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Leaf and Fruit Responses to Kaolin Particle Film Applied onto Mature Olive Trees

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Abstract
Hot dry summers with high irradiance levels and low precipitation are typical in Mediterranean areas where olive trees are cultivated. Kaolin particle films (PF) are used with various crops to alleviate heat stress under these conditions. In this study, the effect of kaolin PF application on leaf physiological characteristics and functions of mature field-grown 'Konservolea' olive trees was investigated. Deficit irrigated [around 50% of crop evaportraspiration (ETc)] trees were studied during the 2009 and 2011 fruit growth periods ('on' years) in central Greece. Repeated applications of kaolin PF resulted in a white coating highly reflective in photosynthetically active radiation (PAR) on olive leaf surfaces found to significantly decrease the available PAR on leaf surface underneath the coating. The physiological characteristics of kaolin-treated leaves did not change to support any shade effect, but showed changes related to ameliorating heat stress. Kaolin applications improved leaf gas exchange functions under mild climatic conditions early and late in the season. In midsummer with high vapour pressure deficit and heat and water stressed olive trees, kaolin did not improve leaf gas exchange. However, overall, olive leaves from kaolin-treated trees seemed to manage better their water status and had more stable physiological characteristics and less negative midday stem water potential through the experimental period. Kaolin-treated-trees had similar fruit production, yield variables, fruit and fruit oil content were of similar maturity with control trees, but olive oil from kaolin-treated trees had lower free acidity and peroxide index compared to oil from control trees showing decreased oxidative stress.
Keywords: *Olea europaea*, heat stress, photosynthetic rate, stomatal conductance, chlorophyll, fruit quality

1. Introduction

Olive trees are widely cultivated in Mediterranean areas with summers characterized by high temperatures, high vapour pressure deficit, high irradiance levels and low precipitation. Stomatal closure is one of the first lines of defense against unfavorable climatic conditions such as the ones described above, to reduce water loss. However, when stomata close, leaf and plant carbon assimilation significantly decrease in various plants including olive (Chaves 1991; Nogués & Baker 2000).

Olive tree is considered to be sclerophyllous drought-tolerant species with photosynthetic apparatus resistant enough to moderate water stress, while the stomata are the main limiting factor to carbon assimilation. Olive leaves have certain structural features and possess active mechanisms for drought tolerance, thus allowing certain degree of control over water loss (Angelopoulos *et al.* 1996; Fernández *et al.* 1997; Bacelar *et al.* 2004). But, when water availability is low and high temperatures and irradiance levels occur during the growing season, the olive tree undergoes significant stress resulting in reduced productivity (Angelopoulos *et al.* 1996).

In recent years, kaolin particle film (PF) technology has been used for heat stress alleviation in many crops. Kaolin creates a white coating on tree surfaces that is highly reflective, thus reducing solar radiation reaching the exposed plant surfaces (and often causing shading of these plant surfaces), but redistributes this radiation throughout the plant reaching both leaf surfaces and shaded plant canopy areas (Lombardini *et al.* 2005; Rosati *et al.* 2006; Rosati *et al.* 2007). According to the above, kaolin PF was found to reduce heat stress and solar injury in the whole apple tree canopy, leaf and fruit (Glenn *et al.* 2003) and, in particular, leaf and fruit temperature (Glenn *et al.* 2002). Furthermore, kaolin application to apple trees increased leaf carbon assimilation, reduced canopy temperature and improved fruit quality characteristics at semiarid environments under heat stress by reducing canopy temperature and improved fruit quality characteristics the sooner kaolin applications started (Glenn *et al.* 2001). Jifon & Syvertsen (2003) reported that kaolin PF decreased leaf temperature and leaf-to-air vapour pressure deficit at midday hours and increased leaf carbon assimilation and gas exchange in grapefruit trees, while Gindaba & Wand (2007) found no effect on midday leaf photosynthetic rate in 'Cripps' Pink' apple. Finally, kaolin PF application on pecan trees (Lombardini *et al.* 2005) and on almond and walnut trees (Rosati *et al.* 2006) did not improve leaf gas exchange. Thus, kaolin PF applications were found to alleviate heat stress in most cases, but its effects on gas exchange are contradictory for the different crops and different regions studied.
Kaolin PF has also been used in many crops to control insects as in olive trees to control main pests such as olive fruit fly (*Bactrocera oleae)* (Saour & Makee 2004; Pascual *et al.* 2010). Application of kaolin PF to control the above pest in olive trees coincides with the summer period, when heat stress is substantial. In addition, since kaolin PF has been approved for organic agriculture (European Economic Community 1991), it is important, as

kaolin is useful for olive fruit fly control, to evaluate the effect of kaolin PF application on olive tree heat stress alleviation.

The only published information about the effects of kaolin PF on olive plant physiological functions was conducted on two-years-old potted olive plants with two major Greek olive cultivars (Roussos *et al.* 2010; Denaxa *et al.* 2012). With 'Koroneiki' olive trees, kaolin PF did not have a significant effect on gas exchange and stress alleviation, but reduced yield under water stressed or well-irrigated conditions (Roussos *et al.* 2010). With 'Chondrolia Chalkidikis' olive trees, kaolin PF improved leaf functions alleviating water stress and even improving leaf functioning under drought conditions (Denaxa *et al.* 2012). There is no work published on the effect of kaolin PF on olive leaf physiology of mature field-grown olive trees with crop, where kaolin PF could be used for olive fruit fly control.

