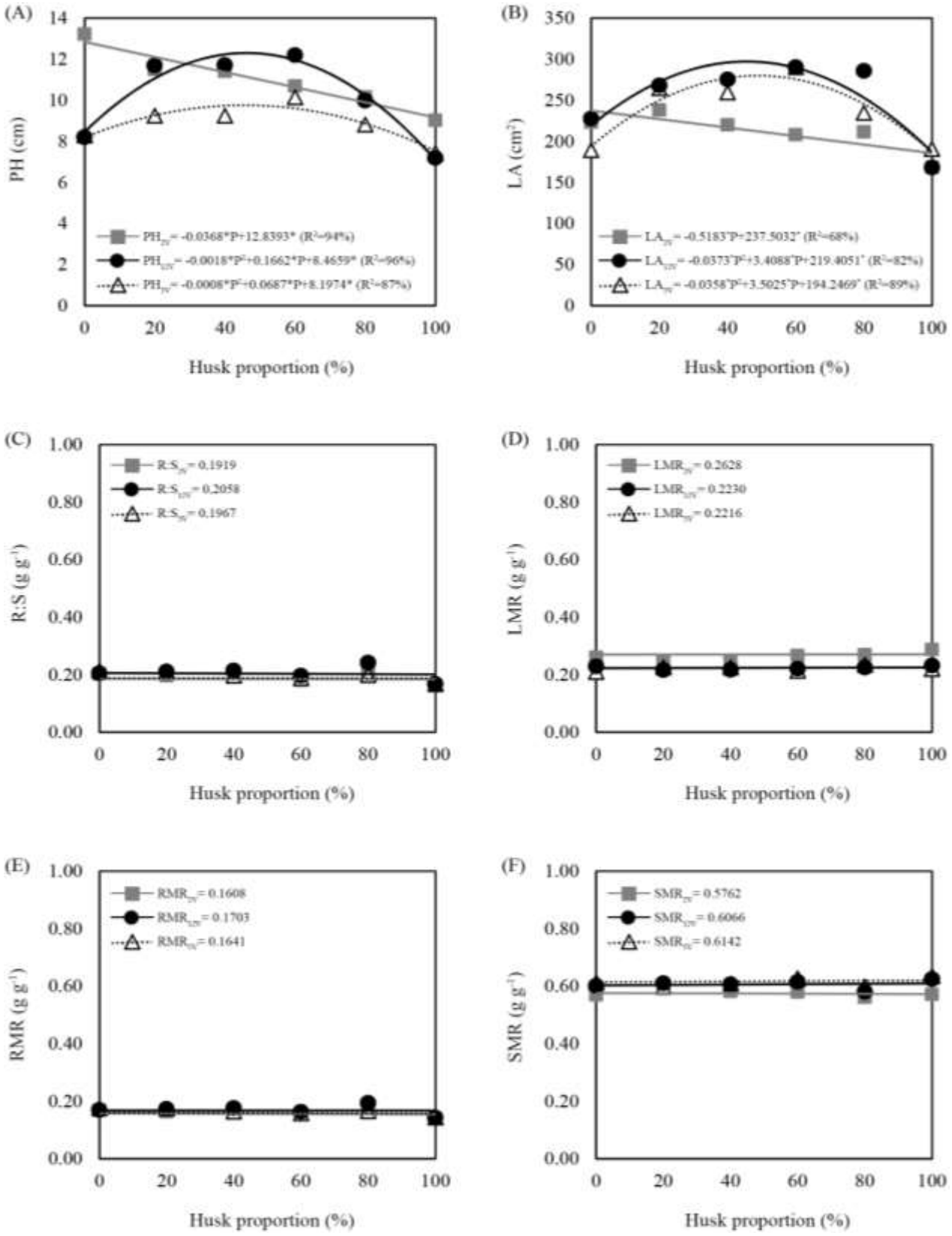

Figure 4. Regression analyzes for plantlet height (PH), total leaf area (LA), mass ratio between roots and shoot (R:S), leaf mass ratio (LMR), root mass ratio (RMR) and stem mass ratio (SMR) of plantlets of *C. canephora* grown with different proportions of coffee husk in the substrate, considering three different genotypes (Coefficients followed by * are signifi cant by the t-test at 5% of probability).

Table 2. Means of stomatal conductance (gs), transpiration rate (E) and intrinsic water use efficiency (WUEi) of plantlets of three genotypes of *C. canephora* for each level of coffee husk used in the substrate.

