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Effects of two reflective materials on gas exchange, yield, and fruit quality of sweet orange tree (*Citrus sinensis* (L.) Osb.)

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Abstract

This study was conducted in an orange orchard (Navelina ISA 315 cv) located in southern Italy. Leaf temperatures, Photosynthetic Photon Flux Density (PPFD), and UV-B reached high values during many days in summer, causing photosynthesis (*An*) in the outer layers of the canopy to decrease. This reduction was caused due to depression of stomatal conductance (*gs*), photoinhibition, and damage to the photosynthetic system. By contrast, *An* was lower in the inner layer because the PPFD was dramatically reduced from outer to inner layers of the canopy.

Reflective materials, either kaolin or calcium carbonate, were sprayed onto the canopy of the trees every month from June to September. The behaviour of the treated trees was compared with that of the untreated trees. The light reflected from the kaolin- (TK) and calcium carbonate-treated trees (TCC) reduced the leaf temperature to an optimal value for photosynthesis (below 30 °C), and increased the stomatal conductance (*gs*). Furthermore, treatments reducing PPFD played an important role in protecting the chlorophyll pigments and increasing the maximum efficiency of photosystem II (treated, ~0.67; untreated, 0.48). Consequentially, in the outer layer of the canopy *An* was higher in treated trees (TK, 9.2 µmol CO^2 m^{-2}•s^{-1}; TCC, 7.6 µmol CO_2 m^{-2} s^{-1}) than in the control trees (TCN, 5.6 µmol CO_2 $m^{-2}s^{-1}$). In the inner canopy layer, the reflective action of kaolin and calcium carbonate improved light penetration and photosynthesis compared to the untreated trees. The total average yield per tree over two years was 15% and 25% higher in TCC and TK, respectively, compared with TCN. The fresh weight, total soluble solids, titratable acid, and the ratio between these quantities were better in the treated groups than in the control, whereas no differences were observed for nutraceutical parameters. Kaolin and calcium carbonate also improved the peel colour of the fruits produced by the treated trees. In conclusion, reflective materials improved the physiological, productive, and qualitative performance of orange trees, and kaolin was the more effective of the two reflective materials. Even considering the cost, the use of these reflective materials can be justified for citrus trees that yield high profits, such as the Navelina ISA 315 used in our experiment.

Keywords: calcium carbonate, kaolin, nutraceutical, Navelina, PPFD

1. Introduction

Under natural conditions, solar radiation is the most important source of energy for plants (Larcher 1995). Light is a key factor for photosynthesis; however, excess light energy is deleterious to the photosynthetic system. In many areas of the Mediterranean Basin, Photosynthetic Photon

Flux Density (PPFD), a component of light with a wavelength range of 400-760 nm, can exceed the photosynthetic capacity of a leaf for many hours during clear summer days. Moreover, the reduction of the ozone layer has also increased the terrestrial amount of ultraviolet (UV) radiation, a component of light with a wavelength range of 280-400 nm. Increased UV-B radiation (wavelength 280 to 315 nm) also affects the photosynthetic process through direct or indirect damage (Balakumar et al., 1993; Potters et al., 2009; Lidon et al., 2012; Mpoloka, 2008; Fedina et al., 2010).

The activity of photosystem II (PSII) declines rapidly when the leaf is exposed to high light intensity. This phenomenon is called photoinhibition (Long et al., 1994), and it involves a slow and reversible reduction of photosynthetic efficiency that leads to a partial loss of capacity to convert radiant energy into dry material (Long et al., 1994; Kitao et al., 2000). Photoinhibition can be caused by ultraviolet light (UV-B), by visible light (V), or by their interaction (Powles, 1984).

Prolonged exposure of leaves to excessive radiation leads to photooxidation. This is a secondary phenomenon occurring after photoinhibition has progressed as a function of light intensity and exposure time (Powles, 1984; Long et al., 1994). Photooxidation causes the destruction of photosynthesizing pigments (Powles, 1984), indicated by discolouration of these pigments, and it may also cause cell death. Pigment bleaching happens when a certain degree of photoinhibition has already occurred (Hendrey et al., 1987).

Under unfavourable conditions, such as thermal stress induced by IR radiation (a component of light with a wavelength range of 760-4000 nm), the light saturation point of leaves occurs at lower intensities compared to optimal conditions. Heat stress negatively influences the carboxylation of ribulose biphosphate (RuBP) in photosynthesis; because of low RuBisCO activity in the thylakoidal stroma, the light saturation lowers, and then photoinhibition phenomena start sooner than in leaves at optimal temperature (Crafts-Brandner and Salvucci, 2000; Cen and Sage, 2005). Furthermore, recent studies have shown that thermal stress inhibits repair of the damage caused to PSII by photoinhibition (Sharke, 2005; Allakhverdiev et al., 2008).

The leaf controls its temperature by reducing heat absorption through different mechanisms. Among these, transpiration is utilized most often, controlled by adjusting the stomata openings (Allakhverdiev et al., 2008; Yamamoto et al., 2008). However, this mechanism may be insufficient for leaf cooling when leaf overheating is the result of a strong inflow of solar radiation, and the temperature of the surrounding environment is high, even if water availability in the soil is optimal (Nahal, 1981).

In the canopy profile, PPFD is higher on the canopy surface than on its under-layers because the overlying canopy layers shade the ones underneath (Jackson et al., 1988). As a result, the photosynthetic activity of leaves in the inner layers is low. The shaded environment of the forest understorey is the original habitat of citrus species (Syvertsen and Lloyd, 1994; Davies and Albrigo, 1994; Spiegel-Roy and Goldschmidt, 1996), and thus the leaf has a low light saturation point (Kriedemann, 1968; Sinclair and Allen, 1982). However, citrus trees are cultivated in a subtropical climate where high solar radiation leads to high temperatures and light availability.

In recent years, many agriculture strategies have sought to mitigate the negative effects of high irradiance and extreme temperature. Application of reflective material is one commonly used approach. This treatment reduces heat and light stress in plants by reflecting solar radiation from the foliar surface (Lombardini et al., 2004, Glenn et al. 2005, 2010). The aim of this work was to study how high PPFD and high temperature affected leaf gas exchange, photosynthesis, and yield production in citrus trees in full field, and to test whether new agronomic strategies, such as reflective materials, can improve leaf physiology in the canopy and drive productive performance of citrus trees during hot summers in the Mediterranean Basin.

2. Materials and methods.

2.1 Orchard

The experiment was conducted over two years, from 2016 to 2017, on the farm of Cooperative OP Frujt, Serrata (RC), Italy, (latitude, 38°30'30.1"N – longitude, 16°03'10.4" E).

