











ISSN 1678-3921

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# Morphophysiological responses of Conilon coffee matrix plants in a super-dense clonal garden

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Received

August 08, 2022

Accepted

October 18, 2023

How to cite

SILVA, J.A. da; ESPOSTI, M.D.D.; SENRA, J.F. de B.; COMÉRIO, M.; CONCEIÇÃO, A.O. da; ZACARIAS, A.J.; MILHEIROS, I.S.; SILVA, U.R.; SILVA, F.G. da; RAIMUNDO, E.G. Morphophysiological responses of Conilon coffee matrix plants in a super-dense clonal garden. *Pesquisa Agropecuária Brasileira*, v.58, e03078, 2023. DOI: <https://doi.org/10.1590/S1678-3921.pab2023.v58.03078>.

**Abstract** – The objective of this work was to evaluate the morphophysiological responses and cutting production of clones of Conilon coffee (*Coffea canephora*) cultivars in a super-dense clonal garden in the state of Espírito Santo, Brazil. The super-dense clonal garden was built in 2019 using 39 clones: 9, 9, 9, and 12 of cultivars Centenária ES8132, Diamante ES8112, ES8122 (Jequitibá), and Marilândia ES8143, respectively. The experiment was carried out in a randomized complete block design, with three replicates. Cutting production and the following morphophysiological traits were evaluated at 9 and 18 months after planting: chlorophyll index, normalized difference vegetation index, plant height, canopy height, canopy diameter, number of shoots, number of viable cuttings, number of leaves, fresh leaf mass, and plant fresh and dry matter mass. The super-dense clonal garden caused different morphophysiological responses among the studied clones. In general, clones C2, C5, C6, C8, D1, D8, D9, J8, M2, M9, M10, and M12 showed a higher mean cutting production, whereas C4, J1, J4, M4, and M5 were the most sensitive to the super-dense regime. Under these conditions, it is recommended to increase the proportion of matrix plants of the latter clones.

**Index terms:** *Coffea canephora*, clones, propagation by cutting, spacing.

## Respostas morfofisiológicas de plantas matrizes de cafeeiro conilon em jardim clonal superadensado

**Resumo** – O objetivo deste trabalho foi avaliar as respostas morfofisiológicas e a produção de estacas de clones de cultivares de café conilon (*Coffea canephora*) em jardim clonal superadensado, no estado do Espírito Santo, Brasil. O jardim clonal superadensado foi implantado em 2019, com 39 clones: 9, 9, 9 e 12 das cultivares Centenária ES8132, Diamante ES8112, ES8122 (Jequitibá) e Marilândia ES8143, respectivamente. O experimento foi realizado em delineamento em blocos ao acaso, com três repetições. A produção de estacas e as seguintes características morfofisiológicas foram avaliadas aos 9 e 18 meses após o plantio: índice de clorofila, índice de vegetação por diferença normalizada, altura da planta, altura da copa, diâmetro da copa, número de brotações, número de estacas viáveis, número de folhas, massa fresca de folhas, e massa fresca e seca da planta. O jardim clonal superadensado promoveu diferentes respostas morfofisiológicas entre os clones estudados. Em geral, os clones C2, C5, C6, C8, D1, D8, D9, J8, M2, M9, M10 e M12 apresentaram maior produção média de estacas, enquanto C4, J1, J4, M4 e M5 foram os mais sensíveis ao regime superdenso. Nessas condições, recomenda-se aumentar a proporção de plantas matrizes destes clones.

**Termos para indexação:** *Coffea canephora*, clones, propagação por estacas, espaçamento.



## Introduction

Clonal gardens of Conilon coffee (*Coffea canephora* Pierre ex A.Froehner) are vegetative multiplication fields where matrix plants are cultivated to produce segments of orthotropic branches, popularly known as cuttings, for the production of clonal plantlets of commercial cultivars, both in nurseries and research institutions (Ferrão, 2019b).

Since 1985, the *C. canephora* genetic breeding program of Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (INCAPER) has developed the following nine clonal cultivars: Emcapa – 8111 – Precoce, Emcapa 8121, Emcapa – 8131, Emcapa 8141 Robustão Capixaba, Vitória Incaper 8142, Diamante ES8112, ES8122 (Jequitibá), Centenária ES8132, and Marilândia ES8143 (Ferrão et al., 2019a). The institution also keeps three other cultivars: BRS Ouro Preto, developed by Embrapa Rondônia (Marcolan & Espindula, 2015); Andina (Partelli et al., 2019); and Tributun (Partelli et al., 2020). For farmers to have fast access to these clonal cultivars, INCAPER uses clonal gardens (Bragança et al., 2001; Fonseca et al., 2019).

