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Nitrogen availability modulating the growth of improved genotypes of *Coffea canephora*

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Studies quantifying the effect of the nitrogen over the growth of genotypes of Conilon coffee contribute to improve the fertilization management. Thus, this study was developed to evaluate the growth of improved genotypes of *Coffea canephora* Pierre ex Froehner submitted to nitrogen fertilization. Six improved genotypes of *C. canephora*, from different groups of ripening cycles, were grown without nitrogen fertilization or with fertilization at level of 0.625 g kg^{-1} of soil, cultivated in greenhouse, following a completely randomized design, with four replications. The plant growths were evaluated considering the plant height, stem diameter, number of leaves and relative growth rate. Nitrogen fertilization was able to promote higher growth, as well as, in general, a higher relative growth rate of all genotypes. Differentiation among genotypes was observed for their responses to each of the evaluated parameters. Noteworthy are the genotypes 77, in leafiness, and 67 in plant height and stem diameter, which presented higher growth compared to other genotypes, with or without nitrogen fertilization.

Key words: *Coffea canephora*, response, deficiency, mineral nutrition.

INTRODUCTION

Nitrogen (N) is the macronutrient with higher accumulation in the tissues of plants of Conilon coffee (*Coffea canephora* Pierre ex Froehner), corresponding to approximately 38% of all macronutrients distributed among the vegetal organs. It is a highly demanded nutrient and there are records showing increases in the crop yield of near 410%, achieved with the increase of nitrogen supply (Bragança et al., 2008; Bragança et al., 2010; Clemente et al., 2013).

The adequate supply of nitrogen is essential for the

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formation of vegetative structures, such as leaves, stems and roots; as well as for flowering and fruit filling; also interfering with productivity (Quintela et al., 2011; Taiz and Zeiger, 2013).

The growth rate of the aerial part of coffee plants varies seasonally due to environmental factors (Amaral et al., 2006; Ronchi and DaMatta, 2007). For Conilon coffee, studies that provide information on the growth rate are necessary to allow optimization of management practices, especially fertilization, pruning and irrigation.

For Hunt (1990), the growth analysis is a very important tool to study the performance of cultivated species growing in natural or controlled environments. The expression of physiological characteristics and the evaluation of its net production can be observed by growth analysis, since they are products of photosynthesis and result from the assimilatory system performance over a period of time. Intrinsic and extrinsic factors to the plant may influence this performance, causing variation of growth and development of plants (Dardengo et al., 2010).

Due to the large variability existing for many agronomic characteristics of genotypes of Conilon coffee, the breeding programs use the ripening cycle to classify groups of genotypes. It is important to highlight that there is differentiation in growth patterns among genotypes (Fonseca et al., 2006; Rodrigues et al., 2012; Martins et al., 2013b), as well as variation regarding the response to fertilization with nitrogen (Colodetti et al., 2014). Therefore, it is important to characterize the response of genotypes to the fertilization, in order to improve the recommendation and to establish differentiated schemes of fertilization for plantations using improved clonal cultivars.

According to this scenario, this study aims to evaluate the response in growth of improved genotypes of *C. canephora* Pierre ex Froehner submitted to nitrogen fertilization.

MATERIALS AND METHODS

The experiment was conducted in greenhouse, located at the experimental site of the Centro de Ciências Agrárias da Universidade Federal do Espírito Santo (CCA/UFES), in the municipality of Alegre, southern Espírito Santo State, in latitude 20°45'S, longitude 41°33'W and at 136 m of altitude.

The experiment followed a triple factorial scheme 6×2×5, with a completely randomized design and four replications. The first and second factors being, respectively, 6 improved genotypes of Conilon coffee and 2 conditions of nitrogen availability in the soil: no addition of nitrogen and fertilization with 0.625 g kg^{-1} of nitrogen (which, according with preliminary tests, was the level of fertilization that promoted the expression of higher variability for plant growth). The third factor was 5 evaluation periods: 30, 60, 90, 120, and 150 days of cultivation.