Orange trees (cultivar Navelina ISA 315) grafted onto sour orange trees were planted in the spring of 1998 in pH 6 (subacidic) sandy soil, with 2.23% organic matter and 1.5 $g \cdot kg^{-1}$ nitrogen content. The plants were spaced 6 m × 4 m apart (417 plants ha^{-1}), and a north-to-south orientation was adopted. Each tree was trimmed into a globe shape. The canopy volume was calculated following the methodology proposed by Barrett and Brown (2012). The orchard was managed using a standard integrated pest management system and stable drip irrigation and fertilisation system.

2.2 Treatment and experimental design

Surround WP and Purshade (both from Serbios Srl, Rovigo, Italy) are commercially manufactured products containing 95% kaolin (kaolinite) and 95% calcium carbonate (calcite), respectively. Both products have good properties for use as a reflective foliar coating. They were applied at concentrations of 50 $g \cdot L^{-1}$ (Surround WP) and 66.8$g \cdot L^{-1}$ (Purshade). Trees sprayed with kaolin and calcium carbonate were compared with control trees (trees sprayed with only water). A total of 36 trees, three blocks of 12 plants with four plants per treatment, were arranged in a randomised block design. Trees with a volume of approximately 30 m^3 were selected. The reflective materials, with 3 L of suspension sprayed per tree, uniformly covered the leaves of the treatment trees. Treatments were applied using a trailed sprayers, every month during the summer season, from June to September. After immature fruits dropped in June ("June drop"), the same number of fruits was left on each tree (~460 fruits $Tree^{-1}$).

We determined the amount of kaolin and calcium carbonate ($g \cdot m^{-2}$) sprayed on the leaves in the outer, intermediate, and inner layers of the canopy using a methodology similar to that adopted by Jifon and Syvertsen (2003). Tester plates (polymethyl methacrylate , 3 cm × 3 cm) were randomly distributed: ten testers were weighed and hung on each layer of the treated and untreated trees. The testers were removed and reweighed after treatment when the reflective materials had dried. The amount of reflective material was determined by the weight difference of the testers before and after treatment. Both coated and uncoated testers were exposed to sunlight at noon on a

sunny day, and PPFD and UV radiations were recorded using PAR and UV sensors, respectively (Spectrum Technologies Inc., Aurora, Illinois, USA), placed 10 cm below the testers.

2.3 Measurements

PAR and UV radiation were monitored on each tree using sensors installed on the three layers. The sensors were connected to a logger that recorded the measurements every minute. Leaf temperature was also monitored every minute, with fine wire thermocouples (GMR Instruments, Firenze, Italy, UE) connected to a data logger (Spectrum Technologies Inc., Aurora, Illinois, USA). The thermocouples were pressed against the abaxial leaf surface and held in place using lightweight clips.

The leaf reflection (whiteness) was measured by a spectrophotometer (CR700; Minolta Cor., Ramsey, New Jersey, USA). Whiteness measurements (L values ranging from 0 to 100, where black = 0 and white = 100) of the adaxial leaf surfaces were recorded before treatment when the leaf was clean, and after treatment, when the individual leaf was coated with kaolin or calcium carbonate.

Leaf ACO_2, *gs*, leaf-to-air vapour pressure deficit (LAVPD), leaf transpiration, and water use efficiency (WUE; ACO_2/Transpiration) were measured for mature leaves in the outer, intermediate, and inner layers of each tree (3 leaves × 3 plants × 3 layers × 3 blocks). Measurements were taken using a portable photosynthesis system (Li-Cor 6400XT; LI-COR Biosciences, Lincoln, Nebraska, USA) and carried out on a clear, sunny summer day from 11:00 to 13:00, under optimal weather conditions (average CO_2 partial pressure of 38 Pa and average PPFD of 1600 $\mu mol \cdot m^{-2} \cdot s^{-1}$) during the last week of the summer months in both years.

Photoinhibition was also estimated using a chlorophyll fluorometer (Li-Cor 6400-40; LI-COR Biosciences, Lincoln, Nebraska, USA). Measurements were taken after 30 min of dark adaptation using leaf clips on the same leaves used for gas exchange measurements. Leaves from

the outer layers were detached, washed with distilled water, and the SPAD 502 (Spectrum Technologies Inc., Aurora, Illinois, USA) index was measured.

The PAR spectrum light was measured at midday (12:00) using a spectroradiometer (PS-300; Apogee Instruments Inc., Logan, Utah, USA). We used an average of 4 points for each layer.

Soil water potential and plant water potential were continuously monitored (every five minutes, 24 hours/day) with Watermark® sensors (Irrometer Company, Inc., Riverside, U.S.A.) located at soil depths of 30 and 60 cm from the trunk, and with a PSY1 Stem Psychrometer (located at a trunk height of 60 cm), respectively. The water tension value in the soil of the treated and untreated trees was maintained between 30 cbars and field capacity during the experiment.

2.4 Fruit sensorial and nutritional quality

Observations were carried out at the *Colture Arboree* Laboratory of the AGRARIA Department of The Mediterranean University of Reggio Calabria. For each tree, five fruits were randomly selected, and their transversal diameter was measured every 14 days from mid-September until mid-December. Concurrently, fruits from each treatment group and the control were sampled (12 fruits per block). Immediately after harvesting, the colour of the skin and pulp was determined in terms of CIELab and HSB colour spaces. Transversal and longitudinal diameters were measured, and fresh weight (FW), dry weight (DW), and juice yield were determined using an electronic balance (Mettler-Toledo, Grelfensee, Switzerland). The juice was evaluated for total soluble solids (SST) using a handheld digital refractometer (PR-1; Atago, Tokyo, Japan). Titratable acidity (TA) was measured using 10 ml orange juice diluted with distilled water (1:1) and titrated to pH 8.2 with 0.1 N NaOH (mEq. NaOH 100 g^{-1} fresh fruit). Ascorbic acid (AA) content was determined using a procedure based on the reduction of 2,6-dichlorophenolindophenol (DIP) dye by ascorbic acid (mg ascorbic acid 100 g^{-1} FW).

For each repetition, six fruits were placed in polyethene bags and frozen at -80 °C, until the analyses of total polyphenol content (TPC) and total antioxidant capacity (TAC) were performed.

Pulp of the orange sample was homogenised using an Ultra-Turrax blender (20,000 rpm, T 25 Basic; IKA, Werke, Germany, UE). The TPC and TAC were separately analysed using a Lambda 35 spectrophotometer (PerkinElmer Corporation, Waltham, Massachusetts, USA). Before measuring the TPC and TAC, standard curves were prepared for each test. The TPC (mg gallic acid equivalents g^{-1} FW) was determined using the Folin–Ciocalteu method (Slinkard and Singleton, 1997). The TAC was determined using the modified TEAC assay and expressed as mmol Trolox equivalents g^{-1} FW (Pellegrini et al., 1999; Re et al., 1999). The TEAC assay included both the hydrophilic and the lipophilic contributions of the orange samples (Scalzo et al., 2005).

