



Particle films improve photosynthesis of citrus trees under excess irradiance by reducing leaf temperature

Lucas G. P. Bernardi¹ | Rodrigo M. Boaretto¹ | Gabriel Constantino Blain² | Dirceu Mattos-Jr¹

¹Centro de Citricultura Sylvio Moreira, Instituto Agronômico (IAC), Cordeirópolis, SP, Brazil

²Centro de Ecofisiologia e Biofísica, Instituto Agronômico (IAC), Campinas, SP, Brazil

Correspondence

Lucas G. P. Bernardi and Dirceu Mattos-Jr,
Centro de Citricultura Sylvio Moreira, Instituto
Agronômico (IAC), Cordeirópolis SP, Brazil.
Email: lbernardi.agro@gmail.com and ddm@ccsm.br

Funding information

São Paulo Research Foundation, Grant/Award Number: 2020/05381-6

Edited by A. Krieger-Liszakay

Abstract

High irradiance and increased air temperature during extreme weather conditions affect tree crops and impact the yield and quality of fruits. Moreover, flowering and fruit set of *Citrus* are likely impaired by UV radiation and/or reduced carbon assimilation, which increase reactive oxygen species production and damage the leaf photosynthetic apparatus. Particle coating films sprayed on leaves have been offered as a way to minimize crop losses due to the climate change scenario, even though the extent of leaf protection is not characterized. We evaluated the use of two protective films on the oxidative stress and leaf photosynthesis of sweet orange trees exposed to varying daylight levels. Trees were maintained under full sun light, sprayed or not (control) with kaolin or calcium carbonate, and under reduced irradiance using either aluminum shade cloth 50% or anti-UV transparent plastic. Kaolin or calcium carbonate reflected 20%–30% of the incident light on the leaf surface compared to leaves not sprayed and under full sunlight. Leaves with coating exhibited improved CO₂ assimilation and photosystem II efficiency, and lower leaf temperatures over time. In addition, the coating protected leaves against excess irradiance due to dissipation of excess energy into the photosynthetic apparatus (NPQt). Nonenzymatic mechanisms for UV protection, such as carotenoids, were higher in full sun control plants than in leaf-coated plants. Comparable responses were observed on trees maintained covered either by the cloth or the plastic film. Finally, we conclude that the use of suspension particles mitigates the harmful effects of excess UV irradiance and temperature in sweet orange trees.

1 | INTRODUCTION

Crop production is currently being challenged by extreme weather events, such as high irradiance, high temperature, and drought, all of which are associated with global climate changes (Mittler et al., 2012). These changes are frequently characterized as the environmental phenomena known as “El Niño” and “La Niña” (Grimm et al., 2020). Such atmospheric disturbances change climate patterns in tropical and mid-latitudinal regions (de Souza Júnior et al., 2017; Geirinhas et al., 2021), causing great economic impacts on agriculture.

Abiotic stress impairs citrus trees' growth and fruit yield mainly during flowering and fruit set, which occur early spring and summer when heat waves and drought are most common (Blain & Bardini-Camarotto, 2014; Pereira et al., 2018). For instance, one of the most important citrus production regions of the world, the Brazilian citrus belt, was severely affected by such adverse weather conditions, causing losses of more than 1.2 million ton during recent seasons (Fundecitrus, 2021).

On the top of the heat waves effect, excess sunlight and irradiance can affect plant metabolism and photosynthesis, two

fundamental processes for productivity, sustainability and crop quality (Ferrante & Mariani, 2018). The energy reaching the leaves are composed of a large spectrum of radiation, which contains the harmful ultraviolet light (UV, 280–400 nm) (Rai & Agrawal, 2017; Tevini & Steinmuller, 1985). The excess irradiance and UV light cause photoinhibition/photooxidative stress (Santini et al., 2012), which produces reactive oxygen species (ROS) in plant tissue due to the excess of electrons (Guo et al., 2006; Gururani et al., 2015).

One of the ROS produced is hydrogen peroxide (H_2O_2). As much as it is harmful at high concentration, H_2O_2 can play an important signaling role in the plant's stress response (Mittler, 2017), such as Abscisic acid (ABA) signalization for stomatic closure (Xia et al., 2015), at low concentration. At high concentrations, ROS cause cellular damage that requires specific scavenging by enzymatic and nonenzymatic systems to ultimately reduce crop losses (Koussevitzky et al., 2008; Nishiyama & Murata, 2014; Saxena et al., 2016).

The frontline enzyme of the antioxidant system is superoxide dismutase (SOD, E.C 1.15.1.1), which is responsible for catalyzing O_2^- by dismuting it into H_2O_2 (Giannopolitis & Ries, 1977). H_2O_2 is then eliminated by other enzymes, such as peroxidases and catalase (CAT, E.C 1.11.1.6), dismuting H_2O_2 into H_2O and O_2 (Zandalinas et al., 2017). Several other enzymes are involved in the elimination of H_2O_2 , and nonenzymatic systems are also operating. Carotenoids are natural, low-molecular-weight pigments, and one of many nonenzymatic protection mechanisms (Edge & Truscott, 2018; Krinsky, 2001). The role of carotenoids against ROS is important due to the capacity of this metabolite to inhibit singlet oxygen production by quenching both triplet sensitizers and excited chlorophyll; thus, protecting the photosynthetic machinery (Soares et al., 2019).

Kaolin [$Al_2Si_2O_5(OH)_4$] and calcium carbonate ($CaCO_3$) are white clay-type minerals, acting as a reflective particle film when sprayed onto plant leaves. They can reduce the excessive leaf temperature resulting from the described unfavorable environmental conditions, and increase photosynthetic efficiency, hence decreasing crop losses. These particle films have already been tested in grapevine, where it was demonstrated that kaolin usage led to higher flavonoids and anthocyanins in the ripe berries than control (Conde et al., 2018). Particle films also had a thermic protective effect on apple trees (Alvarez et al., 2015), decreased fruit sunburn damages and improved yield and fruit quality of citrus (Ennab et al., 2017; Gullo et al., 2020). Nonetheless, the plant physiological traits affected by these "solar protectors" were not clearly characterized to warrant their ample use in agriculture.

Based on the above discussion, this study hypothesized that the use of clay-type leaf-covering particle films alleviates stress caused by excess irradiance associated with nonstomatal limitations; for instance, the balance of metabolites that disturb plant photosynthesis. Therefore, we investigated how the enzymatic and nonenzymatic mechanisms, gas exchange, and nonstomatal parameters, are affected by kaolin or calcium carbonate suspensions sprayed onto the leaf surface, while plants are subjected to various radiation level without affecting air temperature conditions.

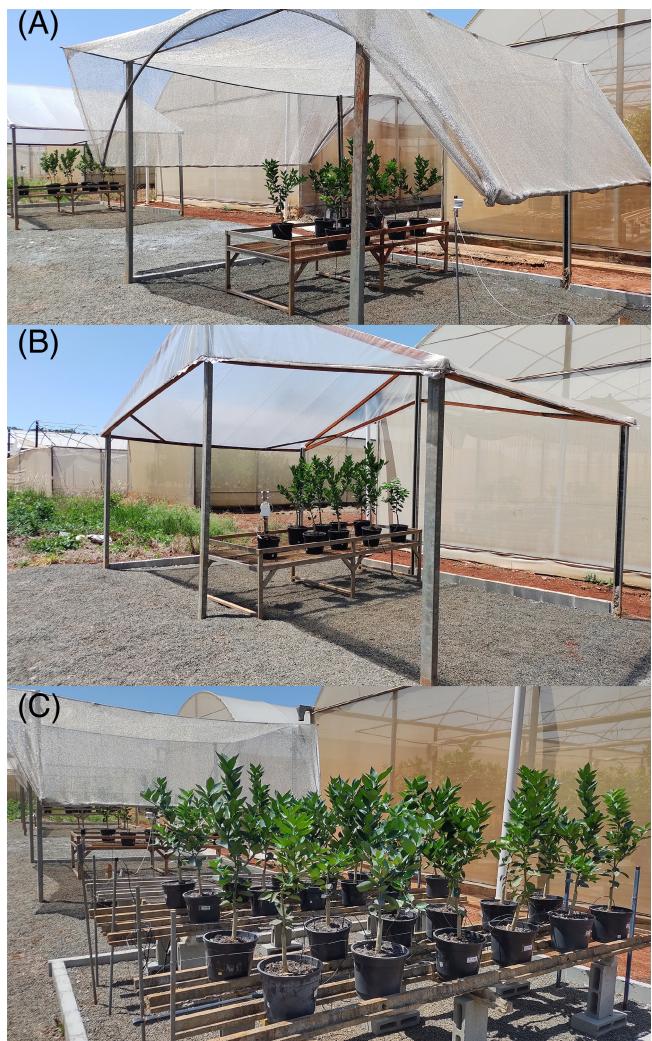


FIGURE 1 Illustration of the experimental setup with trees under full sunlight or covered conditions. (A) Aluminum shade cloth area, (B) transparent anti-UV plastic area and (C) full sunlight area

2 | MATERIAL AND METHODS

2.1 | Plant material and growing conditions

The experiment was carried out at the Centro de Citicultura Sylvio Moreira (22°27'40"S, 47°24'4"O; 639 m height), using 2-year-old sweet orange (*Citrus sinensis* (L.) Osb., cv. Valencia) trees, grafted onto citrumelo "Swingle" [*C. paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.]. Six plants per treatment were grown in 12-L pots containing organic substrate. They were watered and fertilized via controlled drip irrigation to avoid drought stress during the experiment. Trees were also pruned to induce uniform shoot growth.

