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RESEARCH

Kaolin-based particle film mitigates high light stress in native tree seedlings through anatomical and growth adjustments

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Abstract

Deforestation and environmental degradation increase the need for effective strategies to improve seedling establishment in ecological restoration programs, particularly under high irradiance conditions. Although processed kaolin particle film (PKPF) has been widely used to mitigate abiotic stress in agricultural crops, its potential application to native forest species during restoration remains poorly understood. This study evaluated the effects of PKPF on anatomical, ultrastructural, and biometric traits of *Cordia superba* and *Citharexylum myrianthum*, two native Atlantic Forest species commonly used in restoration projects. Seedlings were grown under full sunlight with or without PKPF application and compared with greenhouse-grown plants. PKPF reduced leaf temperature, preserved chloroplast and mitochondrial ultrastructure, and promoted the accumulation of starch grains and plastoglobules. These responses indicate improved maintenance of cellular integrity and energy metabolism under high irradiance, contributing to enhanced stress tolerance during seedling establishment. Growth responses were more pronounced in *C. superba*, whereas *C. myrianthum* exhibited greater intrinsic tolerance to light stress. Overall, the results demonstrate that PKPF mitigates the adverse effects of excessive solar radiation by protecting cellular structures associated with photosynthetic performance and supporting seedling development. This study provides novel evidence for the use of kaolin-based technologies in native forest species and highlights their potential to improve restoration success under increasingly challenging environmental conditions.

Keywords Environmental degradation · Kaolin · Leaf anatomy · Plant growth

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Introduction

The growth of the human population and the expansion of anthropogenic activities have resulted in intense pressure on ecosystems, with approximately 58.4% of the Earth's surface already impacted (Williams et al. 2020). Deforestation, agriculture, and resource exploitation have reduced forest fragments, leading to biodiversity loss and compromising ecosystem functionality and stability (SER 2004; Oliveira et al. 2021). It is estimated that 60% of tropical forests have already been severely altered, aggravating problems such as increased CO_2 concentrations, water scarcity, and the intensification of extreme climate events (IPCC 2021). In this scenario, global initiatives such as the UN Decade on Ecosystem Restoration aim to mobilize governments, scientists, and communities to take action for environmental recovery and climate crisis mitigation (United Nations Brazil 2022). Among the ecosystems most affected by these pressures, tropical forests have become a priority for restoration efforts due to their exceptional biodiversity, ecological importance, and vulnerability to ongoing environmental change (Chazdon et al. 2016; Gann et al. 2019; Lewis et al. 2019).

As one of the world's most threatened tropical forest biodiversity hotspots, the Atlantic Forest (AF) continues to experience substantial habitat loss, with 14,697 hectares of forest cover lost between 2022 and 2023 alone, resulting in the emission of more than 7 million tons of CO_2 (Atlas da Mata Atlântica 2024). To meet international commitments such as the 2030 Agenda, the country has set a target to restore 19 million hectares of forests (IPCC 2021). Environmental restoration, in addition to recovering ecosystem structure and functionality, ensures essential services such as climate regulation, soil fertility, and carbon sequestration (Gann et al. 2019). High solar radiation and other abiotic factors present a significant challenge to seedling survival and establishment (Castro et al. 2009; Pinheiro et al. 2022). Excessive irradiance can impair photosynthetic performance through photoinhibition and structural alterations in chloroplasts and other cellular components, leading to chlorosis, reduced CO_2 assimilation, and ultimately lower field performance (Campos and Uchida 2002; Houter and Pons 2005; Bernardi 2023).

Given the harmful effects of climate change, management strategies and innovative technologies have been studied to mitigate these impacts. One technology that has been used in agricultural plants to counteract abiotic stress — particularly high solar radiation — is foliar spraying with processed kaolin particle film (PKPF) (Glenn 2016; Frioni et al. 2019). Among the technologies investigated to reduce abiotic stress, processed kaolin particle film (PKPF) has been widely studied in agricultural systems worldwide due to its ability to mitigate the adverse effects of excessive radiation, heat, and water deficit (Wünsche et al. 2004; Steiman 2007; Segura-Monroy et al. 2015; Conde et al. 2016; Gharaghani et al. 2018; Abdallah et al. 2019; Mahmoudian et al. 2021; de Abreu et al. 2022; Gómez-Candón et al. 2022). PKPF is a clay-based product, a natural substance composed of kaolinite ($Al_2Si_2O_5(OH)_4$), typically characterized by white particles, which reduces sunburn (chlorosis) and improves plant yield (Kerns and Wright 2000; Colavita and Valdez 2011). Kaolin protection acts against abiotic stress, such as supra-optimal solar radiation, through two main mechanisms. The first involves the formation of a white particle layer when PKPF is sprayed on leaves, creating a physical barrier that reflects ultraviolet (UV) and infrared (IR) radiation, helping to reduce leaf blade temperature and prevent leaf and fruit sunburn (Sarooghinia et al. 2019). The second involves the induction of physiological stress responses (Zaky 2018; da Silva et al. 2025).

PKPF acts as a physical barrier that reflects excess radiation and heat, enhancing the efficiency of photosynthesis and nutrient uptake, while also preventing photo-oxidative stress (Zaky 2018; Semida et al. 2023). Under conditions of high thermal load on leaves due to intense solar exposure, PKPF application helps reduce leaf temperature, mitigating the effects of thermal, light, and water stress (Gindaba and Wand 2008). The attenuation of these factors is directly related to improved plant physiological performance. Furthermore, PKPF helps preserve photosynthetic pigments, enhancing leaf coloration and protecting against damage such as sunburn (Gindaba and Wand 2008; Zaky 2018; de Abreu et al. 2022). These protective effects extend beyond pigment preservation, as the reduction of thermal and radiative loads contributes to the maintenance of photosynthetic activity, cellular homeostasis, and the integrity of chloroplast structures that are particularly susceptible to damage under excessive irradiance (Bernardi 2023).

This research aimed to evaluate the protective effects of PKPF on anatomical and morphological variables of plants relevant to environmental restoration. These variables have not been previously addressed in this context. The lack of information on these interactions hinders the identification of the most suitable timing for PKPF application, considering plant responses to light conditions. Expanding knowledge of this topic in early-stage restoration areas is critical for ensuring long-term success and the proper establishment of native species in the field. (Carvalho 2010) Moreover, such understanding can provide technical support to optimize seedling production, reducing the need for replanting and, consequently, operational costs.

Although the success of plant establishment and development is crucial in environmental restoration projects, studies investigating the use of protective technologies such as PKPF on plants used in restoration programs remain scarce, despite its wide application in agriculture in crops such as persian walnut (*Juglans regia* L.), grapevine (*Vitis vinifera* L.), coffee (*Coffea arabica* L.), cape gooseberry (*Physalis peruviana* L.), apple (*Malus domestica* Borkh.), and tomato (*Solanum lycopersicum* L.) (Wünsche et al. 2004; Steiman 2007; Segura-Monroy et al. 2015; Conde et al. 2016; Gharaghani et al. 2018; Abdallah et al. 2019; de Abreu et al. 2022). Previous studies have demonstrated that PKPF can reduce leaf temperature, improve tolerance to environmental stress, and protect photosynthetic functioning by modifying the interaction between incident radiation and leaf surfaces, as reported for persian walnut and other perennial crops (Mahmoudian et al. 2021; Gómez-Candón et al. 2022). However, its application in native forest species used for ecological restoration remains largely unexplored. This knowledge gap is particularly relevant for *Cordia superba* Cham. and *Citharexylum myrianthum* Cham., two Atlantic Forest species widely used in restoration programs (Agostini and Sazima 2003; Amaral et al. 2013). Previous studies indicate that *C. myrianthum* seedlings are sensitive to elevated temperatures, whereas *C. superba* may experience photoinhibition under excessive irradiance (Souza et al. 2009; Martins et al. 2023; da Silva et al. 2025). These characteristics make both species suitable models for evaluating the potential of PKPF to improve seedling establishment under stressful environmental conditions. However, the limited understanding of how native forest species respond anatomically and ultrastructurally to PKPF application under high irradiance remains an important knowledge gap, restricting the development of strategies to enhance restoration success, optimize seedling production, and reduce replanting costs.

Therefore, this study evaluated the effects of PKPF on seedlings exposed to full sunlight (with and without PKPF application) and greenhouse conditions. Specifically, we investigated whether PKPF promotes anatomical, ultrastructural, and developmental adjustments in two native AF tree species used in ecological restoration, aiming to determine its potential to mitigate high-light stress and improve seedling establishment under under high irradiance conditions. We hypothesized that: (i) PKPF application would mitigate the adverse effects of excessive solar radiation by preserving leaf anatomical and ultrastructural integrity; (ii) seedlings treated with PKPF would exhibit improved growth performance compared with untreated plants under full sunlight; and (iii) species would differ in their responses according to their intrinsic tolerance to light stress.

Materials and methods

Material and cultivation conditions

The species were selected directly from the nursery of Instituto Terra (IT), where they are commonly used in Atlantic Forest (AF) restoration actions developed by IT. The seeds used for seedling production were collected from local mother trees, ensuring regional genetic representativeness. Additionally, criteria such as the difficulty of establishment during the early planting phase in the field, the robust development of the root system, the availability of seedlings, and the logistical feasibility of transportation to the experimental site were considered. Consequently, young plants of two native Atlantic Forest species, *C. superba* and *C. myrianthum* (Table 1), were used in the experiment. The plants were cultivated in a greenhouse with approximately 80% interception of photosynthetically active radiation (PAR) at Terra Institute (TI) in Aimorés, Minas Gerais state, Brazil (19° 29′ 45″ S and 41° 03′ 50″ W), and sent to UENF.