About 90% of the Brazilian *C. canephora* plantlets come from clonal gardens (Mauri et al., 2015; Fonseca et al., 2019). In the state of Espírito Santo, there are approximately 190 clonal gardens, whose production capacity is 50 million plantlets per year, assuring the annual renewal of 7.0 to 8.0% of the *C. canephora* area of the country (Fonseca et al., 2019).

In general, clonal gardens use a plant spacing of 2.0x1.0 m, resulting in a density of 5,000 plants per hectare (Fonseca et al., 2019). However, increasing plant density can increase the productivity of clonal gardens, leading to the testing of the super-dense clonal garden strategy. According to the same authors, in super-dense gardens, plant spacing is 0.4 m between plants, 0.6 m between rows, and 1.0 m between double rows, with a plant density of 31,250 plants per hectare.

Despite maximizing plantlet production, super-dense clonal gardens require a more intensive and more complex management since plants are exposed to high competition for water, light, and nutrients, which can compromise their development, reducing yield, the quality of the cuttings, and the longevity of the matrix plants (Mauri et al., 2015; Fonseca et al., 2019). Therefore, it is necessary to improve nursery techniques by evaluating factors affecting plantlet

development (Trautenmüller et al., 2017; Giuriatto Júnior et al., 2020; Verdin Filho et al., 2021).

The objective of this work was to evaluate the morphophysiological responses and the cutting production of clones of *C. canephora* cultivars in a super-dense clonal garden in the state of Espírito Santo, Brazil.

## Materials and Methods

The experiment was conducted in the Bananal do Norte experimental farm of INCAPER, located in the municipality of Cachoeiro de Itapemirim, in the state of Espírito Santo, Brazil (20°45'S, 41°17'W). The climate of the region is classified as Aw by Köppen-Geiger.

The super-dense clonal garden was built in November 2019, with a spacing of 0.90 m between lines and 0.40 m between plants. A total of 39 clones of the four following cultivars, recommended for the state of Espírito Santo, were used: 9 of Diamante ES8112 (treatments D1 to D9), with early maturation; 9 of ES8122 (Jequitibá) (treatments J1 to J9), with an intermediate maturation; 9 of Centenária ES8132 (treatments C1 to C9), with a late maturation; and 12 of Marilândia ES8143 (treatments M1 to M12), with drought tolerance (Ferrão et al., 2019a).

The experiment was carried out in a randomized complete block design, with three replicates. Each plot had ten plantlets, totaling 1,170 plants evaluated in the experiment (39 clones, three replicates, and ten plantlets per plot). The border had two lines: an inner one of clones of cultivar Vitória Incaper 8142 and an outer one of clones of cultivar Emcapa 8141 Robustão Capixaba.

Fertilization was applied as recommended in the fertilization and liming handbook for the state of Espírito Santo (Prezotti et al., 2007). Cultural and phytosanitary procedures were carried out according to crop requirements (Ferrão et al., 2019b). The following traits were evaluated at 9 and 18 months after planting, i.e., in the first and second cut, respectively: chlorophyll index (SPAD); normalized difference vegetation index (NDVI); plant height; canopy height; two measurements of canopy diameter, parallel and perpendicular to the planting line; number of shoots; number of viable cuttings; number of leaves; fresh leaf

mass; plant fresh matter mass; and plant dry matter mass.

The SPAD was obtained by measuring the right and left mesophyll of two leaves of the third or fourth pair of plagiotropic branches, located in the middle third of the plant, using the SPAD-502Plus portable chlorophyll meter (Konica Minolta, Inc., Tokyo, Japan). To determine the NDVI, measurements were also taken on the right and left mesophyll of two leaves of the third or fourth pair of plagiotropic branches, located in the middle third of the plant, using the PlantPen NDVI 300 portable sensor (PSI Corporation, Drásov, Czech Republic). Plant and canopy height (cm) were measured using a graduated ruler, one from the ground to the apex of the largest orthotropic branch (last node) and the other from the first node with a plagiotropic branch up to the last node. The two measured diameters were: longitudinal, the greatest length of the plagiotropic branch of the canopy parallel to the planting line; and transversal, the greatest length of the plagiotropic branch of the canopy perpendicular to the planting line. The number of shoots was considered as the number of shoots per plant in viable cuttings, the number of viable cuttings was the number of viable cuttings per plant to be used for plantlet production, and the number of leaves was the total number of leaves of viable cuttings. The Adventurer ARC120 0.01 g precision scale (Ohaus, Parsippany, NJ, USA) was used to weigh: fresh leaf mass (g), immediately after leaves were collected from branches with viable cuttings; plant fresh dry matter mass (g), using plant material with viable cuttings; and plant dry matter mass (g), using the same material for fresh mass, but dried in a forced-air oven, at 65°C, until reaching a constant mass.