Treatments were defined as: (1) trees under full sunlight sprayed with water (control); (2) sprayed with kaolin (wettable powder, 1 μ m [30 g L^{-1}]); (3) sprayed with calcium carbonate (wettable powder, <1 μm [30 g L^{-1}]); (4) trees under reduced irradiance using aluminum shade cloth (Aluminet[®] 50%); (5) and trees under reduced irradiance

using 150-μm-thick, low-density polyethylene type (LDPE) transparent plastic (anti-UV additive, >80% transmissivity). Spraying was performed once with a manual pressure sprayer, with 15 s of application per plant, at a flow rate of 220 ml min⁻¹, shortly before placing the plants in the experiment site. Control, aluminum shade cloth and anti-UV plants were sprayed with water at the same period.

Trees were placed on benches 50 cm above the ground and then placed either under full sunlight or under the aluminum cloth or plastic covers. Special structures, 5.0 m × 6.0 m, were built for the covered treatments to avoid shadows overlapping and allow open-air circulation (Figure 1). Air temperature (T_{air} , 29°C ± 0.05), relative humidity (RH, 59.4% ± 0.09), and photosynthetically active radiation (PAR, 1053.9 μmol m⁻² s⁻¹ ± 9.3) were monitored at 1.0 m above the ground, corresponding to the mid-tree dossel, using automatic sensors (S-LIA-M003, HOBO Data login Solution) (Cordeiro-Júnior et al., 2021). Recorded T_{air} and RH did not differ among open and covered treatments during the day and during the experimental period.

2.2 | Gas exchange measurements

The CO₂ assimilation rate (A_{net}), stomatal conductance to water vapor (g_s), and transpiration (E) were determined from fully expanded leaves in the middle third of the most recent vegetative growth flush. The apparent carboxylation efficiency (A_{net}/C_i) was calculated. The evaluations were performed 17 (March 12) and 36 (March 31) days after treatments imposition (DAT), on a clear day between 9:00 a.m. and 10:00 a.m., as well as between 1:00 p.m. and 2:00 p.m., with an open-path infrared gas analyzer system (LI-6800, LI-COR) equipped with an integrated fluorescence chamber head. Ambient measurement conditions were controlled to maintain 400 Pa CO₂ partial pressure and an artificial photosynthetic photon flux density (PPFD) of 1000 μmol photons m⁻² s⁻¹.

Chlorophyll fluorescence measurements were taken with the infrared gas analyzer system (LI-6800, LI-COR) under the same ambient conditions described for gas exchange measurements. Steady-state (F_0') and maximum (F_m') fluorescence yields were assessed in light-adapted leaves between 1:00 p.m. and 2:00 p.m. F_m' was measured after a light saturation pulse ($\lambda < 710$ nm, PPFD ~10.000 μmol photon m⁻² s⁻¹, 0.8 s). The variable fluorescence yield in light-adapted ($\Delta F = F_m' - F_0'$) leaves and the effective quantum yield (Φ_{II}) of PSII [$\Delta F/F_m' = (F_m' - F_0')/F_m'$] were also calculated (Baker, 2008). The apparent electron transport rate (ETR) was calculated according to Schreiber et al. (1998).

Changes in chlorophyll fluorescence yield and changes in light-induced absorbance were also measured using the MultispeQ® V2.0 instrument (PhotosynQ) using undamaged mature leaves (Kuhlgert et al., 2016). The instrument also allows for (1) the evaluation of the linear rate of electrons from the antenna complex to the PSII reaction center (LEF); (2) nonphotochemical quenching (NPQ); (3) the fraction of light energy captured by PSII, which is directed to NPQ, dissipated as heat within the sheet (PhiNPQ); (4) the energy that is lost by unregulated processes and (5) the fraction of photosystem II centers that are in the state considered “open.” The evaluation times were at 9:00 a.m. and 1:00 p.m.

2.3 | Leaf temperature

The temperature of the leaf surface (T_{leaf}) was measured every 1 h on a sunny and hot day (March 23, 28 DAT), from 8:00 a.m. to 4:00 p.m., using an infrared thermometer (Fluke 59 Max, Fluke Co.) positioned perpendicularly at 25–30 cm distance from a leaf blade completely exposed to full sunlight. The temperature of one leaf per tree from each treatment was measured. The average temperatures were calculated for each time and the sum was performed to verify the accumulated temperature throughout the day from each treatment.

2.4 | Chlorophyll content

51 days after treatment, one leaf sample per plant was collected to determine the concentration of total chlorophyll and carotenoids through extraction in dimethyl sulfoxide. According to Lichtenthaler (1987), optical densities measured by a spectrophotometer were used to estimate their concentrations.

2.5 | Leaf hydrogen peroxide and MDA levels

Quantification of H₂O₂ and MDA was performed using the same extraction, in which 500 mg of fresh leaf mass was homogenized in 0.1% (w/v) trichloroacetic acid (TCA) and 4% (w/v) polyvinylpyrrolidone (PVPP). The mixture was then centrifuged at 5590 × g for 15 min at 4°C (Alexieva et al., 2001). For H₂O₂ concentration, the supernatant was mixed with 100 mM potassium phosphate buffer (pH 7.0) and 1.0 M potassium iodide (1:1:4) and incubated at 4°C for 1 h in darkness and then for 20 min at 25°C. Sample absorbance was measured at 390 nm and H₂O₂ content was calculated using a standard curve with known concentrations of H₂O₂.

The presence of MDA was measured to verify lipid peroxidation, according to Heath and Packer (1968). Briefly, to the extracted supernatant, 1 ml of a solution containing 20% (w/v) TCA and 0.5% (w/v) thiobarbituric acid (TBA) was added and then incubated at 95°C for 30 min. The mixture was then quickly cooled at 4°C to stop the reaction. The samples were re-centrifuged for 5 min at 12,100 × g and the supernatant measured at 535 and 600 nm. The absorbance of the formed TBA reactive substances was determined at 535 nm. Measurements were corrected for unspecific turbidity by subtracting the absorbance at 600 nm. The amount of MDA was calculated using an extinction coefficient of 155 mM⁻¹ cm⁻¹.

2.6 | Enzymatic antioxidant analysis

For protein quantification and posterior enzyme activities, 1 g of fine leaf powder was homogenized in 5.0 ml of 100 mmol L⁻¹ potassium phosphate buffer (pH 7.5) with 3 mM dithiothreitol, 1 mM EDTA, and 4% (w/v) PVPP. The suspension was centrifuged at 12,100 × g at 4°C for 35 min, and the supernatant was stored at -80°C until further

analysis. Total protein content was determined using bovine serum albumin as a standard (Bradford, 1976).

The activity of CAT was determined according to Kraus et al. (1995) with modifications (Azevedo et al., 1998). The reaction was initiated with the addition of 20 µl of plant extract to a reaction mixture containing 100 mmol L⁻¹ potassium phosphate buffer (pH 7.5) and H₂O₂ (30% solution) at 25°C. The enzyme activity was determined by following the decrease in absorbance at 240 nm, which represents the decomposition of H₂O₂, for 1 min against a plant extract-free blank. Catalase activity was calculated using an extinction coefficient of 39.4 M⁻¹ cm⁻¹.

SOD activity was determined according to Giannopolitis and Ries (1977) in a spectrophotometer. The extracted sample of 50 µl was added to a glass test tube containing a solution of 13 mmol L⁻¹ of methionine, 75 µmol L⁻¹ of NBT, 100 mmol L⁻¹ of EDTA and 2 µmol L⁻¹ of riboflavin in 3.0 ml of potassium phosphate buffer (5 mmol L⁻¹, pH of 7.8). The preparation of controls followed the same methodology, replacing the plant extract with sodium phosphate buffer. The tubes were placed 15 min inside a reaction chamber (at 25°C) under artificial lighting with 15 W fluorescent lamps to form the blue formazan compound. The readings were determined on a

UV-Vis spectrophotometer at a wavelength of 560 nm. The results were expressed in Unit SOD mg⁻¹ protein.