2.5 Harvest

The yield per tree (in weight and number of fruits) was determined during harvesting.

Statistical analysis

All data were analysed using one-way ANOVA tests for means comparisons with standard errors. The data regarding fruit quality and nutritional analysis are reported as the means from both years. The differences between plant development and production were calculated according to Student-Newman-Keuls tests and were considered significant at $P < 0.05$. The canopy physiology data were subjected to ANOVA tests, and the means were separated by Tukey's tests when the ANOVAs indicated significant ($P < 0.05$) variable effects. All analyses were performed using the IBM® SPSS® Statistics software, version 22 (SPSS Inc. IBM Company, Armonk, New York, USA).

3. Results

In our experiment, an average of 11.2 g of kaolin and 3.8 g of calcium carbonate per square metre were deposited on the testers hanging on the outer layer of the canopy. In the intermediate canopy layer, the reflective material on the testers was 50% lower for kaolin and 80% lower for calcium carbonate, compared with the outer canopy layer. The lowest amount of reflective material

was recorded from the inner canopy layer, at 1.12 $g \cdot m^{-2}$ for kaolin and 0.38 $g \cdot m^{-2}$ for calcium carbonate (Tab. 1). No differences were recorded in the water status of the treated and untreated trees about stem and soil water potential (data not shown).

The leaf temperature and PPFD of the outer layer of the canopy in the control trees followed the same pattern over 24 h. The pattern of leaf temperature from 10:00 to 16:00 was strongly influenced by treatments with kaolin and calcium carbonate compared with the untreated trees in the outer layer of the canopy (Fig. 1). The leaf temperature was 30 °C from 10:00 to 16:00, with a peak of 34 °C from 11:00 to 12:00 (Fig. 1). The two treatments lowered the leaf temperatures of the outer canopy layers to below 30 °C from 9:00 to 18:00. The average leaf temperatures of the outer canopy layers decreased approximately 12% and 9% in TK (-3.5 °C) and TCC (-2.9 °C), respectively, compared with the outer canopy layer of the control trees (Fig. 1). No differences in leaf temperatures of the outer canopy layers were observed during the night between the treated and untreated trees (Fig. 1). Thus, the long-wave emittance was unaffected by kaolin, corroborating observations by Jifon and Syvertsen (2003). We observed the same with calcium carbonate treatment. Leaf temperature was optimal in the intermediate and inner layers of the canopy without significant differences between the treated and untreated trees (~ 24-25 °C, data not shown).

PPFD changed from 900 to 1200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ from 11:00 to 16:00, with a peak of 1900 $\mu mol \cdot m^{-2} \cdot s^{-1}$ around noon in the outer canopy of the trees. After removal of the hanging plates from trees, the PPFD transmittance in full-field was 50% and 18% lower under the plates from the outer layer of the canopies of TK and TCC, respectively, compared with those from the control trees. Under the testers from the intermediate layer of the canopy, PPFD decreased by 39% in TK and 12% in TCC compared with the control trees. Finally, under the testers from the inner layer of the canopy, PPFD decreased by 28% and 5% in TK and TCC, respectively, compared with the untreated trees (Tab. 1).

In the intermediate canopy layer, the PPFD measured under the plates on the trees was 30% and 20% higher in TK and TCC, respectively, compared to the control trees. In the inner canopy layer,

the PPFD was four times higher in TK (394 μmol·m^{-2}·s^{-1}) and three times higher in TCC (287 μmol·m^{-2}·s^{-1}) compared with the control trees (Tab. 1). Thus, in the middle and inner canopy layers of the treated trees, the PPFD received by the leaves was higher compared with the control trees (Tab. 1).

In the outer layers of the canopy, the red-to-blue light ratio was 1:1, with no difference between treatment and control trees. In the inner layer, the red-to-blue light ratio decreased by 33% in the control trees compared to the treated trees (Fig. 3).

In the outer layer of treated trees, the UV-b radiation was 35% lower compared to the outer layer of untreated trees. No substantial differences were found between the middle and inner canopy layers, or for these layers between the treated and untreated trees (Glenn et al., 2007).

We observed that the light reflected [whiteness (L*)] from the leaves coated with reflective materials was greater than the light reflected from the control leaves (Fig. 2). Leaf whiteness as a result of kaolin and calcium carbonate treatment was 71% and 53% higher, respectively, than that of the control trees, according to Glenn et al. (1999). Between the two treatments, whiteness was significantly higher in TK compared with TCC (Fig. 2).

Stomatal opening responds to four independent variables: light, CO_2, temperature, and vapour pressure deficit (Vpd) (Farquar and Sharkey, 1982; Draper and Smith, 1966). The stomatal conductance (*gs*) in the outer layer did not decrease, but was 1.5 and 1.7 times higher in TCC and TK, respectively than *gs* in TC, as reported in Tab. 1. In the intermediate and inner layers of treated trees, *gs* was also higher compared to control. Among treatments, *gs* was higher in TK than in TCC. Furthermore, no significant differences were observed among treatment and control tree with respect to soil water potential or plant water status (data are not shown).

Ci was significantly higher in the outer layers of the control trees compared with TK and TCC. In the outer layer, *An* was 50% and 40% higher in TK and TCC, respectively, compared with the leaves of control trees (5.8 μmol CO_2 m^{-2}·s^{-1}) (Tab. 1). *An* was approximately 60% higher in the intermediate canopy layer of the treated trees compared with that in the untreated trees. No

differences were observed between the kaolin and calcium carbonate treatments. In the inner canopy layer, *An* was 158% and 383% higher in TCC and TK, respectively, compared with the control trees (Tab. 1).

The differences among years were significant for some gas exchange parameters, but no differences were observed about "year x treatment " interaction (Tab.1).

The ratio of R/B light increased in the inner layer compared to the outer layer in TK and TCC treatments, whereas it did not change in the inner layers of the untreated trees (Fig. 3). The SPAD index (Tab. 2) increased by 11% in the leaves of TK and TCC plants compared with the leaves of the control plants. The Fv/Fm values of TK and TCC were 0.67 and 0.66, respectively, whereas the ratio reached a critical value in the control trees, approximately 0.48 (Tab. 2). The treatments in the outer canopy layers also induced a positive effect on *ΦPSII* (Tab. 2), a fluorescence index that allows determination of the amount of light absorbed by the leaf and used for the photochemical reactions of the light phase of photosynthesis (Correia et al., 2012). The *ΦPSII* was 2 and 1.8 times higher in TCC and TK, respectively, compared to the control trees. qP, the proportion of PSII reaction centres that are open, was also higher in the treated trees because a portion of the absorbed light was used in the photochemical reactions (Tab. 2). In the control trees, most of the energy absorbed was dissipated, as shown by the coefficient of non-photochemical quenching (qN). The differences among years were significant because these parameters are very sensitive to climate factors. However, no differences were observed about interaction "year per treatment" (Tab. 2). Compared with the control trees, in the treated trees the WUE was 77% and 54% higher when treated with kaolin and calcium carbonate, respectively (Fig. 4).