At IT, during seedling production, the seeds were placed in 0.28 m³ tubes containing Vida Verde® substrate, mixed with Osmocote® 3 M (19-6−10) and Osmocote® 5 M (18-5−9) fertilizers at a rate of 4 kg.m³, and Yoorin Master® at 3 kg.m³. The seedlings were fertilized and irrigated to pot capacity under 80% PAR interception. Subsequently, these seedlings were sent to UENF, and at the beginning of the experiment, *C. superba* and *C. myrianthum* were 40 days after sowing [*C. superba*: average height of 0.35 m (approximately 9 leaves) and *C. myrianthum*: 0.25 m (approximately 10 leaves)].

Table 1 Characteristics of the species under investigation

Species	Successional group	Botanical Family	Vegetation type	Foliage habit
Citharexylum myrianthum Cham.	Pioneer[2]	Verbenaceae	Low-Mountain Semideciduous and Deciduous Seasonal Forest, Ombrophilous Forest[2,3]	Evergreen
Cordia superba Cham.	Non-pioneer [1]	Cordiaceae	Caatinga, Cerrado, Riparian Forest, Ombrophilous Forest, Semideciduous and Deciduous Forests[1,3]	Semideciduous

([1]APG 2003; [2] Moraes et al. 2013; [3] Flora of Brazil 2024)

The day after their arrival at the UENF greenhouse, the seedlings were transplanted into 5-liter (L) pots made of high-density polyethylene (HDPE) externally wrapped with aluminum foil to reduce substrate heating. Each pot was filled with a clay-textured red-yellow Latosol, previously prepared for the experiment. Under these conditions, the plants remained in the greenhouse for 34 days, after which the treatments were applied (details will be described in the section "Treatment Application"). The substrate used had a medium acidity pH and an organic matter content of 1.01 dag.kg¹, chosen to provide conditions as similares as possible to the planting site at IT. Irrigation was performed daily, according to the evapotranspiration demand of the cultivation site (greenhouse and outdoor area), in order to maintain the soil at pot capacity. The study was therefore conducted in a greenhouse (with approximately 80% PAR interception), simulating the conditions under which seedlings are produced at IT, and also included a field-simulated area, which corresponded to an open-air site (clear sky conditions) at the State University of Northern Rio de Janeiro (UENF), in Campos dos Goytacazes, Rio de Janeiro state, Brazil (Latitude = 21° 19' 23'' S and 41° 10' 40'' W).

Treatment application

After 34 days of cultivation in the UENF greenhouse, one group of seedlings from both species remained under greenhouse conditions (GH Non Treated), whereas the other groups were exposed to clear-sky conditions. The GH Non Treated treatment was maintained under approximately 80% interception of photosynthetically active radiation (PAR) and served as the control, representing the nursery conditions typically adopted for the production of native tree seedlings destined for restoration projects (Rodrigues et al. 2009). Such conditions allow seedling growth under reduced light availability before their gradual transition to more stressful field environments through acclimation practices (Grossnickle 2012a, b). In contrast, the full-sun treatments reproduced the high irradiance conditions commonly encountered by seedlings after planting in restoration areas. While other group another was transferred to clear-sky conditions. On the night prior to transfer (February 20, 2022), a group of young plants from each species received PKPF application on all leaves (Sunlight with PKPF). The other group did not receive the PKPF application (Sunlight Non Treated).

For the application, PKPF was dissolved in water in a 5 L bucket to homogenize the solution, at a concentration of 0.050 kg of PKPF per 1 L of water (5% solution). Before being placed in the sprayer and during application, the solution was constantly agitated by the operator. The amount of PKPF applied per 1 m² of leaf surface was determined using Petri dishes of known mass and area, positioned at the same angle as the leaves of the studied species during application. After spraying, the Petri dishes were collected and placed in an oven at 85 °C for 24 h to ensure complete water evaporation and were then weighed. Based on the weight difference of the Petri dish containing PKPF before and after drying, the amount of product applied per m² was calculated, totaling 1 g dry PKPF $\cdot m^{-2}$ of leaf area (Surround WP®, NovaSource, USA). For PKPF application, a Guarany® pre-compression sprayer with a capacity of 1.2 L per application and an 80° spray angle was used. Prior to analyses, leaves were carefully cleaned with a moist cotton swab to avoid damage or interference in measurements. Previous studies have shown that PKPF exhibits good adhesion to leaf surfaces and remains effective even after partial loss caused by rain (İtmeç et al. 2020; de Abreu et al. 2022). However, reapplications were necessary after heavy rainfall events to ensure uniform film coverage throughout the experimental period.

The maximum and mean PAR values in greenhouse were 578 ± 18.7 and 289 ± 42.8 µmol m^{-2} s^{-1}, respectively. Maximum, mean, and minimum air temperatures and relative humidity (RH) were 44.5 ± 2.7 °C, 28.9 ± 1.4 °C, and 18.6 ± 0.9 °C, and 98.1 ± 2.7%, 75 ± 4.0%, and 35 ± 6.1%, respectively. All micrometeorological variables inside the greenhouse were monitored using a compact weather station (WatchDog Spectrum Technologies, Thayer Court, Aurora, USA). The same micrometeorological variables were monitored for the plants exposed to full sunlight (open-air site, clear sky), after transfer from the greenhouse, using the same compact equipment (WatchDog Spectrum Technologies, Thayer Court, Aurora, USA). Under clear sky conditions, the respective maximum and mean PAR values were 2000 ± 75.9 and 1190 ± 25.0 µmol m^{-2} s^{-1}. Maximum, mean, and minimum temperatures were 42.4 ± 2.6 °C, 28.8 ± 2.0 °C, and 20.7 ± 1.0 °C, and RH values were 98.1 ± 2.7%, 75.0 ± 4.0%, and 35.0 ± 6.1%, respectively (Fig. 1).

Biometry

This analysis consisted of a destructive assay; therefore, plants were evaluated in a randomized block design with eight replicates at 50 days after treatment application (108 days after planting), corresponding to the final harvest of the experiment. At this stage, the entire plant was harvested for biometric analyses. A total of 48 plants were evaluated, comprising eight biological replicates per treatment. For Citharexylum superba, samples were obtained from plants grown under full sunlight without PKPF application (Sunlight Non Treated), under full sunlight with PKPF application (Sunlight with PKPF), and under greenhouse conditions without

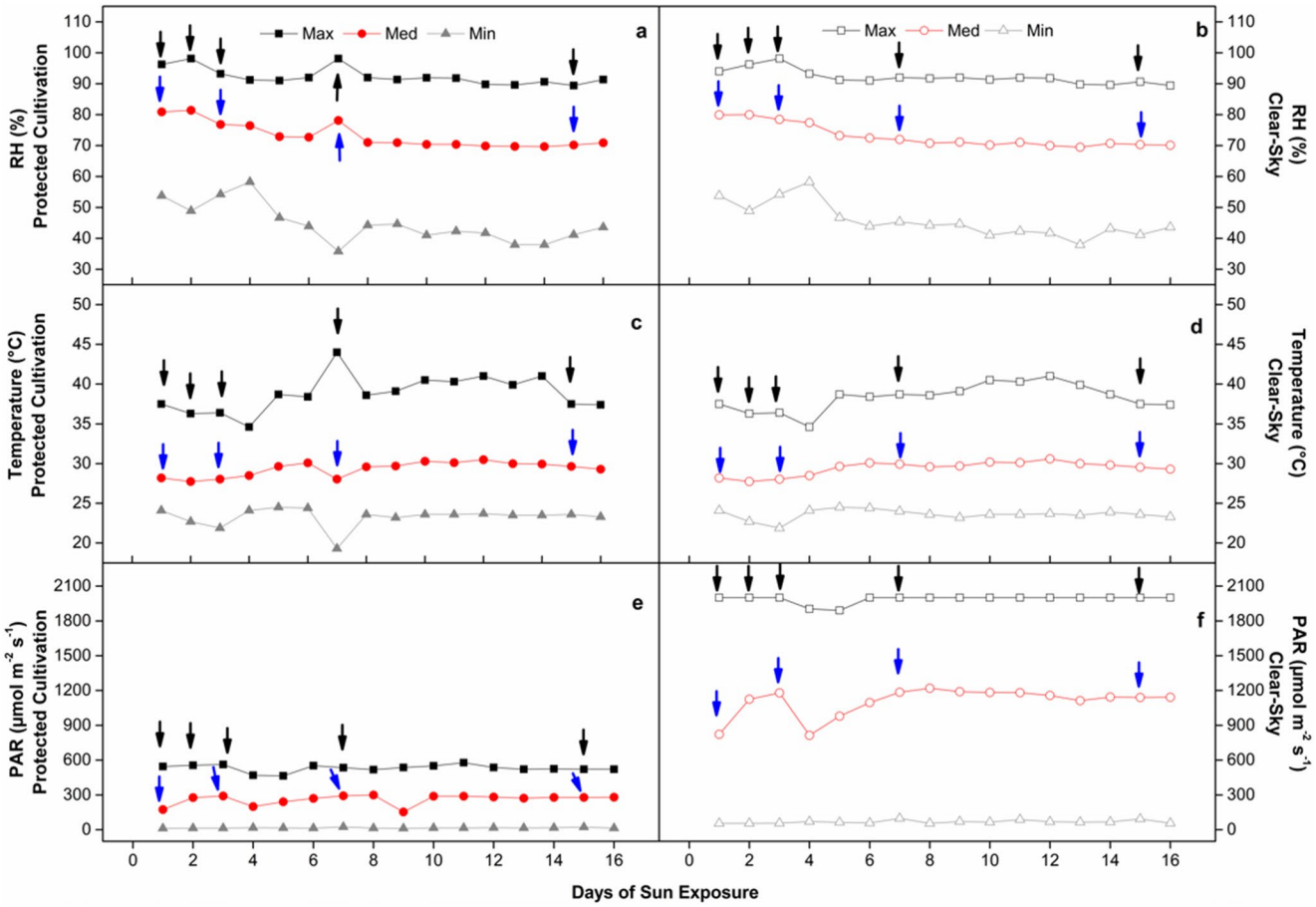

Fig. 1 Relative humidity (RH), air temperature (Tair), and photosynthetically active radiation (PAR), **a**, **c**, and **e** recorded during eco-physiological measurement of young native Atlantic Forest trees (exposure to full solar radiation under clear-sky conditions) in a protected cultivation at 80% PAR interception, and **b**, **d**, and **f** under clear-sky conditions. Blue arrows indicate destructive assessments and black arrows non-destructive eco-physiological assessments