2.7 | Statistical analysis

The experiment was carried out with five treatments and six replications, each being an individual tree, per treatment. Analysis of variance (ANOVA) was performed for each variable evaluated. In case of significance, the Tukey test was used at 5% significance.

3 | RESULTS

3.1 | Environmental characteristics

The average daily T_{air} recorded at each microenvironmental condition did not vary ($28.9 \pm 0.05^\circ\text{C}$) between growth condition treatments (full open sunlight and under aluminum cloth or plastic-covered benches). This was the same for mean air RH ($61.8 \pm 0.09\%$) (data not shown). The large and

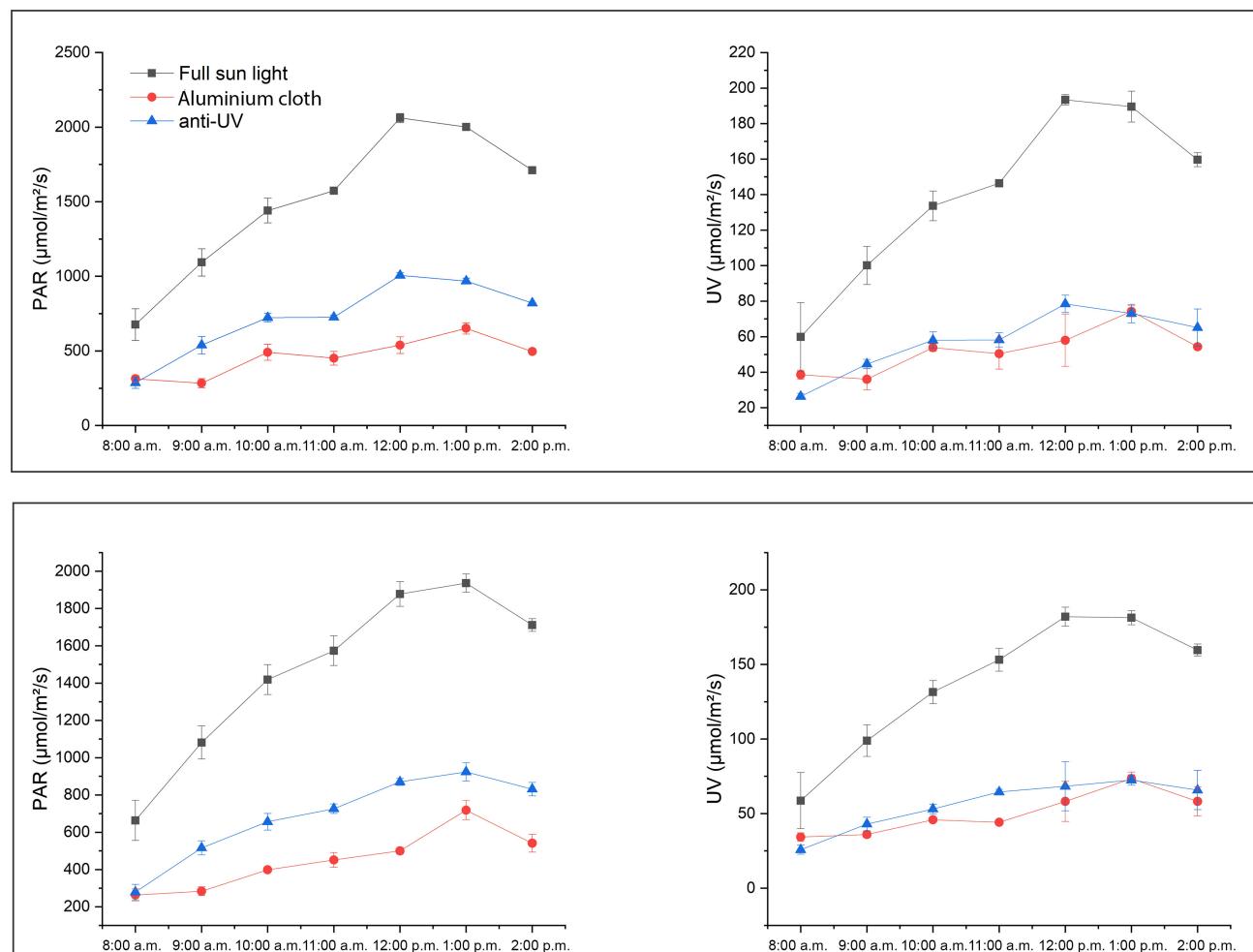


FIGURE 2 Photosynthetic active radiation (PAR) and ultraviolet radiation (UV) during the first period. (A,B) first evaluation day of the first period on March 12 and (C,D) during the second evaluation day (March 13). Values are mean \pm SE ($n = 6$ individual trees).

March 12

March 13

tall covered structures allowed ample movement of air mass in the environment throughout the experimental period, which confirmed our assumption that T_{air} and RH were uniform for different treatments.

PAR recorded from the full sunlight environment was maximum between 12:00 p.m. and 1:00 p.m. during the first evaluation period (17 DAT; March 12 and 13), with $1969.0 \pm 39.7 \mu\text{mol m}^{-2} \text{s}^{-1}$ and UV radiation peaked at $186.5 \pm 2.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Figure 2). The recorded minimum PAR ($602.1 \pm 50.3 \mu\text{mol m}^{-2} \text{s}^{-1}$) and minimum UV ($65.9 \pm 4.5 \mu\text{mol m}^{-2} \text{s}^{-1}$) values were observed under the Aluminet 50%. In contrast, the highest PAR and UV were recorded in full sunlight during the second evaluation period (36 DAT; March 31 and April 1) where they peaked at $1557.4 \pm 48.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $157.5 \pm 15.1 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively (Figure 3).

3.2 | Gas exchange parameters and chlorophyll fluorescence

Net CO₂ assimilation (A_{net}) averaged about $8 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the early morning for all treatments. However, it was lower ($3.5 \mu\text{mol m}^{-2} \text{s}^{-1}$)

at the beginning of the afternoon on 17 DAT (Figure 4A). This trend was uniform, with the exception being the aluminum cloth treatment, in which gas exchange parameters were the lowest compared to those under other treatments. Furthermore, E and g_s varied similarly to A_{net} . The same parameters were lower by the afternoon during the first evaluation period (17 DAT) compared to the early morning measurements (Figure 4). The imposed treatments affected A_{net} , E , and g_s at both morning and afternoon evaluations (Figure 4B,D,E) on 36 DAT. Trees under full sunlight sprayed with kaolin or calcium carbonate, as well trees under the UV-plastic cover, exhibited greater A_{net} compared to either the control or the aluminum cloth treatments in the morning and at 1 pm (32.8% and 51.4%, respectively). Such differences were again mostly related to variations in E and g_s (Figure 4).

The calcium carbonate treatment provided better A_{net}/C_i in the morning and in the afternoon (30%–75%) compared to the control or the aluminum cloth treatments, considering both periods of evaluation (Figure 5).

Treatment impositions affected ETR only for the measurements taken at 9:00 a.m., when calcium carbonate-sprayed trees exhibited a 34% increase rate compared to the control or aluminum cloth-shaded

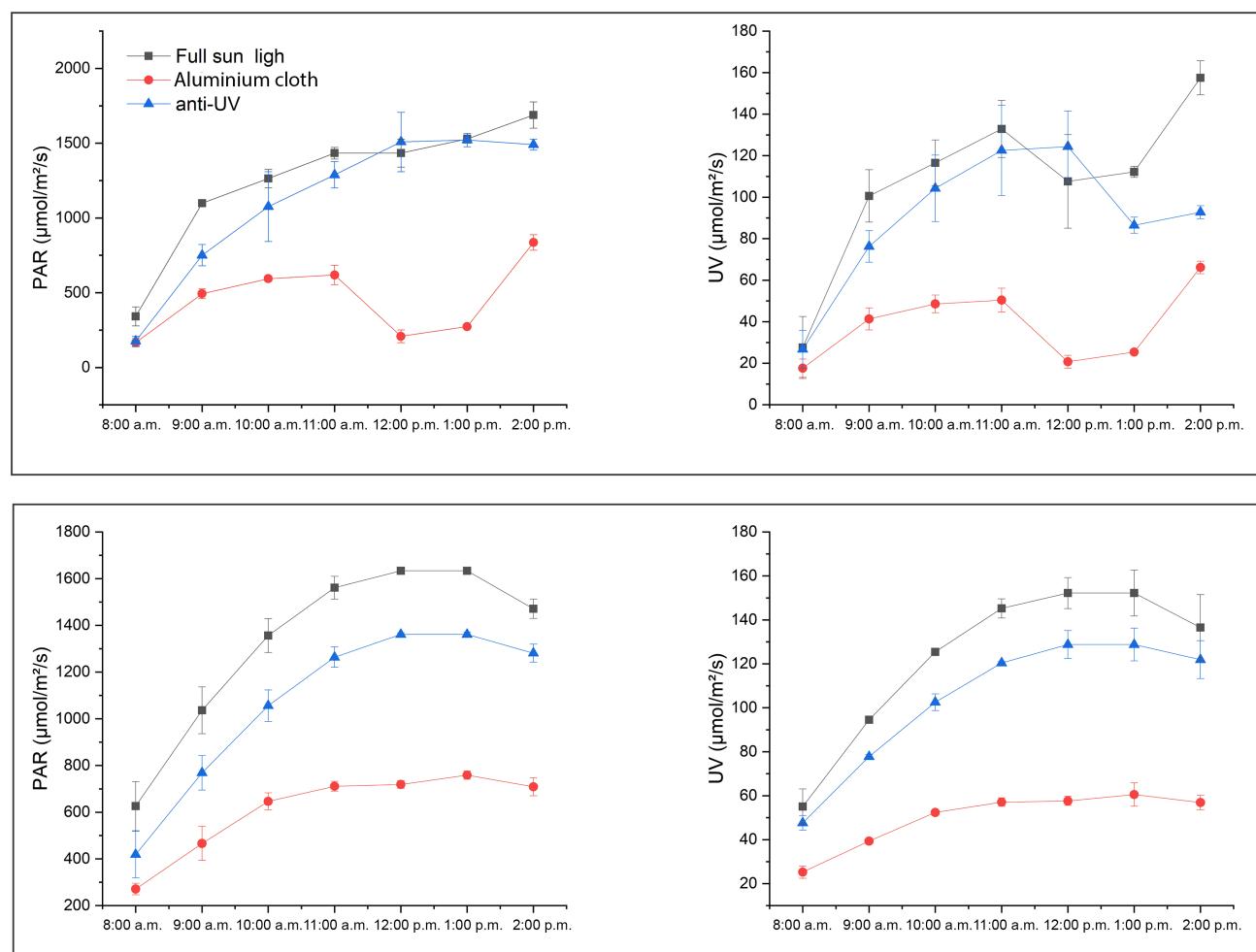


FIGURE 3 Photosynthetic active radiation (PAR) and ultraviolet radiation (UV) during the second period. (A,B) first evaluation day of the second period on March 31 and (C,D) for the second evaluation day (April 1)

March 31

April 1

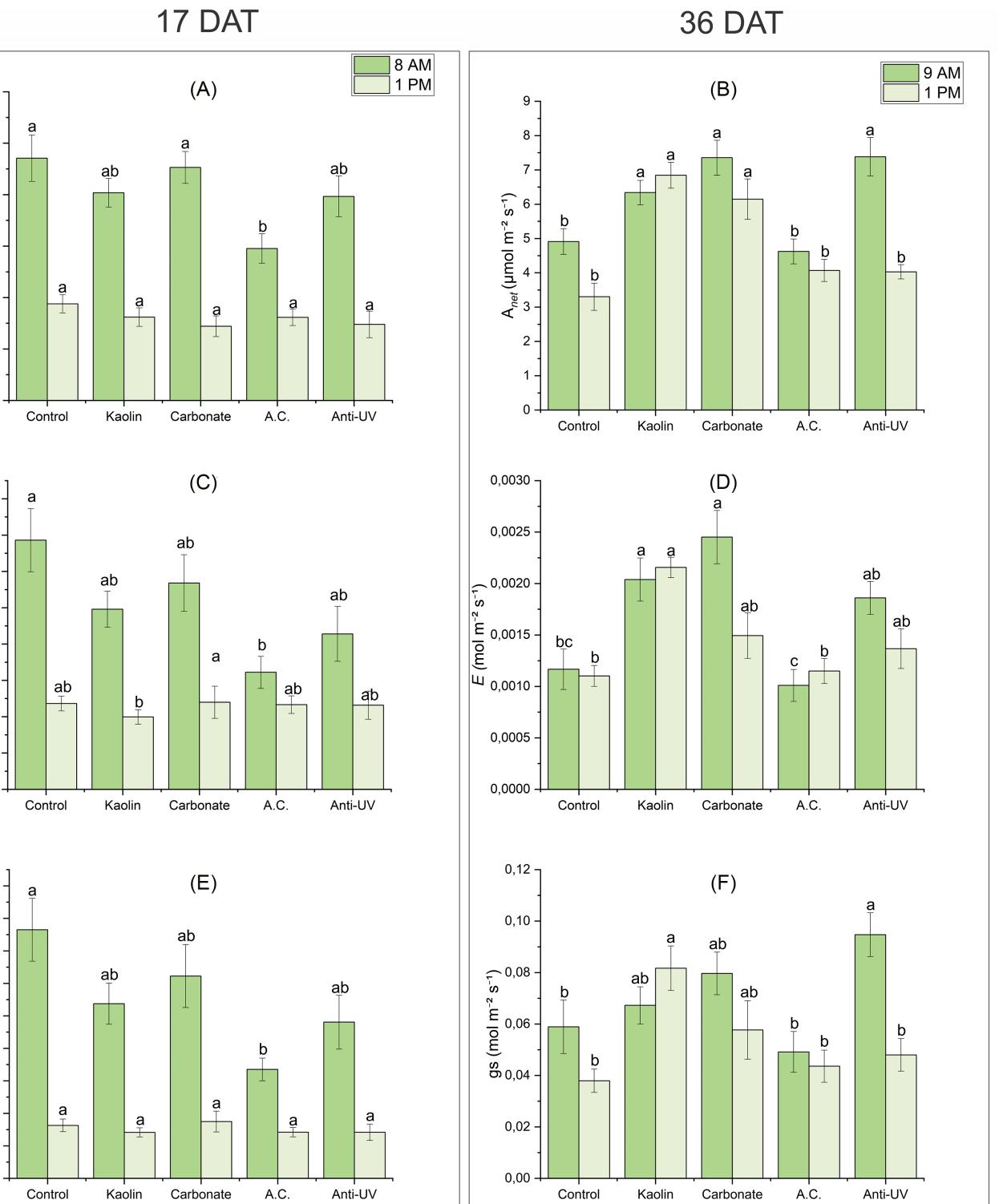


FIGURE 4 CO_2 assimilation rate (A_{net}), transpiration (E) and stomatal conductance (g_s). (A-D) first evaluation (17 DAT), (B-F) second evaluation (36 DAT) of gas exchange on sweet orange leaves for the respective treatments: Control, kaolin, calcium carbonate, aluminum cloth (A.C.), and anti-UV. $n = 6$ individual trees. Tukey test at 5% significance

trees (Figure 6A). Moreover, the effective efficiency of photosystem II (ΦII) was higher during the morning compared with the afternoon with improved tree performance in those sprayed with particle films (kaolin or calcium carbonate).

NPQs (NPQt) were more pronounced in plants treated by kaolin and control plants at 9:00 a.m. on the second day of evaluation. On the other hand, the lowest NPQt value at 1:00 p.m. was registered in control plants. Calcium carbonate exhibited almost the same NPQt

rate between morning and afternoon measurements on the same day (1.5 and 1.7 in the morning and 1:00 p.m., respectively), which was 42.3% higher than the full sunlight control at noon (Figure 7).

3.3 | Leaf temperature

Leaf temperature varied for plants under the different microenvironmental conditions (Figure 8). The sums of the individual values measured from 8:00 a.m. to 4:00 p.m. are shown in Figure 8. Control plants had the highest accumulated temperature, while plants treated with calcium carbonate and anti-UV treatments maintained intermediate values, and kaolin modulated leaf temperature similarly to that

observed in plants under reduced radiation provided by the aluminum cloth.

3.4 | Leaf chlorophyll and carotenoids content

Treatments did not affect the photosynthetic pigments (chlorophyll *a*, *b*, and total chlorophyll) of trees evaluated at the end of the experiment. However, the concentration of carotenoids and the total chlorophyll/carotenoids ratio varied, with lower levels associated with trees exposed to full sunlight (control treatment) (Figure 9).

3.5 | Biochemical analyses

Concentrations of H₂O₂ and MDA in plant leaves varied depending on treatment imposition, with up to 26% higher MDA levels found in trees sprayed with calcium carbonate compared with the control treatment or those under aluminum cloth and anti-UV plastic covers (Figure 10A,B). Interestingly, the enzyme activity of the enzymatic antioxidant system increased, as SOD and CAT were prone to be higher in the kaolin treatment than in the other treatments (Figure 10C,D). Moreover, the lowest levels of SOD were determined in the control treatment.

4 | DISCUSSION

Heat waves have been recorded in the agricultural regions of south-east Brazil, where the maximum air temperature has increased by up to 4.5°C above historical averages, affecting crop production in spring and summer (Dusenge et al., 2018; Geirinhas et al., 2021). The use of agricultural inputs that promote leaf protection during extreme

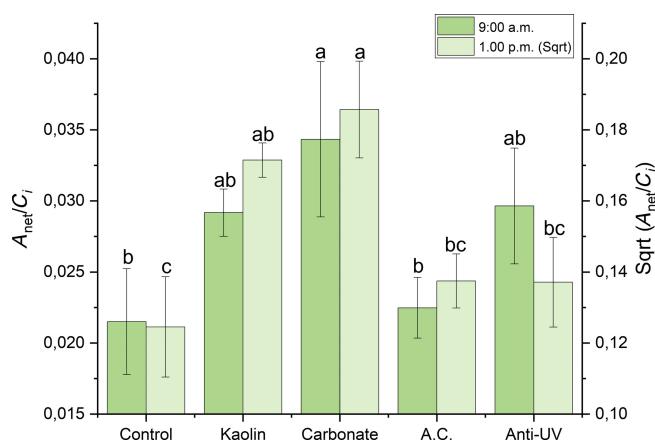


FIGURE 5 Apparent carboxylation efficiency at 9:00 a.m. (A_{net}/C_i) and 1:00 p.m. ($\text{Sqrt}(A_{net}/C_i)$) evaluated at 36 DAT in sweet orange leaves [*Citrus sinensis* (L.) Osb., cv. Valencia], for the respective treatments: Control, kaolin, calcium carbonate, aluminum cloth (A.C.), and anti-UV. $n = 6$ individual trees. Tukey test at 5% significance

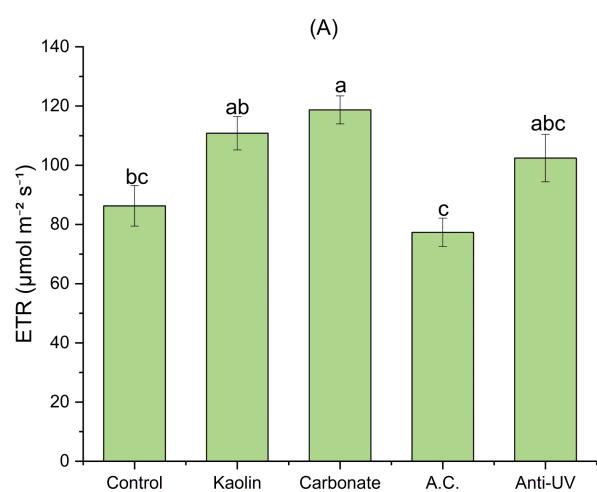
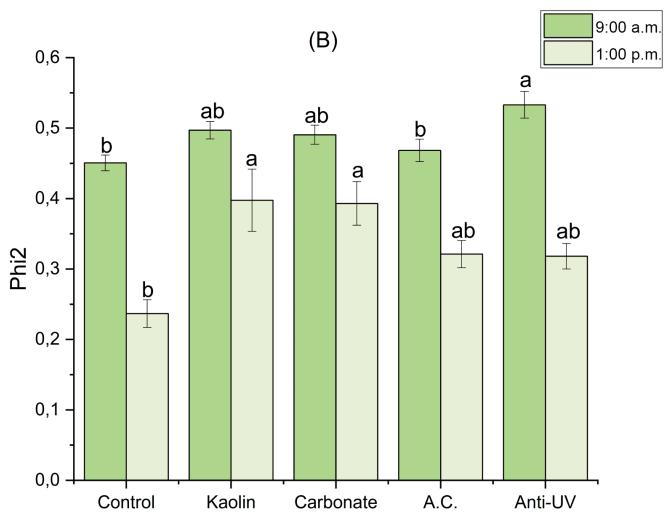


FIGURE 6 Electron transport rate (ETR) (A) and effective efficiency of photosystem II (Phi2) (B) evaluated on 36 DAT in sweet orange leaves [*Citrus sinensis* (L.) Osb., cv. Valencia] for the respective treatments: Control, kaolin, calcium carbonate, aluminum cloth (A.C.), and anti-UV. $n = 6$ individual trees. Tukey test at 5% significance



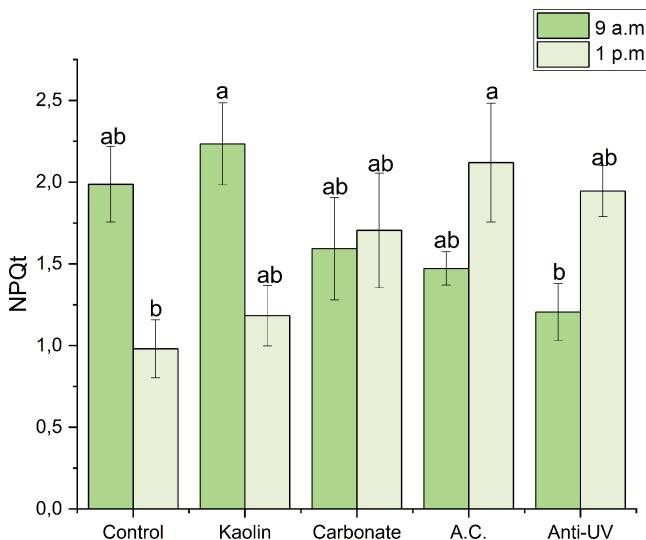


FIGURE 7 Nonphotochemical quenchings evaluated on 36 DAT in sweet orange leaves (*Citrus sinensis* (L.) Osb., cv. Valencia], grafted onto citrumelo Swingle [*C. paradisi* Macf. X *Poncirus trifoliata* (L.) Raf.] for the respective treatments: Control, kaolin, calcium carbonate, aluminum cloth (A.C.), and anti-UV. $n = 6$ individual trees. Tukey test at 5% significance

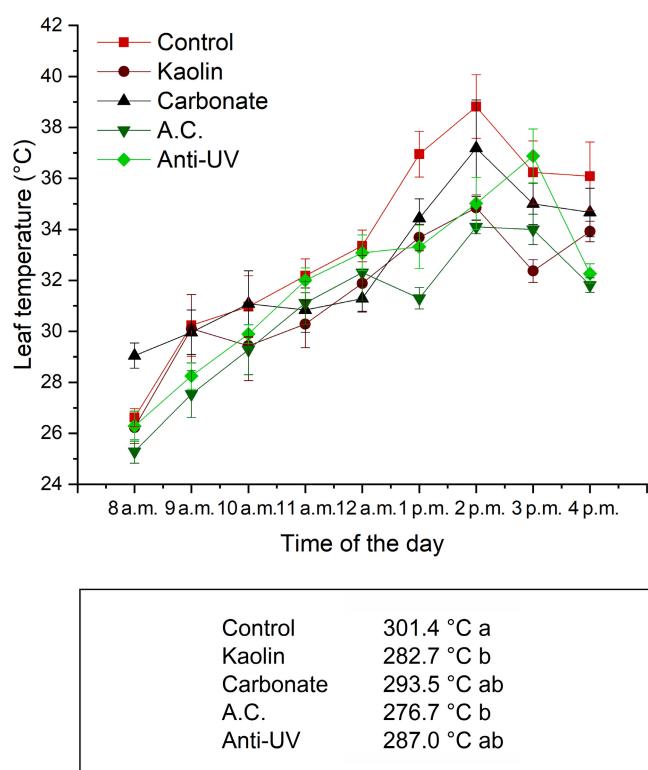


FIGURE 8 Leaf temperature measured from 8:00 a.m. to 4:00 p.m. on 28 DAT for sweet orange (*Citrus sinensis* (L.) Osb., cv. Valencia) trees grown under the respective treatments: Control, kaolin, calcium carbonate, aluminum cloth (A.C.), and anti-UV. Leaf accumulated temperature (box) represent the summation of hourly measurements for the same day. Measurements were made each hour for the sum of temperature means and Tukey test.

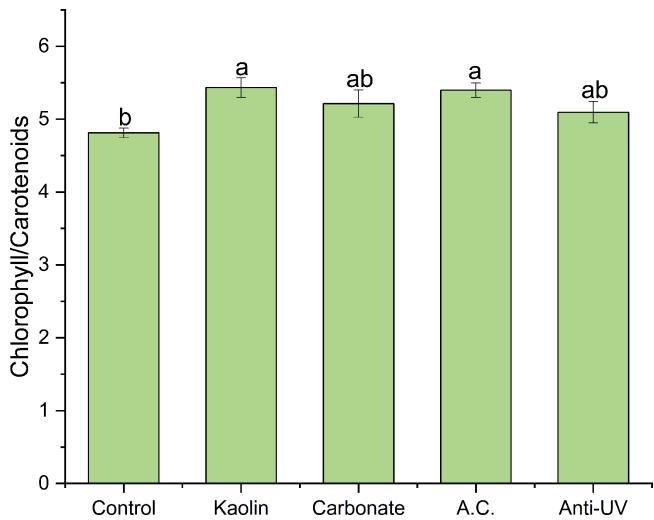


FIGURE 9 Total chlorophyll/carotenoids in sweet orange leaves (*Citrus sinensis* (L.) Osb., cv. Valencia), for the respective treatments: Control, kaolin, calcium carbonate, aluminum cloth (A.C.), and anti-UV. $n = 6$ individual trees. Tukey test at 5% significance

weather conditions has shown the potential to mitigate photosynthesis impairment, which is a temperature-sensitive process (Brito et al., 2019).

Our study demonstrated that leaf temperature was lower in plants sprayed with kaolin particle film compared to plants maintained under full sunlight (control) during the time of highest expected irradiance during the day. For instance, the accumulated temperature on March 23 demonstrated that kaolin application achieved a 270°C value, much lower than that observed for the control treatment, which was 310°C. This highlights the protective effect the film, which simulates shaded conditions for leaves under full sunlight.

We used aluminum cloth cover to reduce irradiance, as well the anti-UV plastic cover to filter a significant portion of the light spectrum that transfers energy to the leaf surface, causing temperatures to rise. In fact, by estimating the heat accumulation in leaves from early morning to late afternoon (Figure 9), particle films favored the maintenance of better tree physiological conditions. Briefly, there was no significant PAR-reducing incident, as observed in the case of aluminum cloth (reduction of 50%). It is also likely that particle film coverage reduced the harmful effects of UV radiation on the photosynthetic apparatus since the estimated heat accumulation was 282°C for kaolin and 293°C for the calcium carbonate treatment, similar to that of the anti-UV treatment (287°C). Therefore, considering our assumption is valid that T_{air} did not vary among the microenvironmental conditions determined by treatments, we provide novel information for the use of particle films as a practice to support plant protection under excess irradiation. Such information will be further detailed with the addition of data collected for gas exchange and other physiological-related traits of plants.

The gas exchange parameters (A_{net} , E , and g_s) measured during the first experimental period were higher in the early morning than in the beginning of the afternoon. Furthermore, they were lowest in

shaded trees, under the aluminum cloth, which was associated with a PAR reduction of 51% compared to the full sunlight condition. The A_{net} of citrus trees saturated with $800 \mu\text{mol m}^{-2} \text{s}^{-1}$ and light above this rate can reduce A_{net} (Alvarez et al., 2015; Tsai et al., 2013). On the other hand, light exceeding the saturation point can harm the photosynthetic machinery, leading to the reduction of A_{net} through a chain of physiological reactions (Alvarez et al., 2015; Brito et al., 2018).

Despite the A_{net} values observed for the control trees being similar to those of trees sprayed with particle films and the ones under the anti-UV cover (particularly during the morning), A_{net} likely increased upon the stress caused by excess irradiance. Consequently, a high T_{leaf} will increase the need for the production of compounds and enzymes associated with plant protection. The stress maintenance energy ends up being high, impairing the normal plant metabolism, as seen on 36 DAT.

Indeed, during the second evaluation (36 DAT), the gas exchange parameters of sprayed trees and those under the anti-UV cover were

greater than the control and shaded trees, either in the early morning or beginning of the afternoon. Gullo et al. (2020) suggested that solar protector products, like calcium carbonate, improve the carbon assimilation of sweet oranges in environments with $1488 \mu\text{mol m}^{-2} \text{s}^{-1}$ of PPFD. The A_{net} increment of plants treated with particles was supported by a high A_{net}/C_i ratio exhibited by trees with kaolin or calcium carbonate (49% more than control; Figure 5). This response shows that calcium carbonate improved light utilization, and it can be explained by nonstomatal parameters like ETR, Phi2, and NPQt.

High ETR values in plants treated with calcium carbonate reflected greater photosynthetic efficiency in our study (Figure 6A). The plant adaptation mechanism for high irradiance is associated with the restoration of PSII, which is dependent on light intensity and leaf temperature for optimal function (Takahashi & Murata, 2008). Therefore, kaolin and calcium carbonate-treated trees presented Phi2 approximately 0.4 at midday (Figure 6B), while shaded and anti-UV exhibited values approximately 0.3, and full sunlight control approximately 0.2 despite similar excess irradiance conditions.

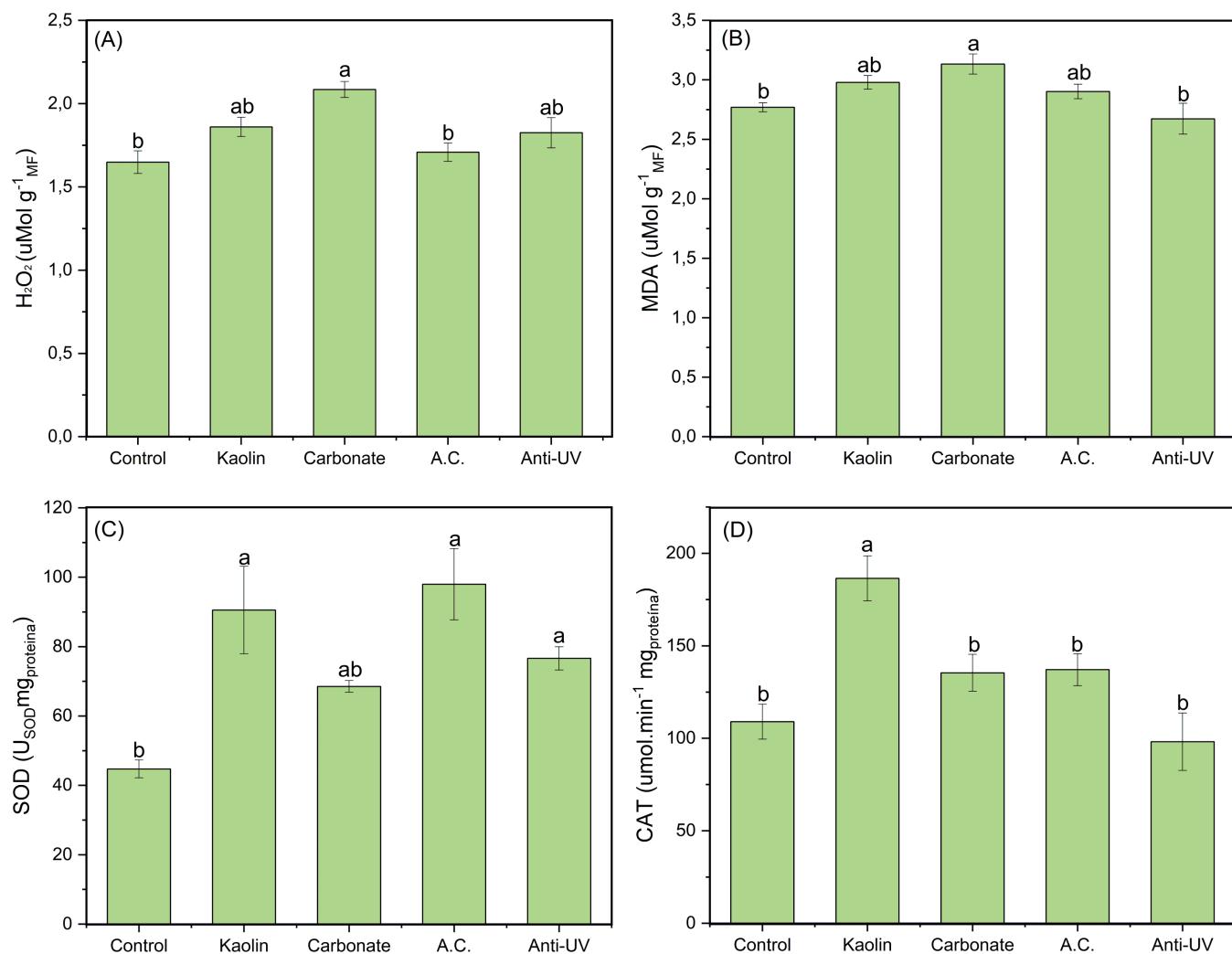


FIGURE 10 (A) H_2O_2 , (B) MDA, (C) superoxide dismutase and (D) catalase analysis in sweet orange leaves (*Citrus sinensis* (L.) Osb., cv. Valencia), grafted onto citrumelo Swingle (*C. paradisi* Macf. \times *Poncirus trifoliata* (L.) Raf.), for the respective treatments: Control, kaolin, calcium carbonate, aluminum cloth (A.C.), and anti-UV. $n = 6$ individual trees. Tukey test at 5% significance



In response to high irradiance, plant photoprotection mechanisms might be characterized by PhiNPQ and NPQt estimates, which represent the amount of energy likely dissipated as heat, to modulate the adverse effects of excess photon absorption in leaves (Esteban et al., 2013; Murchie & Niyogi, 2011). In the early evaluations (17 DAT), PhiNPQ demonstrated high efficiency in protecting the plant against excess irradiance (data not shown). However, after only 15 days, the NPQ mechanism differed among the treatments: the NPQ mechanism is more efficient in plants having some type of protection; hence, maintaining high levels of photosynthetic efficiency (Phi2) and Rubisco carboxylation efficiency (A_{net}/C_i).

