

Full Length Research Paper

## Biometric evaluation of monthly growth rate as a criterion to study the genetic diversity in *Coffea canephora*

Wagner Nunes Rodrigues<sup>1\*</sup>, Tafarel Victor Colodetti<sup>1</sup>, Lima Deleon Martins<sup>1</sup>, Sebastião Vinícius Batista Brinate<sup>1</sup> and Marcelo Antonio Tomaz<sup>2</sup>

<sup>1</sup>Programa de Pós-Graduação em Produção Vegetal, Centro de Ciências Agrárias, Universidade Federal do Espírito Santo. Alto Universitário, Postal Box 16, 29500-000, Guararema, Alegre, Espírito Santo, Brazil.

<sup>2</sup>Departamento de Agronomia, Centro de Ciências Agrárias, Universidade Federal do Espírito Santo. Alto Universitário, Postal Box 16, 29500-000, Guararema, Alegre, Espírito Santo, Brazil.

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The variability of growth patterns and capacity to resist to environmental stresses that exists in populations of *Coffea canephora* Pierre ex Froehner makes it possible to select genotypes for different types of cultivation conditions. The objective of this study was to evaluate the monthly growth rate as a criterion to measure the genetic diversity of genotypes and to estimate the direct and indirect effects of the monthly growth rate, by path analysis, over the length of orthotropic stems. The experiment followed a randomized complete block design, studying 10 genotypes of *C. canephora* Pierre ex A. Froehner, with four replications and six plants per experimental plot. The magnitudes of the direct and indirect effects observed in the path analysis are consistent indicatives that the growth rate during recovery months, in the peaks after periods of slower growth, is highly important to determine the length of the stems during the final of the season. There is a considerable level of similarity between the growth of genotypes from the same group regarding ripening cycle; however, the high variability makes possible to identify genotypes from different behaviors regardless of the group.

**Key words:** Conilon coffee, orthotropic stems, genetic parameters, variability.

### INTRODUCTION

Coffee is one the most valuable commodities traded in the world, and Brazil is the country with the highest production of coffee if considered the amount produced by both of the main cultivated species: *Coffea arabica*

Lineu and *Coffea canephora* Pierre ex A. Froehner (Conab, 2015). Due to the importance of this agricultural product, Brazil keep advancing in the genetic improvement of both coffee species, being considered

\*Corresponding author. E-mail: [rodrigues@phytotechnics.com](mailto:rodrigues@phytotechnics.com).

the world leader in the development of improved coffee cultivars for the renovation of the coffee scenery (Borém and Miranda, 2005). Specifically, for *C. canephora*, the breeding programs have been seeking to improve several agronomic traits, such as crop yield, crop stability, resistance for the main phytosanitary problems, beverage quality, and drought tolerance (Ferrão et al., 2004; Carvalho, 2008).

Regarding drought tolerance, studies aiming to evaluate the capacity of the plants to tolerate water deficit are extremely important, especially considering the actual scenario of climate change. To enhance the crop yield regardless of the occurrence of more frequent dry periods, the breeding programs need to understand the behavior of plants grown under water deficit to be able to identify genotypes capable of expressing mechanisms of drought tolerance (Cattivelli et al., 2008; Lawn and Likoswe, 2008; Blum, 2005).

For coffee, several studies have been developed to understand the behavior of genotypes cultivated with reduced water supply (Dias et al., 2007; Rezende et al., 2009; Fialho et al., 2010; Miranda et al., 2011; Cavatte et al., 2012).

These studies justified the need to evaluate and select genotypes with higher level of resistance to water deficit, in order to characterize their traits and better understand the expression of drought tolerance mechanisms. The efforts of the breeding program of *C. canephora* in the Espírito Santo State, Brazil, to seek to improve the drought tolerance in the species resulted in the development of the clonal cultivar named “Emcapa 8141 - Robustão Capixaba”, which group genotypes able to grow and yield satisfactorily without irrigation (Ferrão et al., 2000).