The aim of this study was to investigate the effect of kaolin PF application on the available PAR and UV radiation to leaf and canopy and on olive leaf physiological functions and leaf characteristics. In particular, we examined if kaolin application could alleviate heat stress in olive trees, generate a shade effect on leaves or block stomata and thus modify physiological functions.

2. Material and methods

2.1 Plant and treatment

The experiment was carried out in a deficit (around 50% of ETc, calculated with Kc=0.5 for July and August) drip-irrigated grove with 40-year-old olive trees (*Olea europaea* L. cv. Konservolea) grafted on wild olive seedlings and planted 6 m × 6 m in Dimini (Latitude 39°13΄N, Longitude 22°48΄E), central Greece from early June to late September 2009 and 2011, both 'on' years. Data from 2009 are shown. The trees were well pruned each year to keep their height below 2.5 m with open canopy. The experimental design included two treatments and twelve trees per treatment and guard trees between each treatment. 'Konservolea' olives are processed mainly as green table olives (harvested in late September), but are also destined for black-ripe table olives or for oil extraction.

Kaolin PF (commercial product SURROUND WP, Engelhard Corp., Iselin, NJ, USA) was applied at 5% w/v in water and 5 L per tree, periodically from early June to mid August (five applications) with a pressurized knapsack sprayer covering the canopy (adaxial and abaxial leaf surfaces) thoroughly enough without runoff. The aim of the repeated applications was to result in more uniform distribution of kaolin particles on leaf surfaces, to ensure the replenishment of total coverage after precipitations and to resemble practical application for olive fruit fly control. After mid August, no kaolin applications were performed to examine the quantity of kaolin residues removed by autumn rainfalls and the subsequent changes of leaf functions. Control trees were sprayed with water on each spray event. The experiments were conducted from early June to late September in 2009 and 2011.

2.2 Climatic measurements

Climatic data, the hours 9:00-13:00 (during leaf gas exchange measurements), were collected from a meteorological station situated 5 Km away from the experimental site. Air vapor pressure deficit (VPD) was calculated from air temperature and relative humidity.

2.3 Photosynthetically active radiation available to the leaves

To measure the transmitted photosynthetically active radiation (Actual PAR) through kaolin onto the leaf surface a similar method to the one described by Jifon & Syvertsen (2003) was used. PAR was measured with a portable quantum meter with three sensors on a 40 cm bar (Model LQS-QM, Spectrum Technologies Inc, Plainfield, IL, USA) and expressed as $\mu mol\ m^{-2}\ s^{-1}$. Highly transparent LDPE film was secured onto a 40 cm × 40 cm wooden frame and kaolin PF was applied in various quantities to simulate the kaolin content found on the experimental leaves. Incident (above the frame) and transmitted through clear film and transmitted PAR light through the kaolin-covered film and kaolin content (per unit film surface) were quantified in late August and used to calculate Actual PAR. With the same instrument, incident and reflected PAR were measured in the field at canopy level with six tree and 12 leaf replicates per treatment (kaolin or control trees) in mid August during 10:00 to 12:00. For the reflected PAR, the sensors of the instrument were positioned towards the canopy about 50 cm away at a height of 2.5 m, while for the incident PAR the sensor(s) was positioned towards the sun.

2.4 Plant measurements

After early June kaolin PF application, leaf functions were measured every around 20 d in four leaves of fruiting shoots of each of the 12 trees and at each treatment from 09:00-13:00, using a photosynthesis unit (model LCpro, ADC Bioscientific Ltd., Herts, England) with a leaf chamber operated at a flow rate of 300-350 mL min^{-1} under natural conditions. The unit was used to measure or calculate PAR, Actual PAR in kaolin-treated leaves, leaf temperature, leaf stomatal conductance (g_s), net photosynthetic (P_N) and transpiration (E) rates, intercellular CO_2

(Ci), water use efficiency (WUE, P_N over E), quantum yield (QY, P_N over PAR) in kaolin-treated and control leaves and Actual QY (P_N over Actual PAR) in kaolin-treated leaves. Chlorophyll fluorescence (presented as the intrinsic photochemical efficiency of PS II, Fv/Fm) was measured on 12 sun-exposed leaves (one per experimental tree) per treatment with a chlorophyll fluorometer (model OS-30p, Optisciences Inc., Tyngsboro, MA). Midday stem water potential (Ψ_{stem}) was measured on 12 leaves (one per experimental tree) situated in the shade close to the trunk per treatment. The leaves were covered with aluminum foil from 12:00 to 14:00, cut and then Ψ_{stem} was measured with a pressure chamber (model SKPM 1400, Skye Instruments Ltd., Isle of Skye, Scotland).

After the above measurements, 12 leaf replicates per treatment (with six sun-exposed current season fully-expanded leaves per replicate) were collected from fruiting shoots, placed in plastic bags and transported to the Laboratory of Pomology. Initially, kaolin residues were carefully removed with a brush from the adaxial and the abaxial side of the leaves and weighed to quantify kaolin residues. Leaf surface was measured using a scanner and the DT-Scan program and kaolin residues were expressed as g per m^2 leaf surface. The leaves were then washed with deionized water and free water was removed with paper towel. Leaf discs were taken with 9 mm diameter corer, their fresh weight and surface were measured, dried at 80 ^oC and reweighted. Water content (%) (WC) and specific leaf weight (g dry weight per m^2) (SLW) were then calculated. Similar leaf discs were macerated and extracted in 95% ethanol, chlorophyll a and b were measured spectrophotometrically and expressed as mg m^{-2} leaf surface and the ratio chlorophyll a over chlorophyll b (chla/chlb) was calculated (Wintermans & Mots 1965).