PKPF application (GH Non Treated). The same three conditions were also applied to the species *C. myrianthum*, resulting in a comparable set of groups. The dry mass of the roots, stems, and leaf discs was determined after the samples were dried in an oven at 60 °C for 72 h. To prevent interference from air moisture, the samples were rapidly weighed on a precision scale immediately upon removal from the oven. Leaf disc area was determined using a puncher with a known area of 4.15 cm². Plant height was measured with a graduated tape from the soil surface to the ligule of the first fully expanded leaf (leaf + 1), following the Kuijper system (Horschutz et al. 2022; Figueiredo et al. 2015). Stem diameter was measured using a caliper at three points at the plant's base, and an average was calculated. The central vein length was measured using a millimeter ruler. Specific leaf mass (SLM) was calculated using Eq. [1]:

$$\begin{aligned} &Specific\ leaf\ mass \\ &= Leaf\ dry\ mass\ of\ the\ disc \\ &\div Leaf\ area\ of\ the\ disc \end{aligned} \quad (1)$$

Leaf pigments (greenness, anthocyanin, and nitrogen-associated indices)

A leaf chlorophyll content (Chl index) (leaf greenness) were estimated with a Dualex® portable leaf pigment meter (Scientific, FORCE-A, Orsay, France). These analyses were performed on the same leaves used for the anatomical analyses. To that end, random readings were taken at three equidistant points on the equatorial axis of the surface of three leaves per plant, with 6 repetitions per treatment.

Anatomical and ultrasturctural analyses

Leaves from plants were evaluated in a randomized block design with 5 replicates at 1 day of exposure to full solar radiation (DES), 3 DES, 7 DES, and 15 DES (corresponding to 49, 51, 58, and 73 days after planting, respectively) for scanning electron microscopy (SEM). The analyses of transmission electron microscopy (TEM) and the images obtained using the light microscopy performed at 15 DES.

A total of 30 plants were analyzed, with five samples from each of the six experimental treatments. These treatments included *C. superba* under full sun (Sunlight with PKPF and Sunlight Non Treated), *C. superba* in a greenhouse (GH Non Treated), *C. myrianthum* under full sun (Sunlight with PKPF and Sunlight Non Treated), and *C. myrianthum* in a greenhouse (GH Non Treated).

After collection, mature leaf fragments approximately 1–2 mm² were sampled from the central region of the leaf blade, avoiding major veins, then fixed for 48 h in a solution of 2.5% glutaraldehyde and 4.0% formaldehyde buffered with 0.05 mol L^{-1} sodium cacodylate buffer, pH 7.2, at room temperature (modified Karnovsky 1965). Samples were taken to the Structural Botany Laboratory of the Rio de Janeiro Botanical Garden (LBE), Brazil. Samples were removed from fixative (modified Karnovsky 1965), washed three times with the same buffer, dehydrated in an acetone series (Johansen 1940). In the Structural Botany Laboratory (Rio de Janeiro Botanical Garden, Brazil), the samples were washed three times for 30 min in phosphate buffer, dehydrated in ascending ethyl series (Johansen 1940), infiltrated, and embedded in LeicaR historesin, in line with the manufacturer's recommendations. Next, the embedded samples were sectioned to a thickness of 3 µm using a Leica RM2245 rotary microtome and glass knives. The sections were then adhered to slides using a hot plate and stained with 0.05% Toluidine Blue in 0.1 M phosphate buffer (Feder and O'Brien 1968). The slides were analyzed and images captured using cellSens software and an Olympus BX-50 optical microscope equipped with a DP73 digital câmera for light microscopy. The critical point dried with CO_2 using a Leica EM CPD030 device (Bozzola and Russel 1999), and coated with a 20 nm gold layer using an Emitech K550X Sputter Coater (Ashford, Kent UK). Subsequently, images of the abaxial and adaxial leaf surfaces were obtained using a Zeiss EVO 40 scanning electron microscope operating at an accelerating voltage of 20 kV.

Transmission Electron Microscopy (TEM) samples were washed three times with the same buffer, post-fixed in 1% osmium tetroxide in 0.05 M sodium cacodylate buffer for one hour, washed three times with the same buffer, and dehydrated through a graded acetone series (Johansen 1940), followed by infiltration and embedding in Spurr resin according to the manufacturer's specifications. Ultrathin sections were obtained using a Leica EM UC7 ultramicrotome with a diamond knife, mounted on copper grids (300 mesh), and contrasted with uranyl acetate and lead citrate (Reynolds 1963) at room temperature. Images were acquired using a Tecnai Spirit FEI 120 kV transmission electron microscope at National Center for Structural Biology and Bioimaging (CENABIO)– Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil.

Statistical analyses

A factorial experiment was conducted in a randomized complete block design with additional treatments following a (2 × 2; species × treatment) + 2 designs. This approach combines the investigation of multiple factors and levels with the inclusion of treatments not part of the factorial combinations but considered relevant to the study. This design allowed a detailed study of factor effects and comparisons with additional treatments such as control treatments (k = 2, species cultivated in nursery conditions).

The statistical model used was:

$$Y_{ijk} = m + B_k + T_i + S_j + TS_{ij} + e_{ijk}$$
$$Y_k = m + A + e_k$$

Where Y_{ijk} represents the observed values, m is a constant common to all observations, B_k is the block effect, T_i represents the effect of the treatment levels, S_j represents the effect of species levels, TS_{ij} is the interaction effect between treatment and species levels, A is the effect of the additional treatment, and e_{ijk} and e_k are random errors. The data was subjected to analysis of variance (ANOVA). The assumption of normality of the residuals was verified by the Lilliefors test ($\alpha = 0.05$), while the homogeneity of variances was assessed by the Bartlett test ($\alpha = 0.05$). Tukey's and Dunnett's tests were used as post-hoc methods in the ANOVA to determine which means were significantly different. Tukey's test was used to compare all pairs of factorial treatments (species and treatments Sunlight with PKPF and Sunlight Non Treated), while Dunnett's test was specifically applied to compare each factorial treatment with the control treatments (Tukey 1949; Dunnet 1955, 1964). The Genes program (Cruz 2016) was used for this purpose, and means were compared at 5% significance. Graphs were generated using OriginPro software version 8.5.

Pearson correlation analysis ($p < 0.05$) was performed to evaluate possible linear relationships between growth variables (Table 4) and chlorophyll index in *C. myrianthum* and *C. superba* plants. Analyses were conducted separately for treatments Sunlight with PKPF and Sunlight Non Treated under full solar radiation. Significance of the phenotypic correlation coefficient was evaluated by the t-test, with only the most significant results presented. The Genes program (Cruz 2016) was used for this purpose, and means were compared at 5% significance. Graphs were generated using OriginPro version 8.5 software.

Results

Plant height was influenced primarily by species, with *C. superba* exhibiting greater height than *C. myrianthum* regardless of treatment (Tables 2 and 3). PKPF application did not significantly affect plant height in either species. In contrast, *C. myrianthum* generally maintained a greater number of leaves than *C. superba*, particularly under full-sun conditions without PKPF. Moreover, *C. myrianthum* showed a significantly greater leaf number under Sunlight Non Treated conditions compared with GH Non Treated plants, indicating a positive response of leaf production to full-sun exposure in the absence of PKPF (Table 3).