Husk proportion (%)	Genotype	Gs (mmol m^{-2} s^{-1})	E (mmol m^{-2} s^{-1})	WUEi (µmol mol^{-1})
0	12V	124.57c	4.93[a]	72.94[a]
	2V	293.20[a]	4.94[a]	37.15c
	5V	225.98[b]	4.53[a]	47.65[b]
20	12V	243.52[b]	4.68[a]	43.66[a]
	2V	361.24[a]	4.32[a]	28.95[b]
	5V	223.18[b]	4.53[a]	47.58[a]
40	12V	237.96[b]	4.30[a]	44.85[a]
	2V	298.48[a]	3.95[a]	35.97[a]
	5V	218.60[b]	4.30[a]	44.23[a]
60	12V	228.93[a]	4.14[a]	45.55[a]
	2V	245.88[a]	3.88[a]	35.96[b]
	5V	193.46[a]	3.92[a]	52.08[a]
80	12V	191.84[a]	3.92[b]	42.10[a]
	2V	192.83[a]	3.80[b]	45.60[a]
	5V	200.60[a]	4.73[a]	40.43[a]
100	12V	154.32[a]	4.63[b]	50.18[a]
	2V	202.29[a]	5.96[a]	37.59[b]
	5V	178.42[a]	5.29[b]	46.76[a]

Means followed by the same letter do not differ statistically by the Scott-Knott test at 5% probability.

were not affected by the use of coffee husk regarding the water use efficiency; while the genotype 12V presented adjustment to a linear regression model of 2nd degree with minimal point at the level of 61% of coffee husk being used in the substrate, increasing again after this point (Figure 5C).

Overall, the use of a small proportion of coffee husk in the substrate seem to have some beneficial effects over the initial growth of the clonal plantlets in nursery, and most its detrimental effects started being observed for proportions above 38% of replacement. This result may be related to the composition of the coffee husk, as the main secondary metabolites present beneficial antioxidant properties (Farah and Donangelo, 2006; Shemekite et al., 2014). However, the high content of tannins and phenolic compounds presented in the chemical composition of coffee husk can inhibit the root growth (Shemekite et al., 2014; Fan et al., 2003; Murthy and Naidu, 2012), causing detrimental effects over the development of the plantlets. This effect was observed in this experiment, especially for the biomass accumulation and the quality of the plantlets, which were initially enhanced by the use of coffee husk in the substrate but decreased when high proportions of coffee husk were used.

The search for alternatives of use and disposal of the coffee husk has a great environmental importance, as the anaerobic decomposition of this residue potentially leads to an increase in greenhouse gas emissions. The chemical composition and richness of mineral nutrients of coffee by-products, such as coffee husk, allow them to be used as potential fertilizers, especially after treatment through oxygen-driven biological methods (Murthy and Naidu, 2012; Insam and Bertoldi, 2007). Another important aspect is the possibility of decreasing the dependency to chemical fertilization and non-renewable sources, allowing the exploration of nutrient and energy cycling to enhance economic, agricultural and environmental efficiencies of the crop (Higashikawa et al., 2010).

The use of coffee husk as nutrient source is possible due to its rich organic matter, being a high-quality source of nutrients such as potassium. Some advantages of using coffee husk for partial replacement of the chemical fertilization are enhancing the soil fertility, improving the absorption of nutrients, promoting the growth rate and coffee yield (Dzung et al., 2013). Furthermore, organic residues, such as coffee husk, have potential to be used as partial substitute of vermiculite and other non-renewable materials for the

