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The effects of calcite silicon-mediated particle film application on leaf temperature and grape composition of Merlot (*Vitis vinifera* L.) vines under different irrigation conditions

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ABSTRACT

This study examined whether the application of calcite-silicon mediated particle film (CaPF) at veraison can mitigate a drought-induced increase in leaf temperature on grapevine, thus contributing to improved leaf functionality, yield and grape composition traits.

A total of 48 five-year-old Merlot (*Vitis vinifera* L.) vines grafted onto SO4 were grown (in 20 L PVC pots) under Mediterranean conditions (Southern Italy). The vines were pruned to two spurs with two winter buds irrigated daily to 100 % field capacity, and fertilised weekly.

At veraison and using a 2×2 factorial experimental design, the two main factors, thermoregulation and water, were imposed at two levels: spraying with a thermoregulation compound (CaPF) and no spraying (NS); irrigation (WW) and drought stress (D)). A group of 24 vines was subjected to a 15-day drought period by receiving, every day, 25 % (D) of the daily water consumption of WW vines. The other 24 vines continued to be fully irrigated on a daily basis (WW). Twelve vines per group were sprayed (WW+CaPF, D+CaPF) with calcite-silicon mediate (3 % V/V) at the beginning of drought imposition, the remaining 24 vines were not sprayed (WW-NS, D-NS). Soil water moisture and stem water potential values were monitored from 11.30 to 13:30 nearly every week, and other vegetative and reproductive parameters were also measured.

During the experiment, air temperature peaked at ≈35 °C at midday, *VPD* at about 3.7 kPa and PAR reached ≈2000 µmol m^{-2} s^{-1}. Results show that in CaPF sprayed vines, leaf-air temperature differences were lower than in unsprayed vines in both irrigated and drought stressed groups. WW+CaPF vines retained significantly more leaf area and showed the highest value of accumulated vine transpiration.

Calcite-silicon mediated particle film could enhance the resilience of grapevine to adverse environmental conditions and may contribute to preserve terroir elements in highly reputed wine grape growing areas.

The study showed that foliar application of calcite silicon-mediated processed particles films can be used in arid regions to mitigate leaf temperatures in grapevines.

KEYWORDS

leaf area, abiotic stress, Merlot/SO4, particle films, stem water potential, vine transpiration

INTRODUCTION

Grapevine (*Vitis vinifera* L.), is among the most cultivated perennial fruit species (Food and Agriculture Organization of the United Nations, 1997) with more than 10,000 cultivars, of which some are widely grown around the world and almost all are of local importance (OIV, 2017). Many highly reputed wine grapes are grown under semi-arid climatic conditions in Mediterranean-type areas, where soil, weather, cultivar and farmers can all contribute to generating specific wine traits and, in turn, to pinpointing a terroir (Vaudour, 2002; van Leeuwen and Seguin, 2006). Nowadays, climate change poses a challenge in terms of crop productivity, economic sustainability (which also applies to viticulture, mainly due to an increase in air temperature), short rainy seasons and the increasing frequency of extreme climatic events, such as heatwaves, storms and heavy rains (Intergovernmental Panel on Climate Change - IPCC, 2014).

It has been observed that the phenological stages (e.g., bud-break and veraison) of grapevines are accelerating as a result of the rise in air temperature, thus potentially causing different types of damage (Jones, 2006; Webb *et al.,* 2012); for example, early bud-break may expose the vines to late spring frost, which will have detrimental effects on buds or the vitality of young shoots (Leolini *et al.,* 2018). Earlier veraison may impact the chemical composition of berries and wine quality, because grape maturation may occur during the hottest period of the season (Jones and Davis, 2000; Keller, 2010; Young *et al.,* 2016). Increasing air temperatures throughout the growing season will also influence evapotranspiration demand and soil water availability, which both impact vine water status (Zavaleta *et al.,* 2003; van Leeuwen and Destrac-Irvine, 2017), and in turn several ecophysiological leaf traits (e.g., stomatal conductance, photosynthesis leaf temperature, berry phenols and sugar accumulation) (Flexas and Medrano, 2002; Castellarin *et al.,* 2007) that can collectively and negatively impact yield and its components (Fraga *et al.,* 2012; Gambetta, 2016).

Supplemental irrigation is increasingly used as an adaptive strategy to address drought stress, as can be seen by the increasing proportion of irrigated vineyards: from 4 % (end of the 80s) up to ≈50 % (2011-2015) (Ayuda *et al.,* 2020). However, the adoption of irrigation to overcome drought events can be debated, mainly because of limited freshwater availability, increased management costs and possible negative consequences on grape quality (Chaves *et al.,* 2010, Ayuda *et al.,* 2020; Gambetta *et al*., 2020). Changes in grape quality traits (and in turn in wine) due to irrigation consequently have an impact on the terroir (van Leeuwen, 2010); therefore, in order to accommodate drought and adverse thermo-radiative conditions, new mitigation/adaptation strategies alternative to irrigation are highly desirable (Fraga *et al*., 2012; van Leeuwen and Destrac-Irvine, 2017).

In this context, the application of processed mineral particle films (e.g., kaolin and calcium carbonate) on the surface of leaves and fruit can protect them from higher temperatures, especially when directly exposed to solar radiation (Glenn and Puterka, 2004). In grapevine, kaolin has been tested for several purposes, including the control of some pests (Tubajika *et al*., 2007), improving fruit quality (Ou *et al*., 2010; Lobos *et al*., 2015), reducing leaf or fruit surface temperature (Shellie and Glenn, 2008), and increasing water use efficiency (Glenn *et al*., 2010; Brillante *et al*., 2016). Calcite particle film (CaPF), however, has received little attention. Processed CaPF fits the criteria proposed by Glenn and Puterka (2004) for a chemical useful for mitigating drought. Briefly, CaPF is chemically inert with a particle diameter of < 2 μ, and it is formed in such a way so as to create a uniform film on the treated surface that does not interfere with stomata functionality, as well as to modify the radiative budget of the leaf, and to alter plants/insect/pathogen interaction; furthermore, it can be washed away from the fruit's surface (Glenn and Puterka, 2004; Alvarez *et al*., 2015; Hagagg *et al*., 2019). Hence CaPF could be a reliable tool in the face of drought stress.

The effect of CaPF on some gas exchange parameters (e.g., photosynthesis) has been previously tested in both well-watered and drought stressed conditions (Attia *et al*., 2014), as well as in apricot nutrition and fruit quality (Martinez *et al*., 2010). Moreover, CaPF applications has been reported to be beneficial to several annual or perennial crops, including grapevine, especially under drought conditions (technical data sheet for Megagreen®: https://dokumen.tips/documents/megagreen-study.html, accessed on 21/08/2020). However, to our knowledge, the thermoregulation effect of applying processed CaPF, along with its impact on yield components and grape quality, has not been adequately studied. Improving knowledge in this specific field would support the viticulture industry in mitigating climate change and preserving terroir reputation. Therefore, this study examined the effects of the application to grapevine of CaPF on vine water relations, leaf area, vine transpiration, yield, and berry composition in well-watered and drought stressed grapevines.

MATERIALS AND METHODS

1. |Experimental site and plant material

The trial was carried out at the 'Metapontum Agrobios' Research Centre of the Basilicata Agency for Innovation in Agriculture (ALSIA), located in Metaponto, Southern Italy (40°23'31.4''N, 16°47'10.9''E) during the 2018 growing season in outdoor conditions. Meteorological values air temperature (°C), air humidity (%), global radiation (W m^{-2}) and wind speed (km/h) were recorded once an hour by an automatic standard weather station located within 100 m (40°23'23.29''N, 16°47'06.65''E) from the experimental site. The air vapour pressure deficit (*VPD*) was calculated according to Goudriaan and van Laar (1994) and reference evapotranspiration (ET_0) was retrieved from the local weather station.

A total of 48 five-year-old Merlot (*Vitis vinifera* L.) vines grafted onto SO4 (*Vitis berlandieri* Planch × *Vitis riparia* Michx) rootstock were grown in 20 L PVC pots. They were drip irrigated, with one dripper per pot (4 L/h discharge rate), and covered with a plastic film to minimise the direct evaporation of water from the soil. The substrate was a 3:1 v/v mixture of sandy loam soil (82 % sand, 7 % silt and 11 % clay) and peat. Vines were spur pruned (×2 spur per vine) in the dormant season, with a total of 4 buds per vine being left. After bud break, the water sprouts were periodically eliminated and only four shoots per vine were trained (upward oriented) toward the catch wires. All pots were aligned in 3 rows with a distance of 2 m between rows. At flowering (stage 23 of the modified E-L system; Coombe, 1995; 06/06/2018), each vine was pruned and two bearing shoots (two clusters each) were selected. The selected shoots were trimmed to 12 nodes after the second cluster node, corresponding to 15-16 main leaves per shoot. The laterals formed after trimming were left.

2. Experimental design

From bud-break (27th March) till veraison, all the vines were fully irrigated on a daily basis to keep soil moisture at field capacity; this was done by irrigating the pots in the evening till the water drained out of the pots. The vines were also fertilised weekly with 10 g per pot of NPK fertiliser 13.40.13 (Master, Valagro Spa, Atessa, Italy).

The experiment started at veraison (28th June, stage 33, modified E-L scale) - hereafter referred to as 0 days after treatment (DAT) - by grouping vines according to irrigation water (W, Factor 1). Namely, 24 vines continued to be well watered (WW) by receiving 100 % of daily water consumption, while the other 24 vines were subjected to drought (D), receiving, on a daily basis, 25 % of the water supplied to WW vines according to Briglia *et al*. (2019). After DAT 15, irrigation was resumed for all vines ensuring soil moisture at field capacity.

Following a 2^2 factorial experimental design, the WW and D vines were further split based on the application of the calcite particle film (CaPF, Factor 2), with 12 vines per treatment being grouped. The treatments were: WW-NS (well-watered, no calcite received), D-NS (drought conditions, no calcite received), WW+CaPF (well-watered, calcite received), D+CaPF (drought conditions, calcite received). Details of the final experimental design are summarised in Table 1.

The CaPF was sprayed in a single application on 28th June (0 DAT) as a 3 %vol aqueous solution and without any surfactant according to the product label. The solution was sprayed using a hand-pressure backpack sprayer. The remaining twenty-four vines did not receive the CaPF and were well-watered or drought stressed. The CaPF was the commercial Turn-on®, sourced by Agronutrition (Carbonne, France), which is a processed calcite-silicon mediated particle film obtained from sedimentary limestone rock via a tribomecanic process (EU Patent WO/2000/064586, 2000), and which contains $CaCO_3$ (48 %), SiO_2 (3.4 %), N (4 %), Mn (0.5 %) and Zn (1.5 %).

TABLE 1. Experimental design showing the combinations of Factor 1 (irrigation water) and Factor 2 (thermoregulation).

		Factor 2: thermoregulation	
		CaPF	Not sprayed (NS)
Factor 1: water	Well-watered (WW)	WW+CaPF 12 vines	WW-NS 12 vines
	Drought (D)	D+CaPF 12 vines	D-NS 12 vines

Each factor was applied on two levels, with a total of 24 individuals per level. Interactions groups (WW+CaPF, WW-NS, D+CaPF, D-NS) were of 12 vines each. Four of the 12 vines of each interaction group were placed on the automatic scale.

3. Soil moisture and stem water potential

Soil moisture was measured in all vines late in the afternoon of DAT 1, 9 and 15 just before irrigation. Measurements were carried out by means of a WET-2 sensor (Delta-T Ltd, UK) with an accuracy of ± 0.03 m^3 m^{-3} (± 3 %) on a range of 0 to 1 m^3 m^{-3}.

Vine water status was determined on DAT 1, 9 and 15 by means of stem water potential (Ψ) measured around midday (from 11:30 to 13:30) using a Scholander type pressure chamber (Model 600, PMS Instruments, Corvallis, OR) which was pressurised with nitrogen, according to the protocol by Turner (1981) and Choné *et al*. (2001). Briefly, one fully expanded leaf per vine (×3 vines per treatment) was sampled from the middle part of the main shoot, tagged and sealed in a plastic bag and promptly pressurised. The leaves for Ψ determination were covered with aluminium foil at least 180 minutes before Ψ measurement. After Ψ determination, each leaf was used to compute leaf area and dry weight (after 48 h at 65 °C in a ventilated oven).

4.Vine transpiration and leaf temperature

Four vines per treatment (×4) were singularly placed on 16 electronic and automatic scales (100 kg ± 1 g; FieldScales system, Phenospex, Heerlen, The Netherlands). In order to avoid any influence of the vine supporting structure on weight readings, vines standing on scales were positioned between rows and each shoot was vertically tied to a wooden cane. The FieldScales system was programmed to measure the weight of the pots at 1 min intervals throughout the 0-23 h period, then values were cumulated every 60 min and recorded hourly from 0 to 23 h.

The vine molar transpiration rate per unit of leaf area (E, mol m^{-2} h^{-1}) was automatically calculated in a continuum from the FieldScales data as:

$$E = \frac{|w_2 - w_1|}{LA * 1h * Mw}$$

where w_2 and w_1 (g) were two consecutive hourly pot weights measured at hour t_1 and t_2, respectively over the 0-23 h period and referred to t_2; *LA* was the total leaf area per vine (m^2, see below); and Mw the molar mass of water (18 g mol^{-1}). A maximum 1-2 inconsistent erratic values of Δw due to vine manipulation (e.g., watering and leaf/fruit sampling) were discarded on some of the days and the data gap was filled by assuming a linear water consumption across the time gap.

The total daily *E* of eight well-watered vines (WW+CaPF and WW-NS) was averaged and assumed to be the water consumption of all irrigated vines.

Air and leaf temperature were measured by means of a thermocouple on the leaf clip holder 2030-B of the PAM 2500 fluoremeter (Walz, GmbH, Effeltrich, Germany). Measurements were carried out around midday on the vines placed on the electronic scale. Three well-exposed main leaves per vine were sampled, and the temperature was measured from the central part of the leaf lamina.

5. Leaf area

Initial leaf area of each vine (*LA*) was estimated few days before veraison by counting the total number of leaves of each main shoot (*nMSi*) and of lateral shoots (*nLat*), and by multiplying them by their mean *areaMSi* and *areaLat,* respectively:

$$LA = (\textstyle\sum_1^i (nMSi * areaMSi) + nLat * areaLat)/10000 \quad [m^2\ vine^{-1}]$$

The *areaMSi* was the mean area of the leaf at node *i*. Values of *areaMSi* were destructively determined by collecting the leaves separately from each node of ten main shoots randomly sampled from similar vines not included in the trial. Each leaf at each node was then imaged using a colour digital camera (Panasonic DMC-FS45, mounting a Leica DC Vario-Summarit 1:2.5-6.4/4.3-21.5 ASPH optical zoom with 16 Mega pixel, Panasonic Coorporation, Kadoma, Osaka, Japan), along with a ruler for calibration purposes, and the surface area was determined by ImageJ (Schneider *et al*., 2012). Values for *areaMSi* were calculated as the average of the surface area values of the leaf at the same position *i*.

Similarly, values for *areaLat* were obtained by the digital image of the all lateral leaves divided by their total number.

Thereafter, the *LA* value of each vine was updated daily accounting for the area of leaves sampled for Ψ and for those fallen, which were determined in the way described for *areaMSi*.

6. Berry composition and yield components

Grapes were harvested when their sugar content was over 21 °Brix according to local standard. Hence, 6 randomly sampled berries per plant were monitored for °Brix and berry fresh weight on all the vines once a week.

At harvest (1st August, DAT 33), all the clusters were collected from each vine, enclosed in a plastic bag, stored in a portable refrigerator and then transported to the laboratory where the fresh weight of each cluster was measured. Two samples of about 100 berries per vine were obtained from all the clusters of each vine. These samples were stored in a refrigerator. One sample was squeezed in a mortar and the aliquots from the juice were immediately analysed for total dissolved solids (°Brix), pH, and titratable acidity by titration to a pH end

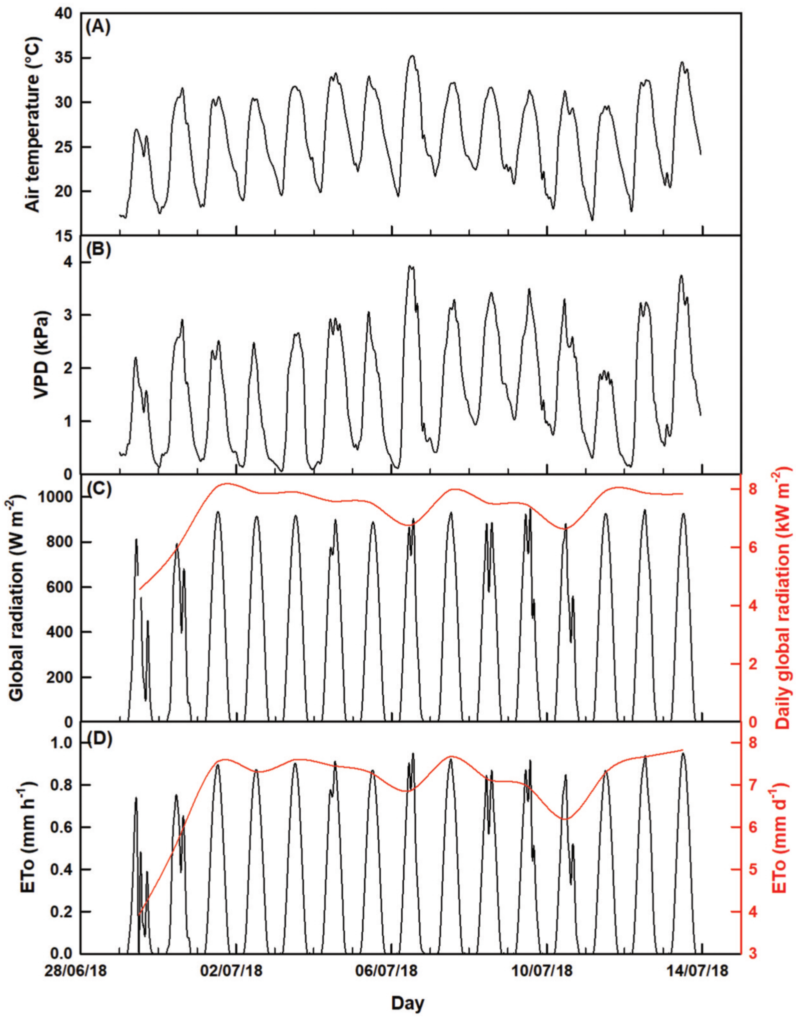

FIGURE 1. Diurnal course of some meteorological variables registered by the local weather station from 29th June (DAT 1) to 13th July (DAT 15) 2018.

TABLE 2. Soil moisture (m^3 m^{-3}) and midday stem water potential (Ψ, MPa) on three sampling days of the experiment as influenced by Factor 1 (W, irrigation water) and Factor 2 (thermoregulation).

Factors	Treatments	Soil moisture (m^3 m^{-3})*			Ψ (MPa)**		
		DAT 1	DAT 9	DAT 15	DAT 1	DAT 9	DAT 15
W	WW	41.62	40.21a	43.59a	-0.40	-0.49a	-0.54a
	D	39.36	25.67b	24.92b	-0.41	-1.32b	-2.14b
Thermoregulation	CaPF	40.42	33.41	35.65	-0.43	-0.93	-1.32
	NS	40.56	32.47	32.88	-0.38	-0.88	-1.36
Interaction W × Thermoregulation	WW+CaPF	40.32	40.00a	46.70a	-0.40	-0.50a	-0.50a
	WW-NS	42.92	40.42a	40.48a	-0.35	-0.48a	-0.58a
	D+CaPF	40.53	26.82b	24.55b	-0.47	-1.38b	-2.14b
	D-NS	38.20	24.52b	25.30b	-0.40	-1.28b	-2.15b

*Soil moisture, Factor 1 and 2, $n = 24$; W × Thermoreg. interaction $n = 12$ **Values for Ψ are the average of 12 measurements. When comparing treatments within the same DAT, factor and interaction, the different letters indicate statistically significant differences (p-value < 0.05) (Holm-Sidak multiple comparisons test). Note that the letters were omitted when means were not statistically different.

point of 7.0 with 0.1 N NaOH (OIV, 2018). Total acidity was expressed as g/L of tartaric acid equivalents.

The other berry sample was used to determine mean berry weight and total phenolic concentration. Berry epidermis and seeds were carefully removed with a scalpel and any mesocarp residue was removed using blotting paper. Number of seeds per berry, berry fresh weight and epidermis fresh weight were then measured using an electronic balance (AE 200 Mettler Toledo, Milano, Italy). After this, the epidermis was lyophilised and stored in a -80 °C refrigerator.

The dehydrated mass was ground with a pestle and mortar in liquid nitrogen. A subsample of 50 mg of epidermis powder was stored and shacked for one night in a 100 % methanol solution (1 mL) (Mazza *et al*., 1999). Before analysis, the mixture was centrifuged and an aliquot (20 µL) of the resulting supernatant was diluted 1:1 with water. Thereafter Folin-Ciocalteu reagent (20 µL) and Na_2CO_3 20 % (20 µL) were added to the mixture. After 10 min, the phenolic content was measured using a microplate spectrophotometer (Multiskan™ GO, Thermo Scientific™, Waltham, Massachusetts) with an absorbance reading at 750 nm. Gallic acid was used to calculate the standard curve (y = 176,81x - 23,242 ppm, R^2= 0.995) results were expressed as ppm of gallic acid (OIV, 2018).

7. Statistical analysis

A two way ANOVA (Sigmaplot® 12.3 software (Systat Software, Inc.) was employed for the data analysis. Before the ANOVA, a Shapiro-Wilk test was performed as a normality test and an equal variance test. Differences among means were identified by the Holm-Sidak Multiple test and p values of < 0.05 were considered to be significant.

RESULTS

1. Weather condition

During the experiment, the maximum hourly air temperature ranged from ≈ 27.0 °C to 35.5 °C with an average value of approximately 32.8 °C (Figure 1A). The mean maximum value of *VPD* was approximately 2.6 MPa, and the highest maximum *VPD* values (> 3.0 kPa) were recorded in the second week of the experiment if DAT 11 is excluded (Figure 1B). During the experiment, the available radiation at noon was close to 900 W m^{-2} on each day, except for the first two days (Figure 1C). The maximum hourly value of reference evapotranspiration was about 0.9 mm/h per day, except for the first two days (Figure 1D). Daily ET_0 was above 7 mm/d from the third day on.

2. Plant water status and soil moisture

The restriction in irrigation water significantly lowered the soil moisture of treatment D

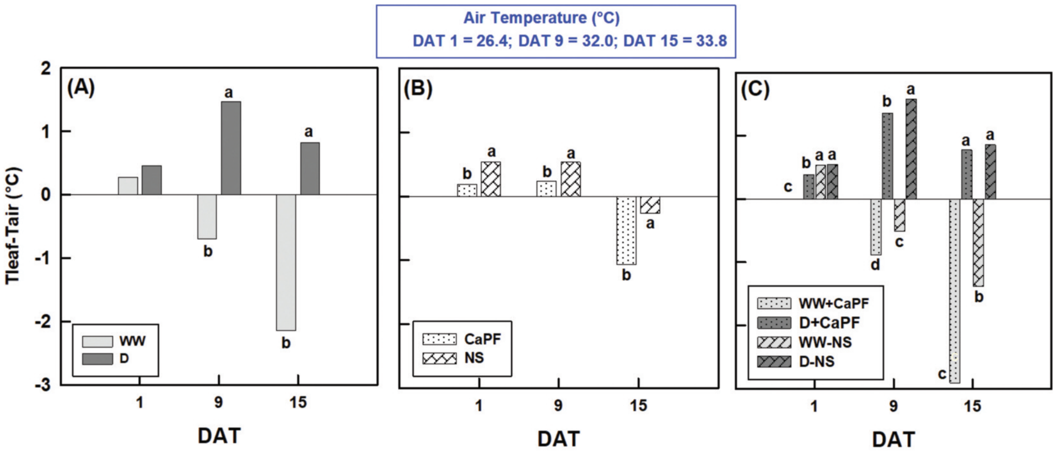

FIGURE 2. Average difference between leaf and mean air temperature (Tleaf-Tair) measured around midday in potted Merlot/SO4 vines. (A) Factor 1 "water" in well-watered (WW) and drought-stressed (D) vines; (B) Factor 2 "Thermoregulation" of the calcite-silicon mediated particle film (CaPF) and unsprayed (NS) vines; (C) W x Thermoreg. interactions. DAT = day after treatment.
Comparing treatments within the same DAT, factor and interaction, the different letters indicate statistically significant differences (p-value < 0.05) (Holm-Sidak multiple comparisons test). Letters were omitted when means were not statistically different. In (A) and (B) $n = 72$, in (C) $n = 36$.

compared to that of WW vines: D vine soil moisture decreased to 26 m^3 m^{-3} (DAT 9), where it remained until DAT 15, while WW vine soil moisture was stable at about 40 m^3 m^{-3} throughout the experimental period (Table 2).

The application of CaPF did not have any statistically significant impact on soil moisture and plant water status (Table 2).

Similarly, the midday stem water potential (Ψ) was at almost -0.4 MPa in all treatments at the beginning of the trial, then it was significantly influenced by the drought imposition and reached -1.32 (DAT 9) and -2.14 MPa (DAT 15) in D vines (Table 2). The analysis of the interaction between the two main factors further confirmed that CaPF did not influence Ψ.

3. Leaf temperature and vine transpiration

On the first day of the trial, mean leaf temperature was nearly 26.4 ± 0.1 °C and 27.0 ± 0.2 °C in WW and D vines, respectively, and their Tleaf-Tair difference was comparable (Figure 2A). On the same day, leaves sprayed with CaPF showed a leaf temperature of about 26.4 ± 0.1 °C while it was about 27.2 ± 0.1 °C in leaf of unsprayed vines (Figure 2B). This made the Tleaf-Tair difference of the CaPF significantly lower than that of NS ones (Figure 2B).

On DAT 9 and DAT 15, mean air temperature was higher than that recorded on DAT 1, reaching 32.0 and 33.8 °C respectively (Figure 2). In these conditions, the effect of the irrigation factor was statistically significant, having a leaf cooling effect on WW vines; that is, the leaf temperature of WW vines was about 0.7 °C and 2 °C below the air temperature on DAT 9 and DAT 15 respectively. Meanwhile, the leaf temperature of D vines was 1.5 °C (DAT 9) and 0.8 °C (DAT 15) higher than air temperature (Figure 2A).

On DAT 9, the application of CaPF significantly lowered the Tleaf-Tair difference compared to that of NS vines across all vines independently of their water status (Figure 2B). On DAT 15, CaPF induced an overall cooling effect on leaves, which was significantly higher than that of unsprayed vines (Figure 2B).

The analysis of the CaPF × W interaction revealed that a significant cooling effect of CaPF was detected in vines exposed to drought on DAT 1 and 9 (Figure 2C). For WW vines on DAT 15, the application of CaPF induced a significantly lower Tleaf-Tair difference, which approached 3 °C (WW+CaPF) and 1.2 °C (WW-NS) (Figure 2C).

Cumulated vine transpiration was similar among all treatments during the first week of the study. Interestingly, differences between WW and

D vines started 8 days after the beginning of the experiment and became statistically significant from DAT 9 till the end of the experiment (Figure 3). By contrast, the application of CaPF on D vines did not have any statistically significant effect compared to D-NS (Holm-Sidak multiple comparison test, $p = 0.05$).

A total of 2,708 molH_2O m^{-2} was transpired in 15 days from WW+CaPF vines, which was 11 % and 45 % higher than that transpired from WW-NS and D+CaPF, respectively.

4. Leaf area

At the beginning of the experiment, leaf area per vine was similar in all treatments at about 1 m^2 (Figure 3A, 3B, 3C). Thereafter, leaf area decreased slightly. However, leaf area reduction was less pronounced in vines receiving CaPF independently of their water status (Figure 3A); that is, from veraison to harvest, CaPF sprayed vines lost about 19 % of leaf area, while non-sprayed vines lost about 29 % of the initial leaf area.

No significantly different leaf area reduction was detected between well-watered vines and drought stressed vines, even if WW vines showed about 0.1 m^2 more leaf area per vine than D vines did (Figure 3B).

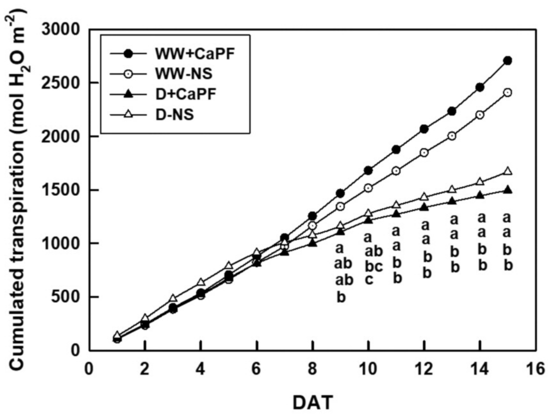

FIGURE 3. The effects of processed calcite-silicon mediated film and water treatments on cumulated transpired water in potted Merlot/SO4 vines. DAT = day after treatment.
WW stands for well-watered vines, D for drought stressed vines, +CaPF indicates that vines were sprayed with the thermoregulation product, NS indicates that vines were not sprayed. Statistical analysis refers to the interaction of factors. When comparing treatments within each DAT, different letters indicate statistically significant differences (n = 4, p-value < 0.05, Holm-Sidak multiple comparisons test).

The interaction of the W × T factors shows that WW+CaPF vines had the largest leaf area at harvest with 0.94 m^2 per vine, which was not statistically different from that estimated for the same treatment at veraison (Figure 3C). In particular, the leaf area of WW+CaPF vines at harvest was 16 % larger than that of D+CaPF and WW-NS, and 24 % larger than that of D-NS vines (Figure 3C).

5. Yield efficiency and berry composition

Well-watered vines showed a significantly higher cluster weight, while CaPF application induced a higher leaf area-to-yield ratio compared to that of NS vines (Table 3).

The number of berries per cluster and the mean cluster weight were significantly lower in D+CaPF vines (Table 3). Treatments did not have any statistically significant impact on yield per vine, or on the ratio between the amount of grape harvested and the water transpired from veraison until the restoration of full irrigation (Table 3).

Independently of the application of the thermoregulation compound, the grapevine responded to well-watered conditions with a significantly higher berry weight and lower pH (Table 4) than that of drought stressed vines. Well-watered vines also showed a significantly higher pulp weight and a lower skin to berry weight ratio than that of D vines (data not showed).

CaPF significantly increased the concentration of dissolved solids in the grape, both in well-watered and in drought-stressed vines (Table 4). There was no difference among treatments in total polyphenols.

DISCUSSION

This study mainly examined the leaf thermoregulation effect of CaPF on leaf temperature and leaf area from veraison to harvest, when the yield and berry quality were greatly influenced by environmental conditions (Castellarin *et al*., 2007; Keller, 2010; Ou *et al*., 2010). In Southern Italy, as in most Mediterranean-type climates, this period is often the warmest and driest of the year, contributing to an increase in leaf temperature, which rises above that of air in several crops, including grape, even under full irrigation (Sharma *et al.*, 2015).

The application of particle-film-based compounds has been suggested to mitigate the impacts of heat stress on leaf and fruit, because it is able to modify tissue radiative properties by increasing the reflectance of solar radiation, changing the leaf/fruit radiant energy exchange, and in turn influencing leaf and fruit temperature (Glenn and Puterka, 2004; Arkebauer, 2005).

Among these compounds, both CaPF and kaolin are able to increase the reflectance properties of leaves, even though their effect on leaf temperature reduction is still under debate (Glenn *et al.*, 2003; Glenn *et al.*, 2010; Shellie and King, 2013; Attia *et al.*, 2014; Brillante *et al.*, 2016; Tosin *et al.*, 2019). In the present experiment, all sampling data for leaves sprayed with CaPF have shown, independently of the water status, a significantly lower leaf temperature than that of non-sprayed ones

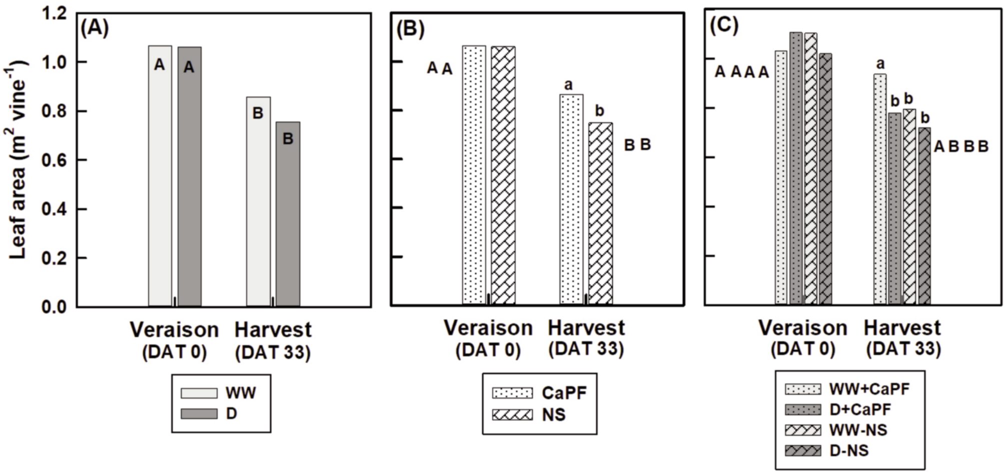

FIGURE 4. Leaf area per vine in foliar sprayed processed calcite-silicon mediated film and water treatments in potted Merlot/SO4 vines. (A) main water effects in well-watered (WW) and drought-stressed (D) conditions; (B) main thermoregulation effect in CaPF sprayed and non-sprayed (NS) vines; (C) W x Thermoregulation interactions. DAT = day after treatment.

CaPF stands for foliar processed calcite-silicon mediated sprayed; NS stands for non-CaPF foliar sprayed vines; WW stands for well-watered vines and D for drought stressed vines. Different capital letters indicate differences between veraison and harvest. Lower case letters indicate differences among treatments within the same phenological stage. Differences were calculated with Holm-Sidak multiple comparisons test at p-value < 0.05 or lower. Each bar represents the average of 24 (A, B) and 12 (C) values.

TABLE 3. Yield and yield components in foliar sprayed processed calcite-silicon mediated film and water treatments in potted Merlot/SO4 vines.

Factors	Treatments	Berries per cluster (n)	Cluster weight (g/cluster)	Yield (g/vine)	Leaf area/yield (cm^2/g)	WUE (g grape/mol H_2O m^{-2})
W	WW	87.04	140.05a	568.2	16.11	0.24
	D	83.31	119.77b	495.8	16.04	0.29
Thermoregulation	CaPF	80.29	122.49	507.1	17.95a	0.24
	NS	90.72	137.33	549.3	14.20b	0.29
Interaction W × Thermoregulation	WW+CaPF	85.22ab	136.28a	545.1	18.40a	0.23
	WW-NS	90.18a	143.82a	575.3	13.82b	0.25
	D+CaPF	75.36b	108.70b	464.2	17.51a	0.24
	D-NS	91.26a	130.84a	523.4	14.57b	0.34

Values are the average of 24 single measurements for the main factors (W, Thermoregulation) and 12 single measurements for the W × Thermoregulation interaction. Values of WUE are the average of 8 vines for main factors and ingle measurements for the interaction.

WUE stands for water use efficiency. Different letters indicate statistically significant differences (p-value < 0.05) (Holm-Sidak multiple comparisons test). Note that letters were omitted when means were not statistically different.

TABLE 4. Berry weight and berry composition in foliar sprayed processed calcite-silicon mediated film and water treatments in potted Merlot/SO4 vines.

Factors	Treatments	Berry weight (g/berry)	° Brix	pH	Titratable acidity (g/L tartaric acid)	Total polyphenols (ppm gallic acid)
W	WW	1.61a	23.30	3.96b	5.64	268.38
	D	1.49b	22.92	4.08a	5.75	254.16
Thermoregulation	CaPF	1.56	23.52a	4.02	5.67	262.27
	NS	1.54	22.70b	4.02	5.72	260.26
Interaction W× thermoregulation	WW+CaPF	1.60a	23.66a	3.94b	5.58	264.86
	WW-NS	1.63a	22.93ab	3.97ab	5.70	271.90
	D+CaPF	1.52b	23.37a	4.10a	5.78	259.69
	D-NS	1.45b	22.47b	4.06a	5.74	248.63

Values are the average of 24 single measurements for the main factors and 12 single measurements for the W × Thermoregulation interaction. Different letters indicate statistically significant differences (p-value < 0.05) (Holm-Sidak multiple comparisons test). Note that letters were omitted when means were not statistically different.

(Figures 2B, 2C); this was conceivably due to the reduction of the absorbed radiation. Although the leaf-to-air temperature difference in CaPF vines was 0.3 (D) and 0.8 °C (WW) lower than that of NS (Figure 2B), a potential improvement in leaf photosynthesis-related metabolisms (e.g., light and dark photosynthetic reactions) might have occurred (Medrano *et al*., 2002; Medrano *et al*., 2003). However, more research effort is required in order to disentangle the thermoregulation effect of CaPF on leaf radiative characteristics and leaf energy budget (Arkebauer, 2005).

As expected, the level of supplied irrigation water resulted in significant differences in soil moisture and vines water status. The WW vines maintained stable soil moisture (≈ 42 m^3 m^{-3}) and midday stem water potential of about -0.46 MPa throughout the experiment (Table 2), while those in which only 25 % of the transpired water was returned, showed a decrease in Ψ to a very low value (-2.14 MPa) on DAT 15 (Table 2). The values of Ψ measured in this trial were categorised according to van Leeuwen *et al*. (2010) as: no water stress (> -0.6 MPa), moderate to severe water stress (from -1.1 to -1.4 MPa) and very severe water stress (< -1.4 MPa).

In this study, soil moisture and midday stem water potential were only impacted by Factor 1 (water) and not by Factor 2 (thermoregulation). These results are in line with previous experiments testing CaPF application on grapevines under glasshouse conditions (Attia *et al.,* 2014). In most other studies carried out on kaolin particle film for biotic or abiotic stress mitigation in different crops, the effects of particle film treatments on grapevine plant water status was negligible (Shellie and Glenn, 2008; Glenn *et al*., 2010; Ou *et al*., 2010; Lobos *et al*., 2015; Brillante *et al*., 2016).

It has been shown that in well-watered conditions leaf temperature is well-correlated with many other metabolic processes of the plant, such as photosynthetic rate, stomatal conductance, (Blonder and Michaletz, 2018). This may explain the high amount of transpired water in well-irrigated CaPF sprayed vines (Figure 3); that is, under well-watered conditions the low leaf temperature of CaPF vines likely increased stomatal opening and in turn water consumption (Figure 3) (Brillante *et al.,* 2016). By contrast, under water stress conditions, CaPF likely contributed to reduced water consumption when compared with D not-sprayed, although differences were not significant (Figure 3) (Blonder and Michaletz, 2018).

During the summer, vines can suffer from anticipated defoliation, even under optimal soil moisture, thus reducing their overall photosynthetic capacity (Chaves *et al.,* 2010; Hochberg *et al*., 2017). In the present study, leaf area decreased in WW vines from veraison to harvest at a similar rate to that reported by Munitz *et al*. (2016) and Charrier *et al*. (2018). Interestingly, the application of CaPF to WW vines contributed to retaining a significantly larger leaf area (25 %) compared to non-sprayed

WW vines; this is a potential advantage in terms of overall vine functioning (e.g., carbon gain). The beneficial effect of CaPF on leaf retention was also observed in D vines (Figures 4B, 4C), even though it was not statistically significant. Leaf area retention induced by CaPF significantly increased the leaf area/yield ratio compared to that of not-sprayed in both WW and D vines (Table 3). This beneficial effect might be due to increased ROS, as observed in tobacco (Tran *et al.*, 2020), which, in turn, might have enhanced the vine acclimation response to high air temperature and radiation (Carvalho *et al.*, 2015; Brito *et al.*, 2019).

According to correlative information reported by Kliewer and Dokoozlian (2005), the larger leaf area might then have caused the high concentration of dissolved solids recorded for both WW and D when sprayed with CaPF (Table 4).

When not sprayed, the WW vines lose leaf area in a similar way to D vines (Figures 4A, 4C). In drought stressed vines, no significant effect of CaPF application on leaf fall was detected, probably because, under severe drought stress, the programmed leaf death which is also triggered by the impairment of the conductive xylem (embolism) (Hochberg *et al.*, 2017; Charrier *et al.* 2018), can dominate over any benefits of CaPF. Non-irrigated vines (D+CaPF and D-NS) experienced very severe water stress ($\Psi < -1.4$ MPa) from DAT 9 to DAT 15, which reduced vine water consumption by about 30 % compared to WW vines. It has been reported (Charrier *et al.*, 2018) that when under severe water stress, loss of turgor and xylem cavitation may produce embolism in xylem vessels and then a reduction in conductivity that could lead to leaf shedding. In this experiment, after two weeks of reduced irrigation volume, D vines lost around 29 % of their leaves (Figure 4); therefore, in order to avoid more severe defoliation on DAT 15, full irrigation in the afternoon was restored and maintained till harvest.

It is well known that under high temperature and solar radiation an excess of reactive oxygen species (ROS) are produced in different cellular components, which may induce oxidative stress (Carvalho *et al.*, 2015). Bernardo *et al.* (2017) have shown a reduction in berry and leaf ROS in kaolin-treated vines compared to untreated ones. Leaf or stem water potential is often employed to identify differences in drought tolerance capability among grapevine cultivars, or to assess the vine response to agronomical practice(s) intended for their drought adaptation (e.g., summer pruning, soil management, etc.) (Schultz, 2003; Charrier *et al.*, 2018).

As expected from many other experiments (see Gambetta *et al.* 2020, for review), the non-irrigated vines showed a significantly lower cluster (14 %) and berry weight (7 %) and higher pH (3 %) and skin/berry ratio (8 %) (data not shown) than for WW vines. Differences in number of berries per cluster do not seem to be related to the effects of treatments, because their number was already fixed at the beginning of the trials. These differences may also have an effect on the significant differences in the cluster weight between irrigated and non-irrigated vines. At the rate used, some CaPF residues (white spots) were visually appraised on about 30 % of berry surfaces, these spots were not present on the unsprayed grapes. As these residues are likely calcium carbonate, they could reduce the acidity of the must during fermentation, potentially influencing aging, as well as some sensorial traits of wine. However, more research is required to test such effects of CaPF. Moreover, the missing statistical differences in total polyphenols may be related to the time and duration of water stress, as highlighted by Gambetta *et al.* (2020) and Mirás-Avalos and Intrigliolo (2017).

Our results show that foliar application of processed calcite silicon-mediated particle film at veraison has a cooling effect on leaves of potted *Vitis vinifera* cv. Merlot, both under well-watered and two-week drought stressed conditions. In addition, the CaPF (*i*) induced high leaf retention during the veraison-harvest interval time, which was beneficial for the °Brix level, and (*ii*) allowed water to be saved, which collectively improved vine resilience. Hence, the CaPF application could favourably be considered as an adaptation strategy for dealing with adverse environmental summer conditions in Mediterranean-type grapevine producing regions.

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