The seasonal variation in the growth rate of coffee is highly related to the environmental conditions, the change in photoperiod, temperatures, light intensity and water availability triggers the metabolic changes, which leads the plant to start different new phenological stages of its life cycle (Ronchi and DaMatta, 2007). The variability of growth patterns and capacity to resist to environmental stresses that exists in populations of *C. canephora* makes it possible to select genotypes with higher vigor and crop yield for different types of cultivation conditions (Bonomo, 2002).

It is possible to identify coffee genotypes that are more adequate to cultivation in different crop systems and in different regions (Fonseca et al., 2006; Ferrão et al., 2008, Rodrigues et al., 2014a, b), as long as enough genetic variability is expressed in the specific conditions. Therefore, it is possible to justify the recommendation of certain genotypes of coffee that are better choices for each farmer, based on the region and system adopted for the plantation.

*C. canephora* still suffer from a smaller library of scientific data when compared to the other major cultivated specie of coffee regarding the growth rate, which makes

it necessary to intensify field studies that allow the exploration of growth variables. These variables can be used to optimize management practices and improve the recommendation of fertilization, pruning and irrigation. It is known that the growth rate of the aerial part of coffee trees suffer seasonal variations due to environmental conditions (Amaral et al., 2006; Ronchi and DaMatta, 2007).

Growth analyses allow the study of the performance of species, and genotypes of the same species, subjected to different kinds of environmental stimulus, making possible to identify vigorous individuals in populations that present better responses to specific conditions (Hunt, 1990; Benincasa, 2003). Larcher (2000) and Dardengo et al. (2010) describe that several intrinsic and extrinsic factors may influence the metabolic performance of plants, causing modifications in the magnitude or pattern of their growth and development.

Therefore, the objective of this study was to evaluate the monthly growth rate as a criterion to measure the genetic diversity of genotypes of *C. canephora* Pierre ex A. Froehner and to estimate the direct and indirect effects of the monthly growth rate on the length of orthotropic stems using path analysis.

## MATERIALS AND METHODS

### Experimental setup

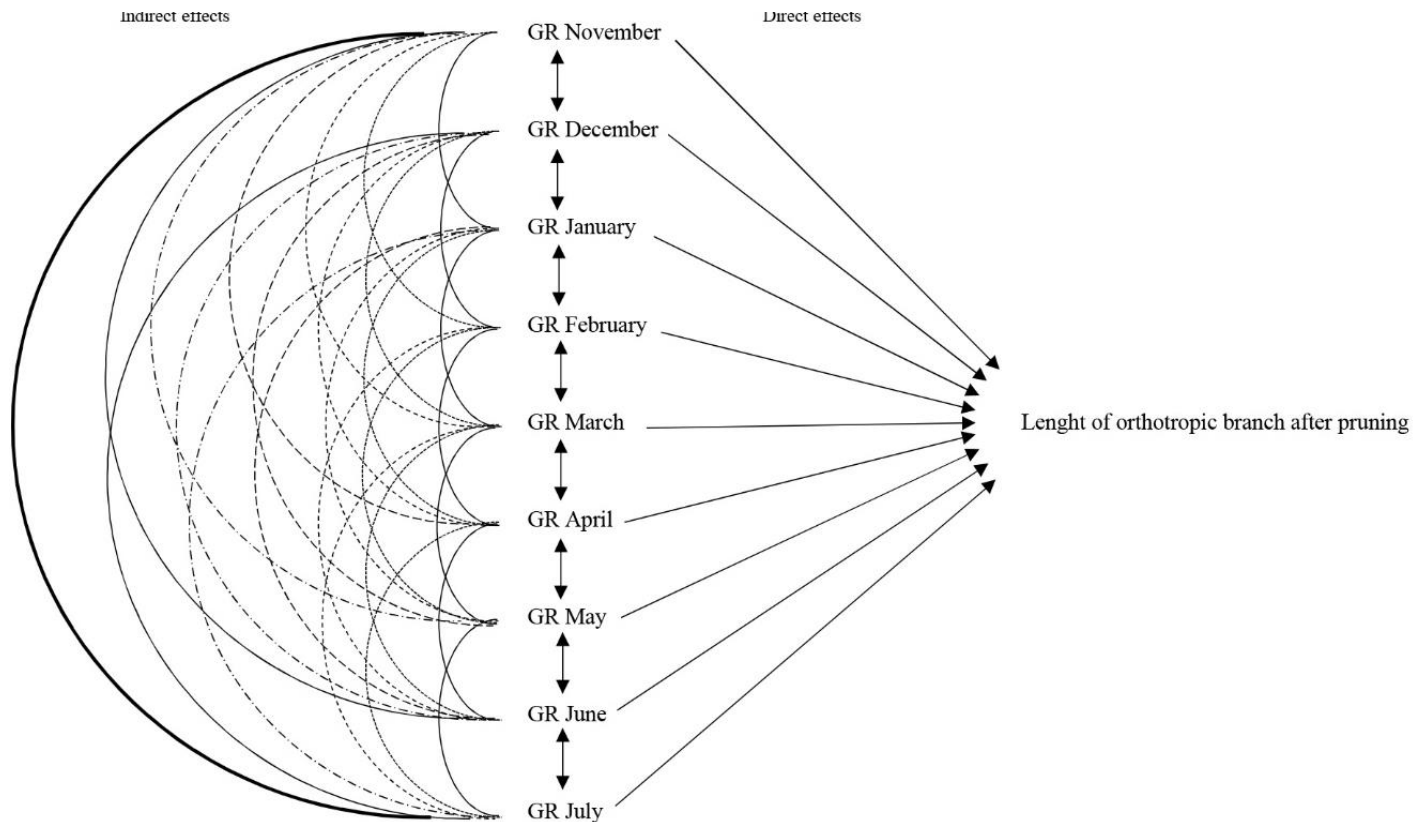
The experiment was conducted in a region where Conilon coffee is typically cultivated, located in the countryside of the municipality of Castelo, Espírito Santo State, Southeast Region of Brazil (20°34'19" S and 41°18'51" W). The area has elevation of 123 m over sea level and presented average temperature of 24°C and accumulated rainfall of 1,080 mm along the year, with the rainy season from October to April and the dry season from May to September.