The presence of reflective materials on the fruit during development and maturation affected its colour. The CIELab colour space for the skin of the fruit, L* was significantly lower in TCC trees, a* was 18% higher in the treated trees compared with the control trees, and b* was lower (less yellow skin) in TCC trees. The a*:b* ratio was also higher in the treated trees compared to the control trees, resulting in a darker orange colour of the skin of the fruit from the treated trees

compared with the untreated trees. This darker colour is recognised as the preferred colour of orange fruits (Tab. 3).

The HSL colour space was statistically different between the skin of the fruit of the TCC trees compared with both TK-treated and control trees (Tab. 3). The °hue was significantly lower in the treated trees, with no significant differences between treatment groups. Furthermore, the reflective materials had a favourable effect on the skin colour by reducing its temperature. The pulp colour was darker orange in the TCC trees compared with the control trees (Tab. 4). Indeed a*, b* (CIELab colour space), a*:b* ratio, and °hue (HSL colour space) were higher in TCC fruit compared to TK and control fruits. No difference was observed between TK and control fruits for these parameters (Tab. 4). Furthermore, L* and chrominance were lower in the TK fruit than in the TCC and control fruits (Tab. 4).

The total average yield per tree over two years was 15% and 25 % higher in TCC and TK, respectively, compared with TC (Tab. 5). The main carpometric indexes (weight and yield in juice) were similar or better in the treated trees compared with the control trees, as shown in Tab. 6. The fruit weight was significantly different between the treated and untreated trees. The fresh fruit weight was 15% and 25% higher in TCC and TK trees, respectively, than in control trees (Tab. 6). The dry weight was also higher in the fruit of the treated trees compared with the control trees, but the dry weight does not change if expressed as the percentage of fresh weight. The percentage of juice yield was not different between the control trees and the TK trees, whereas it was 14% lower in the TCC trees compared with the control trees (Tab. 6). The total amount of juice per fruit was 55.54 and 54.79 g per fruit in the control and TCC fruit, respectively, whereas it was higher in the TK fruit (74.39 g per fruit). Therefore, in the TCC trees, the increased fresh fruit weight compared with the control was attributable to the increased thickness of the peel (data not shown).

The SST values of the treated groups were similar to that of the control. Furthermore, the TCC group decreased its titratable acidity, resulting in an increased SST:AT ratio, whereas no differences were observed between the TCC and TK groups (Tab. 6). Nutraceutical parameters

were also not different according to the treatments (Tab. 7). The ascorbic acid content was not significantly different between the treatment and control groups (Tab. 7). The TAC and TPC contents in the treatment groups were not significantly different from those in the control group, and the values were similar to those recorded by Ramful et al., 2011. Therefore, in our experiment, we have observed that the correlation between AA and TAC was stronger than the correlation between TAC and TPC. Indeed, Pearson's coefficient was higher in the first correlation ($r = 0.89$) than in the second correlation ($r = 0.56$). Ascorbic acid is the major antioxidant in orange juice (Rice-Evans and Miller, 1996).

The yield efficiency of the TK trees was significantly higher than the control trees, whereas no differences were observed between TCC trees, TK, and the control trees (Tab. 5). Ennab et al. (2017) found that kaolin improved some mandarin fruit quality parameters. All carpometric, organoleptic, nutraceutical, and productive parameters shown significant differences among years. Furthermore, no differences were observed between "Year x Treatment" interaction (Tabb. 3,5,6) except the SST, TA parameters of the fruit (Tab. 5).

3. Discussion

Photosynthesis is a highly temperature-sensitive process (Yordanov et al., 1986; Berry and Bjorkman, 1980; Critchey, 1998). The *An* depression was recorded in the outer layer of control trees where light, temperature, and LVPD were high (Fig. 1, Tab. 1). Increased temperature increases LAVPD. This, in turn, increases the conductance, but only up to a certain point, beyond which stomatal conductance decreases. Indeed, high temperatures decreased stomatal conductance in the outer layer of the canopy in the untreated trees where LAVPD likely reached the limit threshold (4 Kpa). This result agrees with previous observations carried out on citrus and other species (Chen and Zhang, 1994; Pons and Welschen, 2003). In contrast, treatments with reflective materials lowered the LVPD in the outer layer of the canopy, increasing *gs* and improved *An* (Tab. 1) in agreement with observation on grapefruit by Jifon and Syvertsen (2003). The lower *An* in the outer

layer of untreated trees is attributable to both stomatal and non-stomatal factors (Flexas and Medrano, 2002). Indeed, PPFD exceeded 1400 μmol $m^{-2}.s^{-1}$ in the outer layer of the untreated canopy during measurements of gas exchange. This value is higher than the light saturation point of the leaf, which for citrus is about 600 μmol m^{-2} $.s^{-1}$ (Jifon and Syvertsen, 2003). Salvucci et al., (2001; 2004) have shown that high temperature lowers the leaf light saturation point.

In addition, excess photons that were not used for the process of photosynthesis caused the production of reactive oxygen species (ROS), which in turn induce photooxidative phenomena (Smirnoff, 1993). Anti-stress protection mechanisms involving the antioxidant system counteract ROS (Brosché and Strid, 2003; Frohnmeyer and Staiger, 2003; Fedina et al., 2007). Furthermore, heat stress induced by high leaf temperature also inhibits the repair of damage caused to PSII (Sharkey, 2005; Allakhverdiev et al., 2008). Initially, ROS inhibits the repair of photodamaged PSII by suppressing the synthesis of proteins such as D1 (Murata et al., 2007), causing photoinhibition primarily by lowering photochemical efficiency (Fv/Fm). The reflective materials we used protected the photochemical process from excess energy as shown by the increasing of Fv/Fm ratio according to Sharkey (2005) and Allakhverdiev et al. (2008).

ΦPS2 shows the same pattern as Fv/Fm. This parameter is a more reasonable criterion to define photoinhibition than Fv/Fm because it reflects the decrease of photochemical efficiency induced by non-photochemical dissipation of excessive photons (Jia, 2001). The other fluorometric parameters are photochemical quenching (qP) and non-photochemical quenching (qN), which increase and decrease respectively in the outer layer of treated trees, and also confirm that the reflective materials have limited the negative effects of high PPFD into the outer layer of the canopy (Tab.2).