Table 2 Biometric structure measurements in *Citharexylum myrianthum* and *Cordia superba* plants grown under full solar radiation without PKPF (Sunlight Non Treated), and treated with PKPF (Sunlight with PKPF)

	C. myrianthum		*C. superba*	
	Sunlight Non Treated	Sunlight with PKPF	Sunlight Non Treated	Sunlight with PKPF
Height [cm] (H)	0.39 ^{Ba}	0.35 ^{Ba}	0.52 ^{Aa}	0.50 ^{Aa}
Number of leaves (NL)	23.75 ^{Aa}	20.38 ^{Aa}	14 ^{Ba}	14.62 ^{Aa}
Root dry mass [g] (RDM)	8.70 ^{Aa}	9.13 ^{Aa}	5.23 ^{Bb}	8.55 ^{Aa}
Stem dry mass [g] (SDM)	4.44	4.55	5.59	5.81
Leaf dry mass [g] (LDM)	4.46	4.57	3.47	3.75
Specific leaf mass [g.m-2 (SLM)	53.92	54.85	52.02	55.06
Central vein length [mm] (CVL)	114.5	124.8	128.8	134.2
Stem diameter [mm] (SD)	9.01 ^{Aa}	9.65 ^{Ba}	9.32 ^{Ab}	11.51 ^{Aa}

Tukey Test *P* (5%): Means followed by different uppercase letters indicate statistically significant differences between species, while lowercase letters compare the protection treatment

Root biomass responses differed between species. In *C. myrianthum*, the highest root dry mass values were observed under the Sunlight with PKPF treatment, although no significant differences among treatments were detected within the species. In contrast, *C. superba* showed a significant increase in root biomass following PKPF application, reaching values close to those observed in greenhouse-grown plants (GH Non Treated). A similar trend was observed for stem diameter, which increased significantly in *C. superba* after PKPF application. These responses indicate a positive effect of PKPF on traits associated with seedling vigor and establishment in this species. Most of the remaining biometric variables, including stem dry mass, leaf dry mass, specific leaf mass, and central vein length, were not significantly affected by PKPF application. However, greenhouse-grown *C. superba* plants exhibited greater stem dry mass than seedlings exposed to full-sun conditions, whereas *C. myrianthum* showed relatively stable growth across treatments (Tables 2 and 3).

Pearson correlation analysis revealed distinct patterns among the full-sun treatments (Table 4). In the Sun Light Non Treated treatment, plant height was negatively associated with chlorophyll index and root dry mass, whereas stem dry mass showed a positive association with plant height. Under Sun Light with PKPF, chlorophyll index also showed a negative association with plant height, while positive correlations were observed between stem diameter and height, leaf number and root dry mass, and, to a lesser extent, between total leaf dry mass and height. The observed correlations between growth traits and chlorophyll index suggest that the relationships among biometric and physiological variables differed between cultivation conditions, revealing distinct seedling response patterns under full-sun exposure with and without PKPF application.

Individuals of the species *C. myrianthum* presented dorsiventral leaves, with two types of trichomes on the adaxial and abaxial surfaces: short secretory trichome, with a uniseriate

Table 3 Biometric structure measurements in *Citharexylum myrianthum* and *Cordia superba* plants grown under full solar radiation without PKPF (Sunlight Non Treated), treated with PKPF (Sunlight with PKPF), and under 80% solar interception in a greenhouse (GH Non Treated)

	C. myrianthum			C. superba		
	Sunlight Non Treated	Sunlight with PKPF	GH Non Treated	Sunlight Non Treated	Sunlight with PKPF	GH Non Treated
Height [cm] (H)	0.39 ^{b}	0.35 ^{b}	0.38 ^{b}	0.52 ^{a}	0.5 ^{a}	0.49 ^{a}
Number of leaves (NL)	23.75 ^{a}	20.38 ^{b}	17.87 ^{b}	14 ^{bc}	14.62 ^{bc}	10.37 ^{c}
Root dry mass [g] (RDM)	8.7 ^{a}	9.13 ^{a}	8.78 ^{a}	5.23 ^{b}	8.55 ^{a}	9.50 ^{a}
Stem dry mass [g] (SDM)	4.43 ^{b}	4.54 ^{b}	4.53 ^{b}	5.58 ^{b}	5.80 ^{b}	7.93 ^{a}
Leaf dry mass [g] (LDM)	4.45 ^{ab}	4.57 ^{ab}	3.61^{b}	3.47 ^{ab}	3.75 ^{ab}	4.87 ^{a}
Specific leaf mass [g.m²] (SLM)	114.5 ^{b}	124.8 ^{ab}	123.2 ^{b}	128.8 ^{ab}	134.2 ^{ab}	147.5 ^{a}
Central vein length [mm] (CVL)	9.01^{b}	9.65 ^{ab}	9.55 ^{b}	9.32 ^{ab}	11.51^{a}	10.66 ^{a}

Dunnet Test *P* (5%): Different letters in the horizontal rows indicate significant differences between the control treatment (GH Non Treated) and the full sun exposure treatments (Sunlight Non Treated and Sunlight with PKPF), according to Dunnett's test at 5%, based on the least significant difference (DMS) calculated for each variable

Table 4 Pearson Correlation Coefficients *(P*<0.05) Between Growth Variables and Chlorophyll Index of *Citharexylum myrianthum* and *Cordia superba* Plants Grown under full solar radiation without PKPF (Sunlight Non Treated), treated with PKPF (Sunlight with PKPF), and under 80% solar interception in a greenhouse (GH Non Treated)

	Pearson Correlation coefficient	*P*-value
Sunlight Non Treated		
Chlorophyll Index and Height [m]	−0.68	<0.01
Root Dry Mass [g] and Height [m]	−0.63	0.02
Stem Dry Mass [g] and Height [m]	0.68	<0.01
Chlorophyll Index and Stem Dry Mass [g]	−0.63	0.02
Stem Dry Mass [g] and Height [m]	0.68	<0.01
Sunlight with PKPF		
Chlorophyll Index and Height [m]	−0.72	<0.01
Basal Stem Diameter [mm] and Height [m]	0.65	<0.01
Number of leaves and Root Dry Mass [g]	0.51	<0.01
Total leaf dry mass [g] and Height [m]	0.38	<0.01

stalk and a spherical multicellular glandular head, and the tector trichome, unicellular, located mainly along the vein on the abaxial surface (Fig. 2). In frontal view, the epidermis exhibits cells with sinuously anticlinal cell walls on both leaf surfaces. Stomata were observed on both surfaces. Those located on the adaxial surface are anomocytic, while those on the abaxial surface are anisocytic. This species also presents an ornamented cuticle with an epicuticular wax crust layer on the adaxial and abaxial epidermal surfaces.

Individuals of *C. superba* exhibit a uniseriate epidermis composed of generally rectangular and polygonal cells, with sinuous cell walls. The abaxial surface is hypostomatic, with stomata predominantly of the anomocytic type, arranged in rows along the midrib axis. Trichomes were observed on both leaf surfaces, being unicellular, filiform, tector types with ornamentations (Fig. 2) On the midrib, some trichomes appear erect or semi-erect, while others, especially on the abaxial surface, have an apex aligned parallel to the leaf surface. Additionally, this species has an ornamented cuticle with an epicuticular wax layer of the crust type on both epidermal surfaces.

No structural damage was observed in leaf cross-sections of *C. myrianthum*. However, scanning electron microscopy (SEM) analyses performed 15 days after treatment revealed alterations in epidermal organization in Sun Light Non Treated plants, particularly on the abaxial surface, where stomata exhibited wilted guard cells (Fig. 3b8). Visible symptoms of leaf scorch were restricted to Sun Light Non Treated plants and coincided with these epidermal alterations. Despite these surface-level changes, no apparent damage to mesophyll tissues was detected in light microscopy observations (Fig. 4).

In transmission electron microscopy (TEM) damage in plastids was observed in both epidermis and mesophyll cells. Plants grown in the greenhouse exhibited chloroplasts with well-organized thylakoids and the presence of evident starch grains and plastoglobules. Chloroplasts exhibited organized thylakoid membranes arranged in grana, together with visible starch grains and plastoglobules. The mitochondria showed well-defined cristae without signs of swelling or rupture. The cytoplasm showed no signs of plasmolysis (Fig. 5, g). Plants exposed to full in Sunlight Non Treated treatment showed plastids frequently lacked visible starch

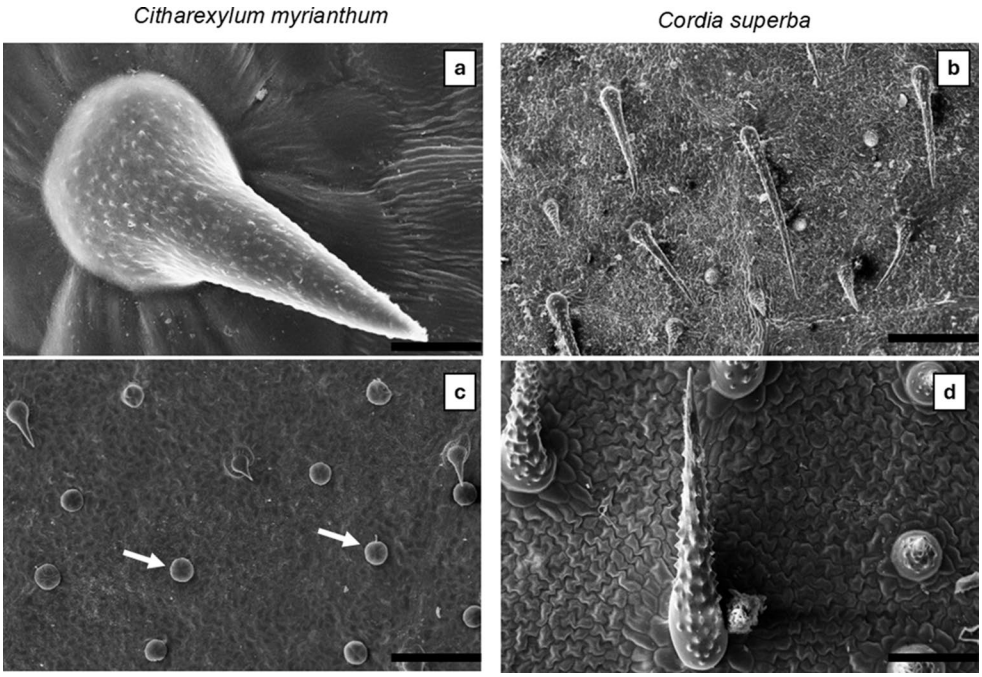

Fig. 2 Foliar anatomical aspects of the adaxial and abaxial surfaces of the studied species. *C. myrianthum* exhibited unicellular tector trichomes, mainly located along the vein on the abaxial surface (**a**), and short secretory trichomes with a uniseriate stalk and a spherical multicellular glandular head, indicated by white arrows on the abaxial surface (**c**). *C. superba* showed trichomes on both leaf surfaces, which were unicellular, filiform, tector-type, with ornamentations on both faces of the leaf (**b**, **d**). Scale bar: 10 µm for a; 100 µm for b and c; 20 µm for d