The soil used in the experiment was collected at a depth of 40 cm, discarding the first 10 cm of in order to reduce the effect of organic matter present in the superficial layer. A sample of this soil was sent to laboratory for chemical and physical analysis, being characterized as a dystrophic oxisol of clayey texture (Embrapa, 2006). After the characterization, the entire volume of soil was dried in shade and homogenized with a 2.0 mm mesh sieve. It was subsequently separated into samples of 10 dm^3 and accommodated into pots of 14 liters of capacity.

Presenting different ripening cycles, the six genotypes of *C. canephora* tested in this experiment were 67 and 23, of early cycle; 77 and 31, of intermediate cycle; and 76 and 153, of late cycle. These genotypes were developed by the breeding program from the Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (Incaper), featuring desirable agronomic characteristics and adaptation to cultivation in the main producer region of Brazil. These genotypes are available for cultivation, comprising some clonal cultivars that were developed by Incaper.

The fertilization, except for nitrogen, was performed according to the recommendation for nutritional studies in controlled environment (Novais et al., 1991). The nutrients were supplied through solutions prepared with salts (KNO_3, KH_2PO_4, NH_4NO_3, $CaHPO_4$) to establish the nutritional balance of the soil.

The genotypes were multiplied asexually through cuttings and the plantlets were formed in nurseries registered and certified by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA). From the early development of the third pair of leaves, the plantlets were transplanted to the pots and subjected to differential nitrogen fertilizations.

The fertilization with nitrogen was performed in coverage, with application of urea, split in four applications, starting 15 days after replanting and spaced at intervals of 30 days. The plants were grown under conditions of absence of nitrogen fertilization and fertilization at the level of 0.625 g kg^{-1}.

Irrigation was performed by keeping the soil moisture near 60% of the total pore volume during the experiment. The total pore volume was obtained using the particle density and soil density, determined by the test tube method, according to Embrapa (1997). The other cultural practices were performed manually according to the need.

The plants were conducted for 150 days and their growths were monthly evaluated, collecting data about: number of leaves (NL), obtained by counting; plant height (PH), measured with graduated ruler (cm); and stem diameter (SD), measured with digital caliper (millimeters). The relative growth rate (RGR) was calculated based on the temporal variation of the plant height, using the methodology described by Embrapa (2000).

All analyzes considered 5% of probability and were done with the statistical software Genes (Cruz, 2013).

RESULTS AND DISCUSSION

According to the analysis of variance, there was significant effect of different interactions between the factors for each dependent variable (Table 1). For plant height, there was a significant interaction between genotypes and nitrogen fertilization, and between days of cultivation and nitrogen fertilization. The interaction between genotypes and the fertilization favored the study of differential performance among the genotypes, where, in the presence of fertilization with N, all genotypes showed gains in height when compared to the cultivation in absence of fertilization. Provided no nitrogen supply through fertilization, the Genotype 67 presented higher growth of orthotropic stem. With nitrogen supply, the plants from the Genotypes 67, 23 and 76 had higher vertical growth (Table 2).

To quantify the speed and intensity of growth in height of coffee plants is extremely important, since the

Table 1. Mean squares (MS), coefficients of variation (CV) and overall means for plant height, stem diameter and number of leaves of improved genotypes of *C. canephora*, modulated by nitrogen fertilization and days of cultivation.

Parameter	Plant height (cm)	Stem diameter (mm)	Number of leaves
$MS_{(genotype\ x\ nitrogen\ x\ time)}$	3.00^{ns}	0.12^{ns}	13.07*
$MS_{(nitrogen\ x\ time)}$	68.10*	0.23^{ns}	324.47*
$MS_{(genotype\ x\ time)}$	2.24^{ns}	0.10^{ns}	18.66*
$MS_{(genotype\ x\ nitrogen)}$	36.13*	0.88*	48.88*
MS_{time}	927.29*	37.71*	1247.99*
$MS_{nitrogen}$	2704.36*	7.06*	2769.08*
$MS_{genotype}$	59.62*	0.84*	119.72*
CV (%)	7.96	9.28	9.28
Mean	27.30	16.28	16.28

^{ns} non significant or * significant, at 5% of probability, by the F test.