The experiment followed a randomized complete block design, studying 10 genotypes of *C. canephora* Pierre ex A. Froehner, with four replications and six plants per experimental plot. Each genotype was propagated vegetatively using cuttings and the plants were spaced 3.00 × 1.00 m, being cultivated with 4 stems per plant, keeping a total population of near 13,000 stems per hectare, following the recommendation of Ferrão et al. (2007).

The agricultural practices were applied in accordance with those normally employed in the region, according to their need and following the current recommendations for the cultivation of Conilon coffee in Brazil (Prezotti et al., 2007; Ferrão et al., 2007; Fonseca et al., 2015).

### Genotypes evaluated

Ten genotypes of *C. canephora* Pierre ex A. Froehner, which were originated from the breeding program of the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper), were used for the study. These genotypes have desirable agronomic traits, high crop yield, average drought tolerance and different durations of ripening cycle. The genotypes are components of the clonal cultivar “Emcapa 8141 to Robustão Capixaba”. The genotypes are: RC01, RC02, RC03, RC04, RC05, RC06, RC07, RC08, RC09, and RC10.



**Figure 1.** Chain diagram for the interrelationship of the direct and indirect effects for the explicative variables (monthly growth rate) and the length of the new orthotopic stems at the flowering (Castelo, Espírito Santo, Brazil, 2014-2015).

### Data collected

The plants were cultivated and pruned for renovation of the canopy in 2014. After the process, the growth of the new stems was evaluated along the vegetative cycle, until the plants entered the phenological stage of vegetative rest of the next cycle (2015).

The monthly growth rate, estimated in  $\text{mm day}^{-1}$  for each month from November 2014 (selection of renewed stems) to July 2015 (induction and maturation of the new flower buds in the plant), was calculated based on the temporal variation of the vertical length of the new stem (from insertion to the apex), using the methodology described by Embrapa (2000).

### Data analyses

The collected data were subjected to an analysis of variance using the F test in order to identify the existence of differences between the growth rates of the genotypes. The genetic parameters were estimated based on the model  $Y_{ij} = \mu + G_i + B_j + \varepsilon_{ij}$ , where  $Y_{ijk}$  represents the phenotypic value of the  $ij^{\text{th}}$  observation,  $\mu$  is the general mean,  $G_i$  is the fixed effect of the  $i^{\text{th}}$  genotype,  $B_j$  represents the effect of the  $j^{\text{th}}$  block, and  $\varepsilon_{ij}$  is the random error related to the  $ij^{\text{th}}$  observation.