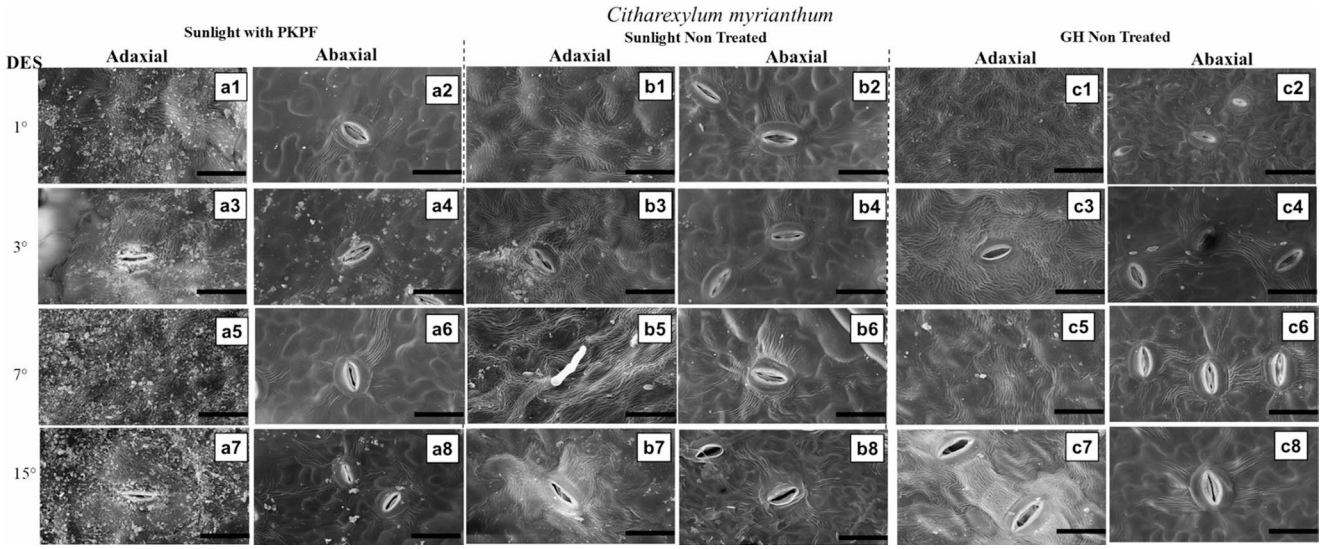

Fig. 3 Anatomical foliar aspects of the adaxial and abaxial surface of *Citharexylum myrianthum* grown in a greenhouse under a shading screen with 80% PAR interception (GH Non Treated) **a1**, **a2**, **a3**, **a4**, **a5**, **a6**, **a7** and **a8**, under full sunlight without treatment (Sunlight Non Treated) **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, **b7** and **b8**, and treated with PKPF (Sunlight with PKPF) **c1**, **c2**, **c3**, **c4**, **c5**, **c6**, **c7** and **c8**. DES indicates the days of solar exposure after PKPF application. Scale bar: 10 µm

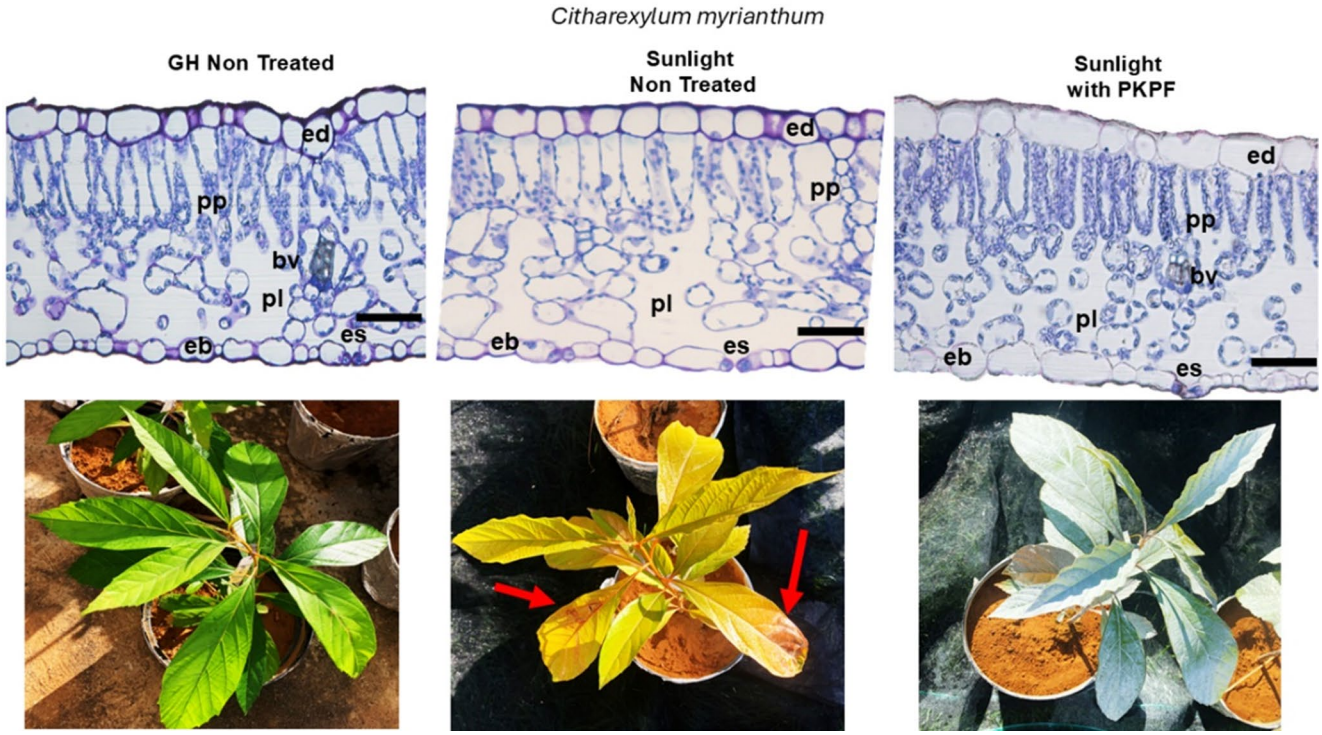

Fig. 4 Cross-section of the leaf blade of *Citharexylum myrianthum* plants grown under 80% solar interception using photoselective shade screens in a greenhouse (GH Non Treated), under full sun treated with PKPF (Sunlight with PKPF), and under full sun without PKPF treatment (Sunlight Non Treated). Red arrows indicate damage/burning of leaf tissue (chlorosis). Ed: adaxial epidermis, pp: palisade parenchyma, bv: vascular bundle, pl: spongy parenchyma, es: stoma, eb: abaxial epidermis. Scale bar: 20 µm

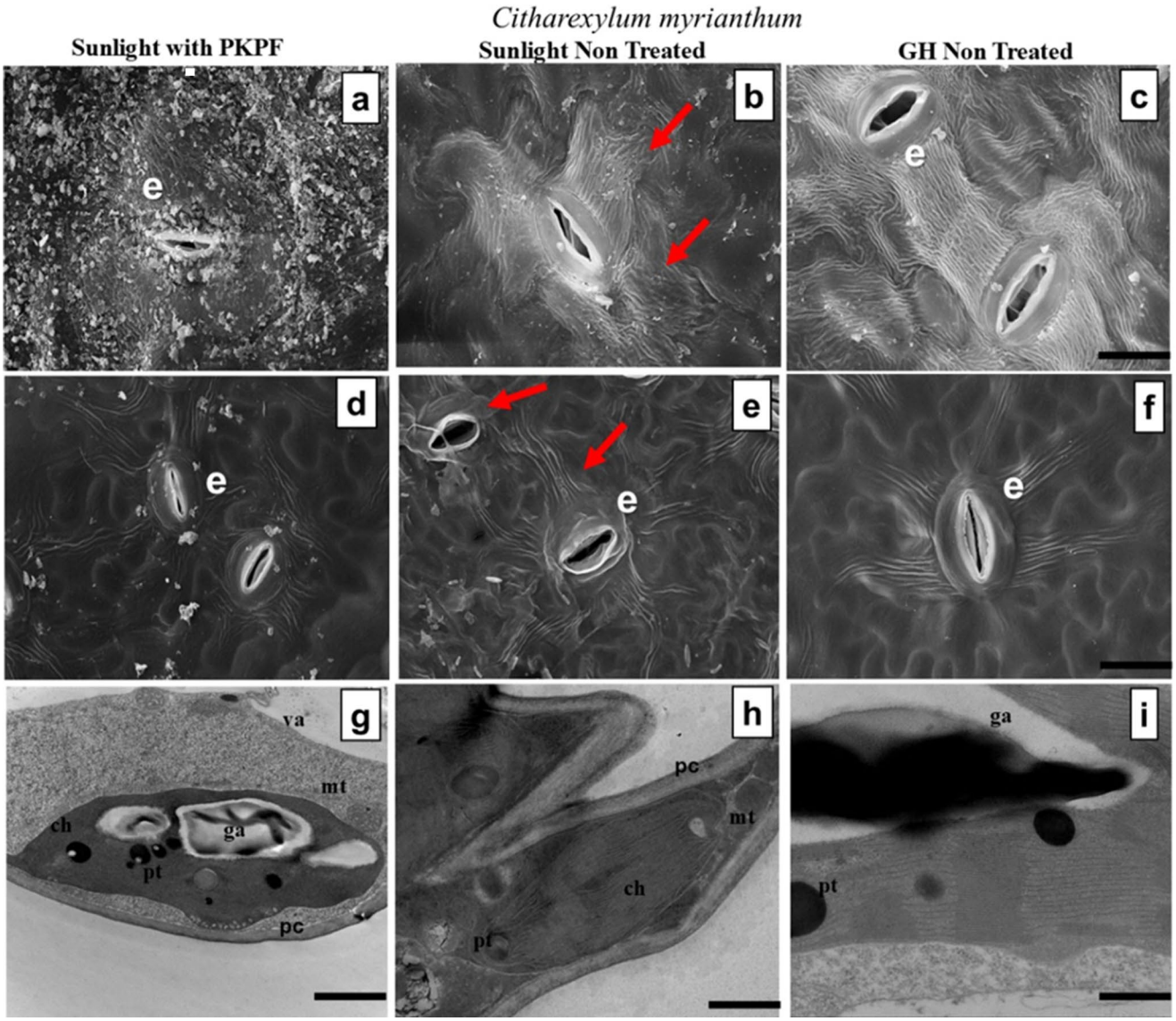

Fig. 5 Anatomical foliar aspects of the adaxial (**a**, **b** and **c**) and abaxial surface (**d**, **e** and **f**). Transmission electron microscopy images (TEM) of chlorophyll cells from the palisade parenchyma (**g**, **h** and **i**) of *Citharexylum myrianthum* grown under full sunlight without treatment (Sunlight Non Treated), and treated with PKPF (Sunlight with PKPF) and in a greenhouse shading screen with 80% PAR interception (GH Non Treated). The images correspond to 15 DES. Pt: plastoglobules, mt: mitochondria, ch: chloroplast, va: vacuole, pc: cell wall, ga: starch grain. Scale bars: 10 µm for a, b, c, d, e and f; 500 nm for h and i; 1 µm for g

grains and exhibited poorly defined plastoglobules, together with structural disorganization of internal membranes, as well as malformed mitochondria with indistinct or absent cristae (Fig. 5, e).

In contrast, chloroplasts from Sun Light with PKPF plants exhibited preserved thylakoid organization, visible starch grains, and plastoglobules. Mitochondria maintained recognizable cristae, and no evident signs of organelle disruption were observed. SEM analyses revealed a continuous superficial layer associated with PKPF deposition on the adaxial surface, while stomatal structures on the abaxial surface remained morphologically preserved. Overall, tissues from PKPF-treated plants displayed fewer visible structural alterations than those observed in Sunlight Non Treated plants.

Structural alterations became evident in leaves exposed to full sunlight without PKPF from 3 DES onward in *C. superba*. On the adaxial surface, epidermal disorganization and loss of the characteristic cell ornamentation were observed. Similar changes occurred on the abaxial surface, where epidermal collapse and disruption of cell wall patterns became progressively more pronounced over time (Fig. 6b3–b7). In contrast, leaves treated with PKPF maintained the overall organization of epidermal cells and

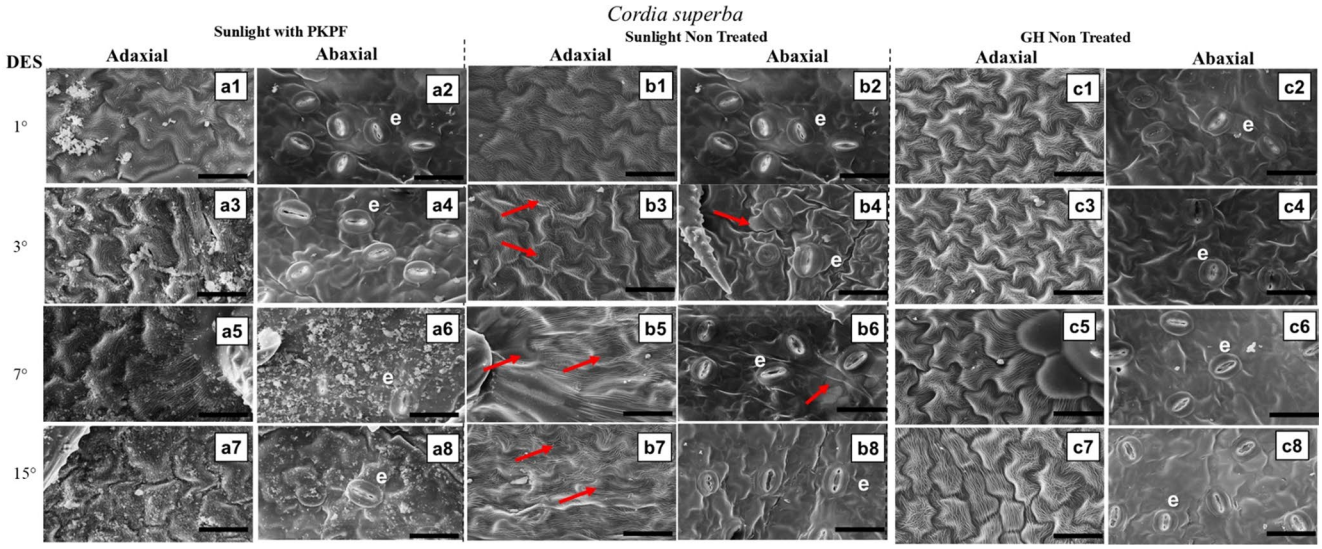

Fig. 6 Anatomical foliar aspects of the adaxial and abaxial surface of *Cordia superba* grown in a greenhouse under a shading screen with 80% PAR interception (GH Non Treated) **a1**, **a2**, **a3**, **a4**, **a5**, **a6**, **a7** and **a8**, under full sunlight without treatment (Sunlight Non Treated) **b1**, **b2**, **b3**, **b4**, **b5**, **b6**, **b7** and **b8**, and treated with PKPF (Sunlight with PKPF) **c1**, **c2**, **c3**, **c4**, **c5**, **c6**, **c7** and **c8**. DES indicates the days of solar exposure after PKPF application. Arrows indicate the convex-shaped ornamentation of the cells. Scale bar: 10 µm

cuticular structures throughout the experimental period, despite the presence of the particle film covering the leaf surface (Fig. 6a1–a8). Cross-sectional analyses further revealed structural alterations in leaves exposed to full sunlight without PKPF, including degradation of palisade parenchyma cells and loss of tissue organization. Such alterations were not evident in greenhouse plants or in leaves treated with PKPF (Fig. 7).

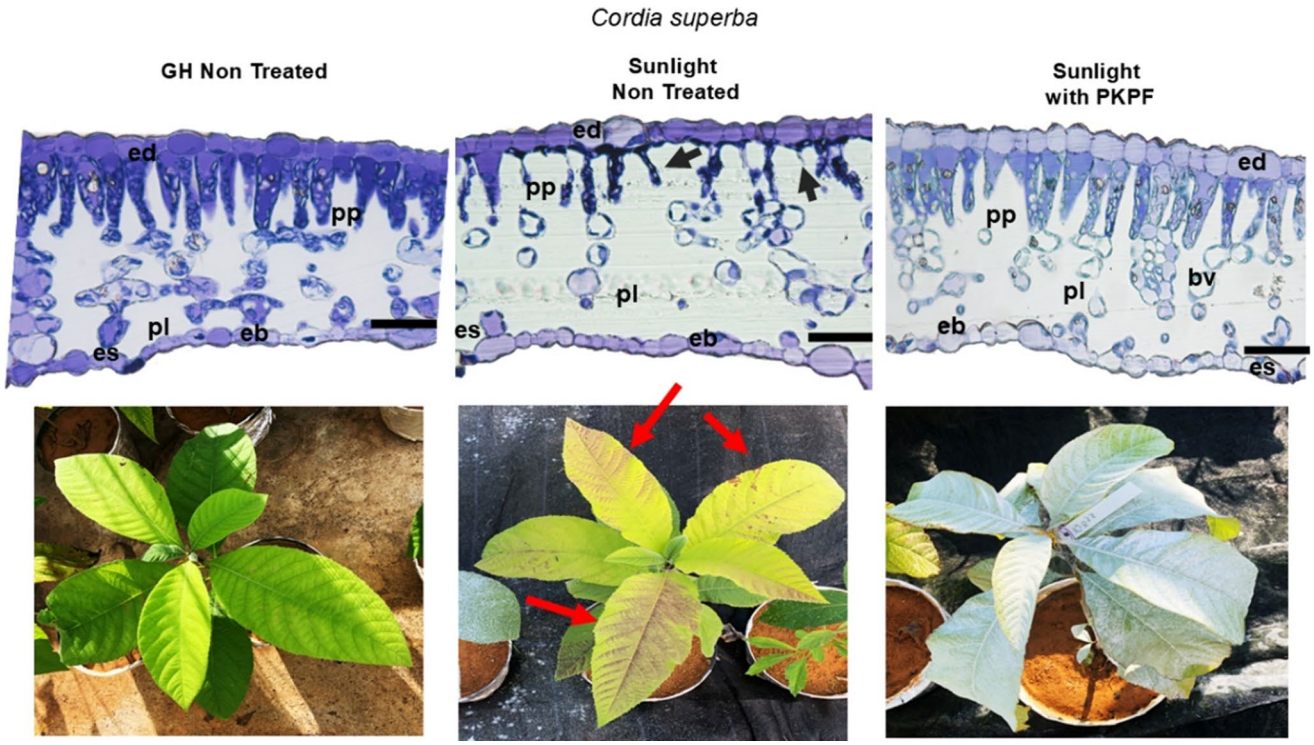

Fig. 7 Cross-section of the leaf blade of Cordia superba, plants under 80% solar interception through photoselective shading screens in a greenhouse (GH Non Treated), under full sun treatment protected with PKPF (Sunlight with PKPF) and untreated with PKPF (Sunlight Non Treated). Black arrows indicate structural damage in the palisade parenchyma and red arrows indicate damage/burning in the leaf tissue (chlorosis). Ed: adaxial epidermis, pp: palisade parenchyma, bv: vascular bundle, pl: spongy parenchyma, es: stomata, eb: abaxial epidermis. Scale bar: 20 µm

