Crop Production

Planting seasons and environments in initial field establishment of yerba mate clonal cultivars in Southern Brazil

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ABSTRACT

Despite the great economic importance of yerba mate (*Ilex paraguariensis* A. St.-Hil.), information about clonal plantations and planting conditions of this tree is still scarce. Thus, we evaluated initial field establishment of five clonal yerba mate cultivars, planted at three seasons of the year (summer, autumn, and spring) in a shaded environment, and in the autumn, we also established blocks in a full sunlight environment, to compare both cultivation environments. We evaluated plants at 3, 6, 9, and 12 months after planting, counting the surviving plants, shoots number, and measuring height and canopy diameter. At 12 months we also analyzed the caffeoylquinic acids (CQA) contents in mature leaves from different environments. Plant survival and growth were not affected by planting seasons. Cultivars Aupaba and BRS 409 had the highest survival rates in all seasons evaluated. The shaded environment provided greater survival and growth than full sunlight, also showing higher CQA levels in the leaves. Clonal cultivars Aupaba, BRS 408, and BRS 409 presented higher growth in both environments. The severe drought in the first year may have affected survival; however, growth was considered satisfactory and plants' initial establishment was better in the shaded environment.

Keywords: *Ilex paraguariensis*; clonal silviculture; shading; cultivation systems; caffeoylquinic acids.

INTRODUCTION

Yerba mate (*Ilex paraguariensis* A. St.-Hil.) is a subtropical tree native to Brazil, Argentina, and Paraguay territories (Oliveira & Rotta, 1983). The species is cultivated for the exploitation of its leaves and represents an important economic activity in these countries (Croge *et al.*, 2020); in 2020, the total production of yerba mate was 1,473,739 tons (FAOSTAT, 2022). Yerba mate leaves are traditionally used in South America to prepare hot and iced beverages (Bracesco *et al.*, 2011; Bracesco, 2019). Recently, yerba mate have become popular worldwide due to their flavor, chemical compounds, and stimulating properties (Cardozo Junior & Morand, 2016). Thus, the high demand for yerba mate leaves requires new plantations to produce high-quality raw material (Gabira *et al.*, 2020).

Yerba mate has a subtropical character, with a geographic distribution majority in Southern Brazil (Da Silva *et al.*, 2018). The southern region of Brazil is classified as having a humid subtropical climate without a dry season (Alvares *et al.*, 2013); however, the years 2019 and 2020 were extremely dry in this region. Yerba mate planting must occur when the soil is humid, preferably on cloudy days (Sturion, 1988), in the rainy season, according to each region (Daniel, 2009). Penteado Junior & Goulart (2019) suggest a preferred season for yerba mate planting between

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September and October due to higher precipitation and less chance of frosts in South Brazil. Despite the recommendations, there are no studies evaluating yerba mate planting at different seasons, even more in extremely dry years. The indication of a better season to plant yerba mate seedlings is required to ensure the success of plantations and to improve the species production systems.

Yerba mate occurs naturally in the subcanopy of subtropical rainforest with *Araucaria angustifolia* (Bertol.) Kuntze (Rakocevic & Martim, 2011), in shaded environments, especially in the early stages of its development (Daniel, 2009). Despite that, it is cultivated in natural forests, agroforestry, or monoculture systems (Penteado Junior & Goulart, 2019; Westphalen *et al*., 2020), adapted to different light conditions (Rakocevic *et al*., 2012). However, studies showed that the environment, especially light availability, strongly affects species' growth and biomass production (Coelho *et al.*, 2007; Poletto *et al.*, 2010; Caron *et al.*, 2014; Borges *et al.*, 2019; Westphalen *et al.*, 2020; Aguiar *et al*., 2022). So far, no studies evaluated the initial establishment of rooted cuttings in different field shading conditions.

To meet the increasing demand for yerba mate leaves, we need to select and adopt genotypes with better silvicultural performance, greater biomass, and secondary metabolites productivity (Vieira *et al.*, 2021). Rooted cuttings obtained by vegetative propagation of genetically superior material provide satisfactory field survival rates, biomass uniformity, and high productivity (Santin *et al.,* 2015); therefore, yerba mate clonal cultivars have been developed in recent years (Wendling *et al.* 2017a; 2017b). However, few studies evaluated clonal rooted cuttings plantation, and there are no data of field establishment of these yerba mate clonal cultivars.

We considered in this study the hypothesis that a) season influences yerba mate clonal cultivars initial establishment and growth; and b) shading condition promotes better clonal cultivars establishment. Thus, our study aimed to evaluate seasons, planting environments, and cultivars on survival and initial growth of clonal yerba mate plants in the field.

MATERIAL AND METHODS

We carried out the experiment in Pitanga, Paraná, Brazil (24º46'31" S, 51º47'17" W). The region is under two climatic domains (Rocha *et al.*, 2014), *Cfa* and *Cfb*, characterized as a subtropical climate without a dry season, with hot or temperate summer, respectively, according to the Köppen classification (Alvares *et al.*, 2013). Plantations were carried out in an environment shaded by native forest, which can be classified as an agroforestry system - yerba mate plantation in an open forest, according to Marques *et al.* (2019). The forest was composed of adult individuals of *A. angustifolia*, *Cabralea canjerana* (Vell.) Mart., *Campomanesia xanthocarpa* (Mart.) O. Berg, *Ocotea* sp. Aubl., and other Brazilian native forest species, providing 60% average shading. Plantation in a full sunlight environment was carried out without any canopy cover, in an area adjacent to the shaded planting – under the same climatic conditions.