In the first cut at 9 months, number of shoots, number of viable cuttings, plant fresh matter mass, plant dry matter mass, SPAD, and NDVI were evaluated. Clones 21, 22, and 32 were not assessed since there was not enough material for the analysis. In the second cut at 18 months, all 39 clones were evaluated for all traits, except plant dry matter mass.

The data was checked for normality, homoscedasticity, and independence of errors using the tests of Shapiro-Wilk, Bartlett, and Durbin-Watson ( $\alpha=0.05$ ).

The data were subjected to the one-way analysis of variance. Means were compared by Scott-Knott's

test ( $\alpha=0.05$ ) using the Rbio software, version 185 (Bhering, 2017). Graphs were built using the ScottKnott package, version 1.3-2 (Jelihovschi et al., 2023), of the R software, version 4.3.0 (R Core Team, 2023).

The assumptions were not met for some variables. A log (x) transformation was used for number of viable cuttings in the first cut, number of shoots per plant in the first cut, and fresh leaf mass in the second cut. A square root transformation of  $x+1$  was used for fresh and dry plant matter mass in the first cut. For discussion purposes, the mean results were presented without transformation.

## Results and Discussion

The production of cuttings and most of the morphophysiological traits in the first and second cuts showed the differentiated behavior of the clones under a high-density planting system (Tables 1, 2, 3, and 4). Only dry matter mass, evaluated in the first cut, did not differ significantly among the studied clones (Table 2).

Considering the production of propagative material, represented by the production of cuttings and shoots, *C. canephora* clones were divided by Scott-Knott's clustering result ( $\alpha=0.05$ ) into two groups in each cut (Figure 1). In the first cut, clones C2, C6, C8, D1, D9, J8, M9, and M12 showed the highest production, and C7, C9, J1, J4, M1, and M6, the lowest (Figure 1 A and C). In the second cut, clones C2, C5, C6, C8, D8, J8,

**Table 1.** Analysis of variance of the chlorophyll index (SPAD) and normalized difference vegetation index (NDVI) of Conilon coffee (*Coffea canephora*) in the first cut, in 2019, in a super-dense clonal garden, located in the Bananal do Norte experimental farm of Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, in the state of Espírito Santo, Brazil<sup>(1)</sup>.

Source of variation	df	SS	MS	F-value	p-value
SPAD (unit)					
Between groups	35	2,543.3	72.66	4.069	7.25e-07
Within groups	62	1,107.2	17.86		
NDVI					
Between groups	35	3,283.0	93.79	45.68	2e-16
Within groups	62	127.0	2.05		

<sup>(1)</sup>df, degrees of freedom; SS, sum of squares; and MS, mean squares.

M2, M9, and M10 presented the highest production, and C1, C3, C4, D4, J4, M1, M6, and M8, the lowest (Figure 1 B and D).

At 9 months after planting, three groups were formed based on the SPAD: one with clones C2, C8, C9, D9, and M12 with the highest averages, one with

**Table 2.** Analysis of variance of cutting production and morphological traits of Conilon coffee (*Coffea canephora*) in the first cut, in 2019, in a super-dense clonal garden, located in the Bananal do Norte experimental farm of Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, in the state of Espírito Santo, Brazil<sup>(1)</sup>.

Traits	Source	df	SS	MS	F-value	p-value
Number of viable cuttings (unit)	Between groups	35	3,115.0	89.0	2.277	0.0023
	Within groups	62	2,423.7	39.09		
Number of shoots (unit)	Between groups	35	194.86	5.568	1.956	0.0104
	Within groups	62	176.44	2.846		
Fresh matter mass (g)	Between groups	35	1.1173	0.03192	1.657	0.0409
	Within groups	62	1.1943	0.1926		
Dry matter mass (g)	Between groups	35	0.16164	0.004618	1.396	0.125
	Within groups	62	0.20511	0.003308		

<sup>(1)</sup>df, degrees of freedom; SS, sum of squares; and MS, mean squares.

**Table 3.** Analysis of variance of the chlorophyll index (SPAD) and normalized difference vegetation index (NDVI) of Conilon coffee (*Coffea canephora*) in the second cut, in 2019, in a super-dense clonal garden, located in the Bananal do Norte experimental farm of Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, in the state of Espírito Santo, Brazil<sup>(1)</sup>.