The estimated values of phenotypic variance ( $\hat{\sigma}_p^2 = \text{mean square of genotypes} / \text{number of blocks}$ ), environmental variance ( $\hat{\sigma}_e^2 = \text{mean square of residue} / \text{number of blocks}$ ), and genotypic variance ( $\hat{\Phi}_g = [\text{mean square of genotypes} - \text{mean square of residue}] / \text{number of blocks}$ ), coefficient of genetic variation ( $CV_g$ ), variation

index ( $CV/CV_g$ ), and coefficient of genotypic determination ( $H^2 = \hat{\Phi}_g / \hat{\sigma}_p^2$ ) were calculated according to the methodology described by Cruz and Carneiro (2006). The correlations were unfolded between direct and indirect effects over the length of orthotopic length, through path analysis (Figure 1), following the methodology described by these same authors.

The diagonal elements of the matrix and the component of residual variance were used to establish the multicollinearity of the matrix. To reduce the effect of high variances, the system of normal equations was modified by the implementation of a constant  $k$ , multiplied by the diagonal elements of the matrix (Hoerl and Kennard, 1970). The value of  $k$  was established following the methodology described by Cruz and Carneiro (2006), using graphics to choose values to which most of the path analysis coefficients were stabilized (Carvalho et al., 2002). The Mahalanobis distance was used as dissimilarity measure to delineate groups using an optimization technique based on a proposal by Tocher applied as described by Cruz and Carneiro (2006). The analyses were performed using the statistical software GENES (Cruz, 2013).

## RESULTS

### Genetic diversity

The results revealed that significant variation was observed for growth rate in all the months. This indicates the existence of variation for growth rate among of the

**Table 1.** Estimative of phenotypic and genetic parameters of the monthly growth rate of genotypes of *C. canephora* Pierre ex A. Froehner (Castelo, Espírito Santo, Brazil, 2014-2015).

Parameter	Growth rate (mm dia <sup>-1</sup> )								
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.
MS <sub>g</sub> <sup>(1)</sup>	4.83*	5.59*	1.86*	2.57*	2.14*	1.50*	0.82*	0.81*	0.71*
CV(%) <sup>(2)</sup>	10.77	11.92	13.91	17.78	10.82	11.06	14.59	16.37	15.39
Minimum	4.03	2.55	1.64	0.80	2.18	1.80	0.92	0.73	1.29
Mean	6.39	4.71	2.80	1.84	3.85	3.24	2.02	1.82	2.04
Maximum	9.06	7.41	4.49	4.28	5.07	4.26	3.02	2.86	3.53
$\hat{\sigma}_p^2$ <sup>(3)</sup>	1.20	1.39	0.46	0.64	0.53	0.37	0.20	0.20	0.17
$\hat{\sigma}_e^2$ <sup>(4)</sup>	0.11	0.07	0.03	0.02	0.04	0.03	0.02	0.02	0.02
$\hat{\Phi}_g$ <sup>(5)</sup>	1.09	1.31	0.42	0.61	0.49	0.34	0.18	0.18	0.15
CV <sub>g</sub> (%) <sup>(6)</sup>	16.33	24.36	23.32	42.60	18.23	18.1	21.20	23.39	19.23
CV <sub>g</sub> /CV <sup>(7)</sup>	1.52	2.04	1.68	2.40	1.69	1.64	1.45	1.43	1.25
H <sup>2</sup> <sup>(8)</sup>	90.20	94.35	91.84	95.83	91.91	91.46	89.41	89.09	86.21

\*Significant by the F test; <sup>(1)</sup>genotypic mean squares (MS<sub>g</sub>); <sup>(2)</sup>coefficient of variation; <sup>(3)</sup>phenotypic variance; <sup>(4)</sup>environmental variance; <sup>(5)</sup>genotypic variance; <sup>(6)</sup>coefficient of genetic variation; <sup>(7)</sup>variation index; <sup>(8)</sup>coefficient of genotypic determination (%).

tested genotypes. Therefore, the MS<sub>g</sub> was significant for all variables at 5% of probability (Table 1).