Table 2. Means of plant height of improved genotypes of *C. canephora* modulated by the nitrogen fertilization.

Genotype	Nitrogen supply (g kg^{-1})	
	0.000	0.625
67	26.12^{Ba}	33.68^{Aa}
23	22.92^{Bb}	32.26^{Aa}
31	23.07^{Bb}	29.52^{Ab}
77	22.40^{Bb}	30.14^{Ab}
76	22.03^{Bb}	32.89^{Aa}
153	24.02^{Bb}	28.59^{Ab}

Means followed by the same uppercase letter in the rows do not differ by the Tukey test, and means followed by the same lowercase letter in the columns do not differ by the Scott-Knott test, both at 5% of probability.

promotion of growth and development of orthotropic stems can positively influence the amount of reproductive branches that the plant can develop, these being related to the capacity of sustaining the fruit production (Carvalho et al., 2010).

The plant height of all genotypes increased linearly along the days of cultivation. However, the plants grown in the presence of nitrogen fertilization developed faster, which can be observed in Figure 1A, by the higher slope coefficient in the regression for the plants cultivated with 0.625 g kg^{-1} of nitrogen. It is worth mentioning that, from the first 30 days of cultivation, the plants supplied with nitrogen fertilization already grew significantly higher than those who did not receive fertilization with N, a behavior that was maintained throughout the whole period evaluated in the study (Figure 1B).

Nitrogen is directly involved in the plant metabolism, acting as a component of amino acids, proteins, amines, amides, amino sugars, purines, pyrimidines alkaloids, coenzymes, vitamins and pigments, directly interfering with photosynthesis, which leads to the conclusion that, due to this interference, the lack of this nutrient limits the proper plant growth. Environments with restricted supply of this nutrient cause blockage in cytokinin synthesis, a hormone responsible for the plant growth, which results in losses in size and development of plant organs (Taiz and Zeiger, 2013).

Only the interaction between genotypes and nitrogen fertilization had significant effect for stem diameter (Table 1). The addition of nitrogen promoted the formation of thicker stems in most genotypes, except for the Genotypes 31 and 153, for which the stem grew to statistically similar values in either absence or addition of fertilization with N (Table 3).

The Genotypes 67, 31 and 153 showed the greatest thickening of the stem when cultivated with absence of fertilization with N, while the addition of the nutrient caused the Genotypes 67 and 23 to express superiority in the radial growth of their stems (Table 3).

The growth of the stems along the days of cultivation followed a linear trend, showing good fit to the regression $SD=0.02^{*}D+3.04^{*}$ ($R^2=0.98$), for which SD represents the stem diameter and D is the number of days of cultivation (up to 150).