We used precipitation data from Pitanga meteorological station (24º45'26" S, 51º45'33" W) – Hydrological Information System (SIH) of the Instituto de Águas do Paraná. Temperature data were obtained from INMET (Figure 1A), meteorological station of Inácio Martins, PR (27º34'12" S, 51º04'48" W). Total rainfall in 2019 was 1467.8 mm in Pitanga, while the average from 2008 to 2018 was 2113.3 mm, representing a deficit of 645.5 mm (Figure 1B). In the year 2020, rainfall was also below average, 1317.8 mm, a deficit of 795.5 mm, even lower than that recorded in 2019.

The three planting seasons in a shaded environment were summer, autumn, and spring, corresponding to the months of March, June, and December 2019. In autumn (traditional plantation season), we established a control plantation in a nearby area without forest cover, in full sunlight, with the same cultivars. The genotypes of *I. paraguariensis* used in this study are clonal cultivars registered within the Brazilian National Register of Cultivars (RNC), namely BRS BLD Yari (36544), BRS BLD Aupaba (36545), BRS 408 (34467), BRS 409 (34470), and a non-commercial cultivar, called Clone 4. Cultivars were propagated by mini-cuttings technique (Wendling *et al.*, 2020) and planted in the field with, on average, 20 cm ± 5 cm in height, 3 mm in stem diameter, and a well-developed root system. Due to the need to protect plants from direct solar radiation in the first months after planting (Penteado Junior & Goulart, 2019), we partially covered plants with pine laminate for up to 6 months in the full sunlight environment.

We planted rooted cuttings at 3 m x 1.5 m spacing in 20 cm deep planting holes manually opened, with the application of 400 mL hydrogel per plant. We performed a soil chemical analysis before planting to calculate fertilization recommendation, using the Ferti-Matte application (Em-

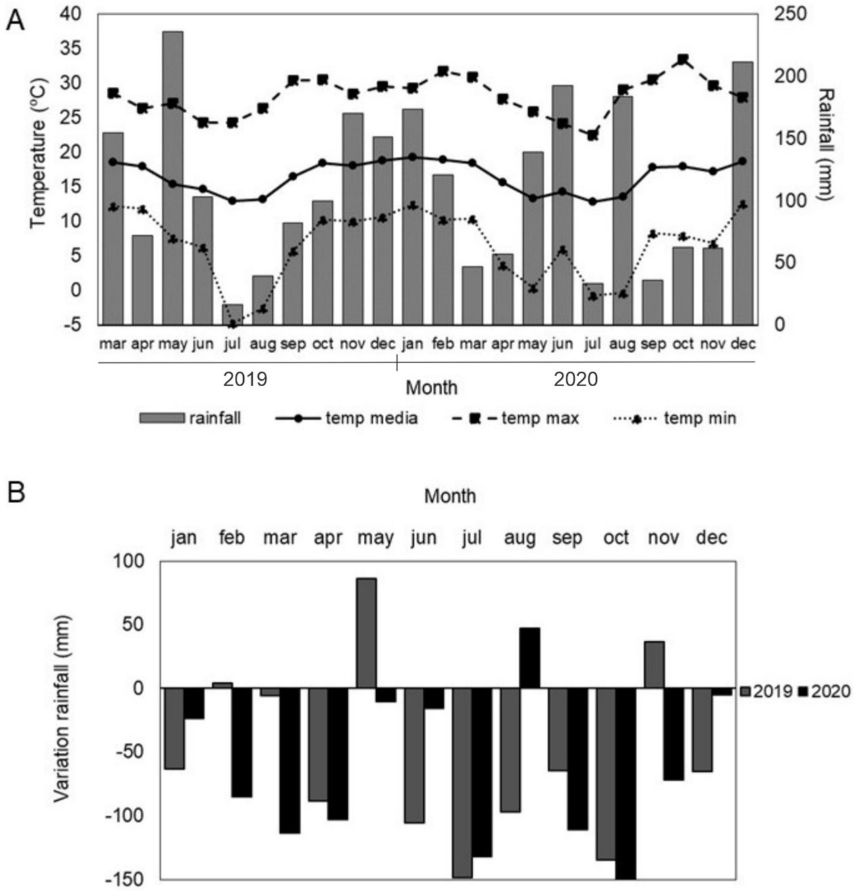

Figure 1: (A) Monthly average, maximum, and minimum temperatures, and monthly precipitation from March 2019 to December 2020; (B) Difference in monthly precipitation from January 2019 to December 2020, concerning the average precipitation between 2008-2018.

brapa, 2019): 28.0 g planting hole^{-1} MAP (monoammonium phosphate) and 4.4 t ha^{-1} calcitic limestone (PRNT 80%) applied in the total area before experiment implementation. Post-planting nutrition, recommended for January and September of each year, was not performed because if we applied this fertilization we would create a mismatch in plant growth at different seasons, which could compromise comparison between planting seasons. We maintained plantation environments with manual crowning and mowing between rows every three months. In case of precipitation absence for a period greater than 15 days, manual irrigation was performed with 2.5 - 3 L water plant^{-1}.