Indeed, the excess photon absorption without corresponding energy dissipation decreases the efficiency of PSII and A_{net} by photo-inhibition and changes the structure of the PSII antenna system (La Porta et al., 2005; Molina-Bravo et al., 2011). Trees under the control treatment exhibited the lowest NPQt values, mostly during the afternoon, indicating a limited ability to dissipate energy, which will need to be compensated by other defense mechanisms against the stress condition.

Other mechanisms for protection against stress in plants stimulated with high irradiance are known, for example the nonenzymatic role of carotenoids. Carotenoids allow plants to absorb excess energy, reducing its transfer to ground-state oxygen and the formation of singlet oxygen (1O_2) that damages plant cells (Edge & Truscott, 2018). Furthermore, carotenoids act like an energy drain and dissipate this as heat (Sandmann, 2019). As observed in the control trees, where the chlorophyll to carotenoids ratio was the lowest in this study, high irradiance increased photoinhibition, which is in line with the discussion above (Figure 6).

Different environmental stress factors, combined or alone, trigger physiological changes in citrus plants (Balfagón et al., 2019). As a result, toxic compounds are produced in the plants, such as O_2^- and H_2O_2 , which need to be eliminated by enzymatic or nonenzymatic systems (Ramel et al., 2012; Zandalinas et al., 2017). Our results demonstrated that plants treated with calcium carbonate exhibited higher H_2O_2 concentration in leaves compared to the control and aluminum cloth (Figure 10A).

Plants protected with particles also exhibited greater photosynthetic efficiency than control and shaded trees. It is then reasonable to assume that increased H_2O_2 did not characterize increased stress. Indeed, H_2O_2 is produced naturally along the photosynthetic chain (Gururani et al., 2015; Saxena et al., 2016), also playing an important role in ABA signaling regulation for stomatal closure (Xia et al., 2015), and activation of proteins involved with high-temperature resistance (Volkov et al., 2006). Furthermore, citrus plants can present variable H_2O_2 and MDA responses under imposed sunlight and acclimatation, depending on exposure time, as reported by Santini et al. (2012).

Increase in H_2O_2 concentration in the carbonate-treated plants can result from the relative increase in SOD-dependent H_2O_2 production accompanied by no improvements in the activity of H_2O_2 -scavenging enzymes, such as CAT (Figure 10). Moreover, H_2O_2 can be generated through the spontaneous dismutation of superoxide anion and activity of distinct enzymes from different metabolic pathways

(e.g., photorespiration, β -oxidation of fatty acids and/or polyamine metabolism) (Soares et al., 2019). In this context, an increased H_2O_2 could be potentially related to mechanisms necessary for plants to cope better with high irradiance, as supported by the good photosynthetic activity (Figures 4, 5) and maintenance of normal leaf anatomy of the carbonate-treated plants under high irradiance.

Interestingly, the H_2O_2 overproduction can cause oxidative stress because of these same characteristics (high stability and diffusibility), yielding oxidation of proteins, fatty acids and nucleic acids, and lipid peroxidation (Soares et al., 2019). The increased MDA level suggested that lipid oxidation was triggered at least once in the leaves of carbonate-treated plants (Figure 10). However, changes in the H_2O_2 concentration may only explain part of the variations in MDA level (e.g., A.C.-treated plants had similar H_2O_2 concentration in comparison to control plants, but increasing trend in MDA levels), indicating that lipid peroxidation was enhanced by other compounds.

Generally, total SOD activity increased in leaves protected with kaolin, shaded or under the anti-UV treatment, compared to the control. In a minor deviation, CAT activity was also associated with kaolin-protected trees. High SOD values were observed in trees having high H_2O_2 values (kaolin, calcium carbonate, or anti-UV), except in shaded plants, which exhibited low values for LEF and ETR, parameters indicative of ROS production (Nishiyama & Murata, 2014). These results suggest an enzymatic role in avoiding ROS accumulation (Koussevitzky et al., 2008).

In conclusion, our findings suggest that suspension sprays with particles that form a thin, reflective film coating on leaf surfaces, such as kaolin or calcium carbonate, reflect incident light and reduce the adverse effects of excess UV irradiance on the physiological traits of citrus trees since plants exhibited improved CO_2 assimilation and photosystem II efficiency, and lower leaf temperature over time. Such effects were related to improved antioxidant enzyme activity, likely associated with excess energy dissipation in the PSII.

AUTHOR CONTRIBUTIONS

The study was designed and conducted by Dirceu Mattos-Jr, Rodrigo M. Boaretto and Lucas G. P. Bernardi. Material preparation, data collection and analysis were performed by Lucas G. P. Bernardi and Dirceu Mattos-Jr. The first draft of the manuscript was written by Lucas G. P. Bernardi and Dirceu Mattos-Jr. Lucas G. P. Bernardi, Dirceu Mattos-Jr, and Rodrigo M. Boaretto commented on previous versions of the manuscript, while Gabriel Constantino Blain contributed the final version.

ACKNOWLEDGMENTS

This study was financially supported by the São Paulo Research Foundation (FAPESP, Grant No. 2020/05381-6). The authors are grateful for the critical contributions of Marcia E.A. Carvalho (Esalq/USP) on the results for the plant antioxidant response system.

CONFLICT OF INTEREST

The authors declare that they have no competing interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

ORCID

- Lucas G. P. Bernardi  <https://orcid.org/0000-0003-2893-7957>
 Rodrigo M. Boaretto  <https://orcid.org/0000-0001-9959-7924>
 Gabriel Constantino Blain  <https://orcid.org/0000-0001-8832-7734>
 Dirceu Mattos-Jr  <https://orcid.org/0000-0002-6149-9189>