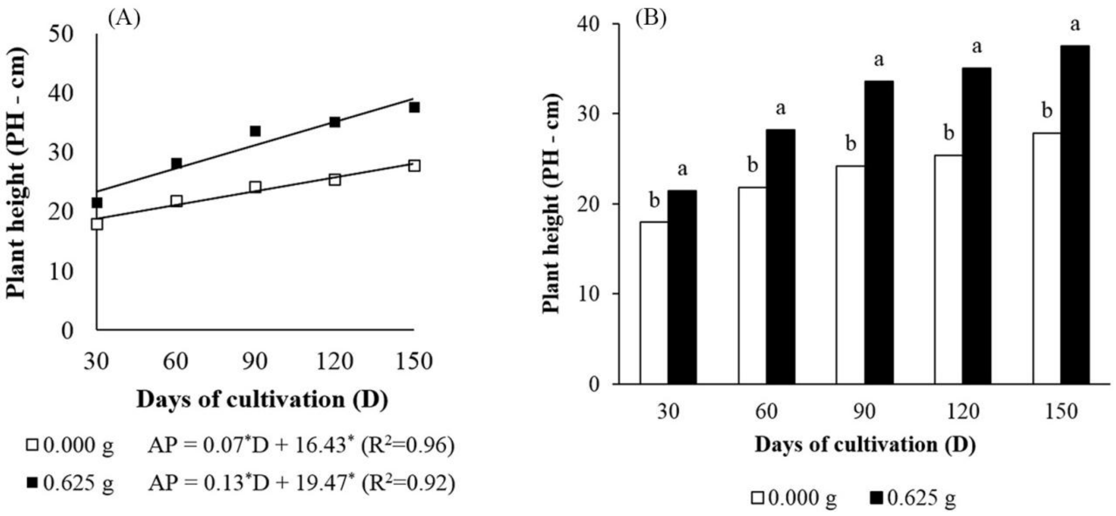

Figure 1. Regression analysis for plant height (A) and means of plant height (B) of improved genotypes of *C. canephora*, modulated by the presence or absence of nitrogen fertilization, along 150 days of cultivation a) *Coefficient is significant by the t-test, at 5% of probability, (b) means following by the same letter in each period of time do not differ by Tukey test, at 5% of probability.

Table 3. Means of stem diameter of improved genotypes of *C. canephora* modulated by the nitrogen fertilization.

Genotype	**Nitrogen supply (g kg^{-1})**	
	0.000	**0.625**
67	4.88^{Ba}	5.61^{Aa}
23	4.54^{Bb}	5.40^{Aa}
31	4.91^{Aa}	5.01^{Ab}
77	4.61^{Bb}	4.99^{Ab}
76	4.64^{Bb}	4.96^{Ab}
153	5.05^{Aa}	5.07^{Ab}

Means followed by the same uppercase letter in the rows do not differ by the Tukey test, and means followed by the same lowercase letter in the columns do not differ by the Scott-Knott test, both at 5% of probability.

The effect of the differences caused by the genotypes of Conilon coffee, the absence or presence of nitrogen fertilization and the period of cultivation modulated the means for number of leaves, and a triple interaction between these factors was significant (Table 1).

At 30 days of cultivation, there was no difference in the number of leaves among the genotypes, regardless of the management of the nitrogen fertilization (Table 4). At 60 days, it was already possible to notice a lesser leafiness of the plants from Genotypes 23 and 31 due to the lack of nitrogen supply, whereas there was no difference for the other genotypes for the condition or absence or presence of fertilization with N (Table 4).

After 90 days of cultivation (for 90, 120 and 150 days), the leafiness developed by plants cultivated with nitrogen fertilization was superior than the observed for the plants kept in absence of fertilization with this nutrient, emphasizing the importance of nitrogen for the development of new leaves in plants of Conilon coffee (Table 4).

The Genotypes 31 and 77 stood out regarding emission of new leaves at 90 days of cultivation, while the Genotype 67 had lesser leafiness than all others, considering the results obtained with nitrogen fertilization. The Genotype 77 was the only one who stood out in the absence of N fertilization, presenting good leafiness for both conditions of fertilization (Table 4).

With addition on nitrogen, the Genotypes 77 and 76 produced more leaves than the other at 120 days of cultivation, while the Genotypes 67 and 153 had the smaller number of leaves at this time. For the condition of absence of nitrogen fertilization, the Genotype 77 was

Table 4. Means of number of leaves of improved genotypes of *C. canephora* modulated by the nitrogen fertilization, along 150 days of cultivation.