In the long run, ROS causes photodestruction of the chlorophyll pigments. The hand-held Chl meter (SPAD) gives accurate estimate of foliar chlorophyll (Torres Netto et al., 2002; Chang and Robison, 2003; Marenco et al., 2009) and is a good tool to diagnose the integrity of the photosynthetic system after exposure of the leaf to high PPFD level (Torres Netto et al., 2005). The

SPAD index was lower in the outer layer of the untreated canopy than in trees treated with kaolin and calcium carbonate**.**

In the intermediate and inner canopy layers, the temperature was within the optimal range for the leaf. Indeed, no differences were observed with regard to LVPD in the internal layer among the treated and untreated trees. Therefore, the different *gs* between the treated and untreated canopy can be exclusively ascribed to better light penetration (Tab. 2), as the effect of reflective action of kaolin and calcium carbonate. The treated trees also had better photosynthetic performances compared to the untreated trees, increasing production as fruit size and dry weight.

The Pearson's coefficient showed a negative correlation among the amount of material deposited on the leaves and PPFD into the canopy (Tab. 1) and a very low correlation among the amount of material deposited on the leaves and gas exchange parameters (Tab. 1).

Therefore, the results obtained could be attributed to the reflective action of the material, rather than its quantity (Tab.1).The spectral distribution of solar radiation changes as the light penetrates and scatters within the tree canopy due to the structure and optical properties of the canopy components, such as the leaves, fruits, and branches (Palmer, 1977; Baldini et al., 1997). The higher levels of red light compared to blue light should promote more formation of flowers in the inner layer of the treated trees compared to the untreated trees. Indeed, the increase in yield of the treated trees was statistically significant when compared with the control trees, similar to what was observed in other species treated with reflective materials (*Olea europaea* L. (Glenn et al., 2001), *Malus communis* L. (Puterka et al., 2000; Wünsche et al., 2004; Glenn and Puterka., 2007), *Prunus dulcis* (Rosati et al., 2006), *Pyrus communis* L. (Chamchaiyaporn et al., 2013), *Mangifera indica* L. (Abd-Allah et al., 2013;), and *Punica granatum* L. (Palitha et al., 2010; Hegazi et al., 2014).

Climate factors affect fruit quality, as was observed by differences in the colour of rinds among citrus fruits cultivated in tropical and subtropical regions (Young and Erickson, 1961). Orange and mandarin fruits do not attain their attractive rind colour under warm temperatures. The

high temperature interferes with the loss of chlorophyll as well as with the build-up of carotenoids. The reflective action of kaolin and calcium carbonate during the first maturation phase (September) lowered the rind temperature, improving peel colour. Regarding the internal quality of the fruit, high temperatures in untreated trees led to lower soluble solids, acidity, and juice content compared to the lower temperatures of the treated trees, as observed by Burns and Albrigo (1997). Therefore, treatments create some improved parameters according to Ennab et al. (2017). Furthermore, Kaolin can contrast fruit fly (Bernardes Ourique et al., 2018) and some disease (Melgarejo et al., 2004).

All observed, parameters are more o less sensitive to climate factors (Utsunomiya et al., 1982), and because these last can be similar among the years but not identical, the differences among years were significant for some of them, whereas "year xtreatment" interaction was observed for only a few parameters.

4. Conclusion

This study suggests that in warm citrus producing regions, where high radiation and VPD can limit photosynthetic capacity, reflective materials such as kaolin and calcium carbonate could improve leaf carbon uptake potential in the outer layer of the canopy.

These materials also improve light penetration inside the canopy where the light is the very limiting factor photosynthetic process, as an effect for shading the layers of upper leaves.

The treated tree has improved yield production and fruit quality as size, colour and maturation index. Among the two reflective materials used, Kaolin gave the best results compared with calcium carbonate for its high light reflective actions. However, for their cost, their use can be justified in citrus trees that yield high profits, such as the Navelina ISA 315 used in this experiment.

Acknowledgements

The research was financed by MIUR: Project PON03PE_00090_03 "Sustainable models and new technologies for the valorisation of Mediterranean vegetable chains".

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Figure

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Figure 1 – Pattern of temperature (°C) and PPFD (micromol. m^2. s^{-1}) in the outer layers of the canopy in treated (Kaolin= TK; calcium carbonate =TK) and untreated (Control =TC) sweet orange tree, cv Navelina ISA 315. The measures reported representing the average of the observations made in 3 days of full summer and clear sky during the last decade of July in both years of observation.

Figure 2 –Leaf whiteness measured on the adaxial leaf surfaces, in the outer layers of the canopy in treated (Kaolin= TK; calcium carbonate =TK) and untreated (Control =TC) sweet orange tree, cv Navelina ISA 315. The measures reported representing the average of the observations made in 3 days of full summer and clear sky during the last decade of July in both years of observation. Different letters indicate statistically significant differences per P<0.05); .n= 18

Figure 3 – The Red (680 nm)/Blue (435nm) light ratio in the external (E) and inner (I) layers of the canopy of treated (TK; TP) and untreated (TC) tree. Different letters indicate statistically significant differences per P≤0.05.

Figure 4 - Water use efficiency (WUE) in the outer layers of the canopy in treated (Kaolin= TK; calcium carbonate =TK) and untreated (Control =TC) sweet orange tree, cv Navelina ISA 315. The measures reported representing the average of the observations made in 3 days of full summer and clear sky during the last decade of July on two years. Different letters indicate statistically significant differences per P≤0.05.

Figure 5- Correlations between Total Antioxidant Capacity (TAC) and Total Polyphenols (P) and between Total Antioxidant Capacity (TAC) and ascorbic acid, in the citrus fruit of sweet orange, cv Navelina ISA 315

