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Full length article

Response to applying kaolinite particles in coffee variety Cenicafé 1 seedlings during the nursery stage

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ABSTRACT

The application of kaolinite has been shown to be a viable strategy for protecting crops against excessive solar radiation and high temperatures. In coffee, this could occur during the nursery stage when the seedlings grow directly in full sun. The objective of the present study was to evaluate the effects of kaolinite particles when applied to coffee seedlings in full sun during the nursery stage and those of seedlings with no kaolinite applied grown directly in full sun and in shade. Two *Coffea arabica* L. variety Cenicafé 1 nurseries were established, and the following treatments were established: 1) K30: full sun with kaolinite at 30 g/L; 2) K60: full sun with kaolinite at 60 g/L; 3) SUN: full sun without kaolinite; and 4) SHADE: under a sunscreen with 60 % shade without kaolinite. The studied variables were dry mass, dry mass distribution, leaf characteristics, leaf physiological parameters, albedo, leaf temperature, and seedling mortality. Except for mortality, the variables were analyzed using a simple factorial design in generalized complete blocks and randomly using the Welch-James statistical test with bootstrapping. Mortality was assessed using a logistic binary response model with a X^2 test. The results showed that in comparison to the SHADE treatment, the SUN and K60 treatments resulted in more dry mass; in turn, the K60 treatment resulted in better RDM/TLA. In comparison to the SHADE treatment, both the K30 and K60 treatments resulted in greater increases in net photosynthesis rates, and in comparison, to the SUN treatment, these treatments also resulted in a greater increase in stomatal conductance, although only when the leaf abaxial temperature was greater than 30 °C. This result may have occurred based on the greater light reflection and the lower leaf temperatures obtained with kaolinite, in the K60 treatment. However, the seedlings that grew in full sun with and without kaolinite died three times more often than the seedlings in the shade. The previous suggests that it is not convenient to establish nurseries in full sun, with or without kaolinite, despite the physiological advantages and tolerance to high temperatures at the plant level.

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Abbreviations: A, net photosynthesis rate of CO₂ ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); Alb, albedo ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$); C_i, internal concentration of CO₂ ($\mu\text{mol CO}_2 \text{ mol}^{-1} \text{ air}$); dat, days after transplantation; g_s, stomatal conductance to water vapor ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$); LAR, leaf area ratio ($\text{m}^2 \text{ kg}^{-1}$); LDMF, leaf dry mass fraction (g/g); LN, leaf number; In, natural logarithm; LUR, leaf unit rate ($\text{gm}^{-2} \text{ d}^{-1}$); MP, mortality probability; PAR, photosynthetically active radiation ($\mu\text{mol of photons m}^{-2} \text{ s}^{-1}$); RDM/TLA, root dry mass/total leaf area ($\text{g/m}^{-2}(-)$); RDMF, root dry mass fraction (g/g); RGR, relative growth rate ($\text{gg}^{-1} \text{ d}^{-1}$); SDMF, stem dry mass fraction (g/g); SLA, specific leaf area ($\text{m}^2 \text{ kg}^{-1}$); SRR, shoot-to-root ratio (g/g); TDM, Total dry mass (g); TLA, total leaf area (cm^2); T_{leaf_abaxial}, leaf temperature in the abaxial part (°C); T_{leaf_adaxial}, leaf temperature in the adaxial part (°C); WUE, water use efficiency in reference to transpiration ($\text{mmol CO}_2 \text{ mol}^{-1} \text{ H}_2\text{O}$).

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1. Introduction

Of the 124 species in the genus *Coffea* (Davis et al., 2011), *Coffea arabica* L. accounts for 58.6 % of the coffee consumed in the world, and its plantations are present in more than 68 countries located between 22°N and 24°S (Lashermes et al., 2008; ICO, 2021). *C. arabica* L. originates from the tropical rainforests of Ethiopia, Kenya, and Sudan (altitude of 1600–2800 m, average air temperature between 18 and 22 °C, and precipitation between 1600 and 2000 mm), where it evolved as a medium-sized tree (Davis et al., 2006). It can live up to 100 years in its natural habitat, while on plantations, its lifespan can be between 30 and 40 years (Gokavi et al., 2019).

Arabica coffee grows in shaded or semishaded forest environments, which is why it has been traditionally classified as a shade-tolerant species (Ayalew, 2018) that requires high and somewhat dense tree cover (Taye and Burkhardt, 2013). However, modern coffee cultivars grow well with full sun exposure and can even have higher yields than cultivars in the shade (DaMatta et al., 2010). Thus, coffee plants are able to acclimate to different light environments due to their plasticity (Cavatte et al., 2012), plant (Rakocevic et al., 2018), and canopy (Rakocevic et al., 2021) characteristics. The leaves of modern coffee crops do not meet all the requirements to be true sun leaves or true shade leaves (Matos et al., 2009). Their behavior is more consistent with shade-tolerant species (Rodríguez-López et al., 2014).

Coffee seedlings are usually produced in nurseries covered with plastic mesh that can block approximately 50 to 75 % of photosynthetically active radiation (PAR), achieving values between 400 and 700 μmol of photons $\text{m}^{-2} \text{s}^{-1}$, which corresponds to the light saturation point in coffee (Matiello et al., 2010; Rakocevic et al., 2021; León-Burgos et al., 2022). Given the theory of functional equilibrium, under shaded conditions, a greater partition of dry matter is observed toward the bud and in particular the leaves than toward other organs (root, trunk, and branches), a fact that directly affects the leaf area (Poorter et al., 2012). In comparison to a smaller leaf dry mass fraction, a higher leaf dry mass fraction indicates a rapid expansion of the leaf area to capture light (Tadesse, 2021), indicating that in comparison to seedlings in the full sun, seedlings in the shade have larger leaves and are greener, which can imply a false perception of greater vigor (Moraes et al., 2010). However, seedlings in the shade can present photooxidative damage when transplanted to the field (Moraes et al., 2010).

On the other hand, some nurseries produce seedlings in full sun to reduce production costs and produce seedlings more acclimated to field conditions after transplanting (Paiva et al., 2003), but scientific studies that demonstrate the advantages of using this method are scarce (Tatagiba et al., 2010). According to Moraes et al. (2010), the amount of dry matter in the seedlings grown in full sun was similar to that found in seedlings grown in the shade, although the foliar photosynthetic performance of the plants grown in full sun was higher net photosynthesis rate of CO_2 (A) and higher leaf unit rates (LUR). In comparison to leaves grown in the shade, leaves grown in full sun have higher A in the presence of light saturation (DaMatta, 2004) and a greater demand for water, which consequently gives the leaves directly exposed to the sun a greater hydraulic conductance per unit of leaf area (Carins Murphy et al., 2012).

The hydraulic conductance of a leaf has shown a strong and positive association with stomatal conductance to CO_2 (g_s) (Sack and Frole, 2006). Similarly, seedlings that grow in full sun are less susceptible to both turnover and photooxidative damage, which decreases the need to replace dead or poorly developed plants after transplanting them to the field (Silva et al., 2000). Therefore, determining the strengths and weaknesses of the different methods used to produce seedlings is necessary so that healthy and well-

developed seedlings can be developed for the establishment of new crops (Tatagiba et al., 2010). In addition, exploring new methods that allow the production of quality seedlings needs to occur, and the additional benefits of new methods need to be compared with those of traditional methods (full sun and shade). An alternative method that should be explored is the application of kaolinite to seedlings being grown in full sun, given that kaolinite can reduce the impacts of heat and excessive light on these seedlings (Steiman et al., 2007; Santos et al., 2021).