At 3, 6, 9, and 12 months after planting, the number of living plants, total height, canopy diameter (mean of two measurements), and number of shoots (basal and in canopy, considering apexes greater than 5 cm) were evaluated. We randomly defined three plants per repetition for evaluation, and in case of mortality, we used the subsequent plant in the next evaluations. The experiments in a 3 x 5 factorial scheme (planting seasons x clonal cultivars) and a 2 x 5 factorial scheme (planting environment x clonal cultivar) used a randomized block design, with four replications.

In September 2020, we collected mature leaves from the five clones from the autumn planting, in both full sunlight and shaded environments. Leaves from 12 plants from each treatment, three plants from each block randomly selected, were collected to form a composite sample. Within 24 hours after harvest, leaves were dried in a microwave oven (Tomasi *et al.*, 2021), crushed, and stored in a -20 ºC freezer. For caffeoylquinic acids' analyses, aqueous extracts were prepared with 500 mg of sample and type 1 ultrapure water (Milli-Q®) heated to boiling temperature. Then, extracts were homogenized in a Genie2® vortex for 15 seconds and in a heated ultrasonic bath (Ultracleaner® 1,400 A, 45 ± 5 ºC) for 30 minutes. After cooling, the extract volume was completed with ultrapure water type 1 up to 100 mL (5 mg/mL concentration) and filtered through a 0.22 µm membrane.

Analyses were performed on an Ultra-Fast Liquid Chromatograph (UFLC) equipped with an automated injector and UV detector (SPD-20A). The compounds were separated by a C18 Shim-Pack CLC-ODS (M) column (250 x 4.6 mm, 5 µm) with a Shim-Pack CLC G-ODS pre-column (100 x 4.0 mm, 5 µm), all from Shimadzu®. 20 µL of aqueous extract were injected and compounds separation occurred in a 0.5 mL min^{-1} flow, with a fixed wavelength of 280 nm. The mobile phase consisted of a gradient of water with a 0.1% acetic acid solution (solvent A) and 100% acetonitrile (solvent B). The identification of caffeoylquinic acids was performed using commercial standards (Sigma®) and quantification by analytical curve of 3-caffeoylquinic acid (CQA3). The contents of each CQA were calculated and expressed as mg of the compound per g of dry mass (mg g^{-1}); after we sum CQA3, CQA4, and CQA5, resulting in total CQA. Samples moisture was determined using an oven at 105 °C for 24 hours. We carried out the analyses in duplicate.

For statistical analyses, we verified the assumptions of normal distribution (Shapiro-Wilk test, $p < 0.05$) and homogeneity of variances (Bartlett test, $p < 0.05$). When necessary, the data were transformed with Box-cox. After further verification, we performed ANOVA and Scott-Knott test ($p < 0.05$). We performed the statistical analysis and graphs using the R software (R Core Team, 2023), with MASS, ExpDes.pt, and ggplot2 packages.

RESULTS

Plants growth in height and canopy diameter through the first year after planting is represented in Figure 2, where we can observe a significant increase in both variables. The clone BRS 409, for example, when planted in summer presented 186.89% increment in height and 127.69% in canopy diameter from the 3rd to the 12 months. Growth is more accentuated from the 6th month after planting, especially in cuttings planted during summer and autumn, indicating that higher temperatures and rainfall superior to 100 mm, especially between October 2019 and February 2020, favored plant growth. There is a difference in growth