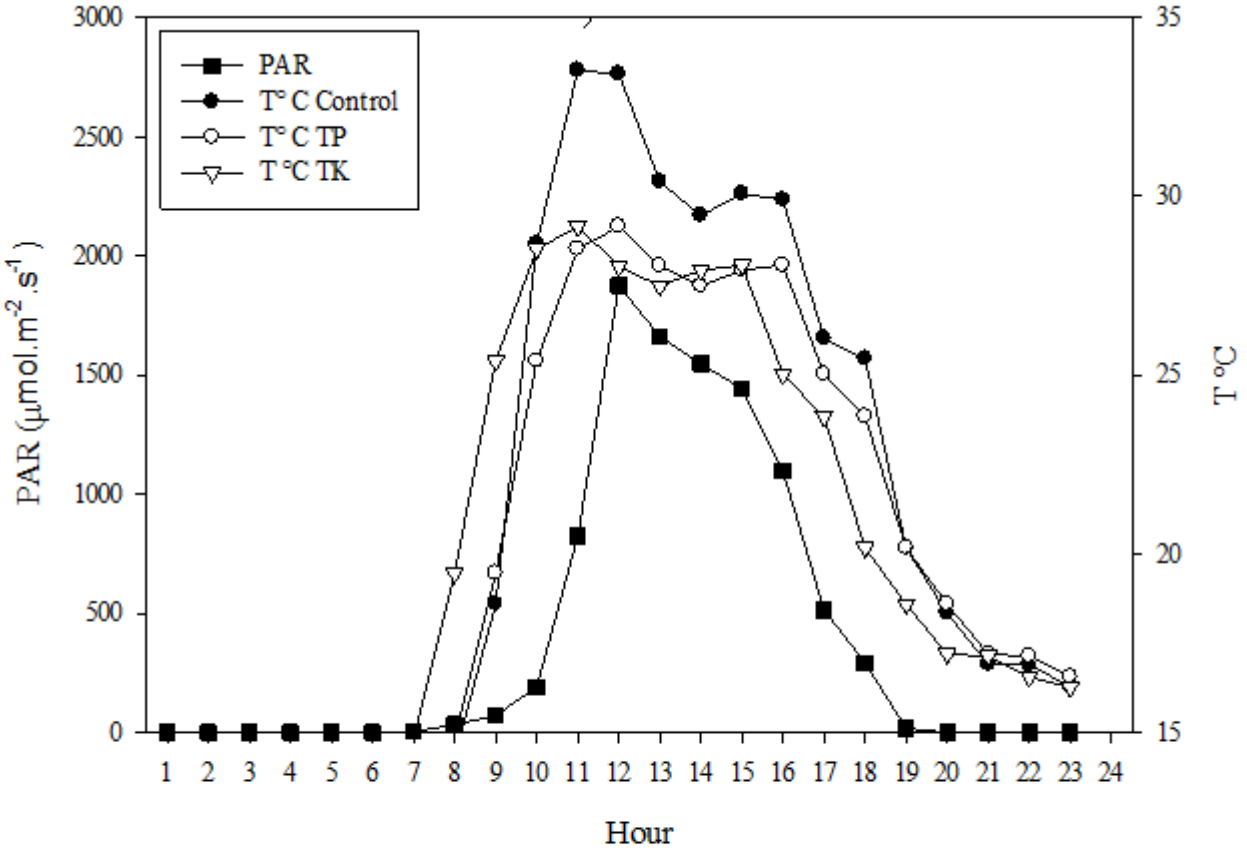

PAR
T° C Control
T° C TP
T °C TK
PAR (µmol.m-2.s-1)
T °C
Hour
3000
2500
2000
1500
1000
500
0
35
30
25
20
15
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Figure 2

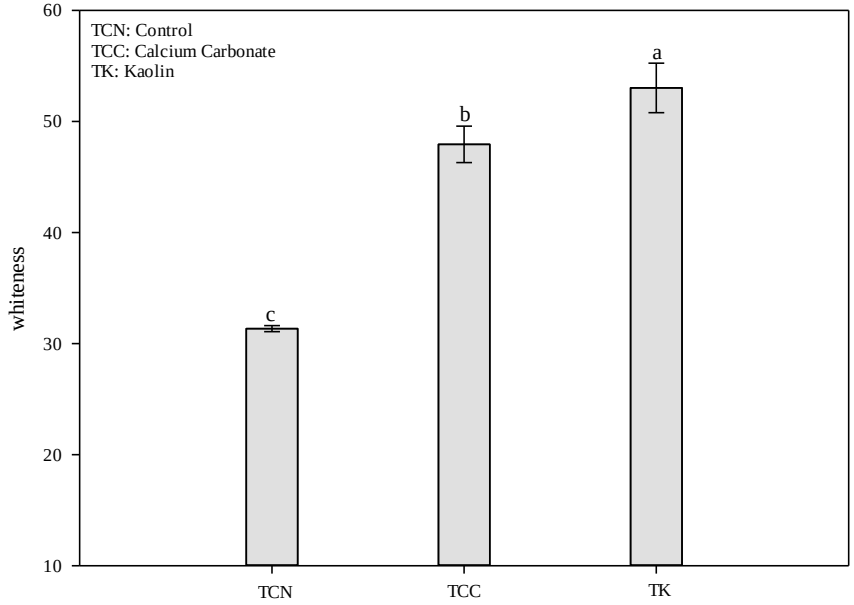

TCN: Control
TCC: Calcium Carbonate
TK: Kaolin
whiteness
60
50
40
30
20
10
a
b
c
TCN
TCC
TK