The use of kaolinite in food production has been certified by the Institute for the Review of Organic Materials (OMRI, 2021). The refractive properties of kaolinite as a particle film, the effect of kaolinite on leaf and canopy temperature, transpiration, and photosynthesis (Sharma et al., 2015; Mphande et al., 2020), and its effect on production through decreases in fruit temperature (Brito et al., 2019) have been investigated since 1970. In general, the absorption of PAR appears to be reduced by between 20 and 40 % with kaolinite (Rosati et al., 2007; Nanos, 2015). The kaolinite particles improve plant water status under stress conditions, regulate the leaf temperature, and increase light inside the canopy which results in carbon gains at the entire plant scale, but it could reduce the net photosynthesis, stomatal conductance, and transpiration at the leaf scale (Glenn, 2009, 2010; Boari et al., 2015). Similarly, in comparison to unprotected plants, plants protected with kaolinite have a lower initial fluorescence (F_0), a higher maximum quantum production of photosystem II (Fv/Fm), and a higher photosynthetic index (PI) (de Abreu et al., 2022). It allows plants with kaolinite to minimize possible damage to their photochemical apparatus and prevent stomatal closure, which would consequently result in higher A values. Research on a wide range of crops and agricultural environments used 30 to 60 g/L of kaolinite (Steiman et al., 2007) with densities of 1 to 4 g m^{-2} of leaf area (Glenn, 2012).

In *C. arabica* variety “Tipica” plants, two and three years after pruning, Steiman et al. (2007) observed that leaf surfaces were 3.4 °C lower when kaolinite was applied and that A increased by 75 %, not even declining after the morning, as occurs with plants in full sunlight, where temperatures are higher than optimal and/or high solar radiation reduces photosynthesis in the leaves. The application of kaolinite during the transition of *C. arabica* and *Coffea canephora* seedlings from the nursery to the field in full sun is a management strategy that has been shown to prevent excessive solar radiation and high temperatures from affecting seedlings that are older than 22 days, resulting a temperature decrease of between 5.6 and 6.7 °C (de Abreu et al., 2022). Acclimatization of seedlings to both irradiance and water supply could decrease seedling mortality and improve growth after transplantation (DaMatta et al., 2018). However, studies in coffee seedlings with kaolinite in nurseries under the full sun are scarce. Therefore, the objective of the present study was to evaluate the effect of applying kaolinite particles to coffee seedlings in full sun during the nursery stage and compare the effects to those of seedlings grown directly in full sun and in the shade without kaolinite in terms of growth, gas exchange, PAR reflection, leaf temperature, and mortality probability (MP).

2. Materials and methods

2.1. Location and plant material

The research was conducted at the Paraguaicito de Cenicafé experimental station (04°24'N, 75°44'W, 1203 m), located in the department of Quindío (Colombia). At the site, two germinators of *C. arabica* L. variety Cenicafé 1 were established with approximately 4000 seeds in each in June 2018 and February 2019 until

the emerging seedlings presented their fully extended cotyledonal leaves (BBCH10 stage) approximately 60 days after sowing seeds. Then, they were transplanted into 2 kg polyethylene bags (17 × 23 cm storage bag). The BBCH phenological state was categorized according to the scale of [Arcila et al. \(2002\)](#). The plant material in the germinators was used to construct the first nursery [transplant date: 09/11/2018 - end date: 02/20/2019, maximum air temperature: 29.9 °C, mean air temperature: 22.0 °C, minimum air temperature: 17.3 °C, rainfall: 1157 mm] and second nursery [transplant date: 05/12/2019 - end date: 5/11/2019, maximum air temperature: 30.5 °C, mean air temperature: 22.3 °C, minimum air temperature: 17.3 °C, rainfall: 672 mm]. The agrometeorological parameters were recorded using a Remote Automated Weather Station, Fire Weather (RAWS-F), Campbell Scientific® installed at 100 m from the experiments. Immediately after transplanting, the seedlings of each nursery (4000) were randomly separated into four groups, each with 1000 seedlings, and of these, 800 seedlings were considered effective in the plots that were subsequently formed.

2.2. Treatments

Four seedling groups were subjected to the following treatments: 1) K30: full sun with kaolinite applied at 30 g/L, 2) K60: full sun with kaolinite applied at 60 g/L, 3.) SUN: sun without kaolinite, and 4) SHADE: under a 60 % shade mesh ([Fig. 1](#)). For each experiment, the seedlings were maintained for 5.4 to 5.9 months of expo-

sure in the treatments, at which time they were ready for transplantation to the field when first pair of primary branches are visible (BBCH20 stage). In the K30 and K60 treatments, the leaves of the plants were sprayed two times in every application with purified kaolinite particles (Surround® WP, TK Inc., AZ, United States) until a uniform protective film was obtained.

To maintain the protective kaolinite layer over time, the applications were conducted two to three times per week throughout the duration of the experiment, according to the percentage of particle coverage, which was visually inspected in 50 leaves randomly selected by treatment. The application was carried out with a manual pump operated at two bars of pressure using a uniform flat jet nozzle (TP8001SE; TeeJet®, IL, United States) at a distance of 70 cm from the leaves. Edaphic fertilization in the treatments was performed according to the technical recommendations for seedlings with 2 g of DAP (18–46–00), applied at two, three, and four months ([Sadeghian, 2022](#)), while the integrated management of weeds, pests, and diseases was performed according to the specifications of [Gaitán et al. \(2011\)](#). Plant irrigation was carried out two to three times weekly according to the observed needs.

In each nursery, the evaluation of the variables was performed at two sampling times: the first sampling time (ST1) occurred between 71 and 84 days after transplantation (dat), when 50 % of the plants reached the BBCH13–14 stage (two leaf pairs unfolded), and the second sampling time (ST2) was performed when 50 % of the plants reached the BBCH15–16 stage (five to six leaf pairs unfolded) between 162 and 177 dat.



Fig. 1. *Coffea arabica* var. Cenicafé 1 seedlings grown under a 60 % shade mesh (a), in full sun without kaolinite (b) and in full sun with kaolinite (c). Image of a traditional nursery with seedlings under a 60 % shade mesh (d) and nurseries in full sun with seedlings without kaolinite (left) and with kaolinite applied at 30 g/L and 60 g/L (right). (e).