Gen.	Days of cultivation									
	30		60		90		120		150	
	0.000 g kg^{-1}	0.625 g kg^{-1}	0.000 g kg^{-1}	0.625 g kg^{-1}	0.000 g kg^{-1}	0.625 g kg^{-1}	0.000 g kg^{-1}	0.625 g kg^{-1}	0.000 g kg^{-1}	0.625 g kg^{-1}
67	8.66^{Aa}	9.66^{Aa}	10.66^{Ab}	12.00^{Ab}	10.66^{Bb}	17.33^{Ac}	10.66^{Bc}	22.00^{Ac}	10.66^{Bd}	34.66^{Aa}
23	8.66^{Aa}	9.66^{Aa}	8.66^{Bb}	12.66^{Ab}	11.33^{Bb}	21.66^{Ab}	12.66^{Bb}	26.33^{Ab}	13.00^{Bc}	28.00^{Ac}
31	7.33^{Aa}	9.33^{Aa}	8.00^{Bb}	15.33^{Aa}	11.33^{Bb}	25.66^{Aa}	13.33^{Bb}	27.00^{Ab}	13.66^{Bc}	31.00^{Ab}
77	8.66^{Aa}	10.66^{Aa}	13.33^{Aa}	13.33^{Ab}	18.66^{Ba}	26.00^{Aa}	22.00^{Ba}	30.66^{Aa}	23.33^{Ba}	35.33^{Aa}
76	7.66^{Aa}	9.33^{Aa}	10.33^{Ab}	10.66^{Ab}	12.00^{Bb}	21.00^{Ab}	13.66^{Bb}	30.00^{Aa}	15.00^{Bb}	31.33^{Ab}
153	8.66^{Aa}	8.66^{Aa}	12.66^{Aa}	12.66^{Ab}	13.33^{Bb}	20.00^{Ab}	15.66^{Bb}	21.33^{Ac}	16.66^{Bb}	23.00^{Ad}

Means followed by the same uppercase letter in the rows (for each period) do not differ by the Tukey test, and means followed by the same lowercase letter in the columns do not differ by the Scott-Knott test, both at 5% of probability.

able to maintain the leafiness less limited, growing a greater number of leaves than the others genotypes; in contrast, the Genotype 67 presented greater limitation, producing a smaller number of leaves (Table 4).

At the end of 150 days of cultivation, after all the parcels of the nitrogen fertilization, the Genotypes 67 and 77 presented greater amount of leaves in response to the fertilization with N, while the Genotype 153 had the smaller number of leaves. The Genotype 77 is highlighted from the group for being able to produce a larger number of leaves even in absence of fertilization (Table 4).

This characteristic of the Genotype 77 is relevant for the breeding programs, since this genotype may be a source of variability to explore the tolerance to cultivation in environments with nutritional limitations or even be studied to seek nutritional efficiency.

The fertilization with N promoted the growth parameters, leading to plants with higher plant height, stem diameter and number of leaves. There is a close relationship between these parameters, as the leafiness is a direct result of the activity of the apical bud of the branches, especially plagiotropics branches. And the growth of these branches is directly related to the growth of orthotropic stem, where the thickening is the result of its secondary growth, playing an important role in plant physical support and in the translocation of solutes between organs. In turn, the leaves are the source of most photosynthetic assimilates essentials to fuel the plant growth (Gomes et al., 2008; Rena and Maestri, 1986).

According to Clement et al. (2013), the nitrogen fertilization mainly influences the characteristics of vegetative growth, and the deficit of this nutrient affects the development of the coffee plant and, consequently, the fruit production.

By regression analysis, it is noted that leafiness increased linearly, for all genotypes, with time when the plants were cultivated with nitrogen fertilization (Figure 2). This same behavior was observed in absence of fertilization, except for the Genotype 67, which was not able to grow new leaves due the nutritional stress (Figure 2A).