Figure 2

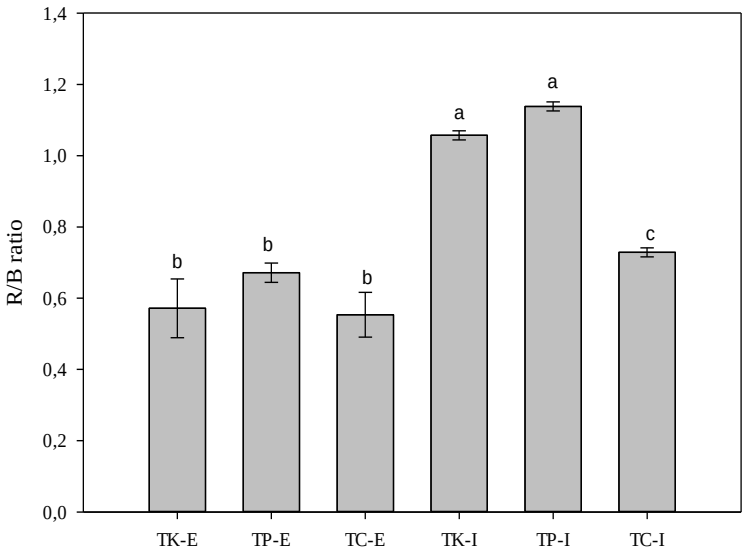


Figure 3

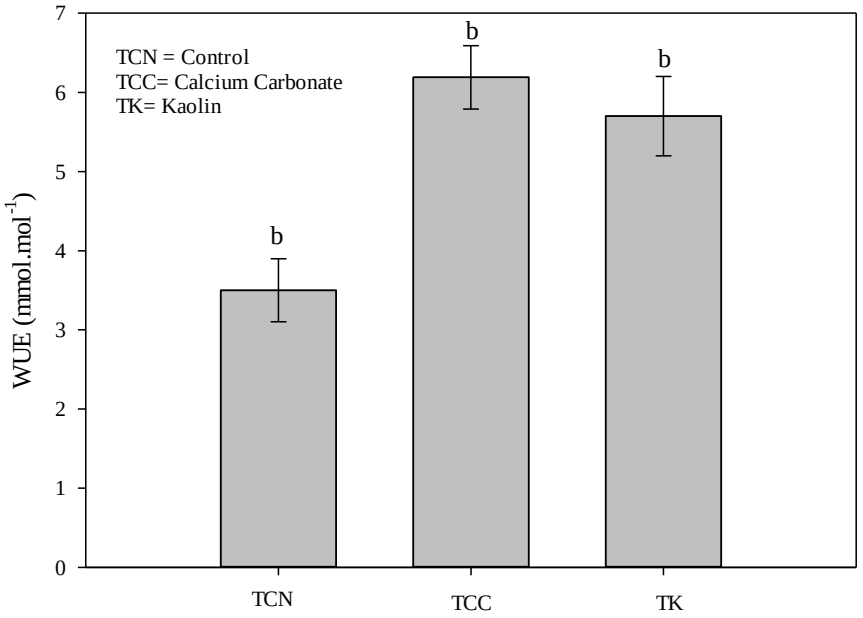
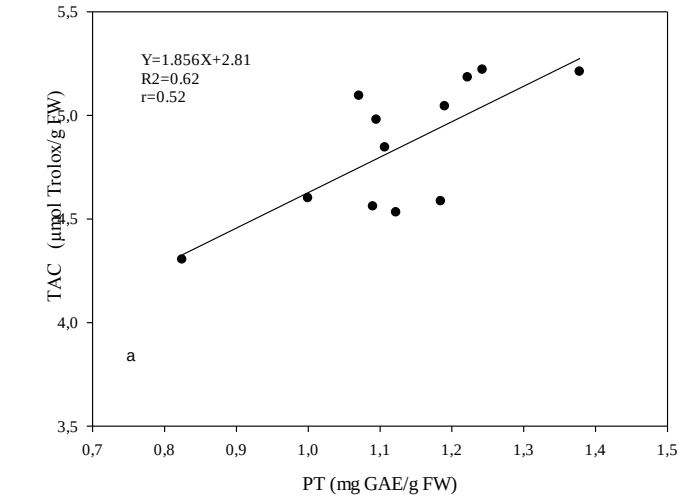

TCN = Control
TCC= Calcium Carbonate
TK= Kaolin
WUE (mmol.mol-1)
0
1
2
3
4
5
6
7
b
b
b
TCN
TCC
TK

Figure 4


Y=1.856X+2.81
R2=0.62
r=0.52
a
TAC (µmol Trolox/g FW)
PT (mg GAE/g FW)
5,5
5,0
4,5
4,0
3,5
0,7
0,8
0,9
1,0
1,1
1,2
1,3
1,4
1,5

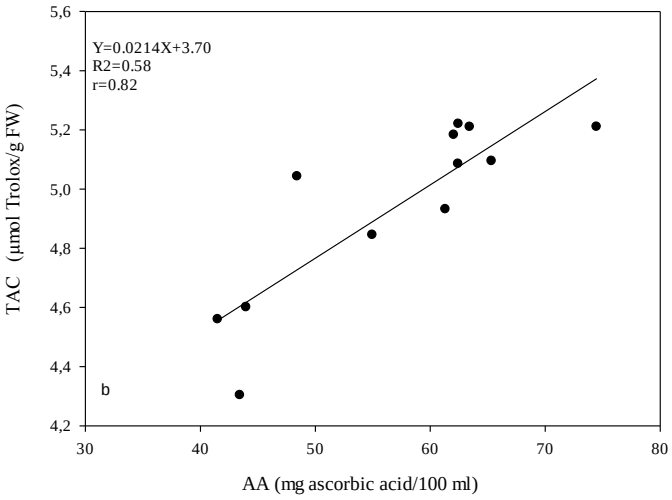

Y=0.0214X+3.70
R2=0.58
r=0.82
b
TAC (µmol Trolox/g FW)
AA (mg ascorbic acid/100 ml)
5,6
5,4
5,2
5,0
4,8
4,6
4,4
4,2
30
40
50
60
70
80

Figure 5

Table

Table 1 - PPFD in three layers of canopy, PPFD under plate in full field and on tree, gas exchange (An, gs, LAVPD,Ci), and Pearson's coefficient in treated *(Kaolin= TK; calcium carbonate =TCC) and untreated (Control =TCN) sweet orange tree, cv Navelina ISA 315. The measures reported representing the average of the observations made in 3 days of full summer and clear sky during the last decade of July on two years. n=54*

	Layer	**Reflective material weight** *g m^{-2}*	**PPFD under plate in full field** *µmol m^{-2} s^{-1}*	**PPFD measured underplate on the tree** *µmol m^{-2} s^{-1}*	**An** *µmol CO_2 m^{-2} s^{-1}*	**gs** *mol H_2O m^{-2} s^{-1}*	**LAVAP** *kPa*	**Ci** *µmol mol^{-1}*
TCN	1	0	1492±102a	1450±98a	5.3±0.2c	0.14±0.1e	4.16±0.01a	310.30±15a
	2	0	1496±109a	480±38e	5.6±0.3c	0. 16±0.08d	2.7±0.02c	240.11±22b
	3	0	1488±105a	102±11h	1.2±0.1e	0.10±0.07f	2.5±0.02d	200.11±32d
TCC	1	3.8±0.09c	933±49c	1180±42b	7.6±0.3b	0.22±0.08b	3.18±0.03b	269.02±32b
	2	1.78±0.08e	1233±37b	605±30e	8.8±0.3a	0.20±0.07c	2.8±0.02c	248.30±25b
	3	0.38±0.08f	1398±11b	286±20g	3.1±0.4d	0.16±0.04d	2.6±0.01d	235.15±10c
TK	1	11.2±0.12a	846±55d	915±46c	8.7±0.4a	0.26±0.03a	3.15±0.02b	247.48±21b
	2	5.5±0.11b	1074±22c	720±33d	9.2±0.3a	0.23±0.04b	2.7±0.01c	250.11±18b
	3	1.12±0.08d	1350±19b	394±21f	5.8±0.2c	0.19±0.03c	2.5±0.01d	230.12±22c
Treatment					*	*	*	*
Year					*	*	*	*
Year x Treatment			n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Pearson Coefficient TCC weight *vs*			0.88	0.95	0.215	0.222	0.341	0.125
Pearson Coefficient TK weight *vs*			0.89	0.96	0.186	0.325	0.284	0.189

*Different letters in the column indicate statistically significant differences per *P≤0.05, ** P≤0.01*** P≤0.001; n.s. = not significant*

Table 2 – Effects of kaolin (TK) and calcium carbonate (TCC) sprays on SPAD index and main indices of fluorescence on Citrus sinensis L. leaf compared to the control (TC) in the outer layers. The measures reported representing the average of the observations made in 3 days of full summer and clear sky during the last decade of July on two years. (SE= standard error)

	SPAD index	**±SE**	**Fv/Fm**	**±SE**	ΦPSII	**±SE**	***qN***	**±SE**	***qP***	**±SE**
TCN	75.445a	2.952	0.48b	0.052	0.102b	0.011	2.07a	0.204	0.298b	0.035
TCC	83.663b	0.813	0.66 a	0.019	0.214a	0.010	1.63b	0.100	0.389a	0.031
TK	83.690b	1.381	0.67a	0.014	0.184a	0.013	1.74b	1.063	0.490a	0.020
Treatment	*		***		***		n.s.		***	
Year	*		**		**		*		**	
Treatment x year	n.s.		n.s.		n.s.		n.s.		n.s.	