Transmission electron microscopy revealed marked differences among treatments. Chloroplasts of greenhouse plants displayed preserved structure, with organized thylakoid membranes, evident starch grains, and plastoglobules (Fig. 8i). In contrast, chloroplasts from leaves exposed to full sunlight without PKPF exhibited thylakoid disorganization, reduced compartment definition, and the absence of visible starch grains (Fig. 8h). Mitochondria were not readily observed in these samples. In leaves treated with PKPF, chloroplasts retained organized grana stacks, visible starch grains, plastoglobules, and recognizable mitochondria (Fig. 8g). Overall, the cellular ultrastructure more closely resembled that observed in greenhouse plants than in untreated sun-exposed leaves.

Discussion

The leaves of plants treated with the kaolin-based particle film (PKPF) showed about 35% reflectance in the PAR range (400–700 nm) compared with untreated leaves maintained under full sunlight or greenhouse conditions

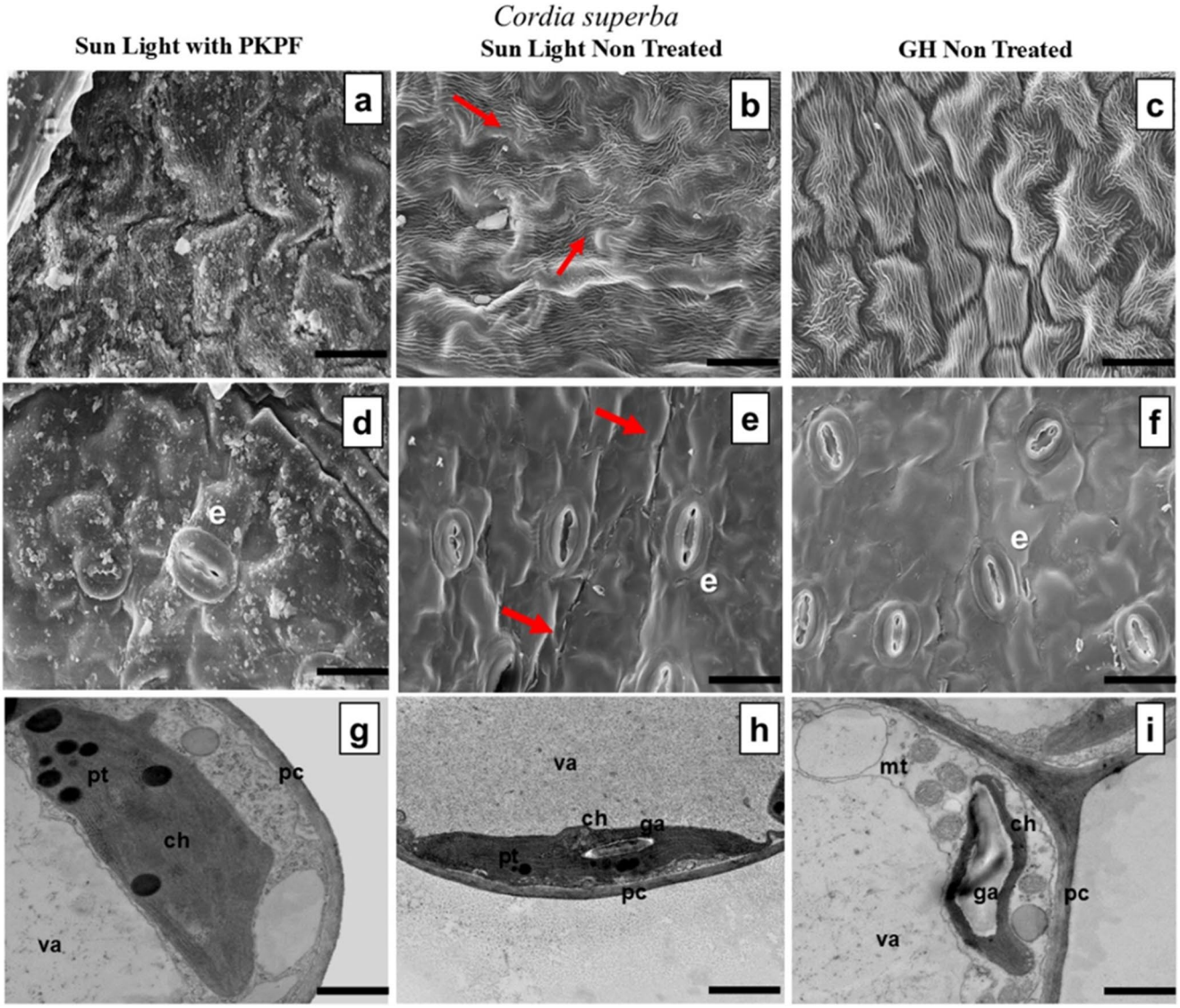

Fig. 8 Anatomical foliar aspects of the adaxial (**a**, **b** and **c**) and abaxial surface (**d**, **e** and **f**). Transmission electron microscopy images (TEM) of chlorophyll cells from the palisade parenchyma (**g**, **h** and **i**) of *Cordia superba* grown under full sunlight without treatment (Sunlight Non Treated), and treated with PKPF (Sunlight with PKPF) and in a greenhouse shading screen with 80% PAR interception (GH Non Treated). The images correspond to 15 DES. Pt: plastoglobules, mt: mitochondria, ch: chloroplast, va: vacuole, pc: cell wall, ga: starch grain. Scale bars: 10 µm for **a**, **b**, **c**, **d**, **e** and **f**; 500 nm for **g**, **h** and **i**

(da Silva et al. 2025). Increased reflectance was associated with lower leaf temperatures, which may contribute to improved plant performance under high irradiance conditions (da Silva et al. 2025). PKPF is widely recognized for its ability to reflect different portions of the solar spectrum, including UV-A radiation (320–400 nm) and PAR, thereby reducing heat absorption by leaf tissues (Glenn et al. 2002; Alvarez et al. 2015). This photoprotective mechanism has been attributed to modifications in leaf optical properties that reduce radiative and thermal loads, minimizing the risk of solar injury under conditions of excessive irradiance (Glenn and Puterka 2004; Glenn 2012). Similar responses were observed by Bernardi (2023) in *Citrus* spp., where reflective efficiency was influenced by particle concentration, size, and distribution on the leaf surface. In the present study, scanning electron microscopy (SEM) analyses revealed a homogeneous distribution of kaolin particles without the formation of concentrated deposits, indicating adequate coverage of the leaf surface. This pattern is consistent with the proposed role of PKPF in increasing reflectance and mitigating the effects of excessive solar radiation on leaf tissues (Gómez-Candón et al. 2022).

The biometric responses indicate that *C. superba* benefited from PKPF application, particularly through increases in root biomass and stem diameter. Additionally for this species, higher stem dry mass was observed in control plants grown under shading, suggesting that reduced solar radiation favored the development of this structure. A similar pattern was found in total root and stem dry mass in control plants, suggesting a greater allocation of biomass to these organs under lower solar radiation exposure (Passos 2023). The basal stem diameter was significantly greater in plants treated with PKPF (11.51 mm) compared to untreated plants (9.32 mm), suggesting that both shading and particle film application contributed to stem thickening (Portela et al. 2019). Overall, growth data suggest that *C. superba* is susceptible when exposed to high solar radiation without PKPF protection. These findings agree with studies by Saour (2005), Bedrech and Farag (2015), Omran (2013), and El-Said (2015), which showed that foliar kaolin spray significantly increased vegetative growth characteristics. Saour and Makee (2004) also observed that olive trees treated with PKPF accumulated more dry matter compared to untreated plants. Such responses have been associated with the ability of particle films to reduce thermal and light stress, creating more favorable conditions for plant growth under high irradiance (Glenn et al. 2002).

Plant morphological characteristics can be analyzed individually; however, their ecological significance is better understood when interpreted in an integrated manner. The correlation patterns observed in plants exposed to full Sunlight Non Treated suggest adjustments commonly associated with stress responses under excessive irradiance. High light availability in taller plants may impose constraints on the maintenance of photosynthetic pigments, particularly when energy absorption exceeds the capacity for its effective utilization, increasing the risk of photoinhibition (Esteban et al. 2015; Bernardi 2023). Under such conditions, changes in chlorophyll index have been reported as part of acclimation adjustments that contribute to the protection of the photosynthetic apparatus and the maintenance of cellular integrity (Ottander et al. 1995; Hashemifar et al. 2025). Taken together, the associations observed between chlorophyll index and growth variables indicate that exposure to intense solar radiation in Sunlight Non Treated plants influenced the balance between carbon acquisition and biomass investment, reflecting morphological adjustments commonly reported in plants subjected to environmental stress (Sturion and Antunes 2000).

