

EFFECTS OF KAOLIN-BASED PARTICLE FILM AND FRUIT ZONE NETTING ON CABERNET-SAUVIGNON GRAPEVINE PHYSIOLOGY AND FRUIT QUALITY

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Abstract

Aims: Long exposure to high temperatures or UV-radiation may induce negative effects on vine physiology and grape composition. Here, the effects of two methods to moderate radiation and temperature in the fruit zone of a Cabernet Sauvignon vineyard were evaluated against a control.

Methods and results: The treatments assessed were: (a) periodical spraying of kaolin on leaves and bunches and (b) fruit zone netting with a Raschell's type mesh. The kaolin-based treatment increased the reflectance of light and moderately reduced fruit temperature (~1°C below the control), whilst the shading net caused a significant reduction in radiation and temperature in the fruit zone (~7°C below the control). The Net treatment showed lower (more negative) stem water potential values than the control, but did not persist until the end of the trial. Also, none of the treatments led to significant changes in stomatal conductance, transpiration or CO₂ assimilation throughout the season. However, the incidence and severity of fruit dehydration was significantly lower in the treated plants compared to the control. Finally, no differences in fruit chemical composition were observed between the treatments and the control.

Conclusion: Under the conditions of this trial, both treatments tested were sufficient in moderating the negative effects of excess radiation or high temperature on grape berries.

Significance and impact of the study: Kaolin-based particle spraying and fruit zone netting were proved to be feasible practical alternatives to lessen the negative effects of excess radiation or high temperature on grape berries, under hot climate.

Key words: grape, midday stem water potential, stomatal conductance, berry shriveling, reflectance

Abbreviations: PAR, photosynthetically active radiation; UV, ultraviolet; NIR, near-infrared; Ψ_s , midday stem water potential; *g_s*, stomatal conductance; *A*, CO₂ assimilation rate; *E*, transpiration; *I*, incidence of berry dehydration; and *S*, severity of berry dehydration.

Résumé

Objectifs: Une exposition prolongée à des températures élevées ou au rayonnement UV peut provoquer des changements de la physiologie de la vigne et de la composition du raisin. Les effets de deux méthodes pour diminuer le rayonnement et la température dans la zone fructifère ont été évalués et comparés à un témoin sur une parcelle de Cabernet-Sauvignon.

Méthodes et résultats: Les traitements évalués étaient: (a) la pulvérisation périodique de kaolin sur les grappes et le feuillage et (b) l'utilisation d'un filet de type Raschell dans la zone fructifère. Le traitement à base de kaolin a augmenté la réflexion de la lumière et a réduit de façon modérée la température de la surface des fruits (~1°C inférieur au témoin), tandis que le filet d'ombrage a provoqué une réduction plus importante du rayonnement et de la température des fruits et des feuilles dans la zone fructifère (~7°C inférieur au témoin). Le traitement avec filet d'ombrage a montré des valeurs moindres (plus négatives) de potentiel hydrique de tige par rapport au témoin, mais qui n'ont pas persisté jusqu'à la fin de l'étude. De plus, aucun des deux traitements n'a conduit à des changements significatifs de conductance stomatique, de transpiration ou d'assimilation du CO₂ pendant la saison. Toutefois, l'incidence et la sévérité de la déshydratation des fruits étaient significativement plus faibles dans les plantes traitées par rapport au témoin. Enfin, aucune différence dans la composition chimique des fruits n'a été observée entre les traitements et le témoin.

Conclusion: Dans les conditions de cet essai, les traitements testés suffisaient à modérer les effets négatifs des forts rayonnements ou des températures élevées sur les baies de raisin.

Signification et impact de l'étude: La pulvérisation périodique de kaolin et l'utilisation d'un filet d'ombrage se sont avérées des alternatives concrètes et réalisables pour diminuer l'incidence négative du rayonnement solaire et de la température dans la zone fructifère, sous climat chaud.

Mots clés: raisin, potentiel hydrique de tige, conductance stomatique, déshydratation des baies, réflectance

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INTRODUCTION

Solar radiation and temperature are essential for vine metabolism and are known to affect grape composition, yet can be harmful when in excess (Bergqvist *et al.*, 2001 ; Spayd *et al.*, 2002). Although difficult to assess independently from temperature, moderate amounts of photosynthetically active radiation (PAR) in the fruit zone ($\leq 100 \mu\text{mol m}^{-2} \text{s}^{-1}$) have been correlated with increased soluble solids and phenolics, and reduced titratable acidity and malic acid (Bergqvist *et al.*, 2001 ; Dokoozlian and Kliewer, 1996). Conversely, higher PAR values are linked with more transpiration and fruit dehydration, coupled with reductions in berry mass and size (Bergqvist *et al.*, 2001). Furthermore, if either radiation or temperature is excessive, tissue damage could be observed (Dokoozlian and Kliewer, 1996 ; Spayd *et al.*, 2002).

To date, several practices have been used to moderate the effects of excessive radiation or temperature in wine grapes, including canopy management and the use of shading nets. These practices have also been reported to reduce plant temperature, soluble solids, anthocyanin accumulation, and stomatal conductance (Chorti *et al.*, 2010 ; Iacono *et al.*, 1995 ; Lobos *et al.*, 2009 and 2012). As these practices can be labor consuming, the use of alternatives such as reflective particle films might be interesting. The most common material used for this purpose is kaolin, a white clay based on layered aluminum silicate, capable of leaving a thin deposit on the surface of the fruit, thus allowing an upsurge in light reflectance (Yazici and Kaynak, 2009). In apple trees, where fruit color development requires direct sunlight, the practice of spraying kaolin-based sunscreens has become a common way to reduce sunburn (Glenn *et al.*, 2002). In grapes, however, the adoption of this technique has been slower. In fact, there are only a few papers in which

this kind of product has been tested, focusing mainly on its protective effect against pests and diseases, and the combined effect with deficit irrigation on fruit composition (Glenn *et al.*, 2010 ; Ou *et al.*, 2010 ; Shellie and Glenn, 2008 ; Song *et al.*, 2012 ; Tubajika *et al.*, 2007).

Based on this knowledge gap, and considering that several studies have reported and projected rises in incident radiation and temperatures for various wine producing regions (Jones *et al.*, 2005 ; Moriondo *et al.*, 2013) as a consequence of climate change, the aim of this project was to evaluate and compare the effects of a kaolin-based sunscreen and a fruit zone netting on selected physiology and fruit quality variables of Cabernet-Sauvignon grapevines.

MATERIALS AND METHODS

1. Plant material and experimental set up

The study was carried out during 2011/2012, in a Cabernet Sauvignon vineyard ($35^{\circ} 06' \text{ S}$, $71^{\circ} 20' \text{ W}$, 230 m.a.s.l., Maule Region, Chile) established in 1994 under a 3x1.5 m frame, with a North-South orientation, trained in a vertical-shoot-positioned system and drip irrigated (drippers $\sim 4 \text{ L h}^{-1}$). The climate is Mediterranean, with an average rainfall of 676 mm per year, and diurnal temperatures during fruit ripening between 3.3 and 29.4°C. Meteorological data of season 2011–2012 is given in Table 1. The soil is sedimentary, dark brown, with texture ranging from loamy-sand to silty clay-loam soil, and a depth of root-growth of 0.6 m (CIREN-CORFO, 1997).

The trial was set in a completely randomized design, with three treatments and three replicates of 20 plants each. To avoid wind drift and edge effects, the treatments were arranged on three non-adjacent rows, separated by two rows each ($\sim 9 \text{ m}$). Similarly, each experimental unit (20 plants) was separated from each other by 25 untreated plants

Table 1. Meteorological data per phenological stage during season 2011–2012

Phenological period	PET ^z (mm)	Rain (mm)	Rain-PET (mm)	Tmax (°C)	Tmin (°C)	Tmean (°C)	GDD
1 st May - Budbreak	155	449	294	13,8	3,2	8	46
Budbreak - Flowering	220	9	-211	22,3	6,8	14,1	258
Flowering - Veraison	365	0	-365	29	11,2	20,1	636
Veraison - Harvest	353	7	-346	27,9	10,8	19	727
Total or Mean	1093	465	-629	23,2	8	15,3	1668

(~38 m). The treatments were as follows: (a) a control, (b) a treatment with seven periodical sprayings of kaolin (Nufresh®, Nufarm, Chile), and (c) a treatment with installation of a black Raschell's type net (35 % shade, 0.6 m tall from the soil) on the west side of the canopy, covering the fruiting zone and a limited portion of the foliage. Based on the manufacturer recommendation, the first spraying of 0.875 kg of