The study of the growth rate during the periods of higher vegetative growth, which happened between the months of November to December, and the period of slower growth, from June to July, helped to identify divergences between the genotypes in higher magnitude. Being especially helpful to evaluate and identify the differences between the genotypes regarding how stable is their seasonal growth patterns. The growth rate during these months returned higher values of estimated relative contribution to the variability between these genotypes.

The use of the growth rate during the period of active growth is especially helpful in this case, since the high estimated values of genetic parameters between November and December favors the identification of differences that are highly related to the genotypic variance (Table 1).

The estimated values of genotypic variances ( $\hat{\Phi}_g$ ) were higher than the values for the environmental variance ( $\hat{\sigma}_e^2$ ) for all monthly rates. Considering this results, it is possible to associate the greater proportion of the phenotypic variance ( $\hat{\sigma}_p^2$ ) to the genotypic differences between the plants (Table 1).

The coefficient of genotypic determination was higher than 86% for all variables, showing that the growth rate was more influenced by genotypic factors than by the environmental conditions during the period of evaluation of this experiment (Ramalho et al., 2004).

The estimated CV<sub>g</sub> was superior to the CV, resulting in a variation index (CV<sub>g</sub>/CV) greater than 1.00 for the growth rate occurred in all months; therefore, genetic factors predominated over environmental factors to determinate this variables (Table 1).

The cluster analysis for the 10 genotypes through the

**Table 2.** Clustering by the Torcher's method, based on the standardized Euclidian mean distance of the monthly growth rate of 10 genotypes of *C. canephora* Pierre ex A. Froehner (Castelo, Espírito Santo, Brazil, 2014-2015).

Groups	Proportion (%)	Genotype
I	40	RC01, RC02, RC05 and RC07
II	30	RC03, RC04 and RC08
III	20	RC09 and RC10
IV	10	RC06

Tocher's method is presented at Table 2. It was possible to identify four groups of genotypes: Group I clustered 40% of the genotypes; Group II grouped 30% of the genotypes; Group III clustered 20% and Group I was formed by only one genotype (RC06).

### Effects of monthly growth rates over the length of the stems

The correction of the distortions was done with the coefficient  $k$  being equal to  $5.07 \times 10^{-2}$  to obtain the multicollinearity diagnostic. The direct effects of the monthly growth over the length of the orthotropic stems after the whole season from pruning to flowering of next cycle followed the decreasing order of magnitude:  $|GR_{December}| > |GR_{January}| > |GR_{November}| > |GR_{March}| > |GR_{April}| = |GR_{July}| > |GR_{February}| > |GR_{May}| > |GR_{June}|$ .

Considering the positive direct effects, the growth rates in December and January presented the higher coefficients (Table 3). During these months, the indirect effects were higher for the month right before, that is, the length of the stems was highly influenced by the growth

**Table 3.** Estimative of direct and indirect effects of nine monthly growth rates of 10 genotypes of *C. canephora* Pierre ex A. Froehner over the length of the new orthotropic stems after pruning, obtained by path analysis, with diagnosis of multicollinearity (Castelo, Espírito Santo, Brazil, 2014-2015).

