

Comparative impact of dusts on peach and olive leaf functions under Mediterranean dry climatic conditions

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Abstract

Peach and olive trees, distinctively different in resistance to heat stress, are grown extensively in areas with hot and dry summers where particulate matter (PM) air pollution is common. Other PMs, such as kaolin particle film (PF), are used as a heat stress alleviating factor. Kaolin PF, soil or cement PM accumulation onto the leaves of both species decreased the available photosynthetically active radiation (PAR) to the leaves, mainly in olive, without causing any shade-related negative effect. Typical differences in leaf gas exchange parameters and leaf characteristics were found between the mesophyte deciduous peach, cv. 'Royal Glory', and sclerophyllous evergreen olive, cv. 'Konservolea'. Kaolin PF accumulation to sufficiently irrigated peach trees improved leaf gas exchange functions acting as significant heat stress alleviating factor, but in the water-stressed olive trees, kaolin PF-covered leaves improved their gas exchange functions only in autumn (mild climatic conditions with improved tree water status). In both species, leaf characteristics and total phenolic content (TPC) of kaolin PF-treated leaves showed partially stress alleviated. Soil and mainly cement PM accumulation onto the leaves of both tree species, in combination with summer heat stress, decreased gas exchange functions indicating stomata blocking and other factors which may reduce carbon assimilation, while increased leaf TPC. In conclusion, comparing the three PMs, kaolin PF did not affect or improved leaf functions and characteristics, while the other dusts, soil and mainly cement, negatively affected leaf functions and modified leaf traits as an adaptation mechanism.

Keywords — *Prunus persica, Olea europaea, kaolin particle film, particulate matter, gas exchange, total phenolic content.*

I. INTRODUCTION

Peach and olive trees are grown extensively in the Mediterranean basin with summers characterized by high temperatures, high vapour pressure deficit, high irradiance levels and low precipitation, phenomena that are exacerbated by climate change [1]. In addition, Mediterranean basin is strongly affected by air pollution, with one of the most important

pollutants to be particulate matter. The term particulate matter (PM) is used to describe a heterogeneous mixture of particles differing in size, origin, and chemical composition and is separated in two categories fine PM_{2.5} (0-2.5 µm) and coarse PM₁₀ (<10 µm) according to the aerodynamic diameter of the particles ([2], [3]). PM is mainly associated with industrial activities, agricultural activities, construction, vehicle emissions and other factors that are characteristically magnified by long droughts, with adverse effects on crop productivity ([1], [3]). In addition, it is reported that mineral dust (or soil dust) consists of an important component of PM with the main sources to be wind-blown dust, dust from agricultural activities and dust re-suspended by road traffic [4].

The effect of accumulation of PM on vegetation depends on the PM quantity, the duration of its presence, its chemical composition and the plant species ([2], [5], [6]). Cement factories are one of the main sources of PM emissions to the environment. Suspended dust particles from cement kiln may harm the plants grown around cement factories and may affect plants either directly through dust deposition on plants or indirectly by interfering with the soil's chemical composition ([5], [7], [8]). The dust from cement kiln forms surface crusts on plant material after hydration. This may cause blocking of stomata and shading of photosynthetic tissue, and, as a result, reduction of leaf (and other tissues) photosynthetic and transpiration capacity ([5], [9], [10], [11], [12]).

Other PMs such as dust from unpaved roads are often highly alkaline and may have toxic effects on plant surfaces after hydration, while others may contain toxic metals such as dust derived from streets and highways with heavy traffic [5]. PM accumulation onto the plants may alter the leaf morphological characteristics or anatomical structure, the concentration of photosynthetic pigments or their ratio among them ([11], [13], [14]). Furthermore, the PMs accumulated on leaves that have originated from cement kiln as well as unpaved and paved roads affect several physiological processes of plants linked to leaf gas exchange, carbohydrate metabolism, chlorophyll and carotenoid contents, leaf morphology, mineral nutrition, heavy metal contamination and the leaf antioxidant mechanism ([10], [11], [13], [14],

[15]). The severity of the above effects depends on the frequency and intensity of rain events, dust quantity, plant species and leaf characteristics [2].

In recent years, foliar applications of mineral products such as kaolin particle film (PF) are extensively used in agriculture to control plant pests and to alleviate plants from heat stress ([16], [17], [18], [19], [20], [21], [22], [23]). More specifically, kaolin PF 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 the reflected radiation throughout the plant reaching both leaf surfaces and shaded plant canopy areas ([24], [25], [26], [27]). As a result, kaolin PF was found to reduce heat stress and solar injury by decreasing leaf and fruit temperature in apples ([16], [17]), peaches [21] and grapevines [23]. Results on the effect of kaolin PF applications on leaf gas exchange functions showed that kaolin PF acts as an alleviating factor to water and heat stresses in most cases, but its effectiveness varies with crop species and different regions studied ([20], [22], [24], [25], [28], [29], [30]).

Peach tree is a mesophyte deciduous species with leaf photosynthetic processes considerably insensitive to temperature changes between 20°C and 32°C, but the photosynthetic capacity is reduced above and below this range of temperatures [31]. As a species that is sensitive to water stress, many late-maturing peach cultivars may suffer from summer heat stress with adverse effect on tree productivity even though sufficient irrigation may be applied. In early maturing peach cultivars summer heat stress may also have adverse effect on bud differentiation and, as a result, reduced yield and increased abnormalities of fruit shape in the following growing season.

Olive tree is an evergreen sclerophyllous drought-tolerant species with photosynthetic apparatus resistant enough to moderate water stress, while stomatal resistance is the main limiting factor to carbon assimilation. Olive leaves have certain structural features and possess active mechanisms for drought tolerance, allowing certain degree of control over water loss ([32], [33], [34]). However, when water availability is low and high temperatures and high levels of irradiance occur during the growing season, the olive tree undergoes significant stress resulting in reduced productivity [32].

Plants have been studied extensively as biomonitors of the overall impact of air pollution and as a remediation medium for removing air pollutants, such as PM, in urban and forested areas. However, studies on the effect of PM on sustainable agricultural production, especially tree fruit production, are limited. The hypothesis is that PMs of different origin accumulated onto tree canopy may affect leaf physiological functions in a different manner for two distinctive in their ecophysiology and anatomy tree species. The aim of this study was to present the

comparative effect of three PMs of different origin, use, composition, optical and hydraulic properties on photosynthetic capacities of leaves and productivity of two different fruit tree species, a deciduous mesophyte tree, the peach, and an evergreen sclerophyllous tree, the olive, under hot and dry climatic conditions of Mediterranean region. For this study, an important early to mid-season table peach cultivar ‘Royal Glory’ and the second most important Greek table olive cultivar ‘Konservolea’ were studied.

II. MATERIALS AND METHODS

A. Plant material and treatments

Two tree species with distinct behavior to hot and dry climatic conditions were selected for the current study, a mesophyte deciduous tree, the peach, and an evergreen sclerophyllous tree, the olive. For the peach, an important mid-season table cultivar ‘Royal Glory’ was studied. ‘Royal Glory’ peach trees (*Prunus persica* L. Batsch), on GF677 rootstock, were planted with 5 m x 5 m spacing in 1999, trained in a vase system and grown with standard locally-applied horticultural practices in the Velestino Experimental Station (Latitude 39°23' N, Longitude 22°44' E), University of Thessaly, central Greece with soil pH at 7.9. Drip irrigation was performed twice a week applying 80-100% of water required based on crop evapotranspiration (ETc).