2.5 Fruit and oil measurements

Healthy green olive fruit were randomly picked from each tree in late September and fruit yield and quality characteristics were measured at five replications of five fruit per treatment. Measurements included fresh weight, pit weight, flesh/pit ratio, flesh firmness, skin color. Fruit skin color was measured after kaolin residues removal with water washing. Color was measured with a colorimeter Hunter LAB (Hunter Associates Laboratory, Inc., Model Miniscan XE plus, Shrewsbury, U.K.). Fruit color was measured at the five fruit of each replication after putting them in a petri and taking five measurements by rotating them. Color is presented as L*, C* and Hue. Fruit flesh firmness (N) was measured with a penetrometer (fruit firmness tester, Turoni®, Italy), after skin removal, with a 3 mm diameter tip.

In the middle of November, black olive fruit (maturity index around 5, International Olive Council, 2011) were harvested from each tree and weighed to measure yield per tree. In addition, healthy olives were picked randomly from each tree (five replications of ten fruit per treatment, in total) to evaluate flesh dry matter, oil content and oil quality characteristics. Dry matter was calculated from flesh samples weighed before and after drying at 80 °C. Oil content was measured in flesh samples after chemical extraction using petroleum ether according to Avidant *et al.* (1997). For the measurement of olive oil quality characteristics, 3 kg of olives were picked from each treatment and oil was extracted mechanically using a blender after pit removal. The crushed fruit were mixed for 30 min at 25 ^oC and then the oil was separated by centrifugation. Olive oil quality characteristics were performed with standard certified methods at the Center for Plant Protection and Quality Control, Central Greece.

2.6 Statistical analysis

Analysis of variance was performed with two factors, treatment and date, using the SPSS statistical package (SPSS 18.0, Chicago, USA). Least significant difference (LSD), standard deviation or Duncan's multiple range test results are shown to the figures or tables. Correlation analysis was performed with the same statistical package using Pearson correlation (r, $p<0.05$ or $p<0.001$) and the regression between kaolin quantity and percent reduction of PAR radiation on leaf surface in kaolin-treated leaves with Excel 2007.

3. Results

3.1 Climatic conditions

During the days of field measurements in the summer 2009 and hours 9:00-13:00, air temperature ranged from 20.3 in late September to 28.1 ^oC in mid August, relative humidity from 36.5 to 64.7% and VPD was 1.94 in mid June, 1.83 in mid July, 2.03 in late July, 2.08 in mid August, 1.69 in early September and 0.91 kPa in late September.

3.2 Photosynthetically active radiation available to the canopy

Photosynthetically active radiation, incident and reflected from the canopy, were measured with 3.47 g m^{-2} kaolin residues on the leaves. The incident PAR to the canopy was 1545±69 μmol m^{-2} s^{-1} (mean ± standard deviation) and similar among treatments. The reflected PAR from kaolin-treated tree canopies was 86% higher than the control tree 104 ± 8 μmol m^{-2} s^{-1} PAR value.

3.3 Photosynthetically active radiation available to leaves

The incident PAR, measured with the photosynthesis unit, onto the control leaves was >1200 μmol m^{-2} s^{-1} until early September with only a slight reduction during the summer (data not shown). In late September, incident PAR decreased significantly reaching values between 1000 and 1200 μmol m^{-2} s^{-1}. Kaolin-treated leaves always had similar incident PAR values to control leaves (data not shown).

To find out if kaolin residues accumulated on olive leaf surface could significantly shade the leaf, the transmitted PAR (Actual PAR) through kaolin PF available to leaf surface was calculated. The repeated applications of kaolin PF from early June to mid August gradually increased kaolin residues on olive leaf surface to high levels ranging from 2.0 to 13.5 g m^{-2}. Kaolin residues on leaf surfaces were highly correlated with the % reduction of the incident PAR above kaolin residues transmitted onto leaf surfaces (R^2=0.98). Thus, until early September the Actual PAR in kaolin-treated leaves exceeded 900 μmol m^{-2} s^{-1}, while in late September it was 589 μmol m^{-2} s^{-1}.

3.4 Leaf physiological variables

During the physiological variables measurement period, the external CO_2 varied from 377 to 409 μmol mol^{-1} and the intercellular CO_2 concentration varied from 148 to 221 μmol mol^{-1} without differences among treatments.
The deficit irrigation of olive trees during the summer drought period decreased Ψ_{stem} in both treatments from the middle of June, -2.75 MPa in control trees and -2.71 MPa in kaolin-treated trees, to the middle of August, -3.52 MPa in control trees and -3.05 MPa in kaolin-treated trees, while in late September Ψ_{stem} became less negative, -2.98 MPa in control trees and -2.74 MPa in kaolin-treated trees. During the experimental period, kaolin-treated trees had less negative Ψ_{stem} compared to control trees from mid July until early September and similar the rest of the period.

Leaf g_s decreased progressively until late July and reached its lowest values in the middle of August (Fig. 1). In September, g_s increased significantly due to rain events, cooler temperatures and, probably, the final olive fruit flesh growth and oil accumulation. Similar changes with time were measured with E (ranged from 1.4 to 2.6 mmol m^{-2} s^{-1}, detailed data not shown). Finally, P_N decreased gradually from the middle of June until the middle of August and then increased until late September (Fig. 2). Thus, g_s, E and P_N had their lowest values in mid August, when VPD was highest and Ψ_{stem} more negative, and their highest values at late September, when VPD was lowest and Ψ_{stem} less negative.