Secondary component	Association	Length of the stems
Growth rate in November	Direct effect	0.20
	Indirect effect through growth rate in December	0.24
	Indirect effect through growth rate in January	0.18
	Indirect effect through growth rate in February	0.05
	Indirect effect through growth rate in March	0.11
	Indirect effect through growth rate in April	0.10
	Indirect effect through growth rate in May	0.03
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	0.01
Growth rate in December	Direct effect	0.31
	Indirect effect through growth rate in November	0.15
	Indirect effect through growth rate in January	0.14
	Indirect effect through growth rate in February	0.09
	Indirect effect through growth rate in March	0.10
	Indirect effect through growth rate in April	0.09
	Indirect effect through growth rate in May	0.00
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	-0.01
Growth rate in January	Direct effect	0.24
	Indirect effect through growth rate in November	0.15
	Indirect effect through growth rate in December	0.18
	Indirect effect through growth rate in February	-0.01
	Indirect effect through growth rate in March	0.13
	Indirect effect through growth rate in April	0.12
	Indirect effect through growth rate in May	0.01
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	-0.05
Growth rate in February	Direct effect	0.13
	Indirect effect through growth rate in November	0.07
	Indirect effect through growth rate in December	0.20
	Indirect effect through growth rate in January	-0.02
	Indirect effect through growth rate in March	0.00
	Indirect effect through growth rate in April	0.00
	Indirect effect through growth rate in May	0.01
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	0.08
Growth rate in March	Direct effect	0.15
	Indirect effect through growth rate in November	0.14
	Indirect effect through growth rate in December	0.19
	Indirect effect through growth rate in January	0.20
	Indirect effect through growth rate in February	0.00
	Indirect effect through growth rate in April	0.15
	Indirect effect through growth rate in May	0.00
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	-0.06

Table 3. Contd.

	Direct effect	0.14
	Indirect effect through growth rate in November	0.14
	Indirect effect through growth rate in December	0.19
	Indirect effect through growth rate in January	0.19
Growth rate in April	Indirect effect through growth rate in February	0.00
	Indirect effect through growth rate in March	0.15
	Indirect effect through growth rate in May	0.00
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	-0.06
	Direct effect	0.06
	Indirect effect through growth rate in November	0.09
	Indirect effect through growth rate in December	0.02
	Indirect effect through growth rate in January	0.03
Growth rate in May	Indirect effect through growth rate in February	0.03
	Indirect effect through growth rate in March	0.01
	Indirect effect through growth rate in April	0.01
	Indirect effect through growth rate in June	0.00
	Indirect effect through growth rate in July	0.10
	Direct effect	0.00
	Indirect effect through growth rate in November	0.07
	Indirect effect through growth rate in December	-0.01
	Indirect effect through growth rate in January	-0.01
Growth rate in June	Indirect effect through growth rate in February	0.04
	Indirect effect through growth rate in March	-0.01
	Indirect effect through growth rate in April	-0.01
	Indirect effect through growth rate in May	0.06
	Indirect effect through growth rate in July	0.11
	Direct effect	-0.14
	Indirect effect through growth rate in November	-0.02
	Indirect effect through growth rate in December	0.02
	Indirect effect through growth rate in January	0.09
Growth rate in July	Indirect effect through growth rate in February	-0.08
	Indirect effect through growth rate in March	0.07
	Indirect effect through growth rate in April	0.06
	Indirect effect through growth rate in May	-0.04
	Indirect effect through growth rate in June	0.00

Determination coefficient = 0.97.

rate of December, with a high indirect effect of the growth rate of November; and directly influenced by the growth rate of January with higher indirect effect of December. In addition, the determination of the length was highly dependent of the growth rate in November, with strong indirect effect of the growth in both December and January.

## DISCUSSION

Between November and December, the plants were in

conditions of longer days and abundant rainfall, achieving higher growth rates (Table 1). In this period, the new stems are in the vegetative stage of their phenological cycle, characterized by the formation of new leaf buds (similar for the coffee species, as described by Camargo and Camargo (2001), a highly active stage for growth and development of vegetative structures, therefore, high growth rates were observed in this period.

A lower GR was observed in February, which is related to the occurrence of a dry period, the water deficit caused by the lack of rainfall for 14 consecutive days slowed the growth of the stems, causing the smaller gain of extension

in this period.

Another period of smaller growth is observed after June, which is related to the phenological cycle of the new stems, which are ending the vegetative stage and starting the rest stage (Camargo and Camargo, 2001; Ronchi and DaMatta, 2007). This moment of the year is characterized by a slow growth of the coffee plants as a whole due to the low metabolism during the start of the dry and cold season.