For olives one of the most important Greek table olive cultivar ‘Konservolea’ was studied. The experiment was carried out in a deficit (around 50% of ETc, calculated with $K_c=0.5$ 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 with 6 x 6 m spacing in Dimini (Latitude 39°13' N, Longitude 22°48' E), central Greece. The trees were well pruned each year to keep their height below 2.5 m with open canopy. The experiment was conducted from early June to late September from 2009 to 2011, with 2010 to be the ‘off’ year and data from 2011 are presented.

In both experiments, the experimental design was a complete randomized design with four treatments, three replications and four trees per replication. In both experiments treatments included control trees sprayed only with tap water, trees receiving kaolin PF, soil or cement kiln dust, every around 20 d, in total five applications, from early June until early August while thereafter no other applications were performed in order to examine the quantity of kaolin, soil or cement dust residues removed by autumn rainfalls and the subsequent changes of leaf functions. Control trees sprayed with tap water with pressurized knapsack sprayer, kaolin PF (Surround WP, Engelhard Corp., Iselin, NJ, USA) was applied, according to the label of the formulation, at 5% w/v in tap water with pressurized knapsack sprayer. For soil dust, used in both experiments, soil from uncultivated areas of Velestino Experimental station

was collected, oven dried at 80 °C, milled and sieved through a 500 µm screen. Cement kiln dust was produced from a local cement factory. For soil and cement dust applications initially tree canopy was sprayed with tap water using the pressurized knapsack sprayer. This procedure was followed in order to promote dust particles deposition on foliar surfaces. Just after wetting the leaves soil or cement dust were applied uniformly using a manually operated duster. Data from middle July, 118 days after full bloom (DAFB) for peach (full bloom at 20 of March) and 86 DAFB for olives (full bloom at 20 of April), late August, 160 DAFB for peach and 128 DAFB for olives, and late September, 189 DAFB for peach and 159 DAFB for olives, 2011, when the rains removed most of the dust, are shown, to better understand the comparative effect of these different PMs during the drought period under heat stress conditions in these two different tree species.

B. Climatic measurements

During leaf gas exchange measurements (09:00-13:00) weather data were collected from the meteorological station of the Experimental Station for the experiment with peaches and from the local meteorological station for the experiment with olives [35]. Mean air temperature (9:00-13:00) ranged from 29.7 °C in the middle of July to 21.0 °C in late September and vapor pressure deficit ranged from 2.24 kPa in the middle of July to 0.85 kPa in late September for the peach experiment. For the olive experiment mean air temperature (9:00-13:00) ranged from 31.0 °C in the middle of July to 21.0 °C in late September and vapor pressure deficit ranged from 2.72 kPa in the middle of July to 0.78 kPa in late September.

C. PAR available to the leaves

The method used to measure the transmitted photosynthetically active radiation (Actual PAR) through kaolin PF, soil or cement PM onto the leaf surface has been described elsewhere [12]. Actual PAR was calculated from the incident PAR to the leaves and the dust quantity deposited onto the leaves.

D. Plant measurements

Leaf functions were measured in the middle of July, late August and late September in 16 leaves per treatment from 09:00-13:00, using a leaf gas exchange system (model LCpro, ADC Bioscientific Ltd., Herts, England) as described before [12]. The system was used to measure or calculate PAR, leaf stomatal conductance (g_s), net photosynthetic rate (P_N), transpiration rate (E) and water use efficiency (WUE, P_N over E), intercellular CO_2 (Ci) in kaolin PF treated, soil- or cement-dusted and control leaves.

After the above measurements, six leaves fully exposed to the sun per tree were collected, placed in plastic bags and transported to the Laboratory of Pomology. Kaolin PF, soil or cement PM residues,

expressed as g per m² leaf surface as described elsewhere [12]. Leaf disks were taken with a 9-mm diameter corer; their fresh weight and surface area were measured, dried at 80 °C, and reweighed. Leaf water parameters such as water content (WC, %) and degree of succulence (SUC, mg H₂O per cm²), leaf sclerophylly parameters such as leaf mass per area (LMA, g dry weight per m²) and leaf tissue density (LTD, g dry weight per kg fresh weight) were then calculated. Similar leaf disks were macerated and extracted in 95% ethanol, and chlorophyll a (Chl a) and b (Chl b) were determined spectrophotometrically (Spectronic 301, Milton Roy Company, Ivyland, PA, USA) at 665 nm and 649 nm absorbance [36]. Total chlorophyll concentration (Total Chl=Chl a+Chl b) was calculated per m² leaf surface, and the ratio of chlorophyll a over chlorophyll b (Chl a/Chl b) was calculated. From the same leaves, 0.5 g of fresh leaf tissue was macerated and extracted with 25 mL methanol [37]. Total phenolic content (TPC) was determined from the leaf extract spectrophotometrically (Spectronic 301, Milton Roy Company, Ivyland, PA, USA) at 760 nm using the Folin-Ciocalteu reagent and expressed as gram gallic acid per kg leaf dry weight.

E. Statistical analysis

Analysis of variance was performed with two factors, treatment and date, using the SPSS statistical package (SPSS Statistics for Windows, Version 20.0, IBM Corporation, Armonk, NY, USA). Replications were different depending on each parameter measured. Least significant difference (LSD) was used to determine significant differences among means at $P \leq 0.05$. For the correlation between PM quantity and percent reduction of PAR on leaf surface in treated with PM leaves was used Microsoft Excel 2013 (v15.0) (Microsoft Office, Microsoft Corporation, Redmond, WA, USA).

III. RESULTS

A. PAR radiation available to the leaves and dust accumulation

The incident PAR, measured with the photosynthesis system, was similar among treatments for both species (Table I). In peach, kaolin PF onto leaf surfaces ranged from 0.2 to 0.9 g m⁻², for soil dust from 0.02 to 5.8 g m⁻² and for cement dust from 1.0 to 11.7 g m⁻² (Fig. 1). In olive, kaolin PF onto leaf surfaces ranged from 1.3 to 4.3 g m⁻², for soil dust from 0.01 to 12.8 g m⁻² and for cement dust from 2.4 to 20.6 g m⁻² (Fig. 1). Dust quantity on leaf surfaces was positively correlated with the % reduction of incident PAR transmitted through dust particles onto leaf surfaces ($y=0.0326x+0.0545$, $r=0.992$ for kaolin PF, $y=0.0146x+0.0289$, $r=0.907$ for soil dust and $y=0.0149x+0.0667$, $r=0.954$ for cement dust). Actual PAR (the transmitted PAR through dust particles available to leaf surface) ranged from 1020 to 1249 µmol m⁻² s⁻¹ in peach, and from 781 to 1126 µmol m⁻²

s^{-1} in olive with the higher value in soil dusted leaves in late September after dust removal by rain and the lower value in cement dusted leaves in late August when dust quantity to the leaves was maximum (Table I, Fig. 1).