*Different letters in the column indicate statistically significant differences per *P≤0.05, ** P≤0.01;*** P≤0.001; n.s. = not significant*

Table 3 - Effect of treatment on fruit peel and pulp colour characteristics (CIE LAb and HSB colour space) in treated (Kaolin= TK; calcium carbonate =TCC) and untreated (Control =TCN) sweet orange tree, cv Navelina ISA 315.. The measures reported representing the average of the observations made in the last three sampling dates on two years. .(SE= standard error)

Treatments	**L***	**±SE**	**a***	**±SE**	**b***	**±SE**	**a/b**	**±SE**	**Chroma**	**±SE**	**°Hue**	**±SE**
TCN (peel)	68.060 a	0.363	17.102 b	1.152	62.247 a	0.486	0.262 b	0.019	66.459 a	0.577	75.229 a	1.295
TCC (peel)	66.710 b	0.358	20.061 a	1.151	59.765 b	0.593	0.327 a	0.021	65.039 b	0.644	72.980 b	1.077
TK (peel)	68.677 a	0.342	19.838 a	1.054	61.271 ab	0.597	0.316 a	0.019	66.072 a	0.628	73.296 b	0.984
Treatment	*		**		**		*		*		**	
Year	*		*		*		*		*		*	
Treatment x Year	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	
TCN (Pulp)	48.838a		0.134		6.586b		0.177		29.586 ab		0.164	
TCC (Pulp)	48.857a		0.158		7.222a		0.174		30.096 a		0.194	
TK (Pulp)	47.442b		0.166		6.618b		0.200		29.044 b		0.190	
Treatment	*		*		*		*		*		*	
Year	*		*		*		*		*		*	
Treatment x Year	n.s.		n.s.		n.s.		n.s.		n.s.		n.s.	

*Different letters in the column indicate statistically significant differences per *P≤0.05, ** P≤0.01;*** P≤0.001; ; n.s. = not significant*

Table 4 – Fruit drop pre-harvest (%), fruit at harvest, yield per plant, canopy volume and yield efficiency in treated (Kaolin= TK; calcium carbonate =TCC) and untreated (Control =TCN) sweet orange tree, cv Navelina ISA 315. The measures reported representing the average of the observations made at harvest in two years. .(SE= standard error)

Treatment	**Fruit drop during summer** *%*	**±SE**	**Fruit** *number.tree*$^{-1}$	**±SE**	**Kg.tree**$^{-1}$	**±SE**	**Volume** *m*^{3}*.tree*$^{-1}$	**Yield Efficiency** *Kg.m*$^{-3}$
TCN	4.470a	0.10	406	2.1	139.186c	0.10	24.2	5.78b
TCC	3.530b	0.09	410	3.2	160.375b	0.09	25.1	6.40ab
TK	2.118c	0.08	416	2.4	177.591a	0.08	25.2	7.05a
Year (A)	*		n.s.		n.s.		n.s	n.s.
Treatment (B)	**		n.s.		**			**
Interaction (AXB)	n.s.		n.s.		n.s.			n.s.

*Different letters in the column indicate statistically significant differences per *P≤0.05, ** P≤0.01;*** P≤0.001; ; n.s. = not significant*

Table 5 - Effect of treatment on fruit weight (FW)t, dry weight (DW), juice yield, total soluble solids (TSS), titratable acidity (TA) and SST/AT in treated (Kaolin= TK; calcium carbonate =TCC) and untreated (Control =TCN) sweet orange tree, cv Navelina ISA 315. The measures reported representing the average of the observations made in the last three sampling dates on two years. .(SE= standard error)

Treatment	**FW** *g*	**±ES**	**DW** *g*	**±ES**	**DW** *%*	**±ES**	**Juice yield** *%*	**±ES**	**SST**	**±ES**	**TA** *%*	**±ES**	**SST/TA**	**±ES**
TCN	145.781c	63.329	26.136b	7.844	15.348ns	0.226	38.315a	1.083	10.465ns	0.278	1.761a	0.157	8.407b	1.116
TCC	166.774b	83.017	32.924a	12.151	16.068	0.558	33.011b	0.894	10.912	0.215	1.354b	0.097	9.692a	0.798
TK	181.711a	78.144	32.927a	9.735	15.105	0.285	41.104a	0.975	10.348	0.145	1.622a	0.079	8.319b	0.822
Year (A)	n.s.		n.s.		n.s.		n.s.		n.s.		***		**	
Treatment (B)	***		***		n.s.		***		n.s.		***		***	
Interaction (AxB)	n.s.		n.s.		n.s.		n.s.		n.s.		*		*	

*Different letters in the column indicate statistically significant differences per *P≤0.05, ** P≤0.01;*** P≤0.001; ; n.s. = not significant*

Table 6- Total Antioxidant Capacity (TAC), Total Polyphenols (TP), and ascorbic acid o(AA) in orange cv Navelina ISA 315 by the treated (TCC and TK) and untreated tree (TCN). The measures reported representing the average of the observations made in the last three sampling dates on two years. .(SE= standard error).

	TAC *µmoli Trolox/g FW*	**±ES**	**TPC** *mg GAE/g FW*	**±ES**	**Ascorbic acid** *mg ascorbic acid/100 ml*	**±ES**
TCN	4.863ns	0.208	1.158ns	0.051	66.330ns	2.350
TCC	4.856	0.166	1.125	0.041	68.748	1.851
TK	4.399	0.341	1.093	0.076	62.802	1.804
Year (A)	n.s.		n.s.		n.s.	
Treatment (B)	n.s.		n.s.		**	
Interaction (AxB)	n.s.		n.s.		n.s.	

*Different letters in the column indicate statistically significant differences per *P≤0.05, ** P≤0.01;*** P≤0.001; n.s. = not significant*

***Credit Author Statement**

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Conceptualization	X			
Methodology	X			
Formal Analysis	X	X		
Validation	X	X	X	X
Software	X	X	X	
Writing original draft	X	X		
Writing Review and Editing	x	X		X
Data Curation			X	X
Visualization	X	X		X
Project Administration	X			X
Funding Acquisition				X

***Declaration of Interest Statement**

Declaration of interests

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

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: