Understanding kaolin effects on grapevine leaf and whole-canopy physiology during water stress and re-watering

Frioni Tommaso[a,*], Saracino Simone[a], Squeri Cecilia[a], Tombesi Sergio[a], Palliotti Alberto[b], Sabbatini Paolo[c], Magnanini Eugenio[a], Poni Stefano[a]

[a] Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122, Piacenza, Italy
[b] Department of Agricultural, Food and Environmental Sciences DSA[3], Università degli Studi di Perugia, Borgo XX Giugno 74, 06121, Perugia, Italy
[c] Department of Horticulture, Michigan State University, 1066 Bogue Street, 48824, East Lansing, MI, USA

ABSTRACT

Kaolin applications have been investigated in grapevines to understand cooling effects on leaves and clusters and the relative impact on gas exchange, leaf biochemistry, water use efficiency, glyco-metabolism and hormonal patterns. Several Almost all previous contributions have relied upon single-leaf measurements, leaving uncertainty on whole canopy performances, depending on the complexity of a canopy system *vs.* individual leaves. In our study, kaolin was sprayed at pre-veraison (DOY 204) on potted mature vines (cv. Sangiovese) and washed off a month later (DOY 233), while control vines were left unsprayed. Within control (C) and kaolin (KL) treated vines, well-watered (WW) and water stress (WS) treatments were also imposed over a 10-day period (DOY 208–217) and all vines were re-watered when the WS reached its peak (stem water potential between −1.3 and −1.6 MPa). Single leaf measurements included leaf surface temperature by thermal imaging (Leaf T_{mean}), assimilation (Leaf A), transpiration (Leaf E), stomatal conductance (Leaf g_s) rates, F_v/F_m fluorescence ratio, predawn and stem water potential. Concurrently, whole canopy gas exchange was monitored continuously from DOY 200–259 using a vine enclosure system and daily net CO_2 exchange rate (NCER) and canopy transpiration (E_{canopy}) were calculated and then normalized *vs.* leaf area per vine. Results report that for any of the parameters recorded at both levels (single leaf and whole canopy), there was good agreement in terms of relative changes. In absence of water stress, KL was able to improve leaf cooling, while slightly reducing photosynthetic and water loss rates. More interestingly, data taken under water deficit and upon re-watering support the hypothesis that KL can turn into a protective agent for leaf function. In fact, the lack of photo-inhibition and the maintenance of leaf evaporative cooling found in KL-WS at the peak of water-stress ($F_v/F_m > 0.7$, Leaf $T_{mean} < 38°C$ and $E_{canopy} > 0.5 mmol\,m^{-2}\,s^{-1}$) warranted a prompter recovery of leaf functions upon re-watering that did not occur in C-WS vines.

1. Introduction

Global warming is challenging geographical distribution of grapevine cultivation as well as fruit ripening dynamics and resulting wine styles (Palliotti et al., 2014; Santillan et al., 2019). In warm environments, climate change is leading to an increased frequency of summer days with air temperature (T) exceeding the 35 °C, which is a critical threshold for vine physiology and grape ripening (Mori et al., 2007). Excessive diurnal temperatures exert a complex effect on vine physiology and must composition (Poni et al., 2018); among the latter

effects, inhibition and/or degradation of the anthocyanin pool at high T and a very fast depletion of the highly respired malic acid, are representing serious oenological issues. Further to increasing evaporative demand, this climatic pattern also exacerbates the occurrence of leaf and berry sunburn, often followed by desiccation and shriveling (Hulands et al., 2014; Rustioni et al., 2015).

Global warming also impacts the dynamics of fruit ripening, with increased earliness being a common trait in several viticulture areas of the world (Jones et al., 2005; Schultz, 2000; Schultze et al., 2016; Webb et al., 2007). Interestingly, the issue of early ripening is not just the

outcome of a given amount of active heat summation required to ripen the crop being accumulated in a shorter period of time, but rather of a series of cofactors including low cropping, dehydration (*e.g.* total soluble solids surge rapidly due to berry water loss, while phenolic accumulation is lagging behind) and early cessation of vegetative growth due to summer drought. Efforts in assessing both long- and short-term mitigation techniques have been fostered over the last decade to adapt to the above described challenges (Palliotti et al., 2014). Mid- or long-term mitigation solutions embrace the use of new rootstocks and/or cultivars; among the short-term options, the use of reflective particle materials, such as kaolin (KL), having the ability to reflect infrared, PAR and ultraviolet radiation is a potentially viable practice in commercial vineyard (Glenn and Puterka, 2005). In fact, it is well known that, due to modifications in the leaf and fruit texture after spraying as well as changes in the reflected light signature of the plant, KL has a repellent action against a number of damaging insects in different crops (D'Aquino et al., 2011; Joubert et al., 2004; Lapointe et al., 2006; Leskey et al., 2010; Pascual et al., 2010). At the same time, KL is also effective in reducing leaf and fruit sunburn damages in several fruit crop species including apple (Wand et al., 2006; Aly et al., 2010), pomegranate (Melgarejo et al., 2004; Sharma et al., 2018) and grapevine (Dinis et al., 2016a, 2016b, Bernardo et al., 2017). Recently, a number of works have been published, especially in olive and grapevine, investigating the physiological mechanism promoted by KL applications against multiple summer stresses. Under the quite stressful conditions of the Douro region, KL sprayed leaves displayed decreased susceptibility to photo-inhibition due to higher efficiency of the PSII system and a more efficient photochemical quenching (Dinis et al., 2018a). Transcriptional analyses and enzymatic activity assays carried out under similar stress conditions (*i.e.* high temperature and irradiance) reported that KL was able to increase sucrose concentration in leaves, sucrose transport and phloem loading capacity (Conde et al., 2016, 2018). In olives, leaves sprayed with KL showed less oxidative damages, requiring a reduced antioxidant adaptation (Brito et al., 2018).

Not too surprisingly, the fruit cooling effect induced by the KL sprays has enhanced their quality, especially in relation of increased and more uniform pigmentation. This effect has been repeatedly reported in apples (Glenn et al. 2005, Aly et al., 2010) whereas, in grapevines, KL sprays increased, over three consecutive seasons, the concentration of total monomeric anthocyanins in the cv. Malbec grown under arid condition with high solar radiation in Idaho (Shellie and King, 2013a, 2013b). In addition, Brillante et al. (2016) have reported a remarkable increase (+35%) of total anthocyanins in Cabernet S. in a KL treatment when compared to an unsprayed control.

Due to its long-lasting ability to change the reflective properties of the sprayed organ and the consequent cooling effects, KL is also expected to significantly affect leaf photosynthesis and water status (namely transpiration, stomatal conductance and water potential) and, consequently, water use efficiency. Yet in regards to such parameters the outcome picture is anything but univocal. Abou-Khaled et al., 1970 were the first to report that particle films can have different effects on leaf gas exchanges, depending on the tissue microenvironment. They found that a kaolinite treatment was reducing photosynthesis and transpiration rates of orange leaves exposed to low light intensities, while under medium-high light exposure, leaf temperature was reduced and leaf photosynthetic rates and water use efficiency were progressively enhanced when compared to untreated leaves. Focusing on two fruit species (apple and grapes) having the most abundant dataset about KL effects, the main discrepancy relies on different conclusions about KL effects that can be drawn depending upon the photosynthetic measurement level adopted (*i.e.* single leaf *vs.* whole canopy). In cv. Braeburn/M9 rootstock apple trees, KL treated leaves had lower assimilation rates than control over a wide range of incident light levels and temperatures; concurrently, though, neither leaf transpiration nor stomatal conductance were affected by the particle film (Wunsche et al., 2004). However, when whole-canopy assimilation was measured

using an open-top cuvette system, such differences disappeared and whole-canopy water use efficiency (WUE_{canopy}) was similar between sprayed and unsprayed trees. In more recent work on cv. Empire, where carbon isotope discrimination ($\Delta^{13}C$) analysis was applied to estimate seasonal WUE, it has been reported that the irrigated, KL treated plants had the greatest $\Delta^{13}C$ and so the lowest WUE compared to the unsprayed irrigated treatment at a leaf area index (LAI) between 4 and 6 (Glenn, 2010, 2016).

In grapevine, variability of kaolin's effect on leaf physiology is even greater and more confusing. Some papers have reported a concurrent increase or invariance of leaf A, g_s, leaf water potential and intrinsic WUE for KL sprayed single leaves (Attia et al., 2014; Brillante et al., 2016; Dinis et al., 2018a, b), whereas other authors (Shellie and King 2013a) have even shown a decrease in leaf assimilation rates due to KL sprays that was unrelated to the magnitude of leaf reflectance of visible light. As a matter of fact, the KL effect on gas exchange over the entire growing season at the whole canopy level is currently unknown.

The aim of this study was to assess gas exchanges differences at leaf level or whole-canopy measuring scale following grapevine foliar kaolin application. Therefore, single leaf and whole canopy functions were seasonally evaluated on fruiting Sangiovese potted vines subjected to a kaolin treatment *vs.* left unsprayed, in combination with a limited water supply period before proceeding with re-watering.

2. Materials and methods

2.1. Plant material and treatment layout

The experiment was conducted in 2018 in Piacenza (44°55′N, 9°44′E), Italy, on five-year-old cane-pruned cv. Sangiovese (*Vitis vinifera* L.) vines (clone R10 grafted on SO4) and grown outdoors in 55 l pots. Canes were ~ 1 m long with 9 dormant buds each. Twelve vines were arranged along a single, vertically shoot-positioned, 35 °NE-SW oriented row and hedgerow-trained with main cane raised 90 cm from the ground and three upper foliage wires for a canopy wall extending above the main cane by about 1.2–1.3 m. The pots were filled with a mixture of loamy soil and peat (80:20 by volume, respectively) and kept well-watered (WW) until the beginning of the water stress. Pots were painted white before the trial started, to limit radiation-induced overheating and each vine was fertilized twice (*i.e.* one week before and two weeks after bud-break) with 4 g of Greenplant 15 (N) +5 (P_2O_5) +25 (K_2O) +2 (MgO) + micro (Green Has Italia, Cuneo, Italy). The twelve vines were then randomly assigned to the following four independent treatments: control well-watered (C-WW), control water stressed (C-WS), kaolin sprayed – well-watered (KL-WW) and kaolin sprayed - water stressed (KL-WS). On 23 July (DOY 204) at 9:00 am KL-WW and KL-WS were subjected to the application of KL. A formulation of 100% aluminum silicate (Baïkal, Agrisynergie, Perigueux, France) was mixed and diluted in water at 3% concentration. The solution was carefully sprayed on both canopy sides with a shoulder pump after a temporary disassembly of the whole canopy gas exchange system. After full evaporation of the aqueous part of the solution, the chambers were set up again. On 21 August (DOY 233) the KL treatment was artificially washed off from the treated canopies after temporary dismantling and subsequent setup of the chambers. All the vines were kept well-watered until DOY 208 (27 July, BBCH77 according to Lorenz et al. 1995) by supplying a daily amount of 3600 ± 424 ml per vine representing 110% actual canopy transpiration (E_{canopy}) concurrently measured by the whole-canopy system described hereafter over DOY 200-207. Re-watering was performed through an automated water-supply system described by Poni et al. (2015). In brief, the system is designed to deliver fractions of E_{canopy} derived from concurrent measurements performed with the whole-canopy gas-exchange system. This approach makes the process sensitive to large fluctuations in water use, which can occur depending upon evaporative demand or simply due to the development of new leaf area during the seasonal vegetative growth.

The replenishment coefficients entered in the program to define the fraction of mean WW to be delivered to WS can also be adjusted according to pre-stress transpiration rates of each WS vine, *i.e.* a more vigorous WS plant will receive more water than one of lower vigor. Starting on DOY 209 (28 July), a constant water deficit was imposed on the WS vines by programming the water-supply system to deliver daily to each vine only 70% of WW E_{canopy} until achievement of severe water deficit conditions. Re-watering of WS vines to restore the 110% E_{canopy} water supply was performed on DOY 218 (6 August) and then maintained until dismantling of the chambers. During water stress, each pot surface of either WW and WS vines was covered with a plastic sheet to prevent infiltration of rain water and to minimize losses due to soil evaporation. A chronological scheme of treatments application and irrigation scheduling is provided in Fig. S1.

2.2. Single-leaf gas exchange and thermal status

To allow undisturbed readings, single leaf gas exchange and temperature measurements were taken on the apical shoot of each cane that was not enclosed in the plastic chamber (Fig. S2). Leaf net assimilation (Leaf A), transpiration (Leaf E) and stomatal conductance (Leaf g_s) rates of two well-exposed, mature primary leaves inserted between node 3–7 were measured on DOYs 204–208, 210–212, 214, 216, 217, 219 and 228, using a CIRAS-2 portable photosynthesis system (PP Systems, Amesbury, MA, USA). Readings were performed in the early afternoon (13:30–14:30) under constant saturating light [$\cong 1500\,\mu mol/(m^2 \cdot s)$] imposed with an additional external lamp mounted on top of the leaf chamber. The unit uses a broad-leaf chamber (2.5 cm^2 window area) and all readings were taken at ambient relative humidity with an airflow adjusted to 200 mL/min. To ensure stability of the inlet reference CO_2 concentration [CO_2], a mini CO_2 cartridge was used to provide automatic control of inlet (CO_2) at ˜400 mmol/L. Instantaneous and intrinsic leaf water-use efficiency (WUE) were calculated as A/E and A/g_s and given as mmolCO$_2$/molH$_2$O.

Chlorophyll fluorescence measurements were carried out on the same leaves sampled for gas exchange using the field-portable pulse modulated fluorometer Handy-PEA (Hansatech Instruments, Norfolk, UK). Portions of leaves were dark-adapted for 30 min to ensure maximal photochemical efficiency by using the leaf-clips provided with the instrument. The fiber optic and its adaptor were fixed to a ring located over the leaf-clip at approximately 1 cm from the sample and the different light pulses were applied following the standard routines as recommended by the user manual. Signal recordings and calculations were performed using the data analyzer and control software provided with the analyzer. For unstressed leaves, the value of F_v/F_m is highly consistent, with values of ˜0.83, and correlates to the maximum quantum yield of photosynthesis (Demmig and Björkman, 1987).

Only on clear-skied days, mean leaf temperature (Leaf T_{mean}) was measured on the same leaves using the FLIR i60 infra-red thermal imaging camera (FLIR Systems Inc., Wilsonville, OR, USA). On each date, one frontal thermal image per leaf (one per vine) was taken under full sunlight conditions at ˜50 cm distance from the leaf itself. Thermal image analysis was carried out with the software FLIR Tools (FLIR Systems Inc., Wilsonville, OR, USA).

Seasonal progression of water stress was monitored by measuring leaf pre-dawn (Ψ_{pd}) and midday stem water potential (stem Ψ_{MD}). Ψ_{pd} was measured before sunrise on DOY 205, 208, 211, 213, 217, 219 and 234 on one leaf per vine. Stem Ψ_{MD} was measured on the same dates of gas exchange readings at 13:00 on a mature mid-shoot leaves per vine. Measures were taken using a Scholander pressure chamber (3500 Model, Soilmosture Equip. Corp., Santa Barbara, CA). Leaves were sampled from the shoots contained in the chamber thanks to a specially created zip-lock lateral access to the chamber. Stem Ψ_{MD} was measured upon wrapping leaves with plastic film and aluminum foil 2 h prior to readings (McCutchan and Shackel, 1992).

2.3. Whole-canopy gas exchange

Whole-canopy net CO_2 exchange rate (NCER) measurements were taken using the multi-chamber system reported in Poni et al. (2014) featuring alternating current, centrifugal blowers (Vorticent C25/2 M Vortice, Milan, Italy) delivering a maximum air flow of 950 m^3/h, plastic transparent polyethylene chambers allowing 88% light transmission, 6% diffuse light enrichment and no alteration of the light spectrum, a CIRAS-EGM4 single channel absolute CO_2 infrared gas analyzer (PP-Systems, Amesbury, MA) set at 0–1000 ppm measurement range, and a CR1000 data logger wired to an AM16/32B Multiplexer (Campbell Sci., Shepshed, UK). To facilitate air mixing and ensure higher stability in inlet CO_2 concentration, air was forced through a buffer tank (500 L) before being directed to the chambers. Switching of air sampling from one chamber to another was achieved at programmed time intervals (90 s) using a set of solenoid valves; the airflow rate to each chamber was controlled by a butterfly valve and measured with a Testo 510 digital manometer (Farnell, Lainate, Italy) after the flow restriction method (Osborne, 1977).

The flow rate fed to the chambers was set at 9.9 l/s and kept constant throughout the whole measuring season. The polyethylene chambers had a volume of 0.580 m^3 ± 0.04 so a complete volume air change occurred at an interval of ˜59 s. Whole-canopy NCER (µmol CO_2/s) was calculated from flow rates and CO_2 differentials after Long and Hallgren (1985). To warrant unbiased comparison *vs.* canopy development, leaf area (LA) per vine was estimated as described in Gatti et al. (2018) and NCER/LA (µmol CO_2/m^{2}s) computed accordingly. Whole-canopy water-use efficiency (WUE) was calculated as NCER/E_{canopy} and given as mmolCO$_2$/molH$_2$O. Since vines assigned to the four treatments had the same shoot number (˜9) and, additionally, shoot growth along the cane was very uniform, E_{canopy} was estimated to be ˜91% of total vine T.

The chambers were set up on each vine and continuously operated 24 h per day from DOY 200 (19 July, four days prior to KL sprays and eight days prior beginning of reduced water supply) until DOY 259 (16 September, 40 days after re-watering of WS plants). Ambient (inlet) air temperature and the air temperature at each chamber's outlet were measured by shielded 1/0.2 mm diameter PFA –Teflon insulated type-T thermocouples (Omega Eng. INC, Stamford, Connecticut) and direct and diffuse radiation were measured with a BF2 sunshine sensor (Delta-T Devices, Ltd, Cambridge, England) placed horizontally on top of a support stake next to the chambers enclosing the canopies. Ambient (inlet) relative humidity (RH) at each chamber's outlet was measured by a HIH-4000 humidity sensor (Honeywell, Freeport, Illinois, USA) mounted upstream of the EGM4. Final dismantling of the chambers occurred 15 September (DOY 258).

After chambers' removal, two shoots per vine were sampled and brought to the laboratory. Leaf area on each node of the shoots was measured with a leaf area meter (LI-3000A, LI-COR Biosciences, Lincoln, NE, USA). Then, average leaf area was calculated for nodes 1–4 (LA 1–4), 5–10 (LA 5–10), for nodes above the 10th (LA 10 +), for lateral shoots (LA LS) and for the entire shoot (shoot LA).

2.4. Yield components and grape composition

Guard vines of the same cultivar/rootstock combination (*i.e.* cv. Sangiovese, clone R10 grafted on SO4 rootstock) and grown under similar conditions were periodically sampled to track their fruit ripening evolution. On 30 August (DOY 242), when guard vines reached a total soluble solids (TSS) concentration of about 22.0°Brix, the experimental vines were harvested and vine yield was measured with a portable field scale. Three representative clusters per vine were sampled, their weight recorded and berries were separated from the rachis and counted, berry weight was then measured and average berry weight calculated. 50-berries per cluster were collected for further analyses and the remaining berries were crushed and the concentration of TSS (°Brix) determined

by a temperature-compensating refractometer (RX-5000 Atago USA, Bellevue, WA, USA). Must pH was assessed with a digital PHM82 pH-meter (Radiometer Analytical s.a.s., Villeurbanne Cedex, France). TA was measured titrating with 0.1 N NaOH (pH 8.2 end-point) and expressed as g/L of tartaric acid equivalents. To quantify organic acids, juice was directly injected after filtration through a 0.22 μm polypropylene syringe into HPLC auto-sampler vials. For this analysis, an Allure Organic Acid Column, 300 × 4.6 mm, 5 μm (Restek, Bellefonte, PA, USA) was used. Separation was performed in isocratic conditions using water, pH adjusted at 2.5 with ortho-phosporic acid. The column temperature was maintained at 30 ± 0.1 °C, 15 μl of sample was injected. The elution was monitored at 200–700 nm and detection by UV–vis absorption with DAD at 210 nm. Organic acids were identified using authentic standards and quantification was based on peak areas and performed by external calibration with standards.

The 50-berries per cluster were used for the determination of anthocyanins and total phenolics. Berries were homogenized at 3584 x G with an Ultra-Turrax T25 (Rose Scientific Ltd., Edmonton, Canada) homogenizer for 1 min, then 2 g of the homogenate was transferred to a pre-tared centrifuge tube, enriched with 10 ml aqueous ethanol (50%, pH 5.0), capped and mixed periodically for 1 h before centrifugation at 959 × G for 5 min. A portion of the extract (0.5 mL) was added to 10 ml 1 M HCl, mixed and let stand for 3 h; absorbance was then measured at 520 nm and 280 nm on a JascoV-530 UV spectrophotometer (Jasco Analytical Instruments, Easton, MD, USA). Total anthocyanins and phenolics were expressed as mg per g of fresh berry mass (Iland et al., 2011).

2.5. Statistical treatment

One-way analysis of variance was carried out and, in case of significance of F test, mean separation was performed by the Student Newman Keuls (SNK) test at $P < 0.05$ and 0.01. Degree of variation around means was given as standard error. Data taken over time for leaf T_{mean}, Ψ_{pd}, stem Ψ_{MD}, leaf F_v/F_m, leaf A, E and WUE (given as A/E) were analyzed with the repeated measure analysis of variance routine embedded in the XLSTAT software package (Addinsoft, Paris, France). Least squared mean method at $p < 0.05$ was used for multiple comparisons within dates. Equality of variances of the differences between all possible pairs of within-subject conditions was assessed via Mauchly's sphericity test.

3. Results

3.1. Weather conditions, leaf and chambers' temperature

Diurnal mean direct Photosynthetically Active Radiation (PAR) and air vapor pressure deficit (VPD) showed quite large fluctuations during the whole-canopy measuring period (DOY200–259) with increased variability over the second part of the season (Fig. S3). A series of clear and warm days characterized the time window of water stress imposition (DOY 209–217) with air VPD ranging between 2.4 and 3.0 K Pa.

Thermal readings taken throughout the season on single leaves showed that, in WW vines, KL application had only occasional and inconsistent effects of leaf cooling (leaf T_{mean} for C-WW was 34.1 °C *vs.* 33.8 °C in KL-WW) (Fig.1). Restricted water supply had a larger impact on T_{leaf}. In fact, leaf heating recorded in the C-WS treatment was +2.9 °C *vs.* the WW plants. WS vines sprayed with KL had a similar heating at the beginning of the water stress period, but prior to re-watering they reported a consistent cooling effect when compared to the C-WS treatment (*e.g.* 2.7 °C less than C-WS on DOY 215). Cooling achieved with KL during the entire drought period was -1.0 °C in relation to C-WS. Re-watering promptly offset relative differences among treatments until the end of the measuring season (Fig.1).

Chamber heating quantified as ΔT_{outlet} C-WW – T_{inlet} was + 1.8 C° over the experimental season (Fig. 2A). Over the same period, cooling
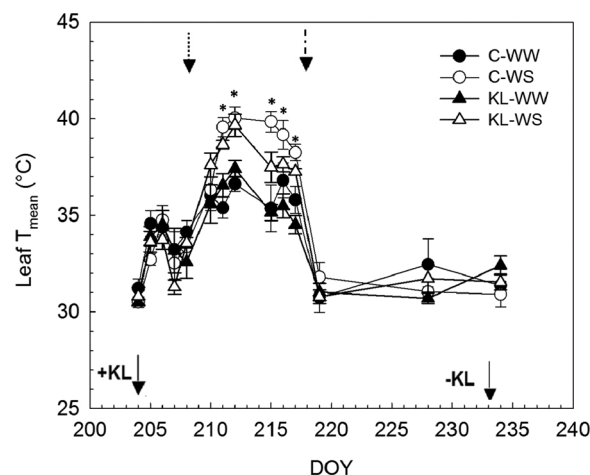

Fig. 1. Seasonal trend of single leaf temperature (leaf T_{mean}, °C) measured using an infrared thermal imaging camera. Readings were taken on two well exposed, basal leaves (node 3–7) per vine inserted on the shoot kept outside the chamber. Asterisk indicates dates within which significant differences among treatment were found according to SNK test (P < 0.05). Repeated measures ANOVA resulted in a significant time x treatment interaction (P < 0.01). Dotted arrow indicates date of beginning of reduced water supply, broken arrow indicates date of full re-watering.

effect due to KL spray (ΔT_{outlet} KL-WW – T_{outlet} C-WW) was -0.5 °C and, even when referred to the time window during which KL was actually on the vines (DOY204–232), it moderately increased to -0.8 °C *vs.* the T_{outlet} C-WW values. During the drought stress period, chamber heating (ΔT_{outlet} C-WS – T_{inlet}) peaked at +3.6 °C, of which +1.4 °C was related to the water supply limitation (ΔT_{outlet} C-WS – T_{outlet} C-WW) (Fig. 2C). Over the same period, with KL, the ΔT_{outlet} KL-WS – T_{inlet} was reduced at +2.5 °C with +1.8 °C resulting as ΔT_{outlet} KL-WS – T_{outlet} KL-WW (Fig. 2B). Direct comparison of the two WS treatments during drought resulted in a −1.1 °C for KL-WS *vs.* C-WS (Fig. 2D).

3.2. Soil and vine water status

In well-watered pots, the KL spray was not able to alter the dynamic of soil water depletion by roots as suggested by the very similar patterns of Ψ_{pd} (Fig. 3A). KL also did not affect significantly the progressive decline of Ψ_{pd} upon stress imposition and the most negative Ψ_{pd} values recorded the last day of stress (DOY 217) were very close (−0.77 MPa in C-WS *vs.* −0.82 MPa in KL-WS). A quite similar response was displayed by the midday stem water potential (stem Ψ_{MD}) that was almost identical in the two WW treatments (−0.65 MPa for data averaged over the entire season, Fig. 3B). Water deficit did not change stem Ψ_{MD} and throughout the whole stress period mean stem Ψ_{MD} scored −1.11 MPa in C-WS *vs.* −1.14 MPa in KL-WS. Such similarity was maintained at the peak of stress (DOY 217) when stem Ψ_{MD} recorded in C-WS and KL-WS was −1.69 and −1.63 MPa, respectively (Fig. 3).

3.3. Single leaf gas exchange and fluorescence

Leaf assimilation (μmolCO$_2$ m^{-2} s^{-1}) recorded on both WW treatments (Fig.4A) reported a mild A limitation (−0.6 μmolCO$_2$ m^{-2} s^{-1}) in KL sprayed vines when compared to C vines as average over the experimental period. However, right after spraying KL (*e.g.* DOY 206 and 208) the A limitation was significant. Conversely, when the two WS treatments were plotted together (Fig.4B) the trend was opposite with KL-WS registering +1.14 and + 1.96 μmolCO$_2$ m^{-2} s^{-1} as compared to C-WS when data were averaged over the entire or drought period, respectively (Fig.4B). In terms of WW *vs.* WS comparisons, in the unsprayed vines the A limitation due to reduced water supply was -66% (3.2 μmolCO$_2$ m^{-2} s^{-1} in WS *vs.* 9.3 μmolCO$_2$ m^{-2} s^{-1} in WW) (Fig.4C),
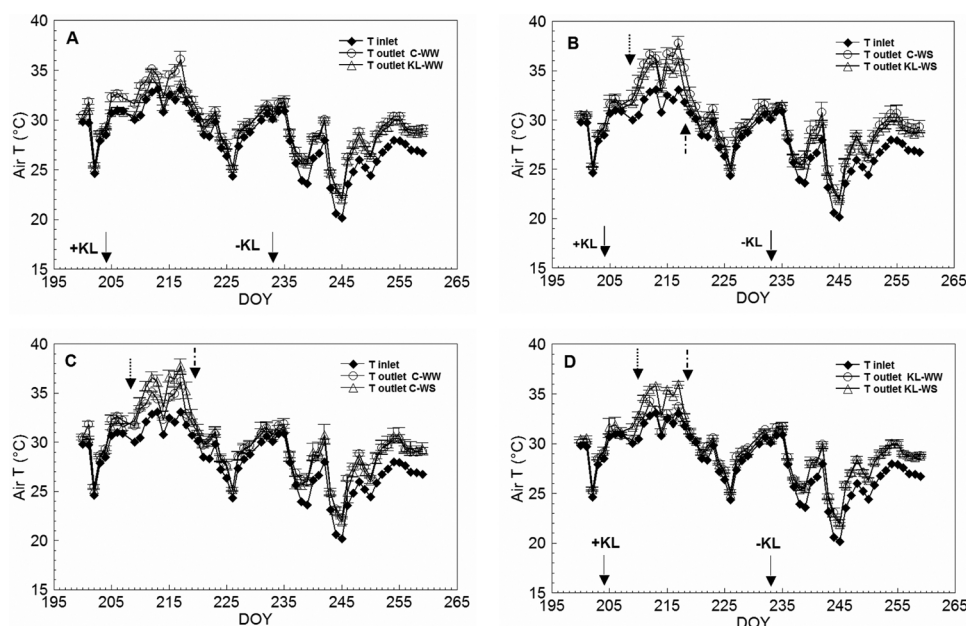
Fig. 2. Seasonal daily trend for the whole chamber measuring period (DOY 200–259) of chambers' T inlet and for chambers' outlets of each treatment. To improve graph readability, patterns are shown in four different panels where, besides T_{inlet}, two treatments at a time are shown. T_{Inlet} daily values are calculated by averaging instantaneous air T_{inlet} taken from dawn to dusk at 24 min intervals. T_{outlet} for the four treatment follows the same calculation and then values are averaged over the three vine replicates per treatment. SE represents standard error (SE), n = 3.
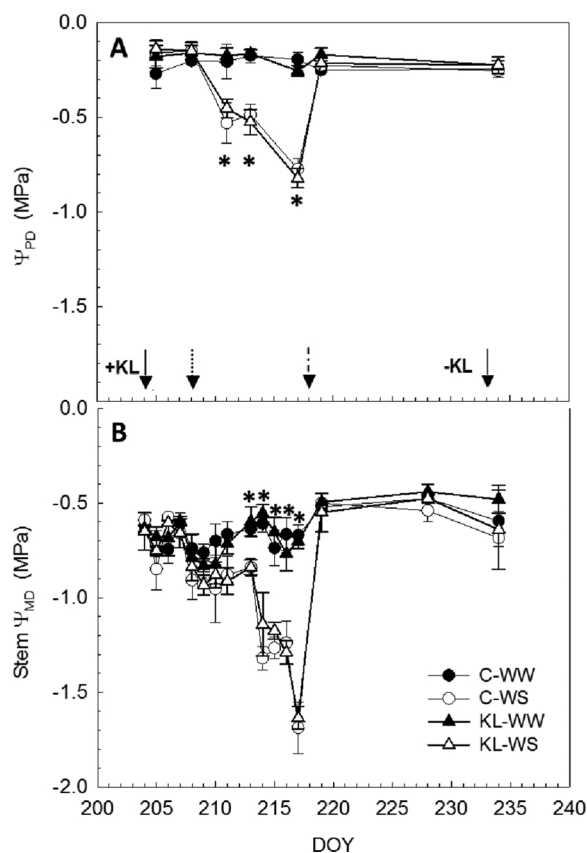

Fig. 3. Seasonal daily trends for predawn leaf water potential (Ψ_{PD}) (A) and stem water potential (stem Ψ_{MD}) (B) measured prior, during and after water shortage on four mature leaves per vine (two for each parameter). SE represents standard error (SE), n = 6. Asterisk indicates dates within which significant differences among treatment were found according to SNK test (P < 0.05). Repeated measures ANOVA resulted in a significant time x treatment interaction (P < 0.01). Dotted arrow indicates date of beginning of reduced water supply, broken arrow indicates date of full re-watering.

whereas on KL sprayed vines such limitation was lowered to -42% (5.1 $\mu molCO_2\,m^{-2}s^{-1}$ in WS *vs.* 8.8 $\mu molCO_2\,m^{-2}s^{-1}$ in WW) (Fig.4D).

Fv/Fm ratio measured on the same dark-adapted leaves prior, during and after drought exhibited close-to-optimum values in all treatments up to DOY 205 (*i.e.* a week after the beginning of water stress) (Fig.5). Thereafter, values recorded on C-WS started to decline, reaching limiting values of about 0.5. The same decline was not observed at the same dates in KL-WS, which maintained a close-to-optimal F_v/F_m ratio albeit statistically lower than the corresponding WW treatments (Fig.5).

Leaf transpiration data averaged over the experimental period and compared to the two WW treatments (Fig.6A), displayed again a mild limitation in KL-WW *vs.* C-WW (−7.2%). Similarly to A rate, such a trend was reversed when the two WS treatments were directly compared (−8.5% in C-WS *vs.* KL-WS) (Fig.6B). In unsprayed vines and for data averaged over six dates during stress, leaf E was limited by 56% as compared to C-WW (Fig.6C), whereas in KL treated vines limited water supply reduced E by only 28% *vis-a-vis* KL-WW (Fig.6D).

Single leaf instantaneous WUE calculated as A/E ratio and compared within WW treatments showed erratic and inconsistent differences throughout the measuring season (Fig.7A) resulting in similar mean WUE (1.47 and 1.53 mmolCO_2/molH_2O) in C-WW and KL-WW, respectively. A quite similar leaf WUE pattern was also shown when the two WS treatments were plotted together although, for data taken at the peak of stress (DOY 216 and 2017), C-WS showed lower leaf WUE than KL-WS (Fig.7B). At several dates during limited water supply, leaf WUE was significantly limited in C-WS as compared to C-WW resulting in 1.28 *vs.* 0.92 mmolCO_2/molH_2O, respectively, if averaged over the six measurement dates during the water stress (Fig.7C). In KL sprayed vines, mean leaf WUE during stress was not significantly affected, although it was apparent that the KL-WS treatment had lower leaf WUE than KL-WW at the beginning of stress and such difference vanished with the increase of the stress severity (Fig. 7D).

3.4. Whole-canopy gas exchange

Over the 60-day experimental period (DOY 200–259), mean NCER of C-WW was 6.7 $\mu molCO_2\,m^{-2}\,s^{-1}$ against 5.6 $\mu molCO_2\,m^{-2}s^{-1}$ for KL-WW (Fig.8A). However, when the above means were recalculated for pre-spraying (DOY 200–203), kaolin-on phase (DOY 204–233) and post KL wash off (DOY 234-259), the $\Delta NCER_{C-WW} - NCER_{KL-WW}$ was 0.6, 1.3 and 1.2 $\mu molCO_2$ m-2 s-1, respectively (Fig. 8A). Within WS

Fig. 4. Seasonal trends of mean leaf assimilation (leaf A) rates measured prior, during and after water shortage on two basal leaves per vine chosen on the shoot outside the chambers. To improve readability, data are shown in four different panels representing the following pair comparisons: C-WW *vs.* KL-WW (A); C-WS *vs.* KL-WS (B); C-WW *vs.* C-WS (C) and KL-WW *vs.* KL-WS (D). Asterisk indicates dates within which significant differences for critical values of each pair contrast were found (P < 0.05). Repeated measures ANOVA resulted in a significant time x treatment interaction (P < 0.01). Dotted arrow indicates date of beginning of reduced water supply, broken arrow indicates date of full re-watering.
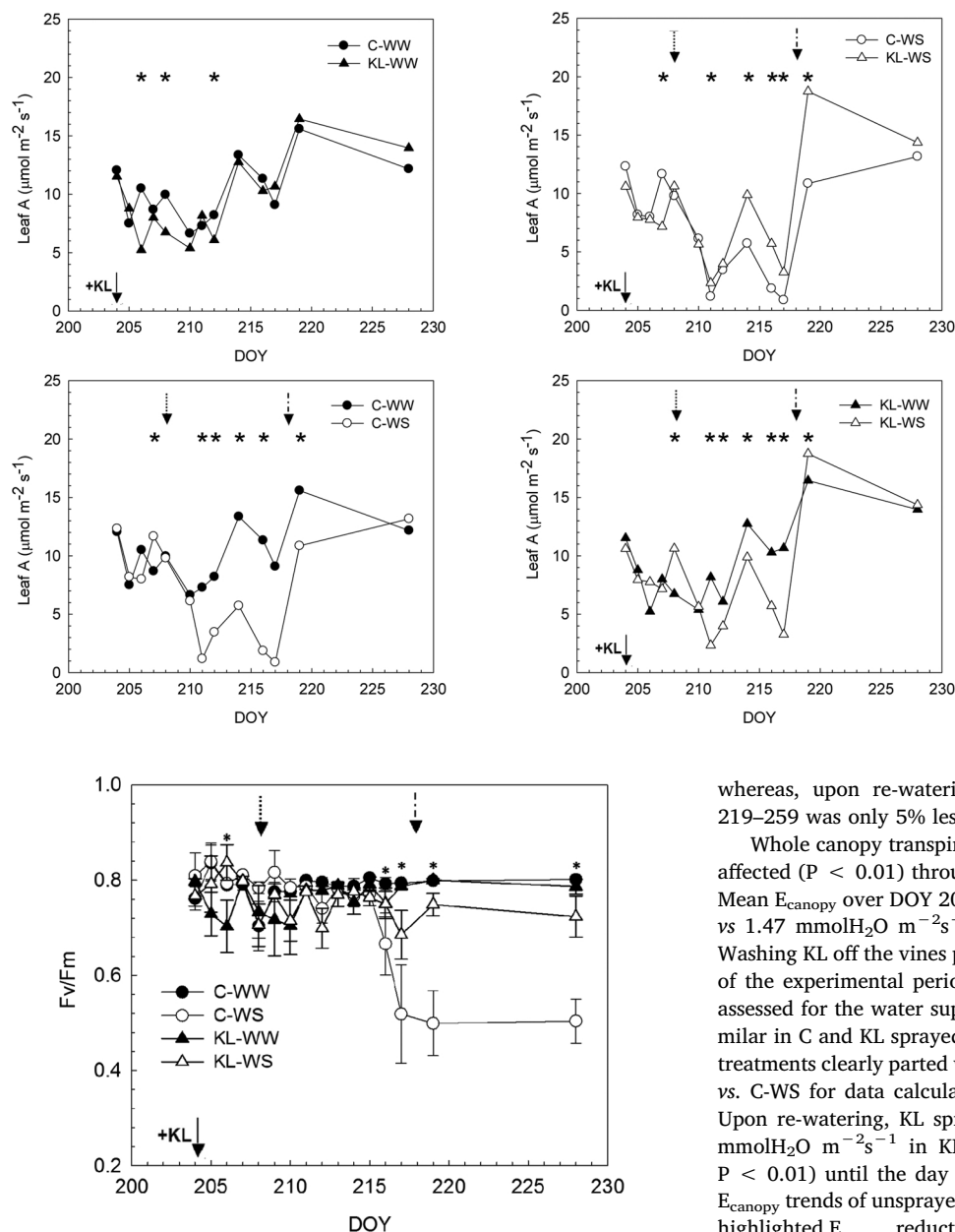

Fig. 5. Seasonal trends for chlorophyll fluorescence Fv/Fm ratio measured prior, during and after water shortage on two mature basal leaves per vine. SE represents standard error (SE), n = 6. Asterisk indicates dates within which significant differences among treatment were found according to SNK test (P < 0.05). Repeated measures ANOVA resulted in a significant time x treatment interaction (P < 0.01). Dotted arrow indicates date of beginning of reduced water supply, broken arrow indicates date of full re-watering.

treatments, whole season NCER rates of C and KL sprayed vines were very similar (4.7 and 4.8 $\mu molCO_2\,m^{-2}\,s^{-1}$, respectively) (Fig. 8B). However, it was apparent that albeit NCER during stress was exactly the same for the two treatments ($\sim$2.2 $\mu molCO_2\,m^{-2}\,s^{-1}$), a clear trend was shown for higher NCER in KL-WS over the last days of water deficit (Fig.8B). Interestingly, NCER resumption upon re-watering was much prompter in KL-WS and mean NCER calculated over the DOY 219–232 time-frame resulted in 6.4 $\mu molCO_2\,m^{-2}\,s^{-1}$ *vs.* 5.4 $\mu molCO_2\,m^{-2}\,s^{-1}$ (P < 0.01) measured in C-WS. In unsprayed vines, mean NCER reduction during stress was 67% of rates recorded on WW vines, whereas, upon re-watering, mean NCER of C-WS calculated over DOY 219–259 was still 22% less than WW plants (Fig. 8C). Same analysis carried out on KL sprayed vines showed that water stress curtailed NCER by 64%

whereas, upon re-watering, NCER of KL-WS calculated over DOY 219–259 was only 5% less than KL-WW (Fig. 8D).

Whole canopy transpiration (E_{canopy}) of WW vines was significantly affected (P < 0.01) throughout the period when KL was on the vines. Mean E_{canopy} over DOY 204–232 was 1.67 $mmolH_2O\,m^{-2}\,s^{-1}$ in C-WW *vs* 1.47 $mmolH_2O\,m^{-2}\,s^{-1}$ in KL-WW (−12%, P < 0.05) (Fig. 9A). Washing KL off the vines promptly restored similar E_{canopy} over the rest of the experimental period. When the same C *vs* KL comparison was assessed for the water supply level, during stress, mean E_{canopy} was similar in C and KL sprayed vines (+7% E_{canopy} in KL-WW) but the two treatments clearly parted with stress severity, reaching +48% in KL-WS *vs.* C-WS for data calculated over the DOY 215–217 period (Fig.9B). Upon re-watering, KL sprayed vines maintained higher E_{canopy} (1.38 $mmolH_2O\,m^{-2}\,s^{-1}$ in KL-WS *vs.* 1.10 $mmolH_2O\,m^{-2}\,s^{-1}$ in C-WS, P < 0.01) until the day of KL wash off. The comparison of seasonal E_{canopy} trends of unsprayed vines as a function of water supply (Fig. 9C) highlighted E_{canopy} reduction in C-WS by 30% and 63% when compared to C-WW over the entire experimental period and for the specific drought period, respectively. Interestingly, for about two weeks after rehydration, previously stressed vines showed a significant lag of E_{canopy} as compared to C-WW. When the same parameters were evaluated for KL sprayed vines (Fig.9D), the above-mentioned differences between KL-WW and KL-WS were reduced significantly to 8% (ns) and 58% (P < 0.01) over the entire experimental period and for the specific drought period, respectively. Upon re-watering, KL-WS showed a very prompt E_{canopy} recovery that was overall maintained over the remainder of the season.

WUE_{canopy} calculated as $NCER/E_{canopy}$ and reported as $mmolCO_2/molH_2O$ was not affected, in well-watered vines, by KL sprays (Fig. 10A). When the same comparison was done for WS vines (Fig. 10B), WUE_{canopy} averaged over the whole recording period was not different. However, KL seemed to play a role at changing WUE_{canopy} during stress progression. In particular, during the first five days of stress, WUE canopy was higher in the C-WS treatment (3.9 $mmolCO_2/molH_2O$ against 3.1 $mmolCO_2/molH_2O$ in KL-WS, P < 0.05). Contrarily, over the last three days of stress, the response was fully reversed with KL-WS setting at 3.7 $mmolCO_2/molH_2O$ *vs.* 1.6 $mmolCO_2/molH_2O$ calculated for C-WS, P < 00.1). In unsprayed vines, water stress
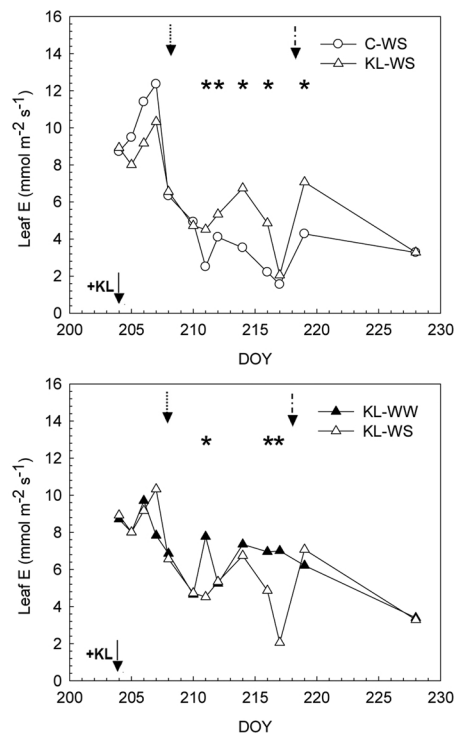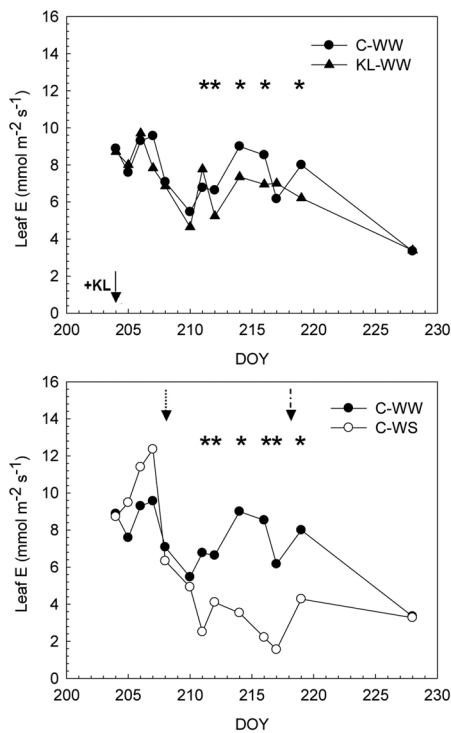
Fig. 6. Seasonal trends of mean leaf transpiration (leaf E) rates measured prior, during and after water shortage on two basal leaves per vine chosen on the shoot outside the chambers. To improve readability, data are shown in four different panels representing the following pair comparisons: C-WW *vs.* KL-WW (A); C-WS *vs.* KL-WS (B); C-WW *vs.* C-WS (C) and KL-WW *vs.* KL-WS (D). Asterisk indicates dates within which significant differences for critical values of each pair contrast were found (P < 0.05). Repeated measures ANOVA resulted in a significant time x treatment interaction (P < 0.01). Dotted arrow indicates date of beginning of reduced water supply, broken arrow indicates date of full re-watering.

markedly reduced WUE_{canopy} (4.5 $mmolCO_2/molH_2O$ in C-WW *vs.* 3.3 $mmolCO_2/molH_2O$ in C-WS, P < 00.1) over the whole water deficit period, although the most consistent reduction was recorded at high stress severity (Fig. 10C). In KL sprayed vines, WUE_{canopy} was likewise significantly reduced throughout the drought stress (4.1 $mmolCO_2/molH_2O$ in KL-WW *vs.* 3.4 $mmolCO_2/molH_2O$ in KL-WS, P < 0.05) (Fig. 10D).

3.5. Vegetative growth, yield components and fruit maturity at harvest

Final total shoot leaf area and leaf area due to lateral shoots were not affected by treatments (Table 1). Total leaf area averaged over different shoot zones (basal, median and apical) showed that, while median and apical shoot sections had no differences among treatments, the basal (*i.e.* node 1–4) shoot zone of the C-WS showed greatly reduced LA as compared to the remaining treatments due to earlier basal leaf senescence and shedding. Yield components (Table 1) and main technological maturity parameters (TSS, TA, pH, tartrate and malate)
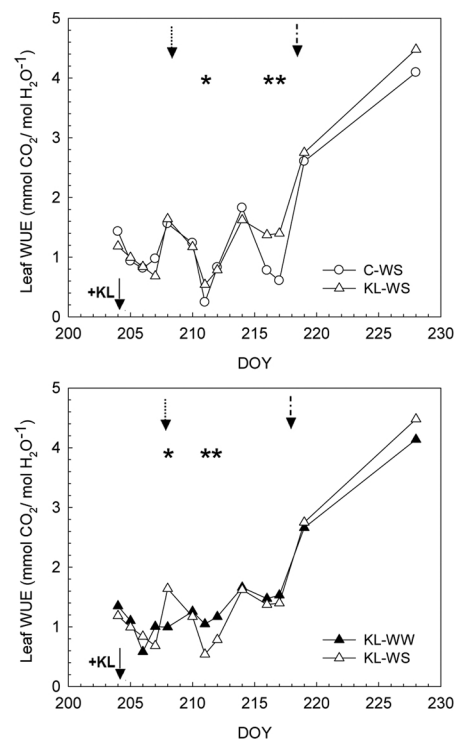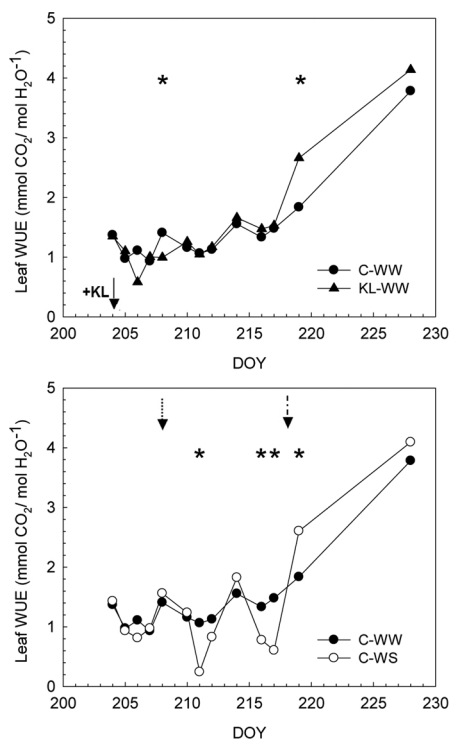

Fig. 7. Seasonal trends of mean instantaneous leaf water use efficiency (leaf WUE) calculated from A and E rates measured prior, during and after water shortage on two basal leaves per vine chosen on the shoot outside the chambers. To improve readability, data are shown in four different panels representing the following pair comparisons: C-WW *vs.* KL-WW (A); C-WS *vs.* KL-WS (B); C-WW *vs.* C-WS (C) and KL-WW *vs.* KL-WS (D). Asterisk indicates dates within which significant differences for critical values of each pair contrast were found (P < 0.05). Repeated measures ANOVA resulted in a significant time x treatment interaction (P < 0.05). Dotted arrow indicates date of beginning of reduced water supply, broken arrow indicates date of full re-watering.

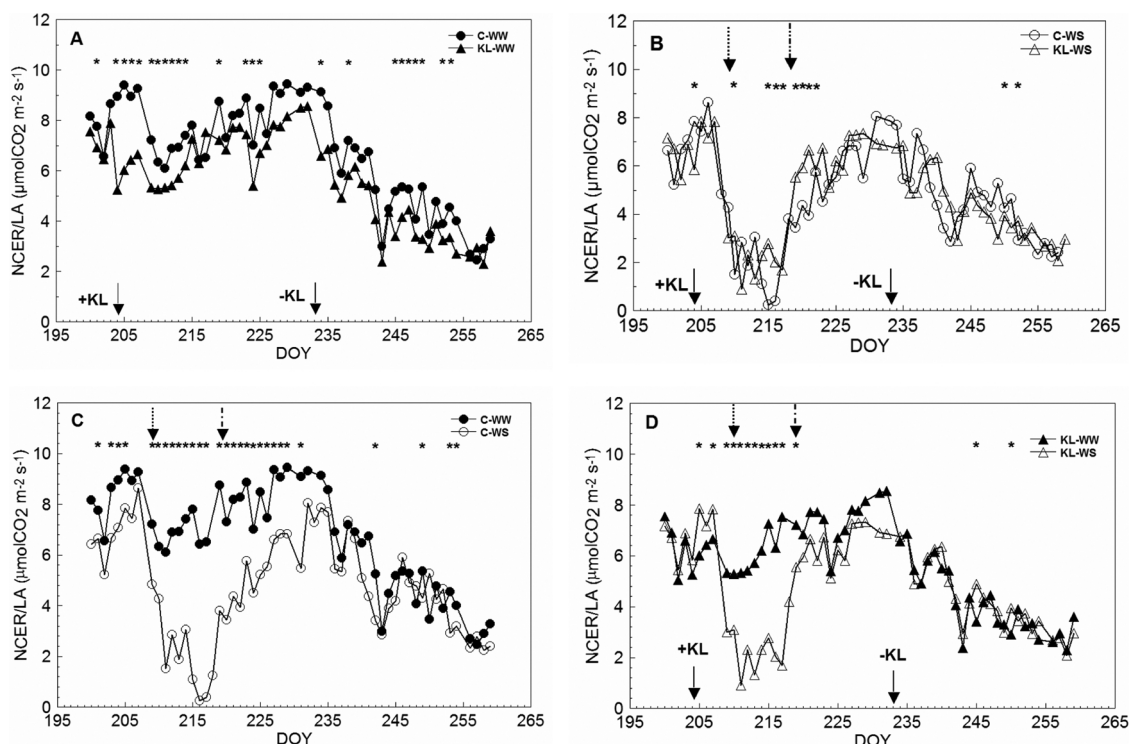**Fig. 8.** Seasonal daily trend for whole-canopy net CO_2 gas exchange rates (NCER) recorded from DOY 200 to DOY 259. To improve readability, data are shown in four different panels representing the following pair comparisons: C-WW *vs.* KL-WW (A); C-WS *vs.* KL-WS (B); C-WW *vs.* C-WS (C) and KL-WW *vs.* KL-WS (D). Daily values are means over three vine replicates per treatment calculated by averaging instantaneous NCER rates taken from dawn to dusk at 24 min intervals. Asterisk indicates dates within which significant differences for critical values of each pair contrast were found (P < 0.05).
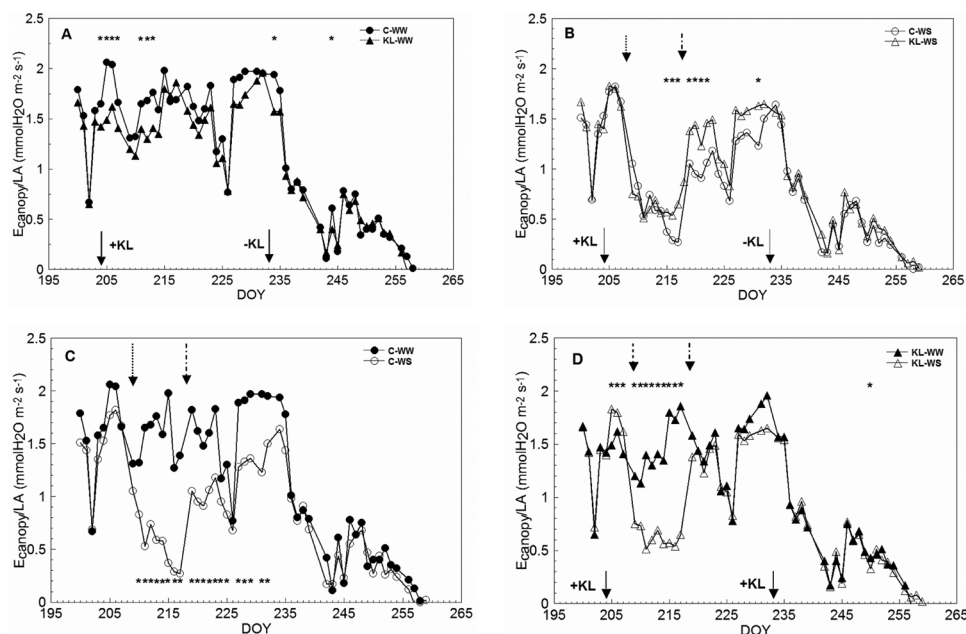
Fig. 9. Seasonal daily trend for whole-canopy transpiration (E_{canopy}) recorded from DOY 200 to DOY 259. To improve readability, data are shown in four different panels representing the following pair comparisons: C-WW *vs.* KL-WW (A); C-WS *vs.* KL-WS (B); C-WW *vs.* C-WS (C) and KL-WW *vs.* KL-WS (D). Daily values are means over three vine replicates per treatment calculated by averaging instantaneous NCER rates taken from dawn to dusk at 24 min intervals. Asterisk indicates dates within which significant differences for critical values of each pair contrast were found (P < 0.05).

reported no differences among treatments (Table 2), whereas it was shown that the KL-WW treatment had higher concentrations of both total anthocyanins and phenolics as compared to C-WS with C-WW and KL-WS setting at intermediate levels (Table 2).

4. Discussion

Based on the clear evidence that KL sprays efficiently reflect solar radiation (Brito et al., 2019), reduction of surface temperature in KL

sprayed organs is somewhat expected. Though, our study suggests that this effect should not be taken for granted and, especially, its magnitude seems to be a function of leaf water status. Brillante et al. (2016), in a three-year study on Cabernet Sauvignon grown in a warm area in the South of Italy, reported that leaf temperature did not differ between C and KL treatments for data pooled over years and KL application caused significant leaf cooling (−1.3 °C) only in stressing vintages. Intriguingly, during the non-stressed vintage of their study (2014) the leaf T of KL sprayed vines was warmer (+1.4 °C) that the unsprayed leaves. A
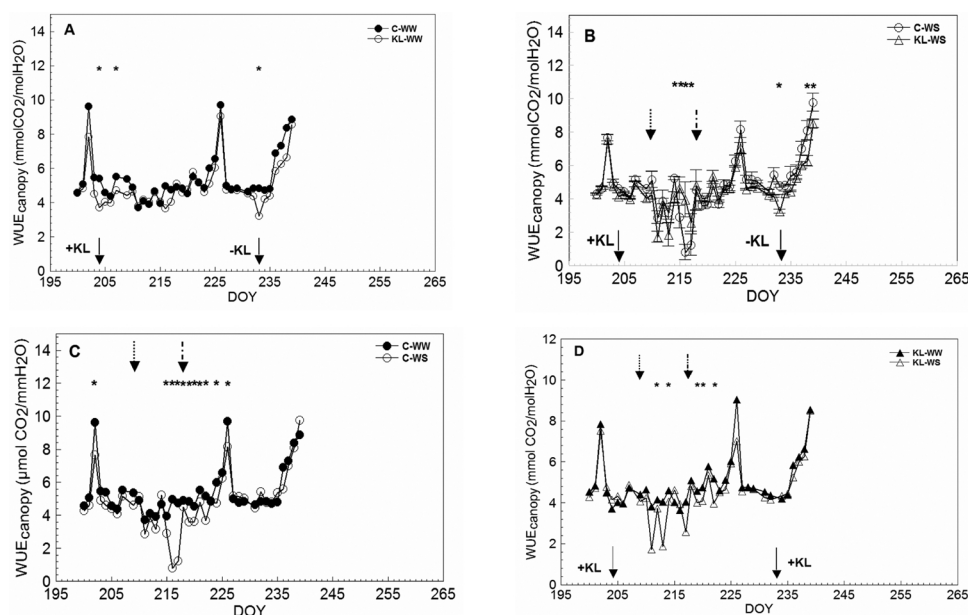
Fig. 10. Seasonal daily trend for whole-canopy water use efficiency (WUE_{canopy}) calculated from daily values of mean NCER and E_{canopy} from DOY 200 to DOY 239. To improve readability, data are shown in four different panels representing the following pair comparisons: C-WW vs. KL-WW (A); C-WS vs. KL-WS (B); C-WW vs. C-WS (C) and KL-WW vs. KL-WS (D). Vertical bars represent standard error (SE), n = 3. WUE_{canopy} values beyond DOY 239 are not reported due to high fluctuations caused by progressive lowering of both NCER and E_{canopy} gas exchange rates and insufficient sensitivity of the measuring system. Asterisk indicates dates within which significant differences for critical values of each pair contrast were found (P < 0.05).

similar scenario is reported by Shellie and King (2013a) who, in cv. Malbec, report that KL had no effect on the cumulated minutes at > 30 °C by leaves of vines under standard irrigation. Under reduced irrigation, leaves with particle film accumulated much less time exposure to T > 30 °C than the untreated leaves. Our single leaf thermal readings confirm overall scant leaf cooling due to KL spray under well-watered conditions and, vice-versa, an increasing cooling effect under an increasingly limited vine water supply. Looking at sustained leaf and canopy transpiration (Figs. 6 and 9) towards the end of stress, it is apparent that KL was effective at assuring some evaporative cooling while preserving partial leaf function and avoiding excessive overheating. In our study, though, we were also able to upscale temperature readings at the whole canopy level and chamber $T_{outlets}$ represent a good integral of the process. Thermal imaging of apple tree (*Malus sylvestris*) canopies showed that trees with a foliar particle film coating (Glenn, 2009, 2012) provided a cooler microclimate throughout the whole canopy. Our whole canopy temperature data nicely confirm the single leaf clues as, under WW condition, KL had little impact on chambers' outlet T whereas more consistent cooling was observed during drought and over the last three days of stress (DOY 215–219) when mean $T_{outlets}$ in KL-WS chambers was 35.4°c vs. 36.9 °C reached in C-WS.

Another unknown is how, in the grapevine, KL application will affect leaf water status. In literature are reported notable increases in midday leaf water potential (up to + 40.7% in 2013 in the Douro region according to Dinis et al., 2018a) with KL application and also no effects or a markedly reduced stem water potential in Cabernet Sauvignon KL treated plants (Brillante et al., 2016). In our study, rate of water uptake

from the soil during drought as well as dynamic of decreasing stem water potential were not affected by KL sprays (Fig. 3), confirming what reported by Shellie and King (2013a). Interestingly, though, at stem water potential values to be considered quite limiting for grapevine leaf function (e.g. lower than − 1.3 MPa peaking at − 1.7 MPa in both WS treatments the day before re-watering), KL sprayed vines assured partial leaf function vs. C vines. Although relative water content was not measured in our study, such behavior agrees with evidence for higher succulence having being previously detected in KL sprayed leaves (Denaxa et al., 2012; Brito et al., 2018) favoring the maintenance of turgor dependent processes. However, turgor maintenance in KL sprayed leaves could also derive from active osmotic adjustment. In terms of glycometabolism, it has been shown in olive trees that KL can favor starch depletion to the benefit of higher concentration of cell compatible soluble sugars (Brito et al., 2019).

Core hypothesis of our study was to determine if whole-canopy assessment of gas exchange parameters in sprayed KL vines, with and without water stress, was confirmatory or, rather, in disagreement with traditional single leaf readings. Literature reporting data on KL effects of single leaf A rates are a good example of variability. In their recent review, Brito et al. (2019) postulates that when KL is used in environments characterized by low irradiance and rainy weather conditions, the effect on A rates is depressing. Conversely, any time the environment shows prevalent limiting factors (i.e. water supply, high light, T and VPD) KL might exert a positive effect on leaf A (Abou-Khaled et al., 1970; Correia et al., 2014; Dinis et al., 2018a). Instead, Brillante at al. (2016) reported reduced A rates in KL sprayed vines, especially in dry years with differences offsetting in the fairly wet 2014 season, whereas

Table 1

Effects of Kaolin spray on final shoot leaf area of well-watered and water stressed vines.

	LA 1-4[1] (cm^2)	LA 5-10[1] (cm^2)	LA 10+[1] (cm^2)	LA LS[1] (cm^2)	Shoot LA[1] (cm^2)	Yield (kg/vine)	Bunch weight (g)	Berry weight (g)
Treatment[2]								
C-WW	283 a[3]	611	567	937	2397	0.53[2]	71	1.2
KL-WW	319 a	537	588	950	2394	0.57	76	1.1
C-WS	68 b	451	711	765	1996	0.63	75	1.0
KL-WS	248 a	652	543	661	2196	0.69	77	1.1

[1] LA 1–4 = Leaf area on shoot nodes 1–4; LA 5–10 = Leaf area on shoot nodes 5–10; LA 10+ = Leaf area on shoot nodes 10 and above; LA LS = Leaf area on lateral shoots; Shoot LA = Total shoot leaf area.

[2] C-WW = Well-watered Control; KL-WW = Well-watered and Kaolin spray; C-WS = Water-stressed Control; KL-WS = Well-stressed and Kaolin spray.

[3] Different letters indicate significant differences at $P \leq 0.05$ (SNK test). The absence of letters means no significant differences found between treatments.

Table 2
Effects of Kaolin spray on fruit composition of well-watered and water stressed vines.

	TSS[1] (°Brix)	pH	TA[1] (g/L)	Malic acid (g/L)	Tartaric acid (g/L)	Anthocyanins (mg/g)	Phenolic substances (mg/g)
Treatment[2]							
C-WW	22.0[3]	3.57	4.31	0.72	7.40	1.02 ab	3.22 ab
KL-WW	22.8	3.54	4.24	0.76	7.37	1.13 a	3.98 a
C-WS	22.7	3.72	4.56	0.89	7.28	0.84 b	2.52 b
KL-WS	21.5	3.57	4.18	0.91	7.55	1.02 ab	2.82 ab

[1] TSS = Total Soluble Solids; TA = Titratable Acidity.
[2] C-WW = Well-watered Control; KL-WW = Well-watered and Kaolin spray; C-WS = Water-stressed Control; KL-WS = Well-stressed and Kaolin spray.
[3] Different letters indicate significant differences at $P \leq 0.05$ (SNK test). The absence of letters means no significant differences found between treatments.

Shellie and King (2013a) found a quite limited leaf A in KL sprayed vines at several times during the season and regardless the water supply. Our single leaf A data fit nicely with the model proposed by Brito et al. (2019); KL sprayed vines, growing in an environment featuring high evaporative demand and high radiation load, yet still benefitting from non-limiting water supply, encountered a mild A limitation. Conversely, reduced water supply was the trigger factor for reversing the response and transforming KL into a leaf-function preserving factor. Naturally, heterogeneity of the single leaf A response to KL applications might also depend on methodology of leaf sampling (age, position along the shoot, placement in the canopy, interference with concurrent phenological stage, crop load, *etc.*) and emphasizes the importance of readings taken at the whole canopy level. For instance, a leaf *vs.* whole canopy assimilation comparison made in apple trees sprayed with KL or left unsprayed (Wunsche et al., 2004) showed that a negative effect of KL on single leaf A rates disappeared when data were scaled up to the whole canopy in light of the reasonable hypothesis that KL, by altering the diffuse and reflective light ratios within the canopy, might cause light enrichment of the inner leaf layers hence increasing their photosynthetic performance. This occurrence was not confirmed in our study as the average reduction of NCER/LA recorded over the entire measuring season in KL-WW was 1.1 $\mu molCO_2\, m^{-2}s^{-1}$ *vs.* C-WW, a value also slightly exceeding the A reduction recorded from single leaf readings (0.6 $\mu molCO_2\, m^{-2}s^{-1}$ less in KL-WW as compared to C-WW). Interestingly, the good correspondence between single leaf and whole canopy assimilation data was confirmed, also, for data taken during the water deficit on in terms of either a similar decrease in CO_2 assimilation rate and in higher rates in KL-WS *vs.* C-WS over the last three days of stress. Such clear correspondence between readings taken at the two different scales finds convincing explanation in the type of canopies we used in our experiment. Based on final shoot leaf area reported in Table 1 and calculating that 9 shoots per vine were retained, total final vine LA varied between 1.97 and 2.15 m^2/m of row length. According to a robust literature (Keller, 2015), such foliage density values are typical of a rather sparse canopy, featuring no more than 1.5 leaf layers, with a high gap fraction and quite good light penetration, even in the inner part of the canopy wall. Under such circumstances, canopy complexity - in terms of relative light exposure of single leaves - is quite low as compared to a dense canopy (*i.e.* > 4m^2/m) and wise single leaf sampling is likely to give reliable assessment of the whole-canopy behavior.

Our CO_2 exchange data, in combination with leaf chlorophyll fluorescence parameters (F_v/F_m) ratio, also support the assumption that, under severe water stress, KL is very effective at preserving the integrity of photosynthetic machinery. Maximum potential quantum efficiency of photosystem II quantified through the chlorophyll F_v/F_m ratio indicates that strong limitation (Fv/Fm approaching 0.5 at the end of stress and held thereafter) was reached only in C-WS, whereas KL-WS still set at sub-optimal values. Such a behavior had a dramatic impact on the longevity of basal leaves in the different treatments. As it can be inferred from Table 1, in C-WS, at harvest, shoot basal nodes had shed most of the leaves whereas, regardless of the seasonal water supply, KL

prevented such occurrence. Another response confirming that KL is effective at increasing leaf resilience upon severe stress events is that photosynthetic recovery upon re-watering, regardless if evaluated on a single leaf or a whole canopy basis, was much prompter in KL sprayed vines.

Apparently, data related to KL effects on amount of transpired water are not very well represented, although some consensus has been reached that KL might increase leaf g_s more than proportionally than leaf E (Correia et al., 2014; Boari et al., 2015), due to the effect of a reduced VPD that, on one side, curtails evaporative demand and, on the other side, enhances g_s (Zhang et al., 2017). However, such results are puzzling since KL should also unwind an anti-transpirant effect (Brito et al., 2019). In our study the ideal comparison to be used was leaf E *vs.* E_{canopy}, both representing water use rates. Leaf g_s, albeit quite similar in trends to leaf E, showed a more erratic pattern (Fig. S4), suggesting that it is not the most reliable parameter. For example, particle film reduced the canopy temperature and stomatal conductance of the cultivars Cabernet Sauvignon, Merlot, and Viognier under well-watered conditions, but cultivar response differed when the vines were under a water deficit (Shellie and Glenn, 2008; Glenn, 2010). Conversely, Brillante at al. 2016, quite surprisingly reported that KL depressed leaf g_s in any season and regardless of different levels of drought reached in each of them. Our concurrent comparison of leaf E *vs.* E_{canopy} (Figs. 6 and 9), while confirming that data taken at the two different scales were in good agreement in terms of relative changes, suggest that, in WW vines KL acted as a mild anti-transpirant (-12% E_{canopy} in KL-WW *vs.* C-WW) as expected. Nevertheless, as hinted by leaf E and E_{canopy}, KL contributed to buffer the transpiration decrease as the drought stress increased.

Effects of KL on leaf WUE are likely the most contradictory. Regardless of chosen leaf WUE expression (intrinsic as A/g_s or instantaneous as A/E) results vary from increase, decrease or invariance (Brito et al., 2019). With WUE_i (A/g_s) being the most common expression, we have examples where leaves with particle film had lower intrinsic WUE than leaves without particle film on two out of five sampling dates (Shellie and King, 2013a) and, on the opposite side, a sharp increase in WUE_i in KL sprayed Cabernet Sauvignon vines especially during dry vintages (Brillante et al., 2016). Such discrepancies might derive from different causes. First, concerning leaf WUE, Medrano et al. (2015), showed that both instantaneous and daily integrals of leaf WUE (as integrals of A/g_s or A/E values) are also highly dependent on the microclimate environment of each leaf position and that WUE values of upper locations were double those of lower ones. These variations were similar or even higher under moderate and severe water stress. In fact, daily leaf WUE proved to be highly determined by the daily intercepted light at each leaf position (with a R^2 of 0.98 for irrigated plants). This suggests that conclusions drawn from measurements taken on well-exposed single leaves do not necessarily reflect the whole-canopy behavior and might lead to unrealistic evaluations in terms of a true water saving strategy. Such hypothesis was also found by Poni et al. (2005) on cv. Sangiovese, where whole-canopy WUE measured throughout the day with an enclosure system was lower

in stressed vines concurrently with leaves tending to assume a prevalent vertical orientation as opposed to higher diurnal instantaneous WUE determined in well-exposed stressed leaves. Unpublished work by Schultz (personal communication, 2017) shows that, for readings taken on leaves in their natural position, there is no increase in extrinsic WUE for non-irrigated 'Riesling' vines as compared to well-watered. Therefore, it is inferred that gas-exchange readings taken on leaves held under saturating light can mask true canopy behavior in terms of water-use efficiency. In our study, such inherent limitation was overcome by a direct comparison between single leaf WUE and whole canopy WUE, the latter calculated as NCER/E_{canopy}. Once again, results were quite consistent with trends shown by instantaneous WUE, suggesting that both WUE expressions showed very little response when the two WW treatments were compared (Fig. 7), whereas it was confirmed that KL is able to increase WUE when the drought stress becomes quite severe (Fig. 7D). This provides stronger evidence that KL enhances leaf performance at severe water deficit by inducing a somewhat near-aniso-hydric behavior; likely cooler leaf T, hence reduced air-to-leaf VPD, prevents full stomatal closure allowing some minimal leaf function. This explains why, under such conditions, leaf E or E_{canopy} seem to be more than proportionally reduced that A and NCER, respectively, hinting to effects bound to the reflective properties of KL; a negative exponential equation describing a light response curve for a grapevine leaf or canopy would suggest that, despite light reflectance of KL sprayed leaves in the PAR region being 2 to 5 times higher than un-sprayed leaves (Shellie and King, 2013a), light absorbed by the leaf was still close to the saturation point. Viceversa, due to linearity of the model describing leaf E variation *vs* intercepted light, it is likely that the same amount of reflected light had a bigger impact on water loss.

Albeit limited to a single season, data taken at harvest on yield components and grape composition support the assumption that color accumulation in berries is sensitive to KL application. In fact, while total anthocyanins and phenolics concentration at harvest were the lowest in C-WS vines, in KL-WS the same parameters were similar to the levels reached in C-WW. In conclusion, our research is in line with previous work on cv. Sangiovese reporting that the genotype can be sensitive to limiting factors affecting berry color accumulation due to overheating (Shellie, 2015; Silvestroni et al., 2016).

5. Conclusions

Our study has provided a comprehensive assessment of cv. Sangiovese grapevines' response to kaolin spraying while also subjected to an abrupt water deficit followed by a full re-watering. Such assessment included an array of physiological parameters evaluated at the single leaf and whole canopy scale. For almost all of the evaluated parameters, there was a good agreement between trends derived from the two sampling methodologies, suggesting that when the grapevine canopy is of moderate vigor and formed by a limited number of leaf layers, judicious single leaf sampling can yield estimates to be realistically extrapolated to a whole-canopy behavior.

Additionally, our trial has consistently shown that kaolin effects on vine physiology might drastically change as a function of the plant water status. Under well-watered conditions, kaolin slightly reduced canopy carbon assimilation and transpiration. If we consider that current kaolin formulations are quite inexpensive and continuous foliage and cluster coverage can be assured for the greater part of the growing season, the option of using kaolin as a long-lasting seasonal anti-transpirant and to protect clusters from overheating and sunburn is a quite realistic and attractive strategy. Moreover, due to the ability of kaolin to preserve berry color through berry skin cooling, the compound seems to have the quite desirable feature of a ripening decoupling agent in red cultivars: *i.e.* no changes in sugar, while color is enhanced.

Based on our data, even more interesting is the role that kaolin might play under a scenario of multiple summer stresses, where high light and thermal stresses often combine with poor leaf water status

that, in most cases, derives from interactive effects of low soil moisture and high evaporative demand. Single leaf and whole canopy physiological assessment have very consistently shown that kaolin sprayed leaves behave much better under severe water deficit (*i.e.* stem Ψ_{MD} between −1.2 and −1.6 MPa) in terms of maintenance of significant rates of carbon assimilation and higher water use efficiency (for both measuring systems). Moreover, kaolin has revealed to be particularly effective at safeguarding the photosynthetic machinery under multiple summer stresses and, most importantly, to assure a quite rapid recovery of full leaf function at re-watering. If all these positive features are taken together, it is quite apparent that, especially in vineyards sited in warm areas prone to summer drought and with limited or no use of supplemental water, kaolin coverage can represent a resilience strategy aimed at preserving leaf function and viability under severe stress while assuring a faster recovery once adequate water supply is replenished.

Declaration of Competing Interest

None. We state that all the authors agree for the submission of this paper and that they have no undeclared competing financial interests.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jplph.2019.153020.

References

Abou-Khaled, A., Hagan, R.M., Davenport, D.C., 1970. Effects of kaolinite as a reflective antitranspirant on leaf temperature, transpiration, photosynthesis, and water-use efficiency. Water Res. Res. 6 (1), 280–289.

Aly, M., El-Megeed, L.A., Awad, R.M., 2010. Reflective particle films effect on sunburn, yield, mineral composition and fruit maturity of 'Anna' apple (*Malus domestica*) trees. Res. J. Agr. Biol. Sci. 6, 84–92.

Attia, F., Martinez, L., Lamaze, T., 2014. Foliar application of processed calcite particles improves leaf photosynthesis of potted *Vitis vinifera* L. (var.' Cot') grown under water deficit. J. Int. Sci. Vigne Vin. 48 (4), 237–245.

Bernardo, S., Dinis, L.-T., Luzio, A., Pinto, G., Meijón, M., Valledor, L., Conde, A., Gerós, H., Correia, C.M., Moutinho-Pereira, J., 2017. Kaolin particle film application lowers oxidative damage and DNA methylation on grapevine (*Vitis vinifera* L.). Env. Exp. Bot. 139, 39–47.

Boari, F., Donadio, A., Schiattone, M.I., Cantore, V., 2015. Particle film technology: a supplemental tool to save water. Agric. Wat. Manag. 147, 154–162.

Brillante, L., Belfiore, N., Gaiotti, F., Lovat, L., Sansone, L., Poni, S., Tomasi, D., 2016. Comparing kaolin and pinolene to improve sustainable grapevine production during drought. PLoS One 11 (6), 1–19 e0156631.

Brito, C., Dinis, L.-T., Ferreira, H., Rocha, L., Pavia, I., Moutinho-Pereira, J., Correia, C.M., 2018. Kaolin particle film modulates morphological, physiological and biochemical olive tree responses to drought and rewatering. Plant Physiol. Biochem. 133, 29–39.

Brito, C., Dinis, L.T., Moutinho-Pereira, J., Correia, C., 2019. Kaolin, an emerging tool to alleviate the effects of abiotic stresses on crop performance. Sci. Hortic. 250, 310–316.

Conde, A., Neves, A., Breia, R., Pimentel, D., Dinis, L.-T., Bernardo, S., Correia, C.M., Cunha, A., Gerós, H., Moutinho-Pereira, J., 2018. Kaolin particle film application stimulates photoassimilate synthesis and modifies the primary metabolome of grape leaves. J. Plant Physiol. 223, 47–56.

Conde, A., Pimentel, D., Neves, A., Dinis, L.T., Bernardo, S., Correia, C.M., Gerós, H., Moutinho-Pereira, J., 2016. Kaolin foliar application has a stimulatory effect on phenylpropanoid and flavonoid pathways in grape berries. Front. Plant Sci. 7, 1150.

Correia, C., Dinis, L.T., Pinheiro, R., Fraga, H., Ferreira, H., Gonçalves, I., Malheiro, A., Moutinho-Pereira, J., 2014. Climate change and adaptation strategies for viticulture. J. Int. Sci. Publ.: Agric. Food 2, 424–429.

D'Aquino, S., Cocco, A., Ortu, S., Schirra, M., 2011. Effects of kaolin-based particle film to control *Ceratitis capitata* (Diptera: tephritidae) infestations and postharvest decay in citrus and stone fruit. Crop Prot. 30 (8), 1079–1086.

Demmig, B., Björkman, O., 1987. Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170, 489–504.

Denaxa, N.K., Roussos, P.A., Damvakaris, T., Stournaras, V., 2012. Comparative effects of exogenous glycine betaine, kaolin clay particles and Ambiol on photosynthesis, leaf

sclerophylly indexes and heat load of olive cv. Chondrolia Chalkidikis under drought. Sci. Hortic. 137, 87–94.

Dinis, L.T., Ferreira, H., Pinto, G., Bernardo, S., Correia, C.M., Moutinho-Pereira, J., 2016a. Kaolin-based, foliar reflective film protects photosystem II structure and function in grapevine leaves exposed to heat and high solar radiation. Photosynthetica 54 (1), 47–55.

Dinis, L.T., Bernardo, S., Conde, A., Pimentel, D., Ferreira, H., Félix, L., Gerós, H., Correia, C.M., Moutinho-Pereira, J., 2016b. Kaolin exogenous application boosts antioxidant capacity and phenolic content in berries and leaves of grapevine under summer stress. J. Plant Physiol. 191, 45–53.

Dinis, L.T., Malheiro, A.C., Luzio, A., Fraga, H., Ferreira, H., Gonçalves, I., Pinto, G., Correia, C.M., Moutinho-Pereira, J., 2018a. Improvement of grapevine physiology and yield under summer stress by kaolin-foliar application: water relations, photosynthesis and oxidative damage. Photosynthetica 56 (2), 641–651.

Dinis, L.T., Bernardo, S., Luzio, A., Pinto, G., Meijón, M., Pintó-Marijuan, M., Cotado, A., Correia, C., Moutinho-Pereira, J., 2018b. Kaolin modulates ABA and IAA dynamics and physiology of grapevine under Mediterranean summer stress. J. Plant Physiol. 220, 181–192.

Gatti, M., Pirez, F.J., Frioni, T., Squeri, C., Poni, S., 2018. Calibrated, delayed-cane winter pruning controls yield and significantly postpones berry ripening parameters in Vitis vinifera L. cv. Pinot Noir. Aust. J. Grape Wine Res. 24 (3), 305–316.

Glenn, D.M., 2012. The mechanisms of plant stress mitigation by kaolin-based particle films and applications in horticultural and agricultural crops. HortScience 47, 710–711.

Glenn, D.M., 2009. Particle film mechanisms of action that reduce the effect of environmental stress in' Empire' apple. J. Am. Soc. Hortic. Sci. 134 (3), 314–321.

Glenn, D.M., 2016. Effect of highly processed calcined kaolin residues on apple water use efficiency. Sci. Hortic. 205, 127–132.

Glenn, D.M., Puterka, G.J., 2005. Particle films: a new technology for agriculture. Hort. Rev. 31, 1–44.

Glenn, D.M., 2010. Canopy gas exchange and water use efficiency of "Empire" apple in response to particle film, irrigation, and microclimate factors. J. Am. Soc. Hortic. Sci. 135, 25–32.

Hulands, S., Greer, D.H., Harper, J.D.I., 2014. The interactive effects of temperature and light intensity on Vitis vinifera cv.' Semillon' grapevines. II. Berry ripening and susceptibility to sunburn at harvest. Europ. J. Hortic. Sci. 79, 1–7.

Iland, P., Dry, P., Proffitt, T., Tyerman, S., 2011. The Grapevine: From the Science to the Practice of Growing Vines for Wine. 2011. Patrick Iland Wine Promotions Pty Ltd, Adelaide, Australia pp.310.

Jones, G.V., White, M.A., Cooper, O.R., Storchmann, K., 2005. Climate change and global wine quality. Clim. Change 73, 319–343.

Joubert, P.H., Grové, T., De Beer, M.S., Steyn, W.P., 2004. Evaluation of kaolin (surround® wp) in an ipm program on mangoes in South Africa. Acta Hortic. 645, 493–499.

Keller, M., 2015. The Science of Grapevines: Anatomy and Physiology. Academic Press.

Lapointe, S.L., McKenzie, C.L., Hall, D.G., 2006. Reduced oviposition by *Diaprepes abbreviatus* (Coleoptera: curculionidae) and growth enhancement of citrus by surround particle film. J. Econ. Entom. 99 (1), 109–116.

Leskey, T.C., Wright, S.E., Michael Glenn, D., Puterka, G.J., 2010. Effect of Surround WP on behavior and mortality of apple maggot (Diptera: tephritidae). J. Econ. Entom. 103 (2), 394–401.

Long, S.P., Hallgren, J.E., et al., 1985. Measurement of CO_2 assimilation by plants in the field and the laboratory. In: Coombs, J. (Ed.), Techniques In: Bio-Productivity and Photosynthesis. Pergamon Press, Oxford, pp. 62–93 1985.

McCutchan, H., Shackel, K.A., 1992. Stem-water potential as a sensitive indicator of water stress in prune trees (*Prunus domestica* L. Cv. French). J. Am. Soc. Hortic. Sci. 117, 607–611.

Medrano, H., Tomás, M., Martorell, S., Flexas, J., Hernández, E., Rosselló, J., Pou, A., Escalona, J.M., Bota, J., 2015. From leaf to whole-plant water use efficiency (WUE) in complex canopies: limitations of leaf WUE as a selection target. Crop J. 3, 220–228.

Melgarejo, P., Martınez, J.J., Hernández, F.C.A., Martınez-Font, R., Barrows, P., Erez, A., 2004. Kaolin treatment to reduce pomegranate sunburn. Sci. Hortic. 100 (1–4), 349–353.

Mori, K., Goto-Yamamoto, N., Kitayama, M., Hashizume, K., 2007. Loss of anthocyanins in red-wine grape under high temperature. J. Exp. Bot. 58, 1935–1945.

Osborne, W.C., 1977. Fans, 2nd ed. Pergamon Press, Oxford (UK) p. 228.

Palliotti, A., Tombesi, S., Silvestroni, O., Lanari, V., Gatti, M., Poni, S., 2014. Changes in vineyard establishment and canopy management urged by earlier climate-related grape ripening: a review. Sci. Hortic. 178, 43–54.

Pascual, S., Cobos, G., Seris, E., González-Núñez, M., 2010. Effects of processed kaolin on pests and non-target arthropods in a Spanish olive grove. J. Pest Sci. (2004) 83 (2), 121–133.

Poni, S., Bernizzoni, F., Reinotti, M., 2005. Whole-canopy and single-leaf gas-exchange responses to partial rootzone drying in potted' Cabernet Sauvignon' grapevines. Acta Hortic. 689, 277–284.

Poni, S., Merli, M.C., Magnanini, E., Galbignani, M., Bernizzoni, F., Vercesi, A., Gatti, M., 2014. An improved multichamber gas-exchange system for determining whole-canopy water-use efficiency in grapevine. Am. J. Enol. Vitic. 65, 268–276.

Poni, S., Galbignani, M., Bernizzoni, F., Talaverano, M.I., Magnanini, E., 2015. A device enabling fully automated water-deficit experiments with potted grapevines. Amer. J. Enol. Vitic. 66, 251–255.

Poni, S., Gatti, M., Palliotti, A., Dai, Z., Duchêne, E., Truong, T.-T., Ferrara, G., Matarrese, A.M.S., Gallotta, A., Bellincontro, A., Mencarelli, F., Tombesi, S., 2018. Grapevine quality: a multiple choice issue. Sci. Hortic. 234, 445–462.

Rustioni, L., Milani, C., Parisi, S., Failla, O., 2015. Chlorophyll role in berry sunburn symptoms studied in different grape (*Vitis vinifera L.*) cultivars. Sci. Hortic. 185, 145–150.

Santillan, D., Iglesias, A., La Jeunesse, I., Garrote, L., Sotes, V., 2019. Vineyards in transition: a global assessment of the adaptation needs of grape producing regions under climate change. Sci. Tot. Env. 657, 830–852.

Schultz, H., 2000. Climate change and viticulture: a European perspective on climatology, carbon dioxide and UV-B effects. Austr. J. Grape Wine Res. 6, 2–12.

Schultze, S.R., Sabbatini, P., Luo, L., 2016. Effects of a warming trend on cool climate viticulture in Michigan. USA. SpringerPlus. 5 (1), 1119.

Sharma, R.R., Datta, S.C., Varghese, E., 2018. Effect of Surround WP®, a kaolin-based particle film on sunburn, fruit cracking and postharvest quality of 'Kandhari' pomegranates. Crop Prot. 114, 18–22.

Shellie, K., 2015. Foliar reflective film and water deficit increase anthocyanin to soluble solids ratio during berry ripening in Merlot. Amer. J. Enol. Vitic. 66 (3), 348–356.

Shellie, K., Glenn, D.M., 2008. Wine grape response to kaolin particle film under deficit and well-watered conditions. Acta Hortic. 792, 587–591.

Shellie, K.C., King, B.A., 2013a. Kaolin particle film and water deficit influence Malbec leaf and berry temperature, pigments, and photosynthesis. Amer. J. Enol. Vitic. 64 (2), 223–230.

Shellie, K.C., King, B.A., 2013b. Kaolin particle film and water deficit influence red winegrape color under high solar radiation in an arid climate. Amer. J. Enol. Vitic. 64 (2), 214–222.

Silvestroni, O., Lanari, V., Lattanzi, T., Palliotti, A., Sabbatini, P., 2016. Impact of crop control strategies on performance of high-yielding Sangiovese grapevines. Amer. J. Enol. Vitic. 67 (4), 407–418.

Wand, S.J., Theron, K.I., Ackerman, J., Marais, S.J., 2006. Harvest and post-harvest apple fruit quality following applications of kaolin particle film in South African orchards. Sci. Hortic. 107 (3), 271–276.

Webb, L.B., Whetton, P.H., Barlow, E.W.R., 2007. Modelled impact of future climate change on the phenology of winegrapes in Australia. Austr. J. Grape Wine Res. 13 (3), 165–175.

Wunsche, J.N., Lombardini, L., Greer, D.H., 2004. "Surround" particle film applications – effects on whole canopy physiology of apple. Acta Hort. 636, 565–571.

Zhang, D., Du, Q., Zhang, Z., Jiao, X., Song, X., Li, J., 2017. Vapour pressure deficit control in relation to water transport and water productivity in greenhouse tomato production during summer. Sci. Reports. 7, 43461.