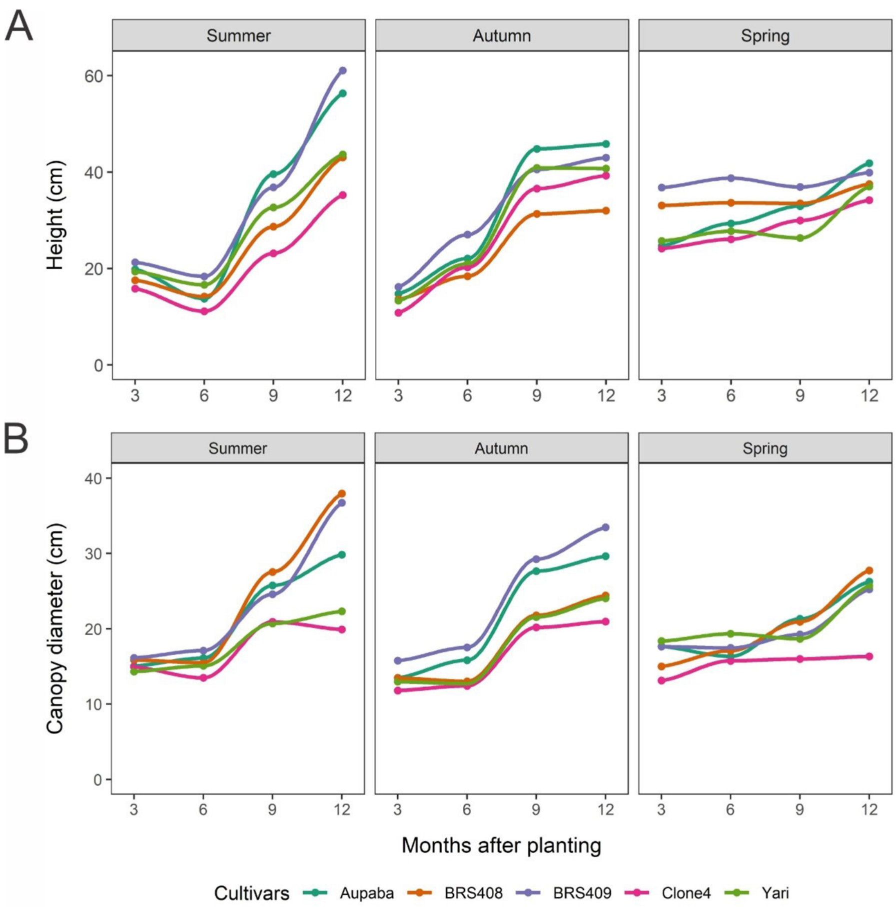

Figure 2: Growth in height (A) and canopy diameter (B) of yerba mate clonal cultivars through 12 months after planting in the field, in different planting seasons.

among the cultivars in each planting season; in summer, for example, cultivar BRS 409 presented 61.08 cm in height after 12 months while cultivar Clone 4 presented 35.23 cm. In general, cultivars BRS 409 and Aupaba presented the highest averages in height and in canopy diameter.

The analysis of variance was performed considering the factors clonal cultivar and planting season for seedlings survival and the growth variables at 12 months after planting (Table S1). We did not obtain significant differences between planting seasons and there was no interaction between factors. The factor clonal cultivar was significant ($p < 0.05$) for all variables. Clonal cultivars Aupaba and BRS 409 presented superior survival and height averages (Table 1). Mean survival was 78.33% for Aupaba and 73.33% for BRS 409, and average height was 48.00 cm for both clonal cultivars. For canopy diameter, Clone 4 presented the lowest average (19.04 cm), significantly different from the other clonal cultivars. For number of shoots, the clonal cultivars Aupaba, BRS 408, and BRS 409 presented the highest averages – 5.05, 5.12, and 6.42, respectively.

As an effect of different environmental conditions, we observed a more accentuated growth of seedlings planted in the shaded environment, especially from the 6th month after planting (Figure 3). The increment in height for the clonal cultivar Aupaba was 209.86% in the shaded environment, while in the full sunlight environment this increment was 106.83%, considering the period between 3 and 12 months after planting. The same growth tendency was observed for the variable canopy diameter, with 119.71% increment in the shaded environment and 24.80% in the full sunlight environment. In the full sunlight environment, the cultivar BRS 408 stands out compared to the others for height, with an average 37.92 cm 12 months after planting; nonetheless, this cultivar presented the lowest height average in the shaded environment – 32.00 cm 12 months after planting.

At 12 months after planting, there was a significant difference in survival between both planting environments (Table S2). The highest survival (59%) was observed in the shaded environment, while in the full sunlight the mean survival was 36% (Table 2). For the height, there was a significant difference between planting environments and an interaction between both factors (clonal cultivar and planting environment). The clonal cultivars BRS 408, BRS 409, and Clone 4 did not differ significantly between both

Table 1: Survival, height, canopy diameter, and number of shoots of yerba mate clonal cultivars planted in 3 planting seasons, evaluated 12 months after planting

Planting seasons	Clonal cultivars				
	Aupaba	**BRS 408**	**BRS 409**	**Clone 4**	**Yari**
			Survival (%)		
Summer	90.00 ± 8.16	65.00 ± 31.09	77.50 ± 9.57	57.50 ± 18.93	75.00 ± 23.80
Autumn	57.50 ± 34.03	65.00 ± 12.91	65.00 ± 20.82	57.50 ± 22.17	50.00 ± 11.55
Spring	87.50 ± 9.57	60.00 ± 11.55	77.50 ± 18.93	45.00 ± 17.32	60.00 ± 14.14
Mean	78.33 a	63.33 b	73.33 a	53.33 b	61.67 b
			Height (cm)		
Summer	56.33 ± 32.25	43.05 ± 19.14	61.08 ± 33.43	35.23 ± 20.69	43.67 ± 21.03
Autumn	45.83 ± 17.68	32.00 ± 17.64	43.00 ± 18.22	39.29 ± 18.80	40.75 ± 11.46
Spring	41.83 ± 15.25	37.50 ± 8.18	39.92 ± 10.65	34.17 ± 20.33	37.00 ± 18.81
Mean	48.00 a	37.19 b	48.00 a	36.26 b	40.47 b
			Canopy diameter (cm)		
Summer	29.84 ± 14.91	37.94 ± 14.78	36.73 ± 20.01	19.90 ± 7.41	22.31 ± 8.46
Autumn	29.62 ± 14.83	24.42 ± 10.80	33.46 ± 18.50	20.96 ± 8.86	24.04 ± 8.58
Spring	26.25 ± 10.26	27.75 ± 14.17	25.25 ± 9.16	16.33 ± 8.26	25.71 ± 11.86
Mean	28.57 a	29.57 a	31.81 a	19.04 b	24.02 a
			Number of shoots		
Summer	5.33 ± 3.72	4.90 ± 3.81	7.67 ± 4.48	2.91 ± 2.12	3.50 ± 1.44
Autumn	4.25 ± 4.88	4.25 ± 3.28	6.08 ± 5.21	2.42 ± 1.50	2.33 ± 1.50
Spring	5.58 ± 3.29	6.17 ± 3.93	5.50 ± 3.75	3.67 ± 2.67	4.83 ± 3.51
Mean	5.05 a	5.12 a	6.42 a	3.00 b	3.55 b

Different small letters represent significant differences between clonal cultivars by the Scott-Knott test ($p < 0.05$).