Index	Source	df	SS	MS	F-value	p-value
SPAD (unit)	Between groups	38	5,774.00	151.95	4.543	0.000000117
	Within groups	75	2,509.00	33.45		
NDVI	Between groups	38	372.20	9.795	4.193	0.00000006
	Within groups	75	175.20	2.336		

<sup>(1)</sup>df, degrees of freedom; SS, sum of squares; and MS, mean squares.

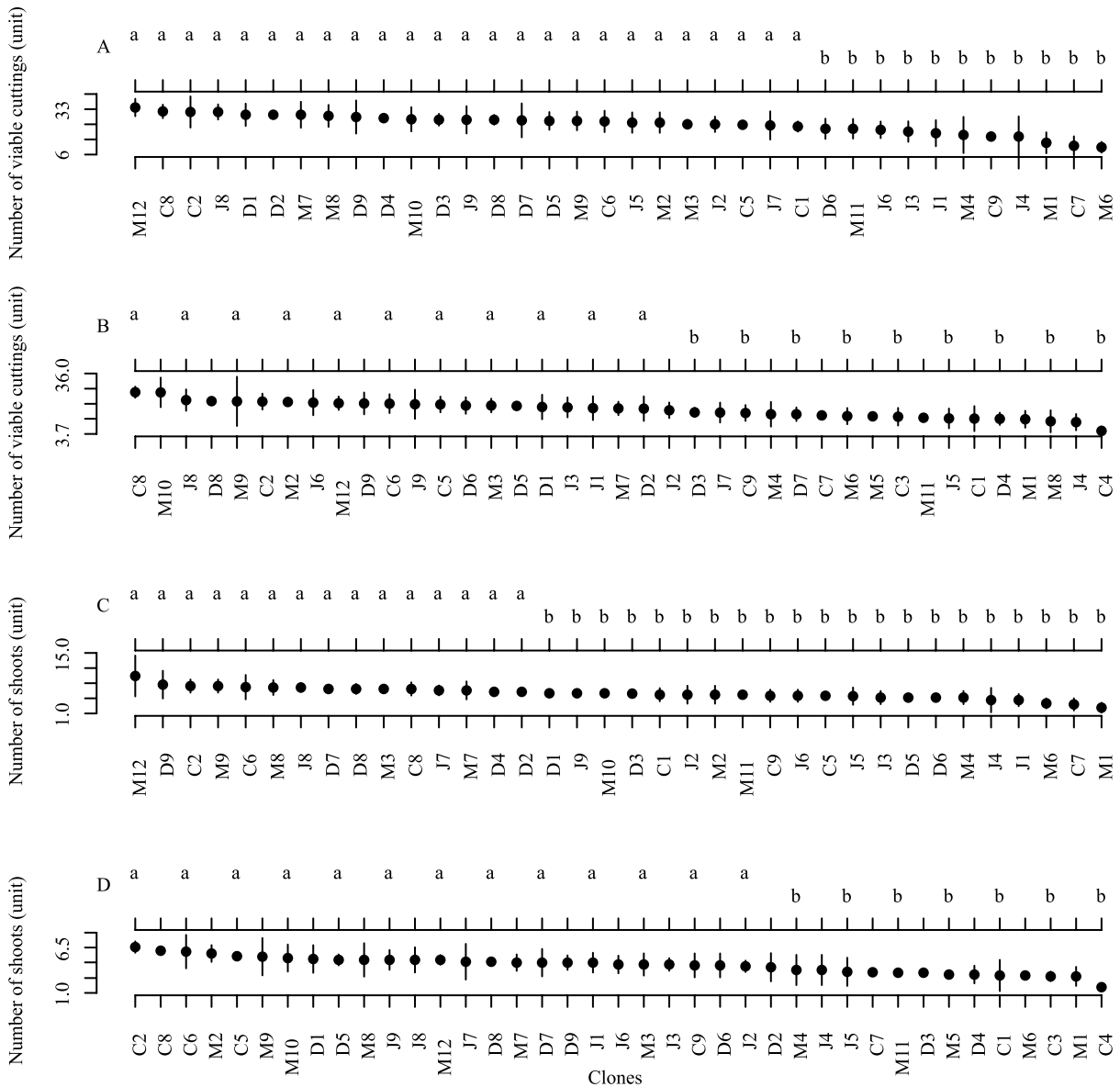
**Table 4.** Analysis of variance of cutting production and morphological traits of Conilon coffee (*Coffea canephora*) in the second cut, in 2019, in a super-dense clonal garden, located in the Bananal do Norte experimental farm of Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural, in the state of Espírito Santo, Brazil<sup>(1)</sup>.