Due to its gametophytic self-incompatibility (GSI) mechanism, with monogenic heritage ruled by a set of three alleles of the gen *S* (Lashermes et al., 1996; Berthaud, 1980), populations of *C. canephora* commonly present high phenotypic and genotypic variability (Fonseca et al., 2006; Ferrão et al., 2008; Rodrigues et al., 2012). For many agronomic traits, the existence of high genetic diversity had been reported, e. g., Colodetti et al. (2014) and Martins et al. (2013c), studying improved genotypes of *C. canephora* in environments with different levels of nutritional stresses, reported the expression of different growth behaviors and nutritional efficiencies.

The existence of variability for crop yield and bienniality between genotypes from different ripening cycles have been studied by Rodrigues et al. (2013), which concluded that there is high diversity intrinsic to each of the groups of genotypes: From early, intermediate and late ripening cycle. This fact is also described by the study of genetic parameters of several traits used in the breeding program of *C. canephora*, which show high variability for genotypes from all of these ripening groups (Rodrigues et al., 2012).

Regarding the growth rate, several authors found different behaviors regarding the increase of leafiness and biomass of improved genotypes of *C. canephora* during the early stages of development, indicating the existence of variability for this trait (Rodrigues et al., 2012; 2013; Silva et al., 2013; Martins et al., 2013a, b, c; 2015; Colodetti et al., 2014, 2015; Menezes-Silva et al., 2015).

Considering the grouping presented at Table 2, most genotypes from the Group I presented early ripening cycle, which normally present length from 34 weeks from flowering to harvest (Bragança et al., 2001; Ferrão et al., 2007). The genotypes from this group also presented higher growth in November and December, lower growth rate on February and a promptly growth retake after May.

Most genotypes from the Group II presented intermediate ripening cycle, which are classified as such for having ripening length near 41 weeks from flowering to harvest (Bragança et al., 2001; Ferrão et al., 2007). The genotypes from this group had slower growth on November and in the period between February and April.

The Group III was formed by genotypes with ripening cycle between intermediate and late, that is, presenting 41 to 45 weeks from flowering to harvest (Bragança et al., 2001; Ferrão et al., 2007). This group was formed by

genotypes with typical slow growth on February and from May to June, with rapid increase in growth rate in July.

The Group IV was singly formed by the genotype RC06, which presented higher growth from November to February. This contrasting genotype has been studied by DaMatta et al. (2003), which concluded that this genotype may present similar physiological traits to others genotypes of *C. canephora* when cultivated with irrigation, but respond differently in environments where it is subjected to periods of drought, being classified as a drought tolerant genotype.

The magnitudes of the direct and indirect effects observed in the patch analysis are consistent indicatives that the growth rate during the highly active months and in the moments of recovery after periods of slower growth, due to low rainfall, may be extremely important to determine the length of the stems during the final of the season. Therefore, genotypes capable of restarting the growth earlier or these able to achieve higher growth rates in the rain season may end up with larger and more vigorous stems.

The growth and therefore the extension of the stems are especially important for fruit bearing species, such as coffee, since these stems are going to support all the plagiotropic branches that will be responsible for the fruit yield. A slow growth may reduce the recovery capacity of the plants, which may not be able to produce properly in the next cycle; and an excessively fast growth of the stem may compromise the growth of the primary plagiotropic stems, which also can limit the crop yield in the next cycle. The pruning management must be adequate to renew the plantation without intensifying such effects, and an interesting recent alternative is the programmed pruning cycle for Conilon coffee (Verdin Filho et al., 2014), which alternate the production and keep the plantation being constantly renewed along the years.

New studies involving growth rates of genotypes of *C. canephora* are important since the specie has high genetic variability for several growth traits. Studies involving other environmental conditions that may regulate the expression of growth traits are especially important to help validating the results, such as studies in other regions and other crop systems with different technological levels, different water and nutritional management and even different pruning managements.

## Conclusion

The monthly growth rate can be a useful tool to identify and to study the variability among genotypes of *C. canephora* Pierre ex Froehner, and the evaluation of the growth rate during recovery months, in the peaks after periods of slower growth, is especially advantageous to study the diversity and the growth rate in these months is highly important to determine the length of the stems during the final of the season.

There is a considerable level of similarity between the growth of genotypes from the same group regarding ripening cycle; however, the high variability makes possible to identify genotypes from different behaviors regardless of the group.

### Conflicts of Interests

The authors have not declared any conflict of interests.

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