REFERENCES

- Alexieva, V., Sergiev, I., Plant, S.M. et al. (2001) The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant, Cell and Environment*, 24, 1337–1344.
- Alvarez, H.L., Di Bella, C.M., Colavita, G.M. et al. (2015) Comparative effects of kaolin and calcium carbonate on apple fruit surface temperature and leaf net CO₂ assimilation. *Journal of Applied Horticulture*, 17, 176–180.
- Azevedo, R. A., Alas, R. M., Smith, R. J., & Lea, P. J. (1998). Response of antioxidant enzymes to transfer from elevated carbon dioxide to air and ozone fumigation, in the leaves and roots of wild-type and a catalase-deficient mutant of barley. *Physiologia Plantarum*, 104(2), 280–292. Portico. <https://doi.org/10.1034/j.1399-3054.1998.1040217.x>
- Baker, N.R. (2008) Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. *Annual Review of Plant Biology*, 59, 89–113.
- Balfagón, D., Sengupta, S., Gómez-Cadenas, A., Fritsch, F.B., Azad, R.K., Mittler, R. et al. (2019) Jasmonic acid is required for plant acclimation to a combination of high light and heat stress. *Plant Physiology*, 181, 1668–1682.
- Blain, G.C. & Bardin-Camparotto, L. (2014) Detecting trends in 10-day rainfall amounts at five sites in the state of São Paulo, Brazil. *Acta Scientiarum: Technology*, 36, 685–692.
- Bradford, M.M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254.
- Brito, C., Dinis, L.T., Ferreira, H., Rocha, L., Pavia, I., Moutinho-Pereira, J. et al. (2018) Kaolin particle film modulates morphological, physiological and biochemical olive tree responses to drought and rewetting. *Plant Physiology and Biochemistry*, 133, 29–39.
- Brito, C., Dinis, L.T., Moutinho-Pereira, J. & Correia, C. (2019) Kaolin, an emerging tool to alleviate the effects of abiotic stresses on crop performance. *Scientia Horticulturae*, 250, 310–316.
- Conde, A., Neves, A., Breia, R., Pimentel, D., Dinis, L.T., Bernardo, S. et al. (2018) Kaolin particle film application stimulates photoassimilate synthesis and modifies the primary metabolome of grape leaves. *Journal of Plant Physiology*, 223, 47–56.
- Cordeiro-Júnior, J.J.F., Pandolfi, H., Filho, J.A.D.B. et al. (2021) Photo-selective shade nets on the production and quality of sugarcane plants. *Revista Brasileira de Engenharia Agrícola e Ambiental*, 23, 366–371.
- CITRICULTURE DEFENSE FUND - FUNDECITRUS. Orange harvest Reestimation 2021/22 in the citrus belt of São Paulo and the triangle/-southwest of Minas Gerais. Available from: https://www.fundecitrus.com.br/pdf/pes_relatorios/0222_Reestimativa_da_Safra_de_Laranja.pdf [Accessed in October 2021].
- Dusenge, M. E., Duarte, A. G., & Way, D. A. (2018). Plant carbon metabolism and climate change: elevated CO₂ and temperature impacts on photosynthesis, photorespiration and respiration. *New Phytologist*, 221 (1), 32–49. Portico. <https://doi.org/10.1111/nph.15283>
- Edge, R. & Truscott, T.G. (2018) Singlet oxygen and free radical reactions of retinoids and carotenoids—a review. *Antioxidants*, 7, 16.
- Ennab, H.A., El-Sayed, S.A. & Abo El-Enin, M.M.S. (2017) Effect of kaolin applications on fruit sunburn, yield and fruit quality of Balady mandarin (*Citrus reticulata*, Blanco). *Menoufia Journal of Plant Production*, 2, 129–138.
- Esteban, R., Fernández-Marín, B., Hernandez, A., Jiménez, E.T., León, A., García-Mauriño, S. et al. (2013) Salt crystal deposition as a reversible mechanism to enhance photoprotection in black mangrove. *Trees - Structure and Function*, 27, 229–237.
- Ferrante, A. & Mariani, L. (2018) Agronomic management for enhancing plant tolerance to abiotic stresses: high and low values of temperature, light intensity, and relative humidity. *Horticulturae*, 4, 21.
- Geirinhas, J.L., Russo, A., Libonati, R., Sousa, P.M., Miralles, D.G. & Trigo, R.M. (2021) Recent increasing frequency of compound summer drought and heatwaves in Southeast Brazil. *Environmental Research Letters*, 16, 034036.
- Giannopolitis, C.N. & Ries, S.K. (1977) Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiology*, 59, 309–314.
- Grimm, A.M., Almeida, A.S., Beneti, C.A.A. & Leite, E.A. (2020) The combined effect of climate oscillations in producing extremes: the 2020 drought in southern Brazil. *Brazilian Journal of Water Resource*, 25, 12.
- Gullo, G., Dattola, A., Vonella, V. & Zappia, R. (2020) Effects of two reflective materials on gas exchange, yield, and fruit quality of sweet orange tree *Citrus sinensis* (L.) Osb. *European Journal of Agronomy*, 118, 126071.
- Guo, Y., Zhou, H. & Zhang, L.C. (2006) Photosynthetic characteristics and protective mechanisms against photooxidation during high temperature stress in two citrus species. *Scientia Horticulturae*, 108, 260–267.
- Gururani, M.A., Venkatesh, J. & Tran, L.S.P. (2015) Regulation of photosynthesis during abiotic stress-induced photoinhibition. *Molecular Plant*, 8, 1304–1320.
- Heath, R.L. & Packer, L. (1968) Photoperoxidation in isolated chloroplasts: II. Role of electron transfer. *Archives of Biochemistry and Biophysics*, 125, 850–857.
- Koussevitzky, S., Suzuki, N., Huntington, S., Armijo, L., Sha, W., Cortes, D. et al. (2008) Ascorbate peroxidase 1 plays a key role in the response of *Arabidopsis thaliana* to stress combination. *The Journal of Biological Chemistry*, 283, 34197–34203.
- Kraus, T. E., McKersie, B. D., & Fletcher, R. A. (1995). Paclobutrazol-induced Tolerance of Wheat Leaves to Paraquat May Involve Increased Antioxidant Enzyme Activity. *Journal of Plant Physiology*, 145 (4), 570–576. [https://doi.org/10.1016/s0176-1617\(11\)81790-6](https://doi.org/10.1016/s0176-1617(11)81790-6)
- Krinsky, N.I. (2001) Carotenoids as antioxidants. *Nutrition*, 17, 815–817.
- Kuhlgert, S., Austic, G., Zegarac, R., Osei-Bonsu, I., Hoh, D., Chilvers, M.I. et al. (2016) MultispeQ Beta: a tool for large-scale plant phenotyping connected to the open photosynQ network. *Royal Society Open Science*, 3, 160592.
- La Porta, N., Bertamini, M., Nedunchezian, N. et al. (2005) Photoinhibition of photosynthesis in needles of two cypress (*Cupressus sempervirens*) clones. *Tree Physiology*, 25, 1033–1039.
- Lichtenthaler, H.K. (1987) Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology*, 148, 350–382.
- Mittler, R. (2017) ROS are good. *Trends in Plant Science*, 22, 11–19.
- Mittler, R., Finka, A. & Goloubinoff, P. (2012) How do plants feel the heat? *Trends in Biochemical Sciences*, 37, 118–125.
- Molina-Bravo, R., Arellano, C., Sosinski, B.R. & Fernandez, G.E. (2011) A protocol to assess heat tolerance in a segregating population of raspberry using chlorophyll fluorescence. *Scientia Horticulturae*, 130, 524–530.
- Murchie, E.H. & Niyogi, K.K. (2011) Manipulation of photoprotection to improve plant photosynthesis. *Plant Physiology*, 155, 86–92.
- Nishiyama, Y. & Murata, N. (2014) Revised scheme for the mechanism of photoinhibition and its application to enhance the abiotic stress tolerance of the photosynthetic machinery. *Applied Microbiology and Biotechnology*, 98, 8777–8796.
- Pereira VR, Blain GC, de Avila AMH, De Matos Pires RC, Pinto HS (2018) Impacts of climate change on drought: changes to drier conditions at



- the beginning of the crop growing season in southern Brazil. *Bragantia*, 77:201–211.
- Rai, K. & Agrawal, S.B. (2017) Effects of Uv-B radiation on morphological, physiological and biochemical aspects of plants: an overview. *Journal of Scientific Research*, 61, 75–101.
- Ramel, F., Birtic, S., Cuiné, S., Triantaphylidès, C., Ravanat, J.L. & Havaux, M. (2012) Chemical quenching of singlet oxygen by carotenoids in plants. *Plant Physiology*, 158, 1267–1278.
- Sandmann, G. (2019) Antioxidant protection from UV-and light-stress related to carotenoid structures. *Antioxidants*, 8, 570–576.
- Santini, J., Giannettini, J., Herbette, S., Pailly, O., Ollitrault, P., Luro, F. et al. (2012) Physiological and biochemical response to photooxidative stress of the fundamental citrus species. *Scientia Horticulturae*, 147, 126–135.
- Saxena, I., Srikanth, S. & Chen, Z. (2016) Cross talk between H₂O₂ and interacting signal molecules under plant stress response. *Frontiers in Plant Science*, 7, 570.
- Schreiber, U., Bilger, W., Hormann, H. & Neubauer, C. (1998) Chlorophyll fluorescence as diagnostic tool: basics and some aspects of practical relevance. In: Raghavendra, A.S. (Ed.) *Photosynthesis: a comprehensive treatise*. Cambridge: Cambridge University Press, pp. 320–336.
- Soares, C., Carvalho, M.E.A., Azevedo, R.A. & Fidalgo, F. (2019) Plants facing oxidative challenges—a little help from the antioxidant networks. *Environmental and Experimental Botany*, 161, 4–25.
- de Souza Júnior, J.A., Nechet, D., Oliveira, M.C.F. & Albuquerque, M.F. (2017) Study of tendency temperature and precipitation in the rainy and less rainy periods in bélém-PA in years of strong el niñ o and la niña. *Revista Brasileira de Climatologia*, 5, 87–101.
- Takahashi, S. & Murata, N. (2008) How do environmental stresses accelerate photoinhibition? *Trends in Plant Science*, 13, 178–182.
- Tevini, M. & Steinmüller, D. (1985) Composition and function of plastoglobuli. *Planta*, 163, 91–96. <https://doi.org/10.1007/bf00395902>
- Tsai, M.S., Lee, T.C. & Chang, P.T. (2013) Comparison of paper bags, calcium carbonate, and shade nets for sunscald protection in “Murcott” tangor fruit. *HortTechnology*, 23, 659–667.
- Volkov, R.A., Panchuk, I.I., Mullineaux, P.M. & Schöfl, F. (2006) Heat stress-induced H₂O₂ is required for effective expression of heat shock genes in *Arabidopsis*. *Plant Molecular Biology*, 61, 733–746.
- Xia, X.-J., Zhou, Y.-H., Shi, K., Zhou, J., Foyer, C.H. & Yu, J.Q. (2015) Interplay between reactive oxygen species and hormones in the control of plant development and stress tolerance. *Journal of Experimental Botany*, 66, 2839–2856.
- Zandalinas, S.I., Balfagón, D., Arbona, V. & Gómez-Cadenas, A. (2017) Modulation of antioxidant defense system is associated with combined drought and heat stress tolerance in citrus. *Frontiers in Plant Science*, 8, 1–10.

How to cite this article: Bernardi, L.G.P., Boaretto, R.M., Blain, G.C. & Mattos-Jr, D. (2023) Particle films improve photosynthesis of citrus trees under excess irradiance by reducing leaf temperature. *Physiologia Plantarum*, 175(1), e13844. Available from: <https://doi.org/10.1111/ppl.13844>