2.3. Measurements of gas exchange in leaves and abaxial leaf temperature

The physiological parameters, A , g_s , internal concentration of CO_2 (C_i), transpiration rate (E) and water use efficiency (WUE) [A/E] were measured between 07:00 and 10:00 in ten healthy and completely expanded leaves of the second or third orthotropic node counted from the apex for ten different plants. Parallel to the physiological measurements, the abaxial temperature of the leaf ($T_{leaf_abaxial}$) was recorded. The gas exchange measurements were conducted in each nursery during ST2 with a CIRAS-3 open system infrared gas analyzer (PP Systems, Amesbury, MA, USA) using two approaches: maintaining the $T_{leaf_abaxial}$ at 25 °C and maintaining it at 30 °C, which is the temperature of the cuvette ($T_{cuvette}$), with the concentration of CO_2 in the chamber ($400 \mu mol CO_2 mol^{-1}$). $T_{cuvette}$ simulates the conditions of the maximum average air temperature on a very hot day. For both approaches, the irradiance was $1000 \mu mol photons m^{-2} s^{-1}$, which was supplied by an RGBW LED light source (PP Systems, Amesbury, MA, USA) attached to the equipment.

2.4. Radiation reflection and abaxial leaf temperature

The albedo (Alb) of the PAR was measured in the nurseries during ST2 using 15 readings of PAR, measured with a Sunscan Canopy ceptometer (Delta-T Devices, Cambridge, UK) inverted toward the leaves (parallel to the edge of the furrow) and with a reference sensor BF5 (Delta-T Devices, Cambridge, UK) on the plants (Alb = PAR refracted by the leaves/PAR measured over the plants) between 11:00 and 13:00 h. In addition, the $T_{leaf_abaxial}$ in each nursery during ST2 was recorded in 50 leaves randomly selected from the plot between 11:00 and 13:00 h using an infrared thermometer (Spectrum Technologies, Inc., Aurora, IL, USA).

2.5. Growth characteristics

At each sampling time, 50 plants per treatment were randomly selected and sectioned into roots, stems, and leaves. The roots were completely washed with water to remove traces of soil, while the leaves per plant were counted (LN = leaf number) and measured with a leaf surface meter (Delta-T Devices, Cambridge, UK) to determine the total leaf area (TLA). Once these processes were completed, the plant tissues were stored in paper bags and dried at 70 °C in an oven to a constant dry mass. Then, the organs of each plant were weighed to determine the leaf dry mass (LDM), stem dry mass (SDM), and root dry mass (RDM) at both the first and second sampling times. Based on previously obtained data, total dry mass (TDM = LDM + SDM + RDM), RDM fraction (RDMF = RDM/TDM), SDM fraction (SDMF = SDM/TDM), LDM fraction (LDMF = LDM/TDM), shoot-to-root ratio (SRR = (SDM + LDM)/RDM), leaf area ratio (LAR = TLA/TDM), specific leaf area (SLA = TLA/LDM) and the ratio of RDM to TLA (RDM/TLA) for ST2. Due to the different impacts of the variability in SDM and RDM on the SRR, this variable was ln-transformed before being analyzed. The RDM/TLA is a representation of the hydraulic conductance of a plant (Avila et al., 2020). The relative growth rate (RGR) and the leaf unit rate (LUR) were calculated with the TDM and TLA data obtained in ST1 and ST2 according to Equations (1) and (2) (Darghan et al., 2022).

$$RGR = \frac{\ln TDM_{ST2} - \ln TDM_{ST1}}{dat_{ST2} - dat_{ST1}} \quad (1)$$

$$LUR = \frac{TDM_{ST2} - TDM_{ST1}}{dat_{ST2} - dat_{ST1}} \frac{[\ln TLA_{ST2} - \ln TLA_{ST1}]}{TLA_{ST2} - TLA_{ST1}} \quad (2)$$

Where: \ln = natural logarithm; TDM_{ST1} = TDM in the first sampling time, TDM_{ST2} = TDM in the second sampling time; dat_{ST1} = dat in the first sampling time; dat_{ST2} = dat in the second sampling time; TLA_{ST1} = TLA in the first sampling time; and TLA_{ST2} = TLA in the second sampling time.

2.6. Seedling mortality

In each nursery, the remaining seedlings per treatment after ST2 ($n = 800$) were classified as alive and dead. Then, the MP (number of dead seedlings/800) was calculated by treatment in each nursery. Due to the need to express the values logistically for the analysis, the data were transformed with $\text{logitMP} = \ln (MP/(1-MP))$. After the analysis, the estimated logistic means obtained were transformed into odds (odds = $\exp(\text{logitMP})$), and then, the ways in which the odds could be expressed in terms of probability (Cerdeira et al., 2013) were transformed again into MPs = odds/odds + 1 to allow an easy interpretation.

2.7. Statistical analysis

The data collected through a simple factorial design in generalized and randomized complete blocks ($y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \epsilon_{ijk}$), with the nurseries acting as blocks (β_j) and treatments as group effects (τ_i) associated with the response (y_{ijk}) were analyzed using the Welch-James (WJ) statistical test with approximate degrees of freedom (ADF), for the variables TDM, LDMF, SDMF, RDMF, SRR, RDM/TLA, TLA, LAR, SLA, LN, RGR, LUR, A , g_s , C_i , WUE, $T_{leaf_abaxial}$, $T_{cuvette}$, and Alb. Given the lack of normality (Shapiro-Wilk test, $p = <0.05$) and/or homogeneity in the variance in the data (Levene's test, $p = <0.05$), the analysis was developed with the "welchADF" package (Villacorta, 2019) in R v. 4.1.0 (R Core Team, 2020). This implements the Johansen formulation of the WJ test, with two improvements: the first is the use of trimmed means and winsorized variances to address nonnormal data, and the second is the use of bootstrapping to calculate an empirical p value (Villacorta, 2017). The post-hoc pairwise contrasts by bootstrapping were adjusted by the Hochberg method ($\alpha = 0.05$; $\alpha = 0.005$) also using the "welchADF" package (Villacorta, 2019). The number of resamples for the bootstrapping process was 10000. In the case of the logitMP variable, a binary response logistic model ($\text{logit}(y_{ijk}) = \mu + \tau_i + \beta_j + (\tau\beta)_{ij}$) was used to determine the effect of the treatments factor using the X^2 statistical test, and comparisons between treatments were performed with the pairwise Z test adjusted by the Shaffer-Simulated method ($\alpha = 0.005$) (Westfall et al., 2011). The analysis of the logistic model was performed with the PROC LOGISTIC procedure in SAS® 9.4 software (SAS Institute Inc, 2018). To reduce the risk of false-positives, the threshold of statistical significance for the differences between treatments was $\alpha = 0.005$, while comparisons with a threshold of $\alpha = 0.05$ were described as suggestive of differences (Ioannidis, 2018; Andrade, 2019; Wasserstein et al., 2019). The box and violin plots were constructed with the "ggplot2" package (Wickham et al., 2021) in R v. 4.1.0 (R Core Team, 2020).

3. Results and discussion

Seedlings grown in nurseries in full sun commonly have a higher assimilate production than nurseries in shade conditions (Moraes et al., 2010; Rodríguez-López et al., 2014; Kufa, 2012). Although, it does not ever occur (Tatagiba et al., 2010; Córdoba et al., 2016). One reason could be a lower assimilate production to high temperatures in nurseries in full sun. In this way, kaolin reduces the leaf and canopy temperature and improves plant water status (Glenn, 2009, 2010; Boari et al., 2015). In the present study,

the coffee seedlings grown in nurseries in full sun without and with kaolinite at 60 g/L have similar accumulate biomass concerning nurseries under shade. In addition, nurseries with kaolinite to high temperatures had high g_s and low $T_{leaf_abaxial}$, which suggests better behavior in these conditions. However, the nurseries in full sun with kaolinite did not reduce the MP of the seedlings.

3.1. Gas exchanges in leaves

For the C_i , WUE, and $T_{cuvette}$ (Fig. 2, c, d, f) when the leaf temperature was kept stable at 25 °C (Fig. 2, e), the treatments showed suggested differences ($p < 0.05$), while for the remaining parameters, no differences were found between treatments

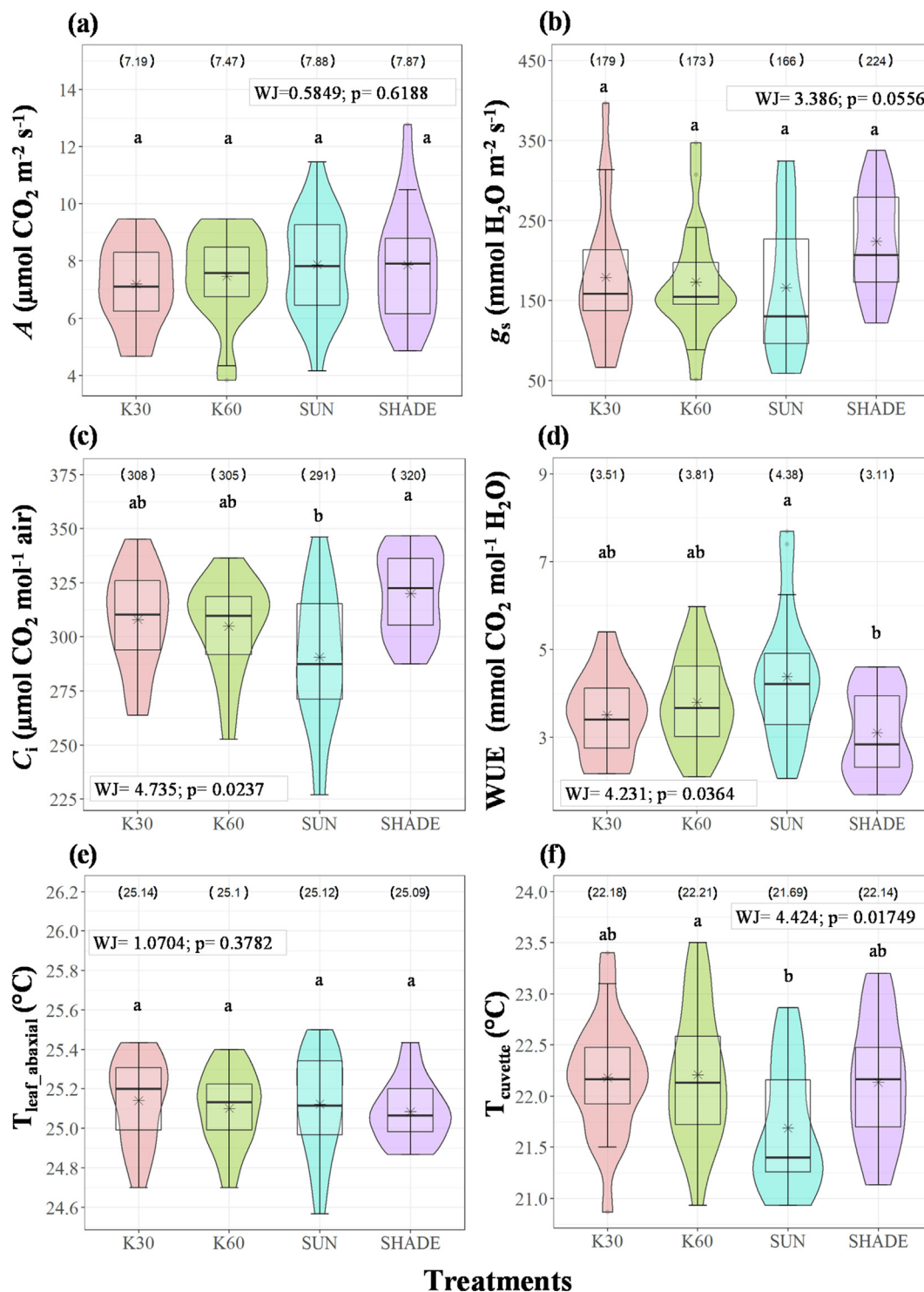


Fig. 2. Leaf gas exchange parameters while maintaining the leaf abaxial temperature stable at 25 °C [A : net photosynthesis rate (a), g_s : stomatal conductance (b), C_i : internal concentration of CO_2 (c), WUE: water use efficiency (d), $T_{leaf_abaxial}$ = abaxial temperature of the leaf (e), and $T_{cuvette}$ = temperature of the tray (f)] for *Coffea arabica* var. Cenicafé 1 seedlings subjected to four treatments [K30: seedlings in full sun with kaolinite applied at 30 g/L (n = 20), K60: seedlings in full sun with kaolinite applied at 60 g/L (n = 20)], SUN: seedlings in full sun without kaolinite (n = 20), and SHADE: seedlings under a 60 % shade screen (n = 20)]. WJ statistic for each characteristic and its p value. Different lowercase letters indicate differences between treatments ($\alpha = 0.05$). The values in parentheses and the asterisk symbol correspond to the mean.

(Fig. 2, a, b). Rodríguez-López et al. (2014) find that the C_i was higher in the treatment in direct sun than in the other treatments in this study. This result was different from that in this study as the C_i was higher in the SHADE treatment (9 %) than in the SUN treatment (Fig. 2, c). On the other hand, the C_i has also been shown to be similar between these treatments in other studies (Marçal et al., 2021), suggesting that this parameter may vary by another factor different from the light gradient at the same leaf temperature (25 °C).

The WUE showed that the seedlings in the SUN treatment were more efficient in incorporating atmospheric CO₂ than the seedlings in the SHADE treatment (Fig. 2, d); in turn, the higher value in the SUN treatment indicated that the humidity conditions were drier. Besides, it should be noted that the seedlings with kaolinite (the K30 and K60 treatments), despite being directly exposed to the sun as those in the SUN treatment, the first ones did not show differences with respect to the seedlings in the SHADE treatment (Fig. 2, d). In *C. arabica* cv. Catuaí Vermelho IAC 44, when the seedlings were protected with kaolinite, the WUE was reduced compared with full-sun plants (de Abreu et al., 2022). The T_{cuvette} was higher in the K60 treatment (2.3 %) than in the SUN treatment (Fig. 2, f), which indicates that the first treatment presented a colder leaf blade, which occurred because an increased temperature was required in the cuvette. Thus, kaolinite reduced leaf temperature, as has been reported in previous studies in *C. arabica* (Steiman et al., 2007; de Abreu et al., 2022). The lack of response in the physiological parameters of A and g_s for treatments in direct sun and shade (Fig. 2, a, b) in *C. arabica* could be linked to the poor physiological plasticity of the leaves when these grow in moderate radiation conditions (i.e., direct sun and shade at 60 %) (Marçal et al., 2021; Rodríguez-López et al., 2014). This also applies to kaolinite because it has a moderate effect on the radiation.

For A , g_s and C_i (Fig. 3, a, b, c), when the cuvette temperature was maintained at 30 °C (Fig. 3, f), the treatments showed suggested differences ($p < 0.05$), while for the $T_{\text{leaf abaxial}}$, there was statistical evidence of differences between the treatments ($p < 0.005$) (Fig. 3, e). The reduced variability in the T_{cuvette} guaranteed that the differences in the photosynthetic parameters and in the $T_{\text{leaf abaxial}}$ were only due to the treatments (Fig. 3, f). The kaolinite treatments (the K30 and K60 treatments) showed an A that was higher than that found in the SHADE treatment (22 and 23 %, respectively), while in comparison to the other treatments, the SUN treatment did not show differences in the A (Fig. 3, a). The g_s and C_i in the kaolinite treatments (the K30 and K60 treatments) were higher than those observed in the SUN treatment (g_s between 27 and 30 %, and C_i between 4 and 5 %) (Fig. 3, b, c). In summary, the kaolinite treatments (the K30 and K60 treatments) performed better in terms of the A , g_s , and C_i when the cuvette temperature was high for leaves. The coffee plant is capable of increasing its A up to temperatures between 30 and 35 °C, as long as g_s is kept under control in response to the increase in the VPD (DaMatta, 2004). In *C. arabica* var. Typica, the A in leaves at full sun with kaolinite was higher than in plants at full sun without kaolinite, but when the leaf temperature was maintained stable at 25 °C (Steiman et al., 2007).

Although the WUE did not show detectable differences among treatments, generally, the SUN treatment had higher WUE values than those in the SHADE treatment (Fig. 3, d). The $T_{\text{leaf abaxial}}$ values in the treatments without kaolinite (the SUN and SHADE treatments) were higher than those in the K60 treatment (between 1.9 and 2.2 %) (Fig. 3, e). This result suggested that the leaves with kaolinite could maintain a cooler temperature during warm days, which is relevant since photosynthesis begins to decrease over 34 °C (DaMatta et al., 2018).

3.2. Radiation reflection and leaf temperature

For Alb and $T_{\text{leaf abaxial}}$, the treatments presented statistical differences between them ($p < 0.005$) (Fig. 4). According to the Alb data, the K60 treatment presented the highest radiation reflection, followed by the K30, SHADE and SUN treatments, with significant differences in all cases (Fig. 4, a). The treatments with kaolinite (the K30 and K60 treatments) presented between 46 and 60 % more reflection than the treatments without kaolinite (the SUN and SHADE treatments). Olive tree canopy treated with kaolinite had a higher refraction the PAR (86 %) than those without kaolinite, which reduced the thermal stress (Nanos, 2015).

For $T_{\text{leaf abaxial}}$, the K30 and SUN treatments showed a leaf surface warmer that recorded in the K60 and SHADE treatments (between 8 and 13 %) (Fig. 4, b). The low $T_{\text{leaf abaxial}}$ in the SHADE treatment was expected since the seedlings grown under the mesh, which decreased the temperature; in contrast, the K60 treatment showed a decrease in $T_{\text{leaf abaxial}}$ (10.8 %) despite being directly exposed to full sun (Fig. 4, b). In coffee var. Typica, the $T_{\text{leaf abaxial}}$ was 9.1 % higher in plants exposed to the full sun than in plants with kaolinite at full sun (Steiman et al., 2007). However, it sometimes may not occur (Russo and Díaz-Pérez, 2005), as in the K30 treatment (K30 vs SUN treatment) (Fig. 4, b). This response would be due to the concentration used, since kaolinite at low concentrations has a limited effect on temperature (Steiman et al., 2007).

3.3. Growth characteristics

The growth characteristics included TDM, LDMF, SDMF, RDMF, RDM/TLA, TLA, LAR, SLA, RGR, and LUR showed statistical differences between treatments ($p < 0.005$), while for the SRR, the differences were only suggestive ($p < 0.05$) (Table 1). In contrast, for the number of leaves, the differences were not detectable (Table 1). In comparison to the SHADE treatment, the K60 and SUN treatments resulted in between 20 % and 21 % more TDM (Table 1). These results indicated that kaolinite did not have a specific effect on the accumulation of seedling dry mass and that the differences were only attributable to the light gradient. In several studies, *C. arabica* seedlings grown in shade have had a lower dry mass than those grown in full sun (Moraes et al., 2010; Kufa, 2012; Rodríguez-López et al., 2014), because under low light conditions, lower ATP synthase activity decreases the amount of ATP necessary for carbon fixation and carbohydrate biosynthesis; as a result, growth decreases (Mathur et al., 2018). Changes in dry matter are a clear indicator of the ability of seedlings to respond and take advantage of variations in light availability (Rodríguez-López et al., 2014). However, there are reports of coffee seedlings that grow better in the shade (<60 %) than in the full sun (Tatagiba et al., 2010; Córdova et al., 2016), and this scenario may occur when the photosynthetic capacity of leaves is overwhelmed by excess light (Mathur et al., 2018). Dry matter is the most direct measure of plant performance as a product of growth (Dawson et al., 2012).

K30: seedlings in full sun with kaolinite applied at 30 g/L (n = 100), K60: seedlings in full sun with kaolinite applied at 60 g/L (n = 100), SUN: seedlings in full sun without kaolinite (n = 100), and SHADE: seedlings under a 60 % shade screen (n = 99).

In comparison to the SHADE treatment, all treatments exposed directly to the sun (the K30, K60 and SUN treatments) resulted in higher SDMF (29, 23 and 19 %, respectively) (Table 1), while only the kaolinite treatments (the K30 and K60 treatments) resulted in higher RDMF, which were 14 % greater than the SHADE treatment RDMF (Table 1). In general, the RDMF increases consistently with the in both herbaceous and woody species (Poorter and Nagel, 2000). In the present investigation, the RDMF tended to be greater when the seedlings were exposed to direct solar radiation, which

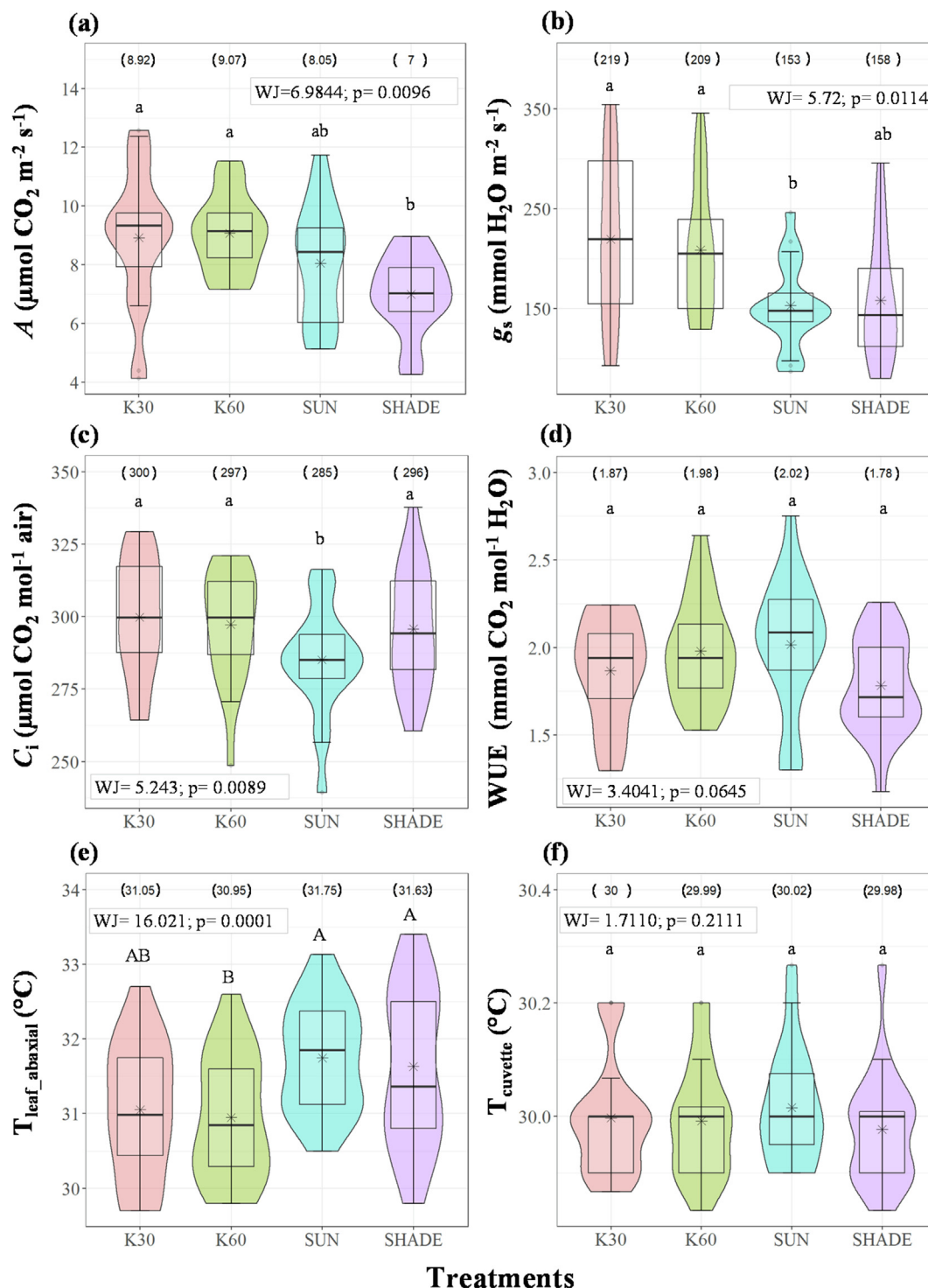


Fig. 3. Parameters of leaf gas exchange while maintaining the temperature of the cell stable at 30 °C [A : net photosynthesis rate (a), g_s : stomatal conductance (b), C_i : internal concentration of CO_2 (c), WUE: water use efficiency (d), $T_{\text{leaf_abaxial}}$ = abaxial temperature of the leaf (e), and T_{cuvette} = temperature of the tray (f)] for *Coffea arabica* var. Cenicafé 1 seedlings subjected to four treatments [K30: seedlings in full sun with kaolinite applied at 30 g/L ($n = 20$), K60: seedlings in full sun with kaolinite applied at 60 g/L ($n = 20$), SUN: seedlings in full sun without kaolinite ($n = 20$), and SHADE: seedlings under a 60 % shade screen ($n = 20$)]. WJ statistic for each characteristic and its p value. Different capital letters denote significant differences between treatments ($\alpha = 0.005$). Different lowercase letters indicate differences between treatments ($\alpha = 0.05$). The values in parentheses and the asterisk symbol correspond to the mean.

has also been reported in coffee (Moraes et al., 2010; Kufa, 2012). On the other hand, seedlings in treatments with kaolinite that invest more dry mass in their roots would have slower growth, but the greater absorption surface could give them an advantage where water and nutrients are limiting (Valladares, 2004) after of transplant at field.

The SRR was higher in the SHADE treatment (15 %) than in the K60 treatment, which implies that the former had a lower capacity to supply water and nutrients given the lower root development (Table 1). In the remaining treatments exposed directly to the sun (the K30 and SUN treatments) the SRR were lower than that in the SHADE treatment but not significantly different (Table 1).

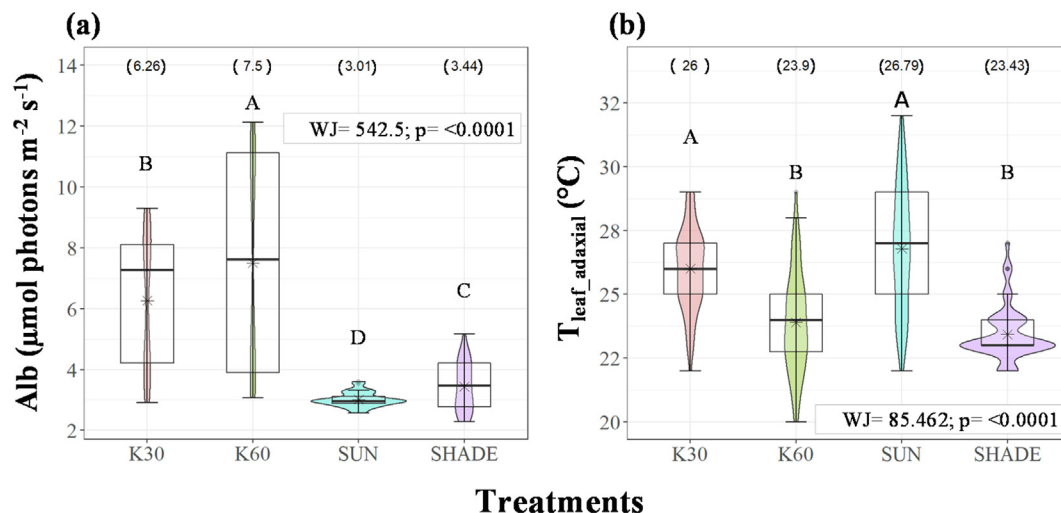


Fig. 4. Albedo (Alb) [n = 30] (a), and leaf temperature in the abaxial part ($T_{\text{leaf_abaxial}}$) [n = 100] (b) in *C. arabica* var. Cenicafé 1 seedlings subjected to four treatments [K30: seedlings in full sun with kaolinite applied at 30 g/L, K60: seedlings in full sun with kaolinite applied at 60 g/L, SUN: seedlings in full sun without kaolinite, and SHADE: seedlings under a 60 % shade screen]. WJ statistic for each characteristic and its p value. Different capital letters denote significant differences between treatments ($\alpha = 0.005$). The values in parentheses and the asterisk symbol correspond to the mean.

Table 1

Growth characteristics [total dry mass (TDM), leaf dry mass fraction (LDMF), stem dry mass fraction (SDMF), root dry mass fraction (RDMF), shoot-to-root ratio (SRR), root dry mass to total leaf area (RDM/TLA), total leaf area (TLA), leaf area ratio (LAR), specific leaf area (SLA), leaf number (LN), relative growth rate (RGR), and leaf unit rate (LUR)] of the *Coffea arabica* var. Cenicafé 1 seedlings subjected to four treatments (Treated). WJ statistic for each characteristic and its p value. Different capital letters denote significant differences between treatments ($\alpha = 0.005$). The values in the table correspond to the mean \pm standard deviation (median).

Trait	Treat.	Value	Trait	Treat.	Value
TDM (g)	K30	3.01 \pm 1.54 (2.93)	TLA (cm ²)	K30	204 \pm 82.4 (198)
	K60	3.47 \pm 1.51 (3.43)		K60	209 \pm 85.5 (206)
	SUN	3.50 \pm 1.38 (3.38)		SUN	243 \pm 89.5 (22.0)
	SHADE	2.76 \pm 1.00 (2.89)		SHADE	274 \pm 80.7 (26.6)
		WJ = 8.245; p = 0.0001			WJ = 14.487; p = < 0.0001
LDMF (g/g)	K30	0.56 \pm 0.12 (0.58)	LAR (m ² kg ⁻¹)	K30	7.6 \pm 2.8 (6.6)
	K60	0.54 \pm 0.08 (0.57)		K60	6.3 \pm 1.6 (5.8)
	SUN	0.59 \pm 0.07 (0.59)		SUN	7.3 \pm 1.9 (7.1)
	SHADE	0.64 \pm 0.05 (0.65)		SHADE	10.4 \pm 2.2 (10.1)
		WJ = 75.16; p = < 0.0001			WJ = 173.21; p = < 0.0001
SDMF (g/g)	K30	0.24 \pm 0.07 (0.22)	SLA (m ² kg ⁻¹)	K30	12.7 \pm 5.9 (10.4)
	K60	0.22 \pm 0.03 (0.22)		K60	11.5 \pm 3.7 (10.6)
	SUN	0.21 \pm 0.05 (0.21)		SUN	12.4 \pm 3.0 (12.1)
	SHADE	0.17 \pm 0.03 (0.16)		SHADE	16.3 \pm 3.2 (15.7)
		WJ = 105.97; p = 0.0001			WJ = 101.406; p = < 0.0001
RDMF (g/g)	K30	0.22 \pm 0.08 (0.21)	LN (#)	K30	12.0 \pm 4.1 (13)
	K60	0.22 \pm 0.06 (0.23)		K60	13.0 \pm 4.7 (14)
	SUN	0.20 \pm 0.06 (0.20)		SUN	12.7 \pm 4.0 (13)
	SHADE	0.19 \pm 0.04 (0.19)		SHADE	12.7 \pm 2.1 (13)
		WJ = 9.935; p = < 0.0001			WJ = 2.207; p = 0.0951
SRR (g/g)	K30	3.73 \pm 1.56 (3.86)	RGR (mg/g d ⁻¹)	K30	16.9 \pm 2.9 (16.5)
	K60	3.64 \pm 1.44 (3.46)		K60	16.8 \pm 4.6 (15.4)
	SUN	4.14 \pm 1.43 (4.01)		SUN	20.8 \pm 6.3 (20.6)
	SHADE	4.26 \pm 1.26 (4.35)		SHADE	16.1 \pm 3.9 (16.2)
		WJ = 7.688; p = < 0.0001			WJ = 186.9; p = < 0.0001
RDM/TLA (g/m ⁻² (- -))	K30	31.1 \pm 11.9 (29.2)	LUR (g/m ⁻² (- -) d ⁻¹)	K30	2.44 \pm 0.67 (2.54)
	K60	36.7 \pm 13.4 (35.1)		K60	2.49 \pm 0.61 (2.50)
	SUN	28.5 \pm 11.7 (27.3)		SUN	2.78 \pm 0.75 (2.76)
	SHADE	19.5 \pm 6.26 (18.5)		SHADE	1.56 \pm 0.32 (1.47)
		WJ = 72.82; p = < 0.0001			WJ = 186.72; p = < 0.0001

In general, the SRR decreased with increasing radiation. This result was expected because with increasing irradiance, the photosynthetic rate per unit mass increases, requiring a smaller allocation of biomass toward the shoot (aerial part); in contrast to maintaining the functional balance of the plant, the allocation of biomass toward the root increases to account for the increase in transpiration and shoot growth with water and nutrients from the soil (Poorter and Nagel, 2000). In coffee seedlings, this response is very consistent, both in the reports where the highest dry matter was achieved in full sun (Moraes et al., 2010; Kufa, 2012) and in those

where the highest dry matter was obtained in the shade (Tatagiba et al., 2010). The SRR was moderate (ranging from 3.6 to 4.3) in comparison to other studies (Paiva et al., 2003; Moraes et al., 2010). Additionally, it is also important to consider that part of this difference may be based on genotype. The SRR values varied between 1.82 and 3.51 in 29 coffee genotypes during their seedling stage (Ramos and Carvalho, 1997).

The TLA of the seedlings in the SHADE treatment was higher than that in the kaolinite treatments (the K30 and K60 treatments) (26 and 24 %, respectively). However, the SUN treatment did not

show significantly different TLA values from those of the SHADE treatment (Table 1). In the shade, leaves maximize light capture by increasing their leaf area (Mathur et al., 2018). This response has been reported in coffee (Córdova et al., 2016), although not always with significant differences (Moraes et al., 2010), as in the present study (the SUN treatment vs the SHADE treatment). Similarly, although some differences in TLA occurred, the LDMF, LAR, and SLA in the SHADE treatment were higher than those in the SUN and K60 treatments (8 and 13 %, 39 and 30 %, and 29 and 24 %, respectively), while those in the SUN treatment were higher than those in the K60 treatment (LMF = 8 %, LAR = 14 %, and SLA = 7 %) (Table 1). The significant differences between the K30 and SHADE treatments showed that the former had a significantly lower LDMF and LAR but not a significantly lower SLA, although in all cases, the K30 treatment showed lower values (Table 1).

The LDMF decreases with the increase in light at the plant level, while RDMF increases, which allows the transpiration rate per unit of root mass to be kept constant, or a greater supply greater of nutrients required for rapid growth in full sun (Evans and Poorter, 2001). It was corroborated in the present study and at others with coffee seedlings under 50 % shade (Moraes et al., 2010; Kufa, 2012). At the leaf level, plants exposed to direct solar radiation have a lower SLA, which indicates a thicker leaf sheet with better photosynthetic capacity per unit area (Evans and Poorter, 2001). In coffee, this response has been documented in both seedlings (Moraes et al., 2010; Rodríguez-López et al., 2014) and plants (Matos et al., 2009; Marçal et al., 2021). The changes in the SLA and in LDMF are accompanied by very similar changes in the LAR, because it is the result of the increase in SLA and the LDMF (Valladares, 2004). The photosynthetic efficiency estimated by the LUR showed that all treatments exposed directly to the sun (the K30, K60 and SUN treatments) had similar values, but these values were higher than those in the SHADE treatment (36, 37 and 44 %, respectively) (Table 1). LUR in coffee seedlings grown in full sun is higher than in seedlings under shade, by lower LAR and SLA (Moraes et al., 2010).

The SUN treatment resulted in a higher RGR in comparison to those of the kaolinite treatments (19 % both) and the SHADE treatment (24 %) (Table 1). This result occurred because the SUN treatment had the lowest TDM during the first sampling but had the highest amount in the second sampling (Table 1), indicating that its gain in size per unit of initial size was greater. On the other hand, the RGR in the K60 treatment was lower than that in the SUN treatment (Table 1), despite achieving a similar TDM (Table 1), which was due to the high amount of TDM in the first sampling with a lower increase in the second sampling, considering that its value was similar to that of the SUN treatment (Table 1), and that scenario could have been associated with the greater allocation of biomass to the roots and lower allocation to the aerial parts in the K60 treatment (Table 1). In this context, the seedlings in full sun (the SUN treatment) had slow initial growth given the initial adaptation the leaves experienced when exposed directly to the sun, but once adjusted, the seedlings grew rapidly with respect to their initial weight. On the other hand, the seedlings in the K60 treatment showed rapid initial growth, possibly due to the initial protection they received from the kaolinite against direct light and temperature. In seedlings coffee grown under shade, the RGR values have been lower than in the full sun, but slightly and no statistical difference (Moraes et al., 2010).

The RDM/TLA of the K60 treatment was greater than that of the other treatments (between 15 and 47 %); in turn, the K30 and SUN treatments had higher RDM/TLA values than that of the SHADE treatment by 37 and 32 %, respectively (Table 1). This result would correspond to the main difference between the K60 and SUN treatments, despite not having differences in terms of dry matter or photosynthetic efficiency. In coffee, an increase in the RDM/TLA

has been associated with a greater hydraulic capacity (Avila et al., 2020), an condition that has been observed in plants in full sun rather than those in the shade (Marçal et al., 2021).

3.4. Seedling mortality

The treatments showed significant differences between treatments for the MP ($p < 0.005$) (Fig. 5). In all treatments exposed directly to the sun (the K30, K60 and SUN treatments), the MP was significantly higher (between 9 and 10 %) that in comparison to the SHADE treatment, indicating that the risk of a plant dying when directly exposed to the sun is 2.7 to 2.8 times the risk of dying in the SHADE treatment (Fig. 5). This factor should be specifically considered when deciding whether to establish a nursery in the full sun or in the shade, as in full sun, a greater loss of seedlings would need to be considered, which would lead to adjusting the initial number of seedlings based on what is needed in the field. Previous reports in Colombia have noted that the implementation of shading will depend on the altitude at which a nursery is located: nurseries established at altitudes lower than 1550 m could be in full light, those between 1550 and 1850 m would need shade, and those above 1850 m should not be established due to plant dwarfism and poor seedling development (Arcila et al., 2007). However, this criterion is rarely applied. The most common practice is establishing seedlings in shaded nurseries before being transplanted to the field to ensure optimum quality seedlings (Mejía, 2021). This practice is justified based on the MP of the seedlings found in the present study. In Colombia is recommended establishing 10 % more coffee seedlings in the shade to address possible losses (Farfan et al., 2015), an amount similar to the maximum MP observed in the present study for the SHADE treatment during the second nursery (11 %).

The highest MP occurred in the treatments exposed directly to the sun (the K30, K60, and SUN treatments), with maximum MP values between 21 and 30 % (in the second nursery). This result indicated that in the treatments exposed directly to the sun (the K30, K60, and SUN treatments), a replacement of up to 30 % of the seedlings to be established in the field should be considered for the location in this study. However, at other locations, the loss

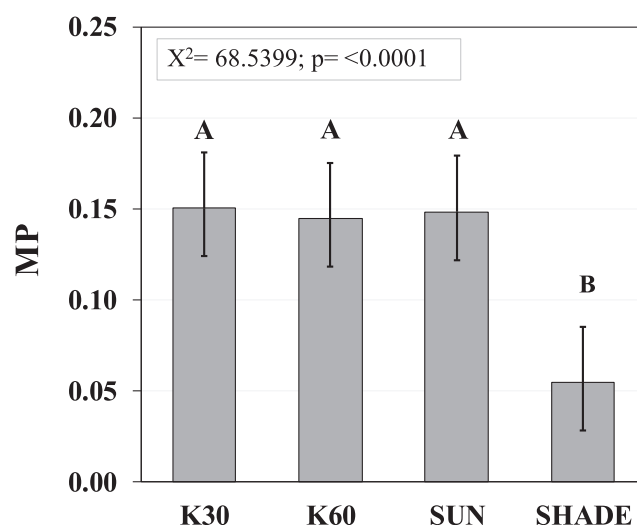


Fig. 5. Mortality probability (MP) of *Coffea arabica* var. Cenicafé 1 seedlings subjected to four treatments [K30: seedlings in full sun with kaolinite applied at 30 g/L ($n = 1600$), K60: seedlings in full sun with kaolinite applied at 60 g/L ($n = 1600$), SUN: seedlings in full sun without kaolinite ($n = 1600$), and SHADE: seedlings under a 60 % shade screen ($n = 1600$)]. X^2 statistic and its p value. Different capital letters denote significant differences between treatments ($\alpha = 0.005$). The bars correspond to the confidence limits.

percentages could be different while maintaining the same risk. Therefore, it is preferable to consider that in full sun, seedling losses could be up to three times those in the shade. Given this information, new techniques that unify the benefits of establishing seedlings in the shade and in full sun with kaolinite need to be developed in future research to take advantage of the greater accumulation of dry matter, *A* and increases in RDM/TLA observed in the treatment with kaolinite at 60 g/L (K60) but with a lower MP, such as that found in the shaded treatment (SHADE). Future research should focus on reducing the MP of the seedlings exposed to full sun with and without kaolinite, mainly during the transplanting of seedlings from a germinator to bags in a nursery.

4. Conclusions

Seedlings grown in nurseries in full sun without and with kaolinite at 60 g/L can achieve a higher TDM than those grown in nurseries in the shade based on changes in the SLA, LA, and dry mass fractions. On the other hand, these seedling production methods differ because seedlings treated with kaolinite at 60 g/L performed better in terms of RDM/TLA than those exposed to full sun without kaolinite. The *A* of the seedlings increased more in the kaolinite treatments (30 g/L and 60 g/L) than in the SHADE treatment, while seedlings had higher g_s in the SUN treatment than in the other treatments; however, this was only observed when the $T_{\text{leaf abaxial}}$ was higher than 30 °C. This result could be associated with the greater light reflection and the lower foliar temperatures, which were particularly notable in the kaolinite treatment of 60 g/L. In summary, in comparison to traditional methods, the application of kaolinite to seedlings during their nursery stage could have benefits in terms of TDM, allometric adjustments and tolerance to high temperatures. However, the use of kaolinite did not reduce the MP of the seedlings. This result suggests that it is not convenient to establish nurseries in full sun, with or without kaolinite, at least using the techniques evaluated, given that the number of replacement plants to sustain the final number of plants required in the field would need to be increased. Future studies with kaolinite in coffee seedlings need to reduce the number of dead plants at full sun in nurseries.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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