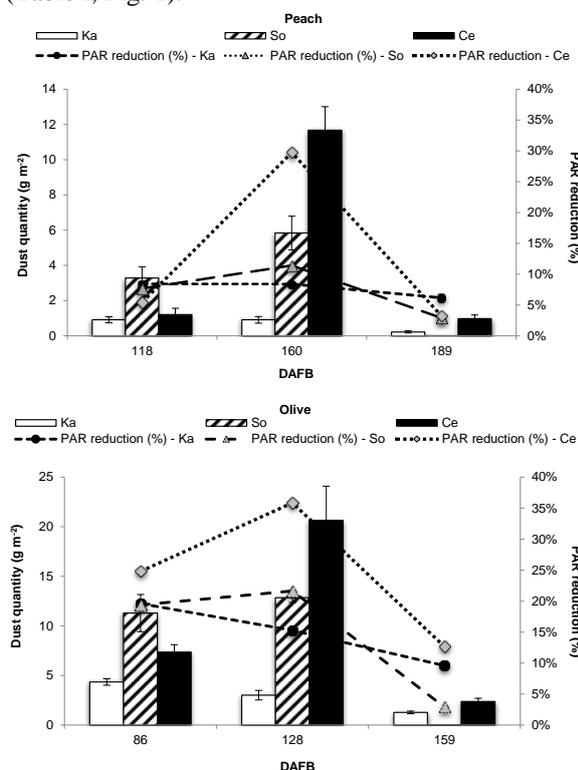


Fig. 1: Dust quantity (n=12) and percentage reduction of incident PAR after transmittance through dust particles (n=16) during the summer period for control, kaolin PF, soil and cement polluted peach and olive trees. Vertical bars represent the standard error

B. Leaf physiological parameters

In both tree species, during the hot summer period, soil-dusted leaves and, even more, cement-dusted leaves in peach, had lower g_s , P_N , E and WUE than control, with no differences between the two PMs (Table I, Fig. 2). In case of kaolin PF, the two species responded differently during the summer period, as peach leaves treated with kaolin PF had slightly higher g_s and P_N , or significantly higher P_N and E only in mid-July, than control, while olive leaves treated with kaolin PF had similar g_s , P_N and E in the middle of July and lower g_s , but similar P_N and E, until late August compared to control leaves (Table I, Fig. 2). In addition kaolin PF applications to peach and olive leaves had no effect on WUE. During the summer period, peach leaves coated with kaolin PF had higher g_s , P_N , E and WUE than the leaves dusted with the other PMs, while olive leaves treated with kaolin PF had higher or similar g_s and E in mid-July and late August, respectively, and always higher P_N and WUE than the leaves polluted with soil or cement dust (Table I, Fig. 2).

In late September, in both tree species, significant rain events removed almost the soil dust, while a portion of cement PM and kaolin PF remained onto the leaves, and, with the mild climatic conditions, g_s , P_N and E increased in all treatments reaching the highest values (Table I, Fig. 2). Under these conditions, peach and olive leaves polluted with soil PM recovered partially in peach and totally in olive. Actually, in peach leaves treated with soil PM g_s was similar with control, P_N was slightly lower and E was significantly lower than control resulting in slightly higher WUE. In olive, soil PM covered-leaves showed higher g_s , P_N and E than control and the other treatments, fact that resulted in a similar WUE with control and kaolin PF treated leaves and lower WUE from the leaves covered with cement dust (Table I, Fig. 2). In late September, the olive leaves polluted with cement PM had similar g_s , but lower P_N and mostly lower E than control which caused the increased WUE compared to control. The peach leaves polluted with cement dust continued to have lower g_s , P_N , E and WUE than control (Table I, Fig. 2). Finally, in late September peach leaves treated with kaolin PF had the highest g_s , P_N and E but similar WUE with the other treatments. Olive leaves treated with kaolin PF had higher g_s , slightly higher E and similar P_N to control, higher g_s , P_N and E than cement polluted leaves and lower than soil-dusted leaves. The last had as a result that olive leaves coated with kaolin PF had similar WUE with control and leaves covered with soil PM and lower WUE than the leaves with cement dust accumulation (Table I, Fig. 2).

C. Leaf water and sclerophylly parameters

Peach leaf LMA gradually increased until late September in kaolin PF treated leaves and cement-dusted leaves, while that of the control or soil-dusted leaves increased until late August and remained unchanged thereafter (Fig. 3). Olive leaf LMA remained unchanged during the measurement period in all treatments (Fig. 3). Peach LTD increased until late August and slightly decreased in late September in all treatments except for cement polluted leaves, where it remained unchanged until late September, while olive LTD gradually increased until late September in all treatments (Table II).

In both tree species, soil- and cement-dusted leaves always had higher LMA than control and kaolin PF treated leaves during the experimental period (Fig. 3). Furthermore, cement-dusted peach leaves had higher LMA than soil-dusted leaves, while the opposite was found for olive (Fig. 3). Kaolin PF-treated peach and olive leaves had similar LMA or lower (in late August) than control leaves (Fig. 3). In most cases and in both tree species, kaolin PF treated leaves had the lowest LTD values, while the other treatments had similar LTD during the whole period (Table II).

TABLE I

Changes in peach and olive leaf incident PAR (PAR), incident PAR transmitted through dust particles (Actual PAR), transpiration rate (E), intercellular CO₂ (Ci) and water use efficiency (WUE) during the summer period for control, kaolin PF, soil and cement polluted peach and olive trees (n=16)

DAFB	Treatment	PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Actual PAR ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	E ($\text{mmol m}^{-2} \text{s}^{-1}$)	Ci ($\mu\text{mol mol}^{-1}$)	WUE (mmol mol^{-1})
Peach trees						
118	Control	1264	-	3.57 d	208 c	3.48 ab
	Kaolin	1358	1249 a ^z	4.15 bc	206 c	3.46 ab
	Soil	1260	1159 a	3.27 de	210 c	3.23 b
	Cement	1297	1232 a	3.18 de	204 c	3.24 b
160	Control	1315	-	3.53 d	211 c	3.82 a
	Kaolin	1183	1088 b	3.85 cd	213 c	3.64 ab
	Soil	1358	1209 a	2.92 e	233 b	2.89 bc
	Cement	1458	1020 b	2.74 e	241 b	2.61 c
189	Control	1231	-	4.53 b	255 a	3.23 b
	Kaolin	1261	1185 a	5.25 a	235 a	3.18 b
	Soil	1212	1176 a	3.92 cd	264 a	3.55 ab
	Cement	1267	1229 a	4.03 c	269 a	2.89 bc
Significance						
DAFB		ns ^y	*	***	***	ns
Treatment		ns	*	***	***	***
DAFB x Treatment		ns	**	ns	***	***
Olive trees						
86	Control	1156	-	2.22 bc	192 cd	3.85 b
	Kaolin	1209	967 bc	2.18 c	199 cd	3.68 b
	Soil	1223	990 b	1.76 d	227 a	2.82 c
	Cement	1232	924 bc	1.74 d	214 b	2.99 c
128	Control	1145	-	2.19 c	182 d	3.60 b
	Kaolin	1252	1064 ab	2.10 cd	172 d	3.47 b
	Soil	1224	955 bc	1.93 cd	228 a	2.57 c
	Cement	1220	781 c	1.87 cd	219 ab	2.55 c
159	Control	1104	-	2.54 b	203 bc	3.80 b
	Kaolin	1048	943 bc	2.77 ab	218 ab	3.44 bc
	Soil	1161	1126 a	2.91 a	220 ab	3.71 b
	Cement	1006	875 c	2.10 cd	216 ab	4.87 a
Significance						
DAFB		ns	ns	***	***	***
Treatment		ns	***	***	***	***
DAFB x Treatment		ns	**	**	***	***

^zThe values represent the mean of 16 replications and values with the same letter within a column are not significantly different, according to LSD at $P \leq 0.05$

^yns, *, **, *** Non significant or significant at $P \leq 0.05$, 0.01, or 0.001, respectively

Peach leaf SUC slightly increased in late September for all treatments except for cement-dusted leaves that remained high during the whole experimental period (Fig. 3). Olive leaf SUC remained unchanged for control and kaolin PF treated leaves during the measurement period, while for soil- and cement-dusted leaves decreased (slightly for cement) in late September (Fig. 3). Cement-dusted peach leaves had higher SUC than the other treatments during all the measurements, but slightly higher than kaolin PF treated leaves in late September. Soil-dusted, kaolin PF-treated and control peach leaves had similar SUC. In olive, soil-dusted leaves

had higher SUC than cement dusted leaves, and the last ones had higher SUC than control and kaolin PF treated leaves during all measurements. Furthermore, peach leaf WC decreased until late August and increased in late September for control and kaolin PF treated leaves or remained unchanged thereafter for soil or cement dust polluted leaves (Table II). In peach, kaolin PF treated leaves had always the highest WC and slightly higher than control and cement dusted leaves, while soil dusted leaves had the lowest WC (Table II). In olive, leaf WC remained unchanged during the measurement period, while

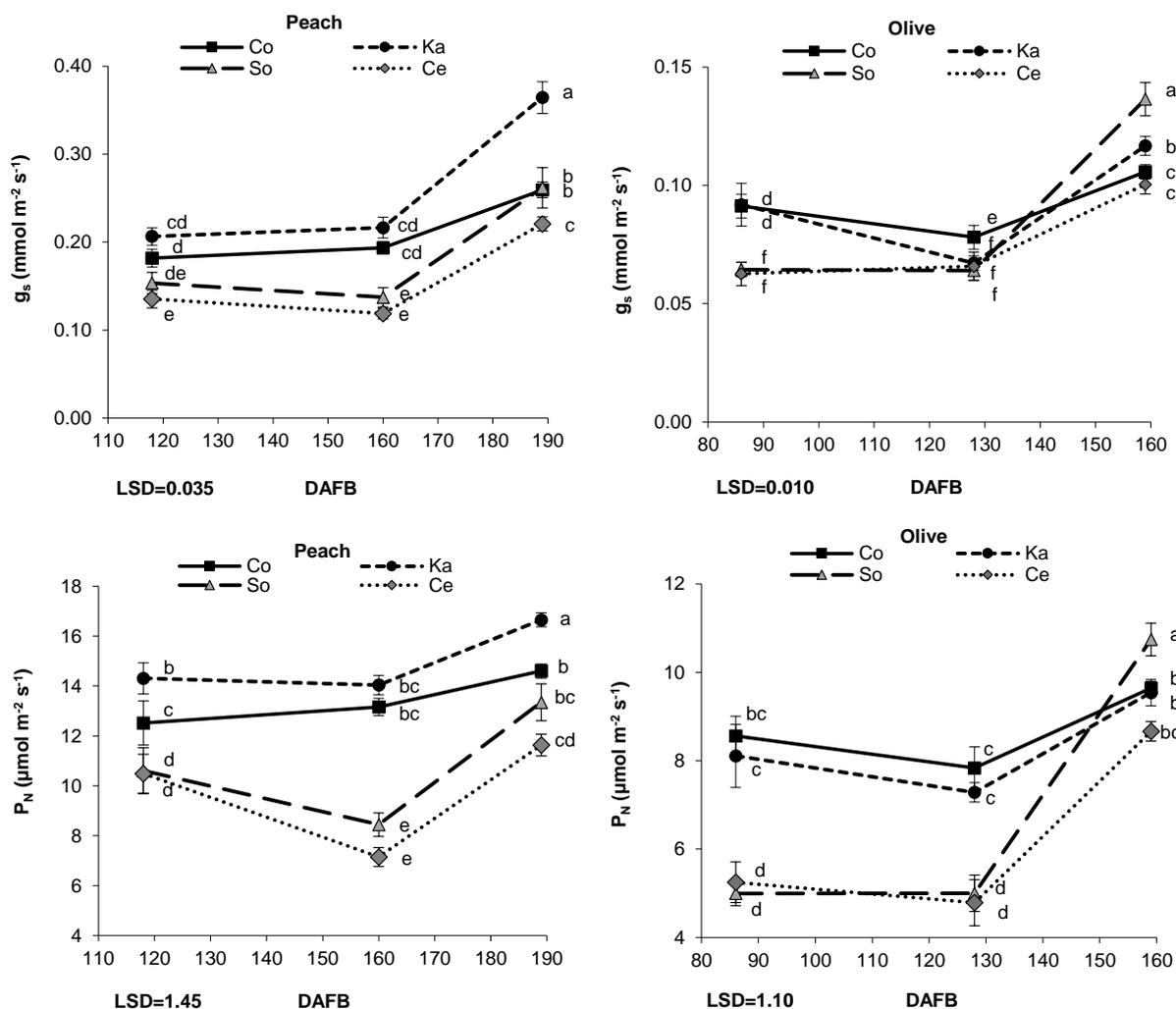


Fig. 2: Seasonal evolution of net photosynthetic rate (P_N) and stomatal conductance (g_s) in control, kaolin PF, soil and cement polluted peach and olive leaves. The values represent the mean of sixteen replications ($n=16$), values with the same letter are not significantly different, according to LSD at $P \leq 0.05$ and vertical bars represent the standard error

kaolin PF treated leaves always had the highest WC compared to the other treatments but the difference was not significant (Table II).

D. Leaf Total Chl and TPC

During the experimental period, peach leaf Total Chl (as mg m^{-2} leaf surface) gradually decreased for all treatments, except for soil-dusted leaves that remained unchanged after late August, while in olive Total Chl gradually increased for all treatments (Fig. 4, Table II). Peach kaolin PF coated leaves had lower Total Chl during the whole measurement period compared to control while the soil- and cement-dusted leaves had lower Total Chl compared to control after late August (Fig. 4). In olive, no significant differences were found in Total Chl among treatments but, after late August, kaolin PF treated leaves had slightly higher Total Chl content compared to the other treatments (Fig. 4).

In peach, leaf Chl a/Chl b ratio gradually increased until late September, while the opposite was found for olive, as leaf Chl a/Chl b ratio decreased in late September for all the treatments (Table II). Kaolin PF

treated leaves had higher Chl a/Chl b ratio than the other treatments during the whole measurement period in peach or until late August in olive. In peach, soil- and cement-dusted leaves had higher Chl a/Chl b ratio than control after late August, while in olive these polluted leaves had lower Chl a/Chl b ratio than control until late August with no differences between the two PMs (Table II).

In peach, TPC had the highest values in late September for PM treatments, while in control the highest value was found in late August (Fig. 4). In olive, TPC had the highest values in late August in all treatments (Fig. 4). In peach, soil and cement polluted leaves had higher TPC than control leaves in mid-July and late September, while in late August after the extended heat stress summer period, control leaves had similar TPC to soil and cement polluted leaves (Fig. 4). In peach, kaolin PF treated leaves had significantly lower TPC than soil and cement polluted leaves during the whole measurement period and lower than control leaves only in late August (Fig. 4). In olive, soil dusted leaves had the highest TPC from all treatments during the whole period. Olive cement

polluted leaves had higher TPC than control and kaolin PF treated leaves and similar to soil dusted leaves only in late August. Olive kaolin PF treated leaves had the lowest TPC content from all treatments except of late August that had similar TPC to control (Fig. 4).

IV. DISCUSSION

A. PAR radiation available to the leaves and PM accumulation

Kaolin PF treated, soil- and cement-contaminated peach and olive leaves had lower

Actual PAR than control leaves and the reduction was proportional to dust quantity. In peach, Actual PAR reaching dusted leaves was always above the light saturation point for peach leaves [31]. In olive, Actual PAR in most cases was lower than the light saturation point for olive leaves [38]. This difference between the two species was due to the higher kaolin PF and soil or cement PM quantity onto olive leaves in comparison to peach leaves, which was probably due to differences in leaf morphology in the two species.

Table II
Changes in peach and olive leaf water content (WC), leaf tissue density (LTD), and the ratio chlorophyll a/chlorophyll b (Chl a/ Chl b) during the summer period for control, kaolin PF, soil and cement polluted peach and olive trees (n=12)

DAFB	Treatment	WC (%)	LTD (g kg ⁻¹)	Chl a/ Chl b
Peach trees				
118	Control	56.1 ab ^z	439 c	2.84 bc
	Kaolin	58.1 a	419 d	2.99 bc
	Soil	55.8 bc	442 c	2.82 c
	Cement	57.2 ab	428 cd	2.83 c
160	Control	51.8 cd	482 ab	2.83 c
	Kaolin	53.8 c	462 b	3.02 b
	Soil	49.9 d	501 a	2.98 bc
	Cement	52.7 cd	473 b	2.94 bc
189	Control	54.1 bc	459 bc	2.95 bc
	Kaolin	56.0 b	440 c	3.25 a
	Soil	51.7 d	483 ab	3.12 ab
	Cement	52.6 cd	474 b	3.00 b
Significance				
DAFB		*** ^y	***	***
Treatment		***	***	**
DAFB x Treatment		ns	ns	ns
Olive trees				
86	Control	44.1 ab	559 b	2.60 ab
	Kaolin	45.9 a	541 b	2.68 a
	Soil	44.3 ab	557 b	2.53 b
	Cement	44.0 ab	560 b	2.50 b
128	Control	42.8 b	572 ab	2.62 ab
	Kaolin	45.1 ab	549 b	2.69 a
	Soil	43.2 b	568 ab	2.54 b
	Cement	44.7 ab	553 b	2.50 b
159	Control	41.3 b	587 a	2.55 b
	Kaolin	44.7 ab	553 b	2.51 b
	Soil	43.1 b	569 ab	2.49 b
	Cement	42.6 b	574 ab	2.45 b
Significance				
DAFB		**	**	**
Treatment		***	***	***
DAFB x Treatment		ns	ns	ns

^zThe values represent the mean of 12 replications (n=12) and values with the same letter within a column are not significantly different, according to LSD at P ≤ 0.05

^yns, *, **, *** Non significant or significant at P ≤ 0.05, 0.01, or 0.001, respectively

It is known that, besides the different properties of the various PMs, their deposition on plant leaves depends on leaf structure, cuticular and epidermis features, pubescence, leaf roughness, the phyllotaxy and the canopy structure and leaf density [15]. Peach leaves are broad and smooth, while olive leaves are small, with a thick cuticle and a continuous rough wax layer, a few stellar trichomes covering the upper epidermis and with the abaxial side with a continuous layer of overlapping stellar trichomes hiding the small and abundant stomata [34], characteristics indicating that olive leaves may accumulate more dust than peach leaves.

The PM and kaolin PF repeated applications during the dry summer period resulted in their accumulation onto the leaf surfaces in both species and especially olive leaves. In September, in both species, significant rain events removed almost totally the soil PM, while a portion of cement PM and kaolin PF remained onto the leaves and mostly onto olive leaves. This is also associated with the different

nature and physical properties of the three PM materials beyond the difference of leaf morphology and characteristics of the two tree species as mentioned above [2]. PMs with hydraulic properties as cement dust may create a crust onto plant surfaces which may also injure plant surface. Kaolin PF contains sticking agents promoting the adherence of these particles onto leaf surface. Soil PM is a natural material without any particular adherence onto the leaf surfaces, and rain water easily removed this PM from leaf surface. Thus, the properties of each PM have different duration of acting on the leaf surfaces. Herein, the result of PM pollution from unpaved roads was studied. PM of Saharan origin arriving with south winds in the European Mediterranean countries is often deposited onto leaf surfaces with light rain events leaving significant volume of sticking material onto these surfaces which remains on the leaf surface until significant rain events occur (G. Nanos, personal observations).

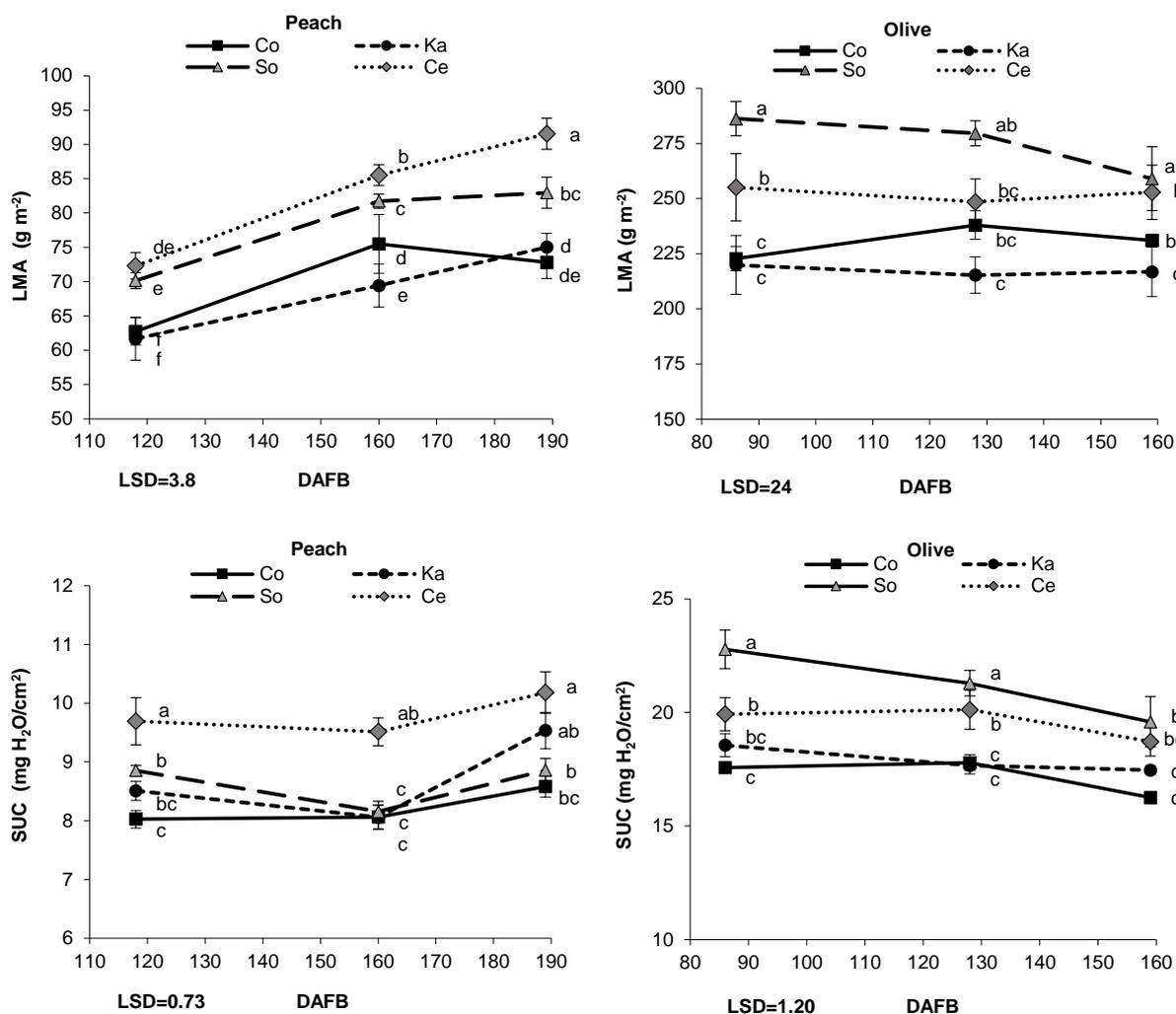


Fig. 3: Seasonal evolution of leaf mass per area (LMA), succulence (SUC) in control, kaolin PF, soil and cement polluted peach and olive leaves. The values represent the mean of 12 replications (n=12) and values with the same letter are not significantly different, according to LSD at P ≤ 0.05 and vertical bars represent the standard error

The deposition of kaolin PF and PM onto the leaves may cause shade effect, but in the case of kaolin PF which is highly reflective, it is suggested that it may redistribute PAR to the inner part of the canopy improving the light status and increasing the whole plant carbon assimilation [24]. It has been reported that shaded olive leaves have lower LMA and P_N, higher Total Chl per unit weight and lower Chl a/Chl b ratio [39]. Similar changes to leaf characteristics due to shade have also been published for peach [40], but it was reported that Chl a/Chl b ratio was not affected [41]. In our study, both peach and olive leaves polluted with soil and cement PM had higher LMA than control leaves, while kaolin PF treated leaves had the lowest LMA. In addition, soil and cement polluted peach and olive leaves had lower P_N compared to control with the cement dusted leaves' P_N being unable to recover even in late September when most of cement dust was removed

by rains. The two tree species reacted differently to the presence of kaolin PF, as in peach leaves PN increased, while in olive leaves it was not affected or slightly decreased.

Total Chl in olive leaves was similar among treatments, while in peach after late August, kaolin PF, soil and cement dusted leaves had lower Total Chl than control, indicating that presence of all three types of PM on the deciduous peach leaf surface may result in leaf senescence acceleration even though in late September P_N increased in all treatments under favorable climatic conditions and dust quantity reduction on leaf surfaces. In addition it has been reported that the decreased photosynthetic pigment content in conifer needles that have been exposed to cement dust was due to shade and to imbalanced Mg, Mn, Fe and N composition of needles, as these elements participate in the biosynthesis of photosynthetic pigments [7].

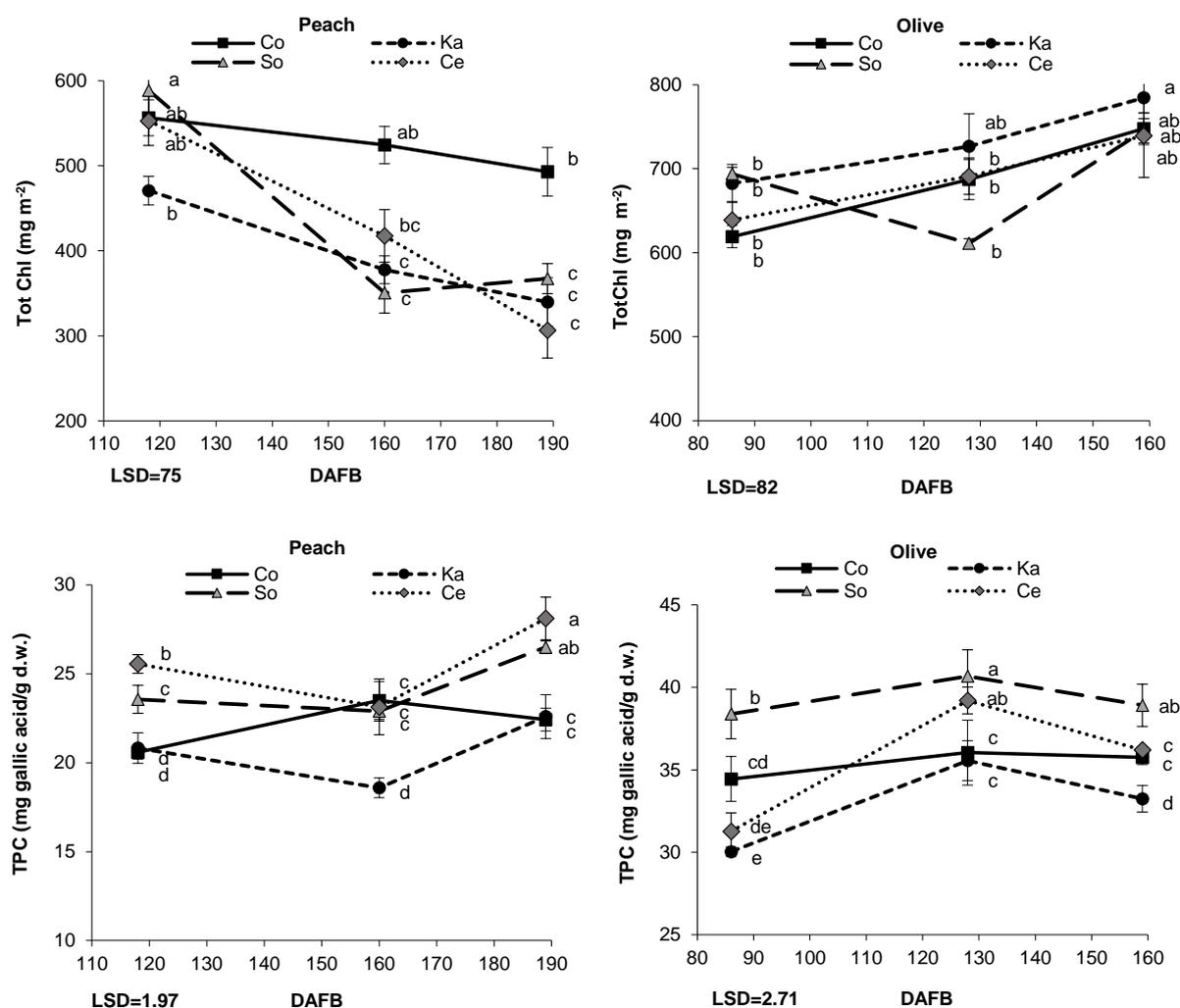


Fig. 4: Seasonal evolution of total chlorophyll content (Total Chl) and total phenolic content (TPC) in control, kaolin PF, soil and cement polluted peach and olive leaves. The values represent the mean of 12 replications (n=12) and values with the same letter are not significantly different, according to LSD at P ≤ 0.05 and vertical bars represent the standard error

According to our previous report, soil and cement PM accumulation onto peach leaves did not cause metal toxicity and serious mineral imbalances to the leaves, but both soil and cement-dusted leaves had decreased K content probably due to lower E [12]. Furthermore, kaolin PF treated leaves had higher Chl a/Chl b ratio than control, soil- and cement-contaminated leaves in both species, but in olive leaves in late September Chl a/Chl b ratio was similar in all treatments. Soil and cement dusted leaves had similar Chl a/Chl b to control in both species. These results show that soil and cement dusted leaves have not shown any modifications of chlorophyll contents due to shading and the reduction of P_N and Total Chl and the increase of LMA compared to control leaves could be due to other factors analyzed below. Alterations to leaf morphology in both species due to presence of kaolin PF are not connected with shade but could be associated with alleviation from heat stress as reported before ([21], [30]).

B. Leaf physiological parameters

In all treatments and both tree species, especially in our deficit-irrigated field-grown olives, g_s , E and P_N had the lowest values during summer, while in late September these parameters reached their maximum values. In olives, similar results have been published previously for other dry-cultivated field-grown olive cultivars [38]. In our study, the high g_s , E and P_N values in late September are probably associated with the favorable climatic conditions and, in case of olives, fruit presence, 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 because of high air temperatures, high irradiance levels, relatively high VPD and minimum rain events, and, in case of olives, low soil water availability as they were deficit irrigated. However, in olives WUE remained quite unchanged with time in control and kaolin PF-treated leaves, while in PM-covered leaves WUE remained the highest in late September, when leaves partially recovered from the stress caused by dust accumulation. In peach leaves, control and kaolin treated leaves had the highest WUE in midsummer and the lowest in late September, while the opposite was found for the leaves dusted with soil or cement, showing the significant effect of soil or cement PM accumulation on the leaves on WUE at midsummer.

It is clear that olive leaves had lower g_s , E, P_N and C_i values compared to peach leaves independently of the treatment. This is a typical difference between a mesophyte deciduous and a sclerophyllous xerophyte evergreen species. The leaves of sclerophyllous species in arid environments are characterized to be thick, tough, and with a higher fibre to protein ratio than mesophytic leaves [42]. In addition, evergreen leaves are characterized by high leaf mass per area with decreased intercellular airspaces and surface

area-to-volume ratio, which may cause lower conductance to CO_2 transfer between the sub-stomatal cavity and chloroplasts, leading to lower availability of CO_2 at rubisco carboxylation sites [43]. Higher internal resistances to CO_2 diffusion in these leaves (including olive leaves) have been related to water and nutrient conservation mechanisms ([44], [45]). It has been reported that leaf photosynthesis in sclerophyllous species is limited by mesophyll conductance, which is influenced by both leaf structure and the environment and found that the mesophyll conductance declines as leaf dry mass per area increases [46]. The fact that mesophyll conductance is constrained by large leaf mass area is mostly related to the thicker cell walls (and lower intercellular spaces) observed in species with high LMA, significantly limiting CO_2 diffusion inside the photosynthesizing cells [47]. In our study olive leaves had higher LMA and LTD compared to peach leaves showing the sclerophylly of olive leaves compared to peach leaves. Similarly, it was found that Mediterranean sclerophyllous evergreen species from the driest part of the environmental gradient showed higher values of leaf mass per area, while deciduous species, at relatively humid sites, showed lower values [48].

Differences of leaf physiological functions between the two species especially during the hot and dry summer period are also associated with the different irrigation status of the two crops, as olive trees were deficit irrigated. Under these unfavorable conditions stomatal closure is one of the first lines of defense, to reduce water loss. However, when stomata close, leaf and plant carbon assimilation significantly decrease in various plants including peach and olive ([49], [50], [51]). Nevertheless in our study olive leaves had similar WUE with peach leaves referring to control leaves.

In both species, leaf functions of soil or cement polluted leaves were further diminished compared to control leaves during hot summer period, which means that soil and cement dust deposition onto the leaves acted synergistically with heat stress. It is possible that soil and cement PM onto peach and olive leaves may have clogged stomata and, thus, polluted leaves had lower g_s , E, P_N and WUE than control leaves. Many authors have reported that PM from different sources blocked stomata and negatively affected gas exchange parameters in various annual or perennial forest plants ([52], [53], [54], [55]). The decrease of leaf gas exchange parameters due to PM accumulation onto peach or olive leaves has been reported before with cement PM on olive leaves [10] and soil PM from unpaved road on nearby trees of various species [13]. Soil or cement polluted olive leaves had in most cases increased C_i , while in peach this was the case only after a long period of heat stress, compared to control leaves. These results showed that decreased

carboxylation efficiency may not be only due to stomata closure or blocking.

Other antioxidant mechanisms are also involved in protecting against oxidative stress under adverse environmental conditions [56]. In our study, soil and cement polluted leaves in most cases, especially in peach, had increased TPC content than control. Furthermore, soil and cement polluted peach leaves had decreased Total Chl content after the long hot dry summer period without recovery in September. On the contrary, soil and cement PM presence onto olive leaves did not decrease Total Chl content compared to control. This is a strong evidence that peach leaves were more adversely affected from these pollutants compared to olive leaves.

It is important that with September rains, the different nature of the two dusts played significant role to stress alleviation. Soil was almost totally removed by rain and, as a result, g_s , P_N and E recovered totally in olive leaves, while in peach leaves g_s recovered, but P_N and E remained somewhat lower than control leaves, as a result of the accelerated senescence (decreased Total Chl content since late August). Cement had developed a crust on leaf surfaces, and, as a result, it was only partially removed by rain. Thus, a quantity of cement remained onto the leaves in late September still negatively affecting leaf gas exchange parameters in both tree species.

Kaolin PF applications onto the leaves had a contradictory effect to the two tree species studied. In our adequately irrigated peach trees, kaolin PF improved leaf gas exchange functions compared to control leaves (but only slightly in late August, after the prolonged period of heat stress) showing that kaolin PF deposition on peach leaves functioned as heat stress alleviating factor. In our previous study, we found that kaolin PF application onto peach trees decreased heat stress by reducing peach leaf and fruit temperature, especially in midday hours, due to reduced PAR and mainly UV radiation reaching the plant surfaces, without causing any negative shade effect [21]. In our deficit irrigated olive trees, kaolin PF did not ameliorate leaf functions, as g_s , E and P_N were similar or lower than control during summer, but in late September when the mild climatic conditions and the most of kaolin still present resulted in higher g_s , slightly higher E and no change in P_N compared to control. These data show that under substantial heat stress, kaolin PF was not able to reduce its negative consequences in olive. In addition, in both species, WUE of kaolin PF treated leaves was usually similar to control leaves, suggesting that kaolin PF did not increase water consumption without increasing CO_2 fixation in olive leaves [27]. When severe drought stress is present, water loss decreases more than P_N resulting in higher WUE [49].

Another significant result is the fact that kaolin PF-treated leaves had increased (in peach) or similar (in olive) g_s to control leaves showing that kaolin

particles did not result in any stomatal blocking causing stomatal malfunctions, as can happen when leaves are polluted with PM of various sources [5]. It seems that the porous nature and the small size of kaolin particles, i.e. $<2 \mu m$, do not interfere with leaf functioning [57].

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 [58]. In addition, it was 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 ([58]). Furthermore, it was revealed the positive effect of kaolin applications on grapevine physiological performance under summer stress conditions in Mediterranean areas [23]. On the contrary, other researchers found no effect of kaolin PF on midday leaf photosynthetic rate in 'Cripps' Pink' apple [29]. Kaolin PF application on pecan trees [24] and on almond and walnut trees [25] did not improve leaf gas exchange. In olive, kaolin PF on two-years-old potted 'Koroneiki' olive plants did not have a significant effect on gas exchange and alleviation from drought stress during dry cultivation cycles [20], while for young potted 'Chondrolia Chalkidikis' olive trees, kaolin PF improved leaf functions alleviating drought stress [30]. Thus, kaolin PF is not sufficient in many cases to soften effects of drought stress and improve leaf functions especially P_N .

C. Leaf water and sclerophylly parameters

In both tree species and mainly in peach, during the hot summer period LMA and LTD increased and WC and SUC decreased as a result of the heat load and leaf maturation. Similarly, it has been reported before that olive leaf WC decreased during midsummer and slightly increased thereafter ([38], [59]). It also found decreased leaf WC in olive plants under drought and heat stress [30].

Leaf sclerophylly parameters showed that olive leaves had higher LMA and LTD than peach leaves, and leaf moisture parameters showed that olive leaves had lower WC, but higher SUC, compared to peach leaves. This is related to the greater fraction of the foliar volume occupied by dry matter, whereas the higher succulence means that thicker leaves contain a greater volume of water per surface unit [60]. In addition, the high leaf mass area is associated with high leaf thickness and density, which appears to be an adaptation to stressful environments like those in the Mediterranean climate [47].

Kaolin PF, soil and cement PM deposition on the surface of peach and olive leaves caused various changes to leaf characteristics. Thus, in both tree species, soil and cement polluted leaves had higher LMA than control, similar LTD to control, similar or lower only for soil PM after late August WC in peach

and similar WC in olive compared to control, slightly higher SUC in soil-contaminated peach leaves or significantly higher SUC in olive and cement contaminated peach leaves compared to control. In addition, in both tree species leaves treated with kaolin PF had lower or similar LMA, lower LTD and increased WC and SUC compared to control. It has been reported that maintenance of relative water content by the plant may be associated with the relative tolerance of plants towards air pollution [61]. In case of kaolin PF, alterations to leaf characteristics showed that kaolin PF may be an alleviating factor from heat stress resulting in better leaf water status [22]. On the other hand, the accumulation of soil or cement PM onto the leaves caused stress to the leaves that altered leaf sclerophylly and moisture parameters as an adaptation mechanism. According to the above, it is clear that the three dusts affected differently the leaf characteristics with similar trend for the leaves of the two tree species studied.

D. Leaf Total Chl and TPC

Peach leaf Total Chl per leaf area decreased (the same was found for Total Chl expressed per dry weight – data not shown) with accumulation of kaolin PF, soil or cement PM on leaf surfaces late in the summer compared to control leaves and this reduction remained until late September. In olive, no decrease was found in Total Chl per leaf area in the presence of any PM. But, when Total Chl were expressed per dry weight it was found that kaolin PF treated olive leaves had higher and soil- or cement-polluted leaves had lower Total Chl compared to control (data not shown). At several studies with forest trees contaminated from unpaved road PM [13], cement PM on olives [10] and cement PM on apple, pear and almond trees [62] it was found lower leaf chlorophyll content compared to uncontaminated leaves. This reduction in chlorophyll content is probably associated with P_N reduction or to alkaline conditions created inside the leaf cells with PM suspension, which may cause degradation in photosynthetic pigment and/or inhibition in activities enzymes for pigment biosynthesis [15].

In the present study, peach leaf TPC increased in soil- or cement-polluted leaves, as antioxidant reaction, compared to slightly stressed control or was similar when all leaves were stressed by the summer heat. The opposite was found for kaolin PF treated peach leaves that had slightly or significantly lower TPC than control after the long hot and dry period. In olive, cement and even more soil PM deposition onto the leaves increased TPC compared to control, while kaolin PF treated leaves had lower, early in the measurement period, and similar TPC to control thereafter. Similarly, it was found that urban traffic pollution increased leaf phenolics in plane trees compared to similar plants at the rural environment [63]. Other researchers found that leaf ascorbic acid content increased as a defense to road PM pollution

stress ([14], [61], [64]). In case of kaolin PF treated leaves, the TPC reduction compared to the other dusted leaves may be related with the high reflectivity of UV from kaolin PF white coated leaves, as UV radiation was found directly connected to leaf phenolic content [65].

V. CONCLUSIONS

Kaolin PF or soil or cement PM accumulation onto the leaves of peach and olive decreased the available PAR to the leaves, and mostly in olive, without causing any shade modifications to the leaves. Kaolin PF accumulation to the sufficiently irrigated peach trees improved leaf gas exchange functions until autumn acting as a significant alleviating factor from heat stress. In the water stressed olive trees kaolin PF deposition onto the leaves did not affect gas exchange functions but only in autumn, under mild climatic conditions and better water conditions, g_s and E were improved compared to control without P_N and WUE modification. Soil and mainly cement PM accumulation onto the leaves of both species significantly decreased g_s , P_N , E and WUE, mostly in peach trees. PM particles, especially cement PM, probably blocked stomata, while cement PM also formed a crust onto the leaf surface, thus limiting gas exchange through stomata. Soil or cement PM deposition onto the leaves acted synergistically with summer heat stress and caused other than stomatal limitations to carbon assimilation, probably accelerated leaf senescence in peach as Total Chl decreased, and caused oxidative stress as TPC increased. In early autumn, rain events removed soil PM partially recovering peach leaf functions or totally in olive, while cement PM created a crust onto the leaves and remained as permanent stress factor until late September in both tree species. Leaf characteristics were differentially affected by the three PMs studied due to their different properties. Thus, in both species kaolin PF treated leaves had improved sclerophylly and leaf moisture parameters showing a better leaf water status and decreased TPC showing less stress. On the other hand, the accumulation of soil or cement PM onto the leaves was a stressful agent, which altered leaf sclerophylly and moisture parameters as an adaptation mechanism. Typical differences in leaf gas exchange parameters and leaf characteristics were found between the mesophyte deciduous peach and sclerophyllous evergreen olive.

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