Traits	Source	df	SS	MS	F-value	p-value
Number of viable cuttings (unit)	Between groups	38	2,118.70	55.76	2.075	0.0036
	Within groups	75	2,015.40	26.87		
Number of shoots (unit)	Between groups	38	110.29	2.902	1.790	0.0161
	Within groups	75	121.60	1.621		
Fresh matter mass (g)	Between groups	38	2.3688	0.06234	2.093	0.00325
	Within groups	75	2.2332	0.02978		
Plant height (cm)	Between groups	38	20,814	547.7	4.036	0.00000128
	Within groups	75	10,178	135.7		
Canopy height (cm)	Between groups	38	2,828	74.42	2.689	0.00013
	Within groups	75	2,075.7	27.68		
Longitudinal canopy diameter (cm) <sup>(2)</sup>	Between groups	38	10,275	270.39	4.305	0.000000354
	Within groups	75	4,711	62.81		
Transversal canopy diameter (cm) <sup>(3)</sup>	Between groups	38	12,043	316.9	4.654	0.000000007
	Within groups	75	5,107	68.1		
Number of leaves (unit)	Between groups	38	796,178	20,952	2.072	0.00364
	Within groups	75	758,323	10,111		
Fresh leaf mass (g)	Between groups	38	0.6813	0.01793	1.698	0.02574
	Within groups	75	0.7918	0.01056		

<sup>(1)</sup>df, degrees of freedom; SS, sum of squares; and MS, mean squares. <sup>(2)</sup>The greatest length of the plagiotropic branch of the canopy parallel to the planting line. <sup>(3)</sup>The greatest length of the plagiotropic branch of the canopy perpendicular to the planting line.

only J4 with the lowest average, and the other with the remaining clones (Figure 2 A). Based on the NDVI, the two following groups were formed: one only with clone J4, with the lowest mean; and the other with the remaining clones (Figure 2 C). At 18 months after

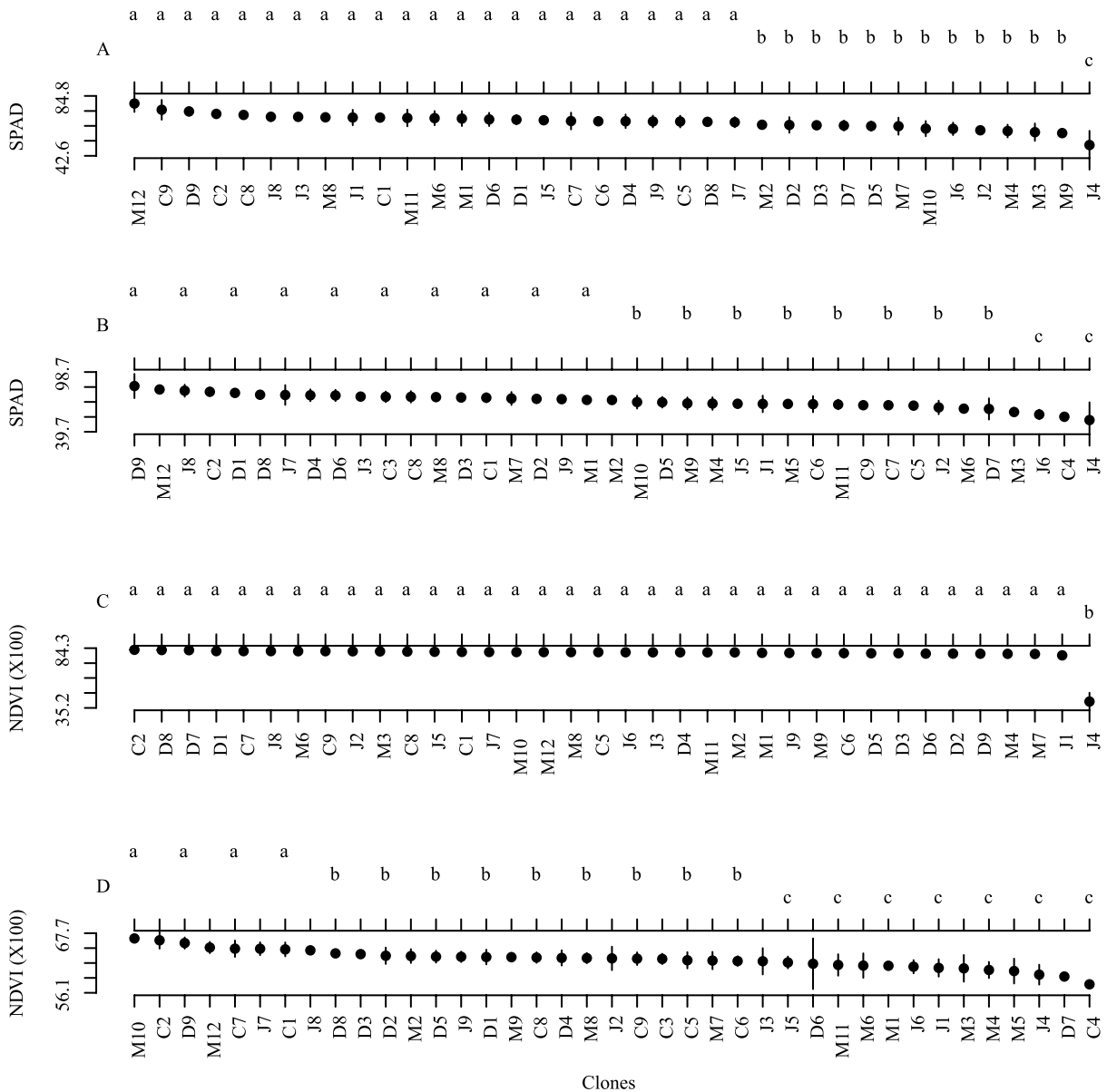
planting, based on the SPAD, the following three groups were formed: one with clones C2, D1, D9, J8, and M12 with the highest averages, one with clones J4, J6, C4 and M3 with the lowest averages, and the other with the remaining clones (Figure 2 B). Based on the



**Figure 1.** Result of Scott-Knott's clustering ( $\alpha=0.05$ ) of clones of Conilon coffee (*Coffea canephora*) cultivars in a super-density clonal garden, showing: number of viable cuttings 9 months after planting (A), number of viable cuttings 18 months after planting (B), number of shoots 9 months after planting (C), and number of shoots 18 months after planting (D). C1 to C9, clones of Centenária ES8132; D1 to D9, clones of ES8122 Diamante; J1 to J9, clones of ES8112 (Jequitibá); and M1 to M12, clones of Marilândia ES8143. Longitudinal diameter, the greatest length of the plagiotropic branch of the canopy parallel to the planting line; and transversal diameter, the greatest length of the plagiotropic branch of the canopy perpendicular to the planting line.

NDVI, three groups were also formed: one with clones C1, C2, C7, D9, J7, J8, M10, and M12 with the highest averages, one with clones C4, D7, J4, M4, and M5 with the lowest averages, and the other with the remaining clones (Figure 2 D).

The average production of cuttings varied 60% between the worst and best groups in the first and second cuts. The number of shoots varied 47.4 and 52.4% between the worst and best groups in the first and second cuts, respectively. The chlorophyll index, represented by the SPAD readings, showed a variation



**Figure 2.** Results of Scott-Knott's clustering ( $\alpha=0.05$ ) of clones of Conilon coffee (*Coffea canephora*) cultivars in a super-density clonal garden, showing: the chlorophyll index (SPAD) 9 months after planting (A), the SPAD 18 months after planting (B), the normalized difference vegetation index (NDVI) 9 months after planting (C), and the NDVI 18 months after planting (D). C1 to C9, clones of Centenária ES8132; D1 to D9, clones of ES8122 Diamante; J1 to J9, clones of ES8112 (Jequitibá); and M1 to M12, clones of Marilândia ES8143.

of 38.5 and 36.2% between the worst and best groups in the first and second cuts, respectively, whereas the NDVI showed a 100 and 7.6% variation between the worst and best groups in the first and in second cuts, respectively.

In *C. canephora* cutting production systems with clonal matrices under a high-density planting system, significant increases in production are expected due to the increase in the population per unit area. However, the use of clones that best adapt to local conditions in this system allows of maximizing the gains with the super-dense clonal garden technology, as observed for clones C2, C5, C6, C8, D1, D8, D9, J8, M2, M9, M10, and M12 with the highest average cutting production. This explains why clonal gardens are the main structures used for the large-scale production of propagative material, maintaining the clones of improved and recommended clonal cultivars and contributing significantly to the sustainability of coffee farming.

The average production of cuttings per plant was 24.71 (686,388 cuttings per hectare) in the first cut and 16.81 (467,000 cuttings per hectare) in the second, i.e., 47% higher in the first cut. According to Fonseca et al. (2005, 2019), a clonal garden of *C. canephora* in a conventional system starts producing cuttings at 24 months, which is increased in super-dense clonal gardens, with a production potential of 2.44 million cuttings 18 months after planting at a density of 31,250 plants per hectare (Fonseca et al., 2019). In the present study, a productivity of 1,153,388.00 cuttings per hectare was reached 18 months after planting of the 39 clones of the most recent clonal cultivars recommended for the state of Espírito Santo, highlighting the importance of this technique for the early production of propagation material in relation to traditional clonal gardens. For the production of clonal plantlets of *C. canephora*, other techniques have also been developed, such as the vertical clonal garden (Espindula et al., 2022), which allows of the production of more than one million cuttings per hectare per year.

In the literature, *C. canephora* genotypes showed genetic variability regarding several traits (Senra et al., 2020; Ferrão et al., 2021). Cogo et al. (2018), for instance, found that shoot emission varied in *C. canephora* genotypes, a trait directly related to the production of propagative material.

Rissini et al. (2015) concluded that plants with a greater vegetative vigor and amount of chlorophyll may present a greater productive potential. The SPAD and NDVI indices are used to measure leaf chlorophyll content, which is related to the photosynthetic capacity and efficiency of plants and, consequently, to their growth and adaptability to different growing conditions (Jesus & Morenco, 2008), such as the super-dense management system for coffee trees. For *C. canephora*, Silva et al. (2022) found positive genetic correlations between vegetative vigor and SPAD and NDVI readings. A similar response was observed in the present study for clones C2, C8, D1, D8, D9, J7, J8, M10, and M12 of the group with highest mean for the NDVI and SPAD indices and for cutting production. Torres Netto et al. (2005), using a portable chlorophyll meter to diagnose the integrity of the photosynthetic system of *C. canephora* leaves, found that SPAD readings lower than 40 indicate damage to the photosynthetic machinery. Since the average values of this index were above this threshold in the present work, the photosynthetic machinery was not compromised despite the stress caused by the high plant population density, which influenced the productivity of cuttings.

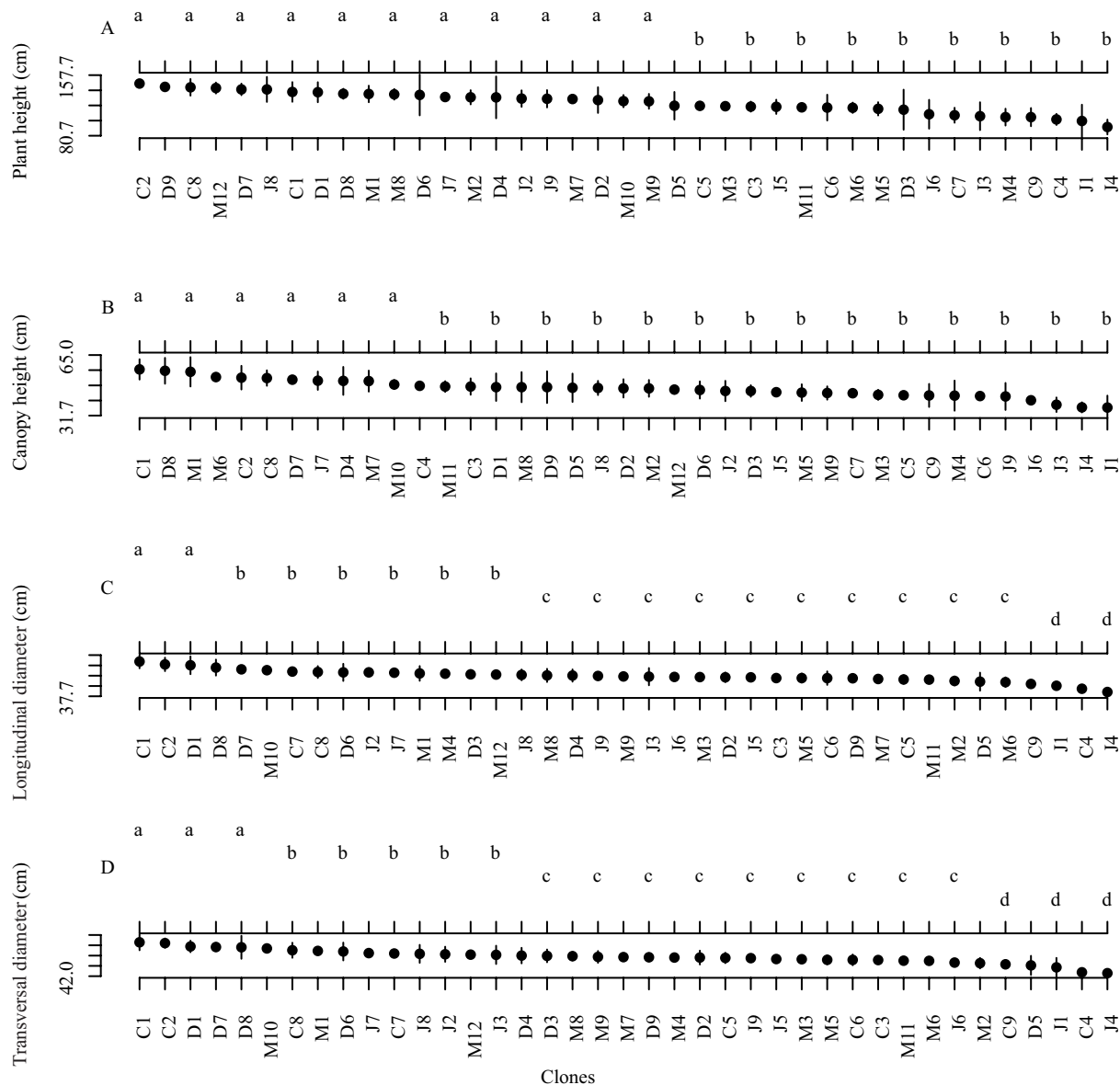
Among the traits evaluated only in the second cut, those that stood out in differentiating clone behavior were plant and canopy height and canopy diameter measurements (Figure 3). Based on plant and canopy height, the clones were classified into two groups: C1, C2, C8, D7, D8, D9, M1, M6, and M12, with the highest averages; and C4, C9, J1, J3, J4, J6, J9, and M4, with the lowest ones (Figure 3 A and B). Based on canopy diameter (longitudinal and transversal), four groups of clones were formed (Figure 3 C and D), with clones C1, C2, D1, D7, D8, and M10 with the highest averages, and C4, C9, D5, J1, and J4 with the lowest ones.

In high-density planting systems, the increased competition for water, light, and nutrients results in a stressful situation that can compromise vegetative development, causing, for example, a significant increase in coffee plant height (Martinez et al., 2007). Pereira et al. (2011) added that this occurs because, as spacing is reduced, the increased competition for the incidence of radiation causes a greater growth or etiolation of orthotropic branches, explaining the low production of viable cuttings observed for clones C1, C7, C9, M1, and M6, which stood out

for morphophysiological traits, but produced little propagative material. Clones C2, C8, D1, D8, M10, and M12 presented a greater plant height, combined with a greater canopy size and greater cutting production.

The SPAD and NDVI indices, combined with plant and canopy height, were able to detect the different

behavior of *C. canephora* matrix plants of the clonal cultivars Centenária ES8132, Diamante ES8112, ES8122 (Jequitibá), and Marilândia ES8143. Clones C4, J1, J4, M4, and M5 were the most sensitive in the super-dense regime, belonging to the groups with the lowest averages for most of the evaluated traits, showing



**Figure 3.** Results of Scott-Knott's clustering ( $\alpha=0.05$ ) of clones of Conilon coffee (*Coffea canephora*) cultivars in a super-dense clonal garden 18 months after planting, for: plant height (A), canopy height (B), longitudinal canopy diameter (C), and transversal canopy diameter (D). C1 to C9, clones of Centenária ES8132; D1 to D9, clones of ES8122 Diamante; J1 to J9, clones of ES8112 (Jequitibá); and M1 to M12, clones of Marilândia ES8143. Longitudinal diameter, the greatest length of the plagiotropic branch of the canopy parallel to the planting line; and transversal diameter, the greatest length of the plagiotropic branch of the canopy perpendicular to the planting line.



a low vegetative vigor and low cutting production. Therefore, if these clones are used in super-dense gardens, the number of matrix plants must be higher than that of the other clones in order to compensate for their reduced vigor. Moreover, the response of each clone under super-dense clonal gardens may compromise the original clonal composition of the cultivars. In general, the morphophysiological traits evaluated in the present work were not hindered in the super-dense regime, only requiring adjustments to improve the performance of some clones, allowing them to reach a production of cuttings similar to that of other clones of the same cultivar.

### Conclusions

1. A super-dense clonal garden causes different morphological and physiological responses in Conilon coffee (*Coffea canephora*) clones.

2. Eighteen months after planting, a total cutting productivity of 1,153,388.00 cuttings per hectare is reached, and clones C2, C5, C6, C8, D1, D8, D9, J8, M2, M9, M10, and M12 show a higher average cutting production.

3. Production is 47% higher in the first cut compared with the second, and the clones with the best and worst performance in each cut generally remain the same.

4. Clones C4, J1, J4, M4, and M5 are the most sensitive to the super-dense regime, which is why it is recommended to increase the proportion of their matrix plants to compensate for their reduced vigor.

### Acknowledgments

To Fundação de Amparo à Pesquisa e Inovação do Espírito Santo (FAPES) and to Consórcio Pesquisa Café, for grants; and to Instituto Capixaba de Pesquisa, Assistência Técnica e Extensão Rural (INCAPER), for the experimental area and research structure.

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