The positive effect of the nitrogen fertilization over the emission of leaves of genotypes of Conilon coffee is easily noticeable in Figure 2. The slope coefficients of the regressions makes possible to hypothesize that cultivating the genotypes with nitrogen fertilization near the level of 0.625 g kg^{-1} promoted the leafiness, resulting in plants with higher vigor, faster growth, and, therefore, presenting a better development, which is necessary on the early stages to grant a possible higher production of fruits.

The analysis of relative growth rate is an important tool to study the speed at which the plant is growing. Using this tool, it was observed that plants of improved genotypes of *C. canephora* showed a similar growth trend, where higher growth rates occurred in the first two periods of evaluation (30-60 days and 60-90 days), indicating that during the first months after planting, the Conilon coffee seedlings express higher gains in plant height. After 90 days of cultivation, the plants presented smaller gains in height, matching the period when they start the radial growth of their canopies. This behavior was easier to identify when the plants were fertilized with N, since the nutritional stress caused limitation to the growth rate (Figure 3).

From 90 to 120 days of cultivation, smaller values of RGR were observed for both conditions of nitrogen fertilization (Figure 3). This behavior is related to the start of development of reproductive branches, thereby the metabolism is directed to the lateral growth of the canopy and the rate of growth in height is reduced. This explains why, after this period, the response in RGR of the fertilization seems to be not as expressive as during other periods.

For the fourth period (120-150 days), the absence of nitrogen fertilization promoted a higher relative growth

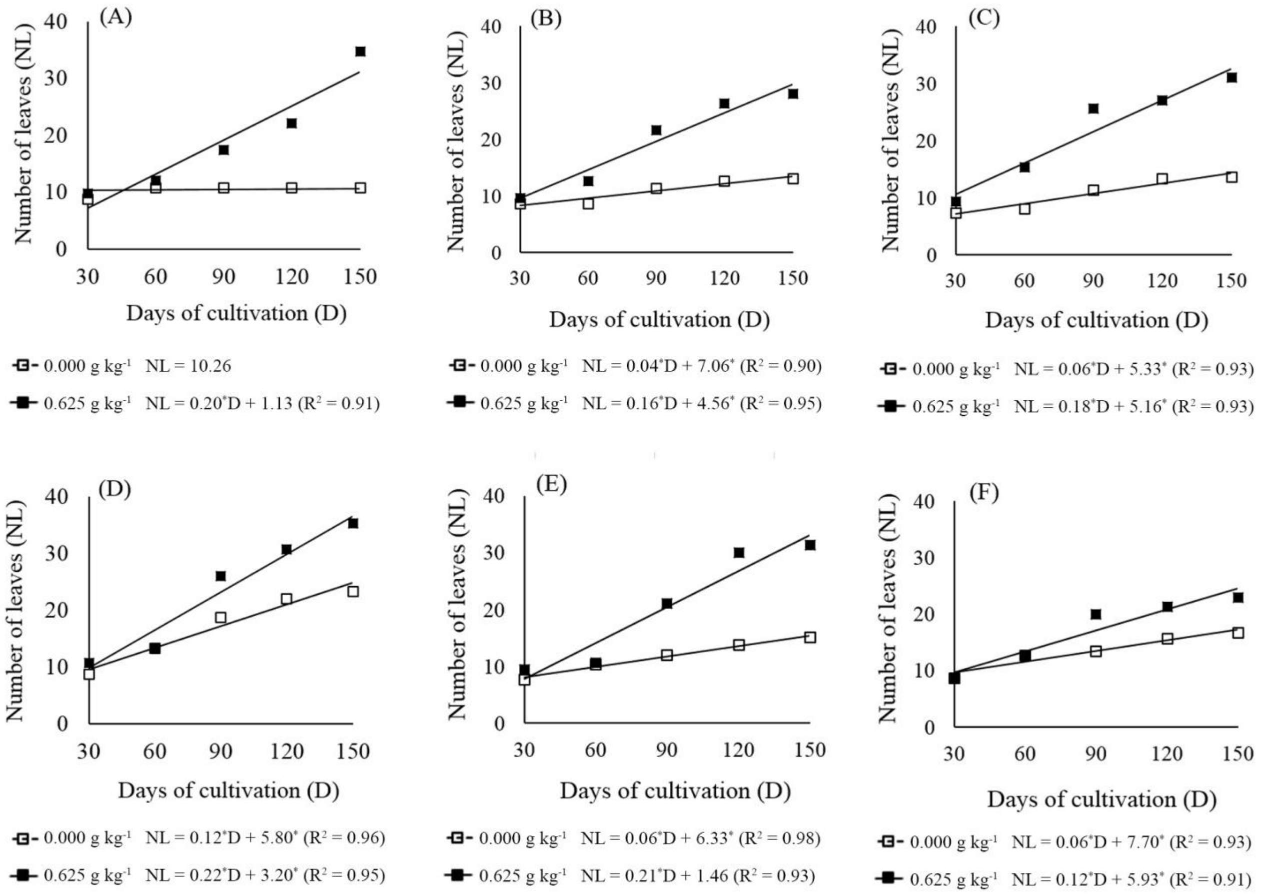

Figure 2. Regression analysis for number of leaves of improved genotypes of *Coffea canephora* (A – 67; B – 23; C – 31; D – 77; E – 76; F – 153), modulated by the presence or absence of nitrogen fertilization, along 150 days of cultivation. *Coefficient is significant by the t-test, at 5% of probability.

rate for the Genotypes 31, 76 and 153, showing that those genotypes were able to grow more in height, relatively to the previous period, in the condition of nutritional stress. This may be a strategy of the plant to accelerate its growth in an adverse environment, aiming to reach further phonologic stages quicker, in order to perpetuate the species.

It is understood that the vegetative growth of Conilon coffee is influenced by the nitrogen supply. The plant growth is a result of photosynthetic process, and it is known that there is a close relationship between the metabolism or carbon and nitrogen. The processes are connected, since the energy required to assimilate nitrogen is directly or indirectly derived from photosynthesis and the photosynthetic capacity itself depends on the nitrogen supply, since this nutrient is largely allocated in leaf proteins involved in photosynthetic processes (Evans, 1983; Evans and Seemann, 1984; Seemann et al., 1987).

The promotion of growth as a function of nitrogen fertilization observed in the genotypes is a result of the proper nutrition promoting the metabolism and the development of plant organs (Marschner, 2012). This growth response can be modulated by the phenotypic expression of different capacities of the genotypes in acquire and utilize the nutrient. Structural and morphological changes as a function of the nutritional efficiency have been already reported in the literature for *C. canephora* genotypes (Martins et al., 2013a; 2013b).

Studies conducted with different genotypes of Conilon coffee have also pointed out the existence of different responses to nitrogen fertilization, such as different accumulation rate of nutrients, accumulation of dry matter, growth, crop yield, nutritional parameters and expression of morphological characteristics (Clemente et al., 2013; Prezotti and Bragança, 2013; Pedrosa et al., 2014, Colodetti et al., 2014).

The combination of this information with the results obtained in this study reinforces the evidence that there is differentiation in the nutritional demands for nitrogen during the formation of seedlings, growth and production of improved genotypes of Conilon coffee with high potential of crop yield. Therefore, it is necessary to modify the management of the fertilization to make sure that the nutritional demands of those highly productive genotypes are satisfied.

Conclusion

The growth of improved genotypes of *C. canephora* is modulated by nitrogen fertilization, being promoted by the increase of nitrogen supply in the soil. Differentiation among genotypes was observed for their responses to each of the evaluated parameters. Noteworthy are the Genotypes 77, in leafiness, and 67 in plant height and stem diameter, which presented higher growth compared to other genotypes, with or without nitrogen fertilization. The relative growth rate of improved genotypes follows the same general pattern as a function of the nitrogen availability, being promoted by it.

Conflict of Interest

The authors have not declared any conflict of interest.

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