Kaolin-treated leaves had increased g_s, E and P_N compared to control leaves from the middle of June until the middle of July and at late September, while from late July until early September had similar values with control leaves (Figs. 2 and 3).

The chlorophyll florescence index Fv/Fm was similar for both treatments and always exceeded the 0.8 value. Based on this parameter, the summer heat stress did not reduce the efficiency of photosystem II.

A significant positive correlation was found between g_s and P_N in control leaves (r=0.86, p<0.05) and in kaolin-treated leaves (r=0.81, p<0.05). The Ci was not correlated with P_N in any case, but it was positively correlated with g_s in control leaves (r=0.76, p<0.05) and in kaolin-treated leaves (r=0.79, p<0.05). In addition, VPD was strongly negatively correlated with g_s in control leaves (r=-0.85, p<0.05) and in kaolin-treated leaves (r=-0.98, p<0.001) and with E only in control leaves (r=-0.95, p<0.05), while VPD was not correlated with P_N in any treatment. Ψ_{stem} was not correlated with leaf physiological variables or VPD. Finally, kaolin content on leaf surfaces was not correlated with any physiological parameter.

In both treatments WUE decreased from the middle of June (around 5 mmol mol^{-1}) to the middle of July and remained constant thereafter until late September with values from 3.7 to 4.2 mmol mol^{-1}. Kaolin-treated leaves had similar WUE to control leaves.

In both treatments, QY only slightly decreased from June to the middle of August and increased thereafter (Fig. 3). Kaolin-treated leaves had similar QY, when incident PAR was used for calculations, to control leaves except in late September, when kaolin-treated leaves had higher QY than control leaves (Fig. 3). When QY was calculated according to the Actual PAR for the kaolin-treated leaves, kaolin-treated leaves had higher Actual QY than control leaves throughout the measurements (Fig. 3).

3.5 Leaf characteristics

Leaf WC (%) changed slightly during the experimental season, but clearly decreased from a maximum in the middle of June to a minimum in August, especially at the control leaves, and partially increased thereafter again until early September and remained unchanged until late September (Fig. 4). In addition, kaolin-treated leaves had increased WC (%) in the middle of July and from the middle of August until early September and similar the other period to the control leaves (Fig. 4).

The SLW of control leaves reached a maximum in August, but changes over time were not significant (data not shown). SLW of kaolin-treated leaves decreased during the summer compared to mid June and September. Overall, kaolin-treated leaves had lower SLW than control leaves during the hot period of summer, especially from late July to late August (data not shown).

Leaf chlorophyll content (expressed as mg m^{-2} leaf surface) slightly decreased during the summer until early September (Fig. 5). In late September, chlorophyll content increased significantly in kaolin-treated leaves, while in control leaves this increase was not significant. In addition, kaolin-treated olive leaves had slightly or significantly higher chlorophyll content than control leaves (Fig. 5).

A significant positive correlation was found between chla and P_N in control leaves (r=0.88, p<0.05) and in kaolin-treated leaves (r=0.83, p<0.05). The chlb and total chlorophyll were not correlated with P_N.
Finally, as the chla/chlb ratio is an index for leaf shading, the calculated ratio showed that kaolin-treated leaves had 4% lower chla/chlb ratio than control leaves.

3.6 Yield and fruit and oil quality

At the harvest of green table olives, fruit from both treatments were of similar maturity as they had similar flesh firmness and skin color (Table 1).

Green olives harvested from kaolin-treated trees had similar weight, length and diameter and flesh/pit ratio compared to olives harvested from control trees (Table 2).

Later at the stage of black ripe olives, harvested yield per tree was similar among treatments with mean values 42.8 kg per tree in control trees to 45.0 kg per tree in kaolin-treated trees. Black olive fruit dry matter (%) and oil content (%) were similar in both treatments (Table 3).

Olive oil obtained by fruit from both treatments is characterized as extra virgin olive oil with olive oil from kaolin-treated trees having decreased free acidity and peroxide index compared to control (Table 4).

4. Discussion

4.1 Photosynthetically active radiation available to the canopy and the leaves

Photosynthetically active radiation measurements of olive tree canopy showed that kaolin PF was highly reflective to PAR radiation compared to control. In grapefruit trees, kaolin PF whiteness at least doubled reflected radiation from the leaves compared to control trees (Jifon & Sylvertsen 2003). In apple trees, kaolin PF was also found to be highly reflective to UV radiation resulting in reduced solar injury to both fruit and leaves (Glenn *et al.* 2002). The reflection of light due to kaolin reflectance could offer some additional radiation to leaf abaxial surface and redistribute this light to the inner canopy. Actually, the reflected PAR after kaolin PF application on almond and walnut tree canopies increased the incident PAR within the canopy and many previously shaded or partially shaded leaves finally received more PAR than in the absence of kaolin PF (Rosati *et al.* 2007). Because of the high reflectance of kaolin PF, the reflected PAR and UV were also found to be redistributed inside the canopy of kaolin-treated pecan trees and increased the whole plant carbon assimilation (Lombardini *et al.* 2005). In addition, leaves in the inner-canopy of the trees may not be uniformly covered with kaolin PF and, thus, shading due to kaolin is minor in addition to the probability of receiving more reflected light than without kaolin (Rosati *et al.* 2007). In our study, this non-uniform kaolin PF distribution was obvious as mature olive trees have relatively dense canopy compared to young trees after the spring flush of vegetative growth.

In the present study, kaolin PF application significantly reduced the Actual PAR reaching the leaf surface compared to control leaves and the reduction was proportional to kaolin residues on leaf surface. The Actual PAR reaching kaolin-treated leaf surfaces always exceeded 900 μmol m^{-2} s^{-1}, except in late September, when it was lower than 900 μmol m^{-2} s^{-1}. Proietti & Famiani (2002) have reported that olive leaf P_N is saturated at 1000-1200 μmol m^{-2} s^{-1} PAR. This means that, in our study, leaves covered with kaolin PF during a long period could possibly adapt to lower PAR values with anatomical and compositional changes related to leaf shading. Shade leaves have lower SLW and P_N, higher chlorophyll content and higher chla/chlb ratio (Hallik *et al.* 2012). Similar changes to leaf characteristics due to shade have been published for olive (Gregoriou *et al.* 2007). From our study, kaolin-treated leaves partially developed shade-related characteristics around a month after kaolin PF initial application, as SLW often decreased and chlorophyll content increased in kaolin-treated leaves, while the chla/chlb ratio and P_N did not change compared to control leaves during the hot summer months. These changes may also be due to the partial alleviation of heat stress from kaolin PF application.
In olive, it has previously been found that the irradiation of both sides of the leaves increased the P_N and QY compared to irradiating only one surface with the same PAR, and decreased the irradiance needed to saturate P_N (Proietti & Palliotti 1997). It is possible that, in our study, more light due to reflection was available to the abaxial surface of olive leaves treated with kaolin compared to control decreasing the negative consequences of shading the adaxial side of the leaf. Actually in our study, kaolin-treated leaves had similar QY compared to control leaves and increased Actual QY compared to control leaves. Thus, kaolin-treated olive leaves used the available light efficiently without any negative consequences in leaf productivity as was also found from the higher P_N compared to control leaves.

4.2 Leaf physiological variables

In both treatments of our deficit-irrigated field-grown olives, g_s, E and P_N decreased in midsummer with the lowest values in mid August and increased again in late September reaching their maximum values. Similar results have been published previously for other dry-cultivated field-grown olive cultivars (Proietti & Famiani 2002) or irrigated young olive plants of various cultivars including 'Konservolea' (Hagidimitriou & Pontikis 2005). In our study, the high g_s, E and P_N values in late September are probably associated with the favorable climatic conditions, better water availability and the presence of fruit, which constitute a major sink for olive trees in September due to their continuation of growth and oil accumulation. On the other hand, the low g_s, E and P_N values during midsummer were due to heat stress and low water availability with Ψ_{stem} significantly negative reaching -3.5 MPa in control trees as olive trees were deficit irrigated.

During the experimental period, kaolin-treated leaves had increased g_s, E and P_N compared to control leaves, but similar values with control leaves in midsummer or, shortly after the hot period, in early September. It seems that with summer drought stress, the plants were more stressed and kaolin PF tended to improve gas exchange of olive leaves but not during the hottest driest period in late summer. Finally, WUE of kaolin-treated leaves was usually similar to control leaves, suggesting that kaolin PF did not increase water consumption without increasing CO_2 fixation. Because when adequate drought stress is present, water loss decreases more than P_N resulting in higher WUE (Chaves *et al.* 2004).

In midsummer the drought stress in combination with high VPD limited gas exchange parameter levels. But the ratio Fv/Fm always exceeded the value of 0.8 showing that heat and drought stress were not severe enough to cause non-stomatal limitations as PSII damage (Maxwell and Johnson 2000) or other photosynthetic metabolic impairment to increase fluorescence dissipation (Flexas & Medrano 2002).

The negative correlation of g_s with VPD in both treatments shows that VPD affects stomatal conductance more than the negative Ψ_{stem}. Water regime of the olive tree plays a significant role to this relation and, more specifically, when Ψ_{stem} was <-4 MPa in mature olive trees there was no relationship between g_s and VPD (Moriana *et al.* 2002). In the present study, even though Ψ_{stem} was relatively low (i.e. always <-2.5 MPa), when climatic conditions were more favorable in June or late September, olive leaves could function well. In addition, E was negatively correlated with VPD only in control leaves showing that kaolin-treated leaves could better manage their water status even at high VPD. Furthermore, Ψ_{stem} was less negative in kaolin-treated trees during midsummer than control trees. It is known that kaolin applications decrease leaf temperature (Roussos *et al.* 2010) and VPD at leaf level (Jifon & Syvertsen 2003) and, thus, at least under mild heat stress, leaves function better keeping stomata open resulting in higher E in kaolin-treated leaves compared to control. P_N was not correlated with VPD in any treatment and it seems that carbon assimilation is less sensitive than g_s to high VPD. On the contrary in apple trees P_N was negatively correlated with VPD, but the combination of kaolin applications and adequate water could maintain maximum P_N (Glenn 2009). In our study, the positive correlation of g_s with E and P_N shows that stomatal closure is the main driving force of leaf functioning. The Ci was also positively correlated with g_s and was not correlated with P_N in contrast to previous work, where Ci was negatively correlated with g_s and P_N (Proietti & Famiani 2002; Denaxa *et al.* 2012). Those authors worked with young olive plants under severe drought stress (Denaxa *et al.* 2012) or with dry-cultivated mature trees of other olive cultivars (Proietti & Famiani 2002). This probably shows that drought stress in our work was not severe enough to alter the photosynthetic functions of 'Konservolea' olive leaves or, most probably, mild drought stress developed gradually up to midsummer and decreased in September.

The only published information about the kaolin PF effects on olive plant physiological functions were conducted on two-years-old potted 'Koroneiki' and 'Chondrolia' olive plants (Roussos *et al.* 2010; Denaxa *et al.* 2012). Kaolin PF applications did not improve leaf gas exchange variables with 'Koroneiki' plants, while the opposite was true with 'Chondrolia' plants under severe drought stress. These results show that kaolin PF effect is cultivar related to olive. Denaxa *et al.* (2012) suggested that the leaves of 'Chondrolia' are larger than 'Koroneiki' and concluded that, in broad leaf olive cultivars, kaolin PF applications may be more efficient, improving P_N under non-favorable water, temperature and irradiation conditions. In our study, even though 'Konservolea' is a broad leaf cultivar (leaf area 895 mm^2 per leaf in our experiments, almost 70% higher than 'Chondrolia' single leaf area), kaolin PF did not improve gas exchange under summer drought stress in deficit irrigated field-grown mature olive trees.

In various tree species kaolin PF applications did not show clear results on leaf gas exchange. In 'Empire' apples the combination of kaolin PF and irrigation maintained midday P_N at maximum levels (Glenn 2009). In addition, Glenn (2009) indicated that benefits of PF treatments would occur in agroecosystems with large VPD and high temperatures and that use of irrigation would further enhance the benefits of kaolin at high PAR levels. On the other hand, Gindaba & Wand (2007) showed that kaolin PF did not significantly affect midday leaf P_N in 'Cripps' Pink' apple under midseason conditions of high air temperature and solar radiation, did not reduce leaf temperature and did not increase g_s or E, but improved WUE compared to control plants. In addition, Rosati *et al.* (2006) found that kaolin application reduced maximum P_N in almond and walnut trees, did not affect g_s, reduced leaf temperature but this reduction did not improve P_N as leaf modifications due to shade were noted.

A final important result from our study is the fact that kaolin PF-treated leaves had increased or similar g_s to control leaves showing that kaolin particles did not result in any stomatal blocking to cause stomatal malfunctions, as can happen when leaves are contaminated with particulate matter of various sources (Farmer, 1993). It seems that the porous nature and the small size of kaolin particles, <2 μm, do not interfere with leaf functioning (Glenn & Puterka 2005).

4.3 Olive leaf characteristics

As it was mentioned before, g_s decreased in midsummer in both treatments due to heat and drought stress. It has been suggested that g_s is more sensitive to rapid climatic or water conditions changes, but, in the long-term, heat and drought stress gradually alter leaf characteristics as adaptive and acclimation responses to avoid water loss (Flexas *et al.* 2008). Probably, this is the reason that no correlation was found between gas exchange variables and leaf characteristics. Leaf WC (%) was positively correlated with WUE only in control leaves ($r=0.97$, $p<0.01$), while in kaolin-treated this relationship was not found suggesting that in kaolin-treated leaves water status was less affected by heat stress and deficit irrigation.

Olive leaf WC (%) decreased during midsummer and slightly increased thereafter in agreement with Proietti & Famiani (2002). Denaxa *et al.* (2012) found decreased leaf WC (%) in olive plants under drought and heat stress. In addition in our study, SLW of control leaves reached a maximum in August, while kaolin PF-treated leaves had more stable SLW during midsummer than control leaves. The increased SLW in August is probably related to drought stress and leaf maturation of this cultivar (Proietti & Famiani 2002; Hagidimitriou & Pontikis 2005).

Total chlorophyll content decreased over the summer period. According to Bacelar *et al.* (2006), total chlorophyll content decreases under low water conditions as a typical symptom of oxidative stress. In late September, leaf chlorophyll content usually increased significantly due to better climatic conditions as has been suggested before (Proietti & Famiani 2002).

In addition in our study, kaolin-treated olive leaves had increased or similar WC (%) to control leaves and decreased or similar SLW compared to control leaves as was also reported by Roussos *et al.* (2010) and Denaxa *et al.* (2012) for young olive plants. Furthermore, kaolin-treated leaves throughout the measurement period had higher chlorophyll content than control leaves as was also found for pecans (Lombardini *et al.*, 2005). These results suggest that kaolin-treated leaves were less stressed than control leaves, as has been previously found for other olive cultivars (Roussos *et al.* 2010; Denaxa *et al.* 2012), but, in our study, this alleviation was not important due to mild stress implemented. Finally, it seems that WC (%) and SLW were more accurate in showing the alleviating effect of kaolin PF on leaves compared to gas exchange variables, as WC (%) and SLW were relatively stable in kaolin-treated leaves compared to changes found in control leaves.

4.4 Yield and fruit and oil quality

Fruit yield was typical of 'Konservolea' yearly pruned medium size olive trees in the study region. Kaolin PF applications on olive trees did not alter olive fruit maturity and did not increase fruit yield, oil content and green fruit quality characteristics in agreement with Roussos *et al.* (2010). On the other hand, it has been suggested that under arid conditions, kaolin applications on mature rainfed olive trees increased fruit production and oil content (Saour & Makee 2003).

In our study olive oil obtained by kaolin-treated trees had lower free acidity and peroxide index, thus improved quality, as has also been found by Saour & Makee (2003). As it was mentioned above, kaolin applications partially alleviated olive leaf heat and drought stress. It seems that the microclimate created in olive tree canopies in the presence of kaolin PF, even if it did not increase fruit production given that P_N was partially increased, could decrease plant oxidative stress and, thus, olive oil from kaolin-treated trees had improved quality with decreased free acidity and peroxide index.

5. Conclusions

In this study, the effect of kaolin PF application on leaf characteristics and gas exchange of mature deficit irrigated fruiting 'Konservolea' olive trees during the summer period was investigated. Repeated kaolin applications created a white coat on leaf surfaces highly reflective to PAR that significantly decreased the available PAR to leaf blade underneath the kaolin layer. Kaolin did not result in any significant leaf structural or compositional changes related to shading, but partially showed changes related to alleviation of heat stress. Kaolin improved olive leaf gas exchange variables only when climatic conditions and water stress were mild. Under more severe water stress late in the summer, high kaolin PF residues present on the leaves were not able to improve leaf gas exchange. However kaolin-treated trees had less negative Ψ_{stem} during the hot and dry summer period and more stable leaf physiological characteristics. Kaolin-treated trees had similar fruit yield and quality characteristics, fruit oil content and fruit maturity to control trees and produced fruit with better oil quality, based on free acidity and peroxide value, than the fruit from control trees, probably due to decreased oxidative stress.

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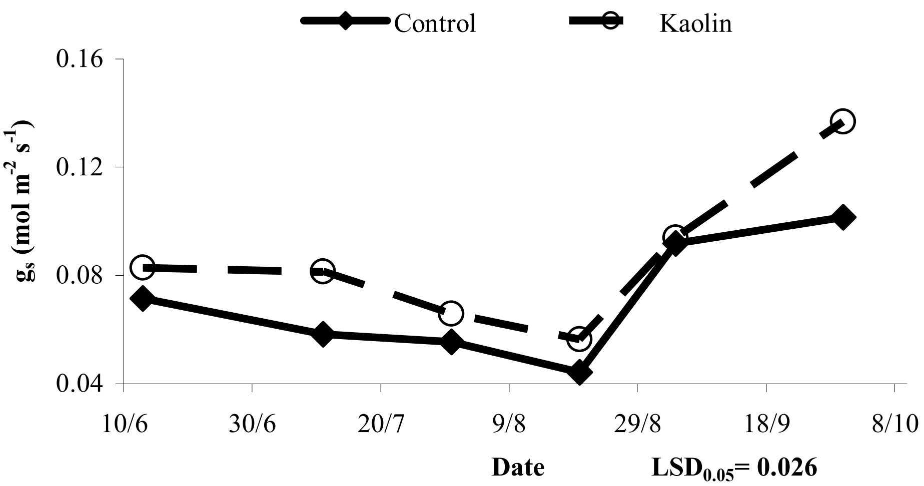

Figure 1. Seasonal evolution of stomatal conductance (g_s) in control and kaolin-treated olive leaves. Each value is the mean of 12 trees. Significant differences were based on LSD test at $p \leq 0.05$

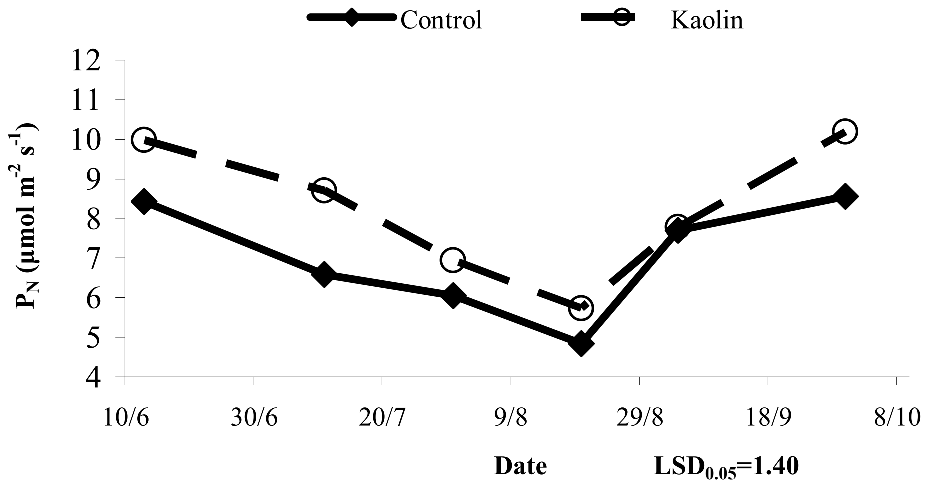

Figure 2. Seasonal evolution of net photosynthetic rate (P_N) in control and kaolin-treated olive leaves. Each value is the mean of 12 trees. Significant differences were based on LSD test at p ≤0.05

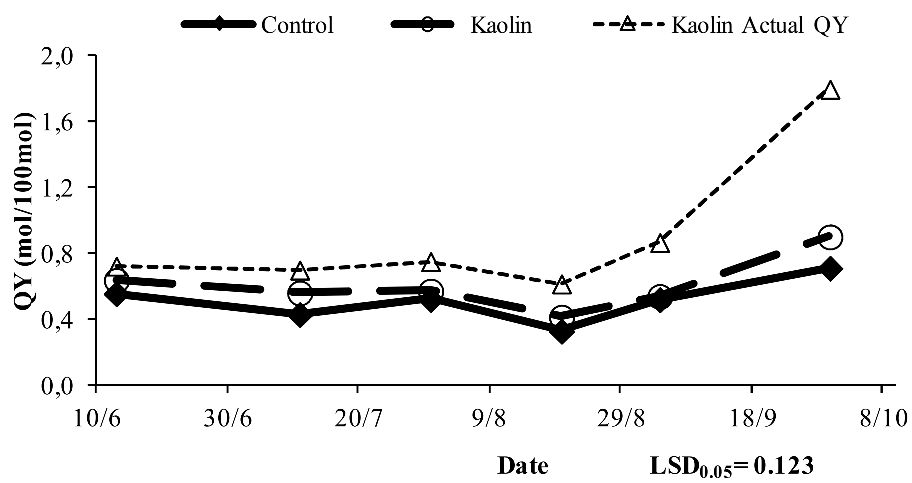

Figure 3. Seasonal evolution of quantum yield (QY) and actual quantum yield (Actual QY) in control and kaolin-treated olive leaves. Each value is the mean of 12 trees. Significant differences were based on LSD test at p ≤0.05

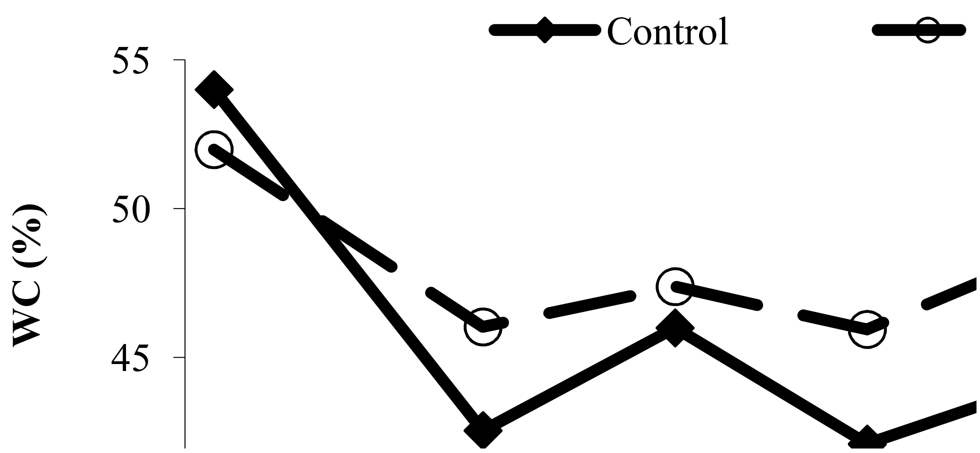

Figure 4. Seasonal evolution of water content (%) (WC) in control and kaolin-treated olive leaves. Each value is the mean of 12 replicates. Significant differences were based on LSD test at p ≤0.05

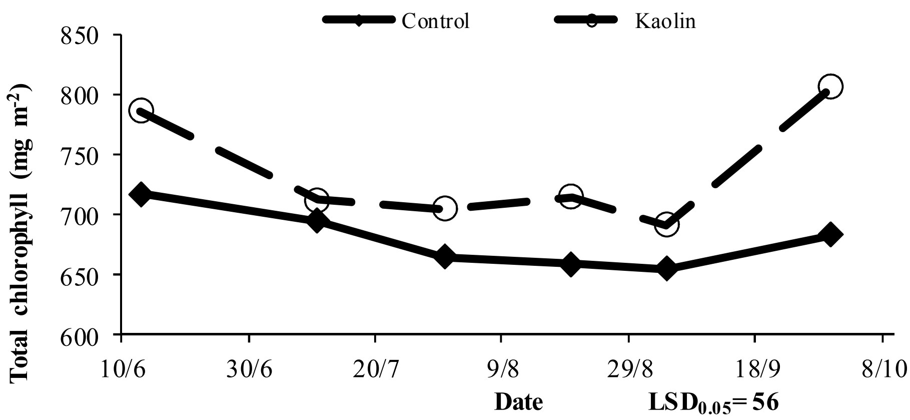

Figure 5. Seasonal evolution of total chlorophyll content in control and kaolin-treated olive leaves. Each value is the mean of 12 replicates. Significant differences were based on LSD test at $p \leq 0.05$

Table 1. Flesh firmness (N) and skin color of green olives

Treatment	Flesh firmness (N)	L*	C*	Hue
Control	6.99 a	42.7 a	31.1 a	107.2 a
Kaolin	7.12 a	43.8 a	32.5 a	107.5 a

Means within columns followed by the same letter are not significantly different using LSD test at $p \leq 0.05$ (n=5)

Table 2. Green olive fruit characteristics

Treatment	Fruit weight (g)	Flesh/pit ratio	Length (cm)	Diameter (cm)
Control	6.30 a	5.39 a	2.42 a	1.89 a
Kaolin	6.63 a	5.14 a	2.43 a	1.95 a

Means within columns followed by the same letter are not significantly different using LSD test at $p \leq 0.05$ (n=5)

Table 3. Black olive fruit flesh dry matter and oil content

Treatment	Dry matter (%)	Oil content (% f.w.)
Control	28.8 a	14.7 a
Kaolin	28.6 a	16.1 a

Means within columns followed by the same letter are not significantly different using LSD test at $p \leq 0.05$ (n=5)

Table 4. Olive oil quality characteristics

Treatment	Free acidity (% oleic acid)	Peroxide index (mEq O_2 kg^{-1})
Control	0.755 a	13.0 a
Kaolin	0.430 b	9.7 b

Means within columns followed by the same letter are not significantly different using LSD test at $p \leq 0.05$ (n=5)