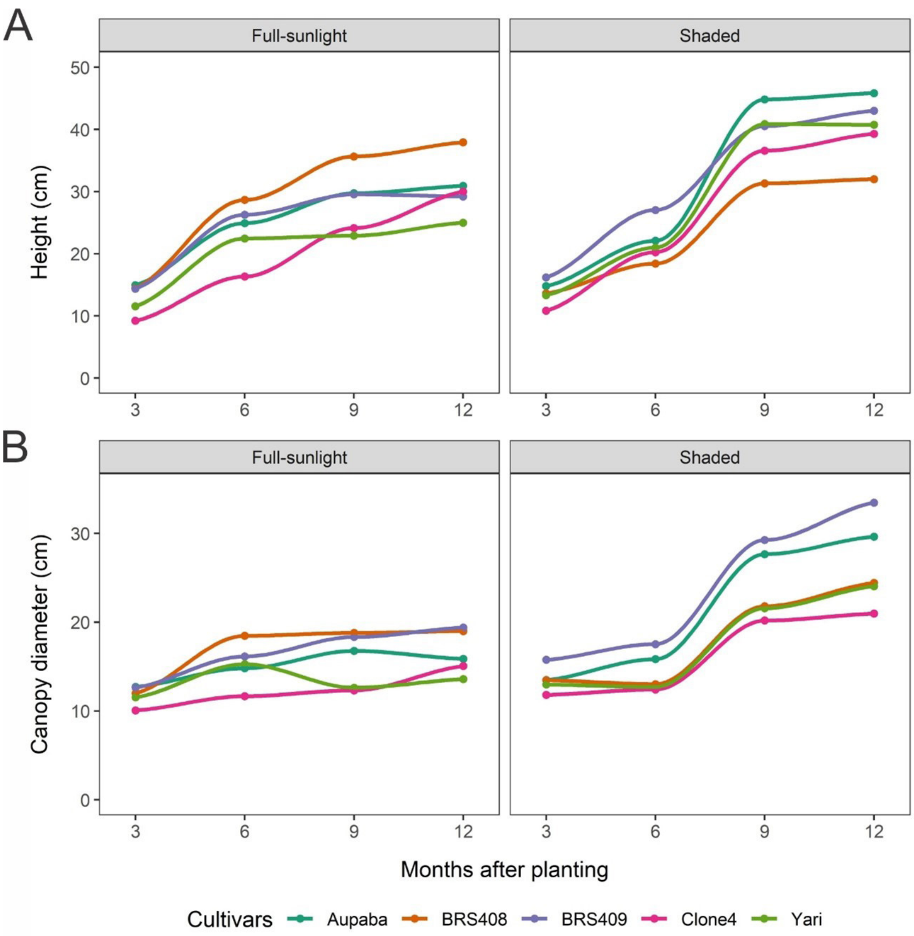

Figure 3: Growth in height (A) and canopy diameter (B) of yerba mate clonal cultivars through 12 months after planting in the field, in different planting environments.

Table 2: Survival, height, canopy diameter, and number of shoots of yerba mate clonal cultivars in different planting environments, 12 months after planting performed in the autumn

Planting environments	Clonal cultivars					
	Aupaba	**BRS 408**	**BRS 409**	**Clone 4**	**Yari**	**Mean**
			Survival (%)			
Full sunlight	36.67 ± 15.27	43.33 ± 11.55	30.00 ± 10.00	30.00 ± 0.00	40.00 ± 10.00	36.00 B
Shaded	57.50 ± 34.03	65.00 ± 12.91	65.00 ± 20.82	57.50 ± 22.17	50.00 ± 11.55	59.00 A
			Height (cm)			
Full sunlight	30.91 ± 11.37 aB	37.92 ± 13.84 aA	29.22 ± 10.19 aA	30.00 ± 11.34 aA	25.00 ± 10.84 aB	-
Shaded	45.83 ± 17.68 aA	32.00 ± 17.64 aA	43.00 ± 18.22 aA	39.29 ± 18.80 aA	40.75 ± 11.46 aA	-
			Canopy diameter (cm)			
Full sunlight	15.86 ± 6.18	19.00 ± 5.37	19.39 ± 5.84	15.05 ± 3.56	13.58 ± 7.02	16.56 B
Shaded	29.62 ± 14.83	24.42 ± 10.80	33.46 ± 18.50	20.96 ± 8.86	24.04 ± 8.58	26.50 A
Mean	23.04 a	21.60 a	27.43 a	18.43 b	18.81 b	
			Number of shoots			
Full sunlight	2.45 ± 1.69	3.38 ± 1.94	2.55 ± 1.33	1.77 ± 0.67	1.92 ± 1.24	2.46 B
Shaded	4.25 ± 4.88	4.25 ± 3.28	6.08 ± 5.21	2.42 ± 1.50	2.33 ± 1.50	3.87 A
Mean	3.39 a	3.80 a	4.57 a	2.14 b	2.12 b	

Different small letters represent significantly diferences between clonal cultivars and different capital letters represent significantly differences between planting environments by the Scott-Knott test ($p < 0.05$).

environments, while the clonal cultivars Aupaba and Yari presented superior mean height in the shaded environment. For canopy diameter and number of shoots, both factors presented significant differences, but there was no interaction between them. The highest averages in canopy diameter and number of shoots were obtained in the shaded environment, with averages over 60% higher comparing to the plants in full sunlight environment. For both variables, the clonal cultivars Aupaba, BRS 408, and BRS 409 presented the highest averages.

Regarding total caffeoylquinic acids, significant effects are observed for both the environment and cultivar, while no significant interaction is observed between these factors (Table S3). Yerba mate leaves from the shaded environment had higher CQA contents when compared to those harvested in full sun (Figure 4A). Among the clonal cultivars, Yari and Clone 4 stood out with the highest CQA content (Figure 4B). Cultivar BRS 408 had the lowest CQA content, 30% lower than Yari. Pearson's correlation between CQA and the other variables was only significant for survival ($p = 0.0177$), with a coefficient of 0.72.

DISCUSSION

Planting seasons did not influence survival and plant growth at 12 months after planting. While yerba mate cultivars differed significantly in survival and in all growth variables, indicating that plants' establishment is more dependent on the genotype than the planting season. The cultivars Aupaba and BRS 409 presented the highest average survival, over 70% at 12 months after planting; contrary, the clonal cultivar Clone 4 presented 53.33% survival. In a study by Santin *et al.* (2015), seedlings average survival was 90% at 12 months after planting. The lower survival observed in our study may be related to the long period of rainfall deficit in 2019 and 2020, which hindered plants establishment in the field. Plant water status immediately after planting is crucial for its establishment after field planting, especially for ensuring root growth (Burdett, 1990). Also, yerba mate is known for requiring a strict regimen of annual rainfall – higher than 1,200 mm, good rainfall distribution throughout the year, and high air and soil humidity (Heck & de Mejia, 2007; Croge *et al.*, 2020); thus, this species is considered drought-intolerant during certain periods (Da Silva *et al.*, 2018).

Cultivars significantly influenced plant growth. We observed maximum mean heights for cultivars Aupaba and BRS 409 – both 48.00 cm – and minimum for Clone 4 – 36.26 cm. Santin *et al.* (2015) obtained a plant height of 35 cm at 12 months after planting, evaluating yerba mate genotypes from different origins. Thus, we can consider that all cultivars evaluated in our study presented satisfactory growth, despite water stress conditions in some periods. Yerba mate tends to close stomata at a relatively high plant water status (Acevedo *et al.*, 2019); it is a strategy to increase species tolerance to water deficit and increase water use efficiency, although missing out potential C assimilation even when environmental conditions are still favorable (Deans *et al.*, 2019). The water deficit also limits root growth because of low photosynthetic rates, and in the future the poor root system may limits water uptake and photosynthesis (Burdett, 1990).

The differences observed in our study on survival and growth among clonal cultivars can be an indicative of genotypes more tolerant to water deficit. To date, there are no records of yerba mate genotypes evaluated under water stress; thus, studies must be carried out with water availability with different genotypes to clarify yerba mate responses to these environmental conditions. Gortari *et al.* (2020) observed a decrease in yerba mate seedlings growth when submitted to low water availability conditions; it indicates that this species growth is highly sensitive to water deficits, which negatively affects plant physiology and, consequently, carbon assimilation (Kumarathunge *et al.*, 2020). Drought tolerance is not only important in natural forests, but it is also essential for perennials plantations, such as yerba mate, where harvested products are mainly the leaves (Acevedo *et al.*, 2019). In South America, climate changes simulations indicate an increase in temperature and significant changes in the distribution and intensity of precipitation under different scenarios, shaping future flood and drought events (Chou *et al.*, 2014). We emphasize that the last years were uncommonly dry, with annual rainfall deficit of more than 600 mm, and it is an indicative of future scenarios for this region due to climatic changes.

We obtained higher survival, canopy diameter, and number of shoots in plants grown in the shaded environment. Yerba mate is a species that occurs naturally in shaded environments, so the growth of young plants can demand a shading level that imitates the natural understory conditions (Rakocevic *et al.*, 2012). Poletto *et al.* (2010) also observed that shaded environments favor yerba mate growth, especially under higher shading levels. For Borges *et al.* (2019), yerba mate plant height did not differ between canopy cover classes in agroforestry systems until the second year after planting, but plants under higher cover rates produced more biomass.

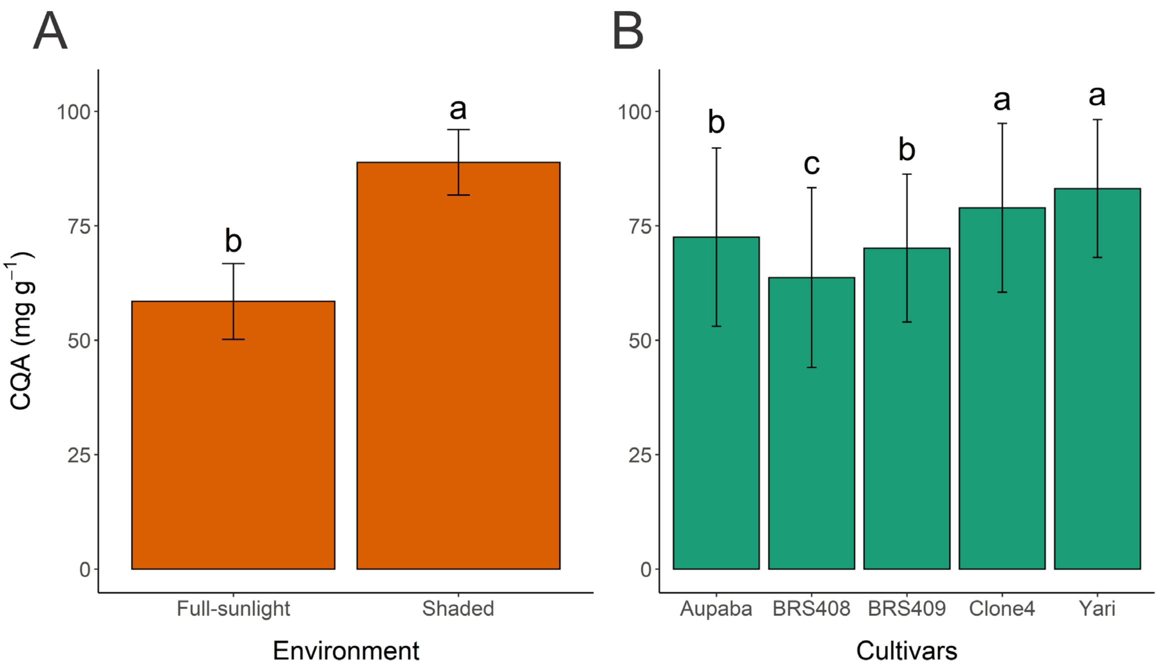

Figure 4: Total caffeoylquinic acids content (CQA - sum of 3CQA, 4CQA and 5CQA contents) in yerba mate mature leaves, as a function of (A) planting environments and (B) cultivars. Different letters indicate a significant difference by Scott-Knott test at 5% probability of error.

Plants absorb sunlight to power the photochemical reactions of photosynthesis; however, high light incidence has the potential to damage the photosynthetic machinery, primarily photosystem II (PSII), thus causing photoinhibition, limiting plant photosynthetic activity, growth, and productivity (Takahashi & Badger, 2011). Furthermore, high light and especially ultraviolet light (UV) can cause stress and damages to DNA, proteins, and other cellular components (Müller-Xing *et al.*, 2014). Phenolic compounds, in particular caffeoylquinic acids, are known to provide protection from excessive UV radiation (Karaköse *et al.*, 2015; Moyo *et al.*, 2022); these compounds are present in high concentrations in yerba mate (Lima *et al.*, 2016; Mateos *et al.*, 2018; Butiuk *et al.*, 2021). We expected a higher concentration of CQA in the full sunlight environment (Dartora *et al.*, 2011; Heck *et al.*, 2008) for protection from direct UV radiation, a result that was not observed. However, the strong relation between survival and CQA content may indicate that these compounds played an important role in plant establishment during the first year in the field.

Despite better growth results in shaded environment, we highlight that yerba mate is classified as a "less shade tolerant" species and may present a shade avoidance behavior (Rakocevic *et al.*, 2012). High shading levels, above 70%, reduce growth and biomass production of yerba mate (Coelho *et al.*, 2007; Caron *et al.*, 2014; Westphalen *et al.*, 2020; Aguiar *et al.*, 2022), not being recommended to ensure long-term economically viable production (Aguiar *et al.*, 2022). Caron *et al.* (2014) – 85% shading, and Coelho *et al.* (2007) – 95% shading. Caron *et al.* (2014) highlighted that despite the higher biomass production in full sun, shade cultivation resulted in greater efficiency in using solar radiation to accumulate leaves dry biomass. Thus, the moderate shading (60%) promoted by the canopy trees in our study may have increased solar radiation use efficiency by plants and favored initial growth. Few studies have been developed about yerba mate plant establishment and initial growth in the field, especially with clonal genotypes. In our study, the initial establishment of plants was favored by shading, although future harvests will confirm if the environmental conditions influence on leaves productivity.

The genetic effect highly influences species establishment, and our study is the first one that isolated the genetic effect using yerba mate clonal cultivars in a field planting. In general, the smallest averages of survival and growth variables were presented by Clone 4, which is not a commercial cultivar so far. Our results indicate that this cultivar does not grant as good initial growth as the others used in our study or that it is not suitable for local environmental conditions. We also emphasize that cultivars must be evaluated in each region before investing in commercial yerba mate plantations, watching for the most vigorous genotypes according to local edaphoclimatic conditions.

Based on our results, we consider cultivars BRS 408, BRS 409, and Aupaba acclimatized adequately to our study environmental conditions, conferring excellent vigor and potential to be planted in the region or under similar environmental conditions. As these genotypes were selected for high biomass productivity (Wendling *et al.* 2017a; 2017b), we already expected that they would show a faster growth and shoot development. Plantation success depends on rooted cuttings that must withstand climatic adversities, present high survival rates, and show good shoot vigor and leaf biomass production (Wendling *et al.*, 2020), as presented by these cultivars. Future studies will prove whether these genotypes will remain superior over subsequent production years and how they will develop under differentiated climatic conditions. We also emphasize the difference between cultivars in terms of CQA levels, indicating that some genetic materials such as Yari and Clone 4 have greater potential for industrial extraction of these phenolic compounds, with high pharmaceutical and commercial value (Butiuk *et al.*, 2021).

CONCLUSIONS

Our study presents unpublished information about yerba mate cuttings in field conditions. There was no significant difference in plant survival and growth between planting seasons, and a significantly difference was observed between cultivars. In general, clonal cultivars Aupaba, BRS 408, and BRS 409 stand out for growth variables, being more vigorous and showing good potential to be planted under similar edaphoclimatic conditions. The shaded environment provides better initial establishment than full sunlight; the greater plant survival in the shade is positively correlated with the total caffeoylquinic acid content. Our results indicate the importance of considering adverse environmental conditions, such as drought, in the silviculture of yerba mate, focusing on silvicultural practices and breeding programs.

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Supplementary Table S1: Analysis of variance (ANOVA) of yerba mate plants 12 months after planting as a function of blocks, planting seasons, and cultivars

Survival					
Source of variation	**DF**	**SS**	**MS**	**Fc**	**Pr > Fc**
Block	3	12.40	5	1.13	0.34
Planting season	2	19.60	6	2.69	0.08
Cultivar	4	47.07	2	3.23	0.02*
Planting season x Cultivar	8	28.23	3	0.97	0.47
Error	42	153.10	4		
Total	59	260.40	1		
Height					
Block	3	2.266	4	1.74	0.16
Planting season	2	1.61	5	1.85	0.16
Cultivar	4	4.88	6	2.81	0.03*
Planting season x Cultivar	8	2.71	2	0.78	0.62
Error	159	69.09	3		
Total	173	80.55	1		
Canopy diameter					
Block	3	2.23	4	2.14	0.09
Planting season	2	1.58	5	2.27	0.11
Cultivar	4	9.50	6	6.84	0.00004*
Planting season x Cultivar	8	2.84	3	1.02	0.42
Error	159	55.21	2		
Total	176	71.36	1		
Number of shoots					
Block	3	104.68	4	2.99	0.03*
Planting season	2	55.40	3	2.37	0.09
Cultivar	4	264.82	5	5.68	0.00027*
Planting season x Cultivar	8	56.71	6	0.61	0.77
Error	159	1853.52	2		
Total	176	2335.13	1		

DF: degrees of freedom; SS: sum-of-squares; MS: mean square; Fc: F value. *Significant at $p < 0.05$.

Supplementary Table S2: Analysis of variance (ANOVA) of yerba mate plants 12 months after planting as a function of blocks, planting environments, and cultivars

Source of variation	**DF**	**SS**	**MS**	**Fc**	**Pr > Fc**
			Survival		
Block	3	0.16	2	0.36	0.78
Planting environment	1	2.09	6	13.84	0.001*
Cultivar	4	0.28	3	0.47	0.76
Planting environment x Cultivar	4	0.31	4	0.52	0.72
Error	22	3.32	5		
Total	34	6.17	1		
			Height		
Block	3	1.26	4	1.04	0.38
Planting environment	1	4.13	6	10.23	0.002*
Cultivar	4	0.84	2	0.52	0.72
Planting environment x Cultivar	4	4.94	5	3.05	0.02*
Error	101	40.82	3		
Total	113	52.00	1		
			Diameter		
Block	3	0.60	2	0.47	0.70
Planting environment	1	12.61	6	29.57	0.00*
Cultivar	4	4.53	5	2.65	0.04*
Planting environment x Cultivar	4	1.95	4	1.14	0.34
Error	101	43.08	3		
Total	113	62.79	1		
			Number of shoots		
Block	3	1.78	4	2.22	0.09
Planting environment	1	1.25	5	4.68	0.03*
Cultivar	4	5.49	6	5.13	0.0008*
Planting environment x Cultivar	4	0.62	2	0.58	0.68
Error	101	27.05	3		
Total	113	36.20	1		

DF: degrees of freedom; SS: sum-of-squares; MS: mean square; Fc: F value. *Significant at $p < 0.05$.

Supplementary Table S3: Analysis of variance (ANOVA) of total caffeoylquinic acids (CQA) in yerba mate mature leaves, as a function of planting environments and cultivars

		CQA			
Source of variation	**DF**	**SS**	**MS**	**Fc**	**Pr > Fc**
Planting environment	1	4621.0	5	417.97	< 0.001*
Cultivar	4	923.4	4	20.88	< 0.001*
Planting environment x Cultivar	4	45.1	3	1.02	0.44241
Error	10	110.6	2		
Total	19	5700.1	1		

DF: degrees of freedom; SS: sum-of-squares; MS: mean square; Fc: F value. *Significant at $p < 0.05$.