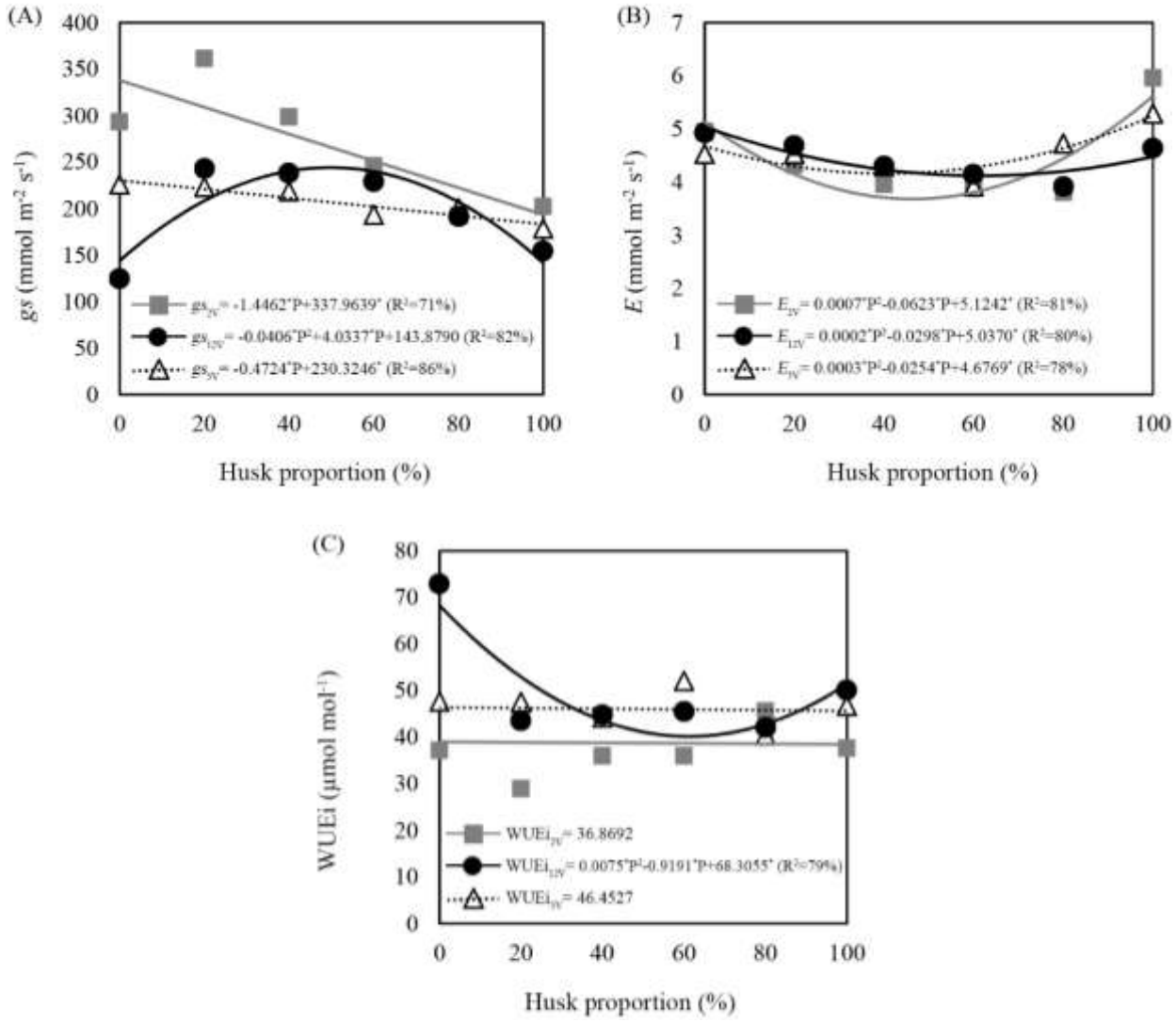

Figure 5. Regression analyzes for stomatal conductance (gs), transpiration rate (E) and intrinsic water use efficiency (WUEi) of plantlets of *C. canephora* grown with different proportions of coffee husk in the substrate, considering three different genotypes (Coefficients followed by * are significant by the t-test at 5% of probability).

production of seedlings and plantlets. Likewise, the agronomic efficiency of the process can be improved by using mixtures or dilutions of the by-product to create a sustainable substrate (Higashikawa et al., 2010; Benito et al., 2006; Melo and Silva, 2008). The results showed that a mixture of commercial substrate with up to 38% of coffee husk might be an adequate initial composition to be explored in further researches aiming to define a proper recommendation. Another fact that may have contributed to the alterations in the gas exchange rates (especially the limitations caused over the stomatal conductance, carbon assimilation and water use efficiency) is the effect of coffee husk over the electrical conductivity of the water in the substrate. The use of coffee husk in higher proportions may increase the electrical conductivity above adequate levels, affecting the osmotic balance, decreasing the efficiency of water absorption and negatively affecting the plant growth and efficiency of their gas exchanges (Higashikawa et al., 2010).

The difference in responses among the genotypes is due to high phenotypic and genotypic variability of the species. Other studies with the same genotypes have showed that beside the existence of diversity of growth patterns among them, there is also different patterns of response to environmental changes related to the soil conditions and fertility (Martins et al., 2013; Contarato et al., 2014; Colodetti et al., 2014). This must be taken in consideration when planning for the multiplication of the plantlets.

Conclusion

The coffee husk is a residue that may be used in mixtures in the substrate to promote plantlet growth and quality; however, this by-product can cause detrimental effects on the net photosynthetic rate, growth and quality of the plantlets if used in high proportions. Largely, the results suggest beneficial effects of the replacement of substrate with coffee husk up to the level of one third of the total volume used in the plastic tubes, with most negative effects showing above the level of 38% of substitution. There are different patterns of response to the use of coffee husk, therefore the before mentioned effects may show with different intensities depending on the genotype, which must be taken into consideration by further researches to help define safety levels and a possible recommendation to use coffee husk in the substrate.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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