In contrast, the association patterns observed in Sunlight with PKPF plants suggest a greater coordination among growth-related traits during seedling development. These results suggest that PKPF application favored a more balanced plant development, promoting greater vegetative growth and biomass distribution. This pattern may be associated with a reduction in photoinhibition induced by intense solar radiation, allowing a more efficient allocation of carbon to both aboveground and belowground organs (Bernardi 2023). Similar responses have been reported in studies showing that particle films modify leaf energy balance and mitigate the effects of excessive solar radiation (Glenn et al. 2002; Bernardi 2023). The positive associations between aboveground and belowground attributes are also consistent with the concept of coordinated resource allocation in young plants, in which shoot and root development are closely interconnected (Behling et al. 2018; Poorter et al. 2012; de Abreu et al. 2022). From an ecological restoration perspective, such integration may be advantageous during the establishment phase, when seedlings must simultaneously sustain shoot growth and acquire water and nutrients under challenging field conditions (Grossnickle 2012a, b; Grossnickle and MacDonald 2018).

High irradiance levels can induce structural changes in leaves at different leaf ontogenetic stages depending on the species, as part of leaf acclimation (Oliveira et al. 2009; Zhang et al. 2022). The contrasting anatomical responses observed between Sunlight with PKPF and Sunlight Non Treated plants suggest that the attenuation of light stress influenced the structural integrity of leaf tissues under high irradiance. Similar effects were reported by Wentworth et al. (2006), who described alterations in mesophyll organization under conditions of high light intensity and elevated temperature. The distinct responses observed between the two studied species also highlight differences in tolerance

to irradiance stress. While *C. superba* exhibited structural alterations in photosynthetic tissues, *C. myrianthum* maintained mesophyll integrity despite the occurrence of leaf scorching. This response may be associated with anatomical characteristics of the species, including the presence of stomata on both leaf surfaces, a feature that has been linked to greater gas exchange capacity and increased flexibility in responding to environmental variation (Mott and Michaelson 1991; Thompson et al. 1992; Pinheiro et al. 2022). The differences observed between the two species also highlight species-specific variation in acclimation to light stress, a phenomenon widely recognized as an important determinant of plant performance across environments with contrasting irradiance levels (Valladares and Niinemets 2008).

Although the anatomical analyses performed by da Silva et al. (2025) did not reveal substantial structural alterations in *C. myrianthum* under light microscopy, the ultrastructural analyses conducted in the present study demonstrated that exposure to high irradiance affected chloroplast organization in both species. These results indicate that cellular alterations may occur before becoming detectable at the anatomical level, highlighting the importance of transmission electron microscopy for the assessment of early stress responses. The foliar degradation observed in untreated plants exposed to full sunlight was accompanied by visible damage to chloroplasts, which is consistent with the effects of excessive radiation and elevated leaf temperatures associated with leaf scorch symptoms (Frioni et al. 2019). In contrast, SEM and TEM analyses indicated that PKPF application contributed to the maintenance of leaf structural integrity under high irradiance. Treated plants exhibited well-organized chloroplasts, preserved thylakoid membranes, starch grains, plastoglobules, and mitochondria with well-defined cristae. These observations are consistent with the lower leaf temperatures recorded in PKPF-treated plants (da Silva et al. 2025) and support the interpretation that the particle film reduced the radiative load reaching leaf tissues. Similar protective effects have been reported in agricultural species, in which kaolin-based particle films increased reflectance, reduced tissue overheating, and mitigated structural damage caused by excessive solar radiation (Glenn et al. 2002; Kerns and Wright 2000; de Abreu et al. 2022; Mohsen and Ibrahim 2021). Taken together, these findings suggest that PKPF may contribute to maintaining cellular organization during seedling establishment under high irradiance, a response that may be advantageous for restoration programs conducted in open-field environments.

In *C. myrianthum*, biomass accumulation remained relatively stable across treatments, with no significant differences in root, stem, or leaf dry mass. This response contrasts with that observed in *C. superba* and suggests species-specific differences in sensitivity to high irradiance during early development. Such behavior may be related to ecological attributes commonly associated with pioneer species, which are often characterized by more conservative morphological responses (Reis et al. 2019). Similar responses have been reported in other species treated with kaolin-based particle films, where growth parameters remained unchanged despite modifications in the leaf microenvironment (Sugar et al. 2005). Despite these morphological responses, ultrastructural analyses revealed clear differences among treatments. In *C. myrianthum* leaves exposed to full sunlight non treated, chloroplasts exhibited characteristics consistent with gerontoplast formation, including thylakoid disorganization and loss of chloroplast structural integrity. Similar features have been described during chloroplast-to-gerontoplast transitions associated with stress and senescence processes (Wise 2016 ; Sadali et al. 2019; Choi et al. 2021). In contrast, sunlight with PKPF plants maintained chloroplasts with a more preserved internal organization, suggesting that the reduction in light stress contributed to maintaining cellular structure under high irradiance. These observations are consistent with the lower leaf temperatures reported for sunlight with PKPF plants (da Silva et al. 2025) and reinforce the potential of particle films as a strategy to mitigate structural damage during seedling establishment in exposed environments.

The preservation of chloroplast organization observed in PKPF-treated plants is consistent with the reduced radiative load previously reported for these leaves (da Silva et al. 2025). In both species, chloroplasts maintained more organized thylakoid systems, well-defined internal compartments, and more evident plastoglobules when compared with untreated plants exposed to full sunlight. Similar structural patterns have been associated with reduced photodamage under high irradiance, whereas prolonged exposure to excessive light has been linked to thylakoid disorganization and progressive chloroplast degradation (Gao et al. 2008; Rottet et al. 2015; Wise 2016; Van Wijk and Kessler 2017; Sadali et al. 2019; Zechmann 2019). Plastoglobules, which are closely associated with thylakoid membranes and participate in chloroplast remodeling under environmental stress, were more conspicuous in PKPF-treated plants and greenhouse-grown controls than in untreated plants exposed to full sunlight (Chabot and Chabot 1977; Tevini and Steinmüller 1985; Djanaguiraman et al. 2011; Rottet et al. 2015; Zandalinas et al. 2017; Van Wijk and Kessler 2017; Zechmann 2019). Considering the structural alterations observed after 15 days of exposure, these differences suggest distinct degrees of plastid preservation among treatments, with PKPF-treated plants maintaining a chloroplast architecture more similar to that observed in greenhouse-grown controls under lower radiative stress.

In addition to differences in chloroplast organization and plastoglobule abundance, mitochondria also varied among treatments. In control plants and in Sunlight with PKPF plants of both species, mitochondria generally exhibited regular morphology and well-defined cristae, whereas untreated plants exposed to full sunlight showed fewer and smaller mitochondria, often with irregular shapes and less distinct internal organization, a pattern also reported by Bernardi (2023). Starch grains were also more evident in the chloroplasts of GH Non Treated plants and Sunlight with PKPF plants, whereas they were absent or scarcely visible in Sunlight Non Treated plants. Similar reductions in starch accumulation have been associated with environmental stress conditions that alter carbon allocation and chloroplast functioning (AbdElgawad et al. 2020). The combined preservation of chloroplast architecture, plastoglobules, mitochondria, and starch grains in Sunlight with PKPF plants suggests that the particle film contributed to maintaining cellular organization under high irradiance. Taken together, these findings reinforce the potential of PKPF as a management tool to mitigate structural damage during seedling establishment, particularly in restoration programs where young plants are frequently exposed to intense solar radiation shortly after field planting (Chabot and Chabot 1977; Djanaguiraman et al. 2011; Zandalinas et al. 2017).

Conclusion

The application of kaolin-based particle film (PKPF) mitigated the effects of high irradiance on young plants of *Cordia superba* and *Citharexylum myrianthum*, contributing to the preservation of leaf anatomical and structural organization. Sunlight with PKPF plants exhibited less structural damage to epidermal tissues, chloroplasts, and mitochondria compared with Sunlight Non treated plants, indicating that the particle film attenuated the impact of light stress during seedling establishment. In addition, positive biometric responses were observed in *C. superba*, whereas *C. myrianthum* exhibited greater morphological stability across treatments, suggesting species-specific responses to PKPF application.

These results demonstrate the potential of PKPF as a promising tool to mitigate light stress during the establishment of native tree seedlings, a critical stage for the success of restoration initiatives. By preserving leaf structural integrity under high irradiance, PKPF may contribute to improving seedling performance in environments characterized by intense solar exposure. Future studies conducted under long-term field conditions and involving a broader range of species will further clarify the applicability of this technology in large-scale restoration programs.

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Author contributions All authors contributed to the conception and design of the study. The preparation of materials, data collection, and analysis were carried out by Amanda Lúcia Pereira Machado da Silva, Guilherme Augusto Rodrigues de Souza, Rosana Maria dos Santos Nani de Miranda, Diesily de Andrade Neves, Larissa Crisostomo de Souza Barcellos, Silas Magno Medeiros Garonce, Paulo Ricardo dos Santos, Cláudia Franca Barros, and Eliemar Campostrini. The first draft of the manuscript was written by Amanda Lúcia Pereira Machado da Silva, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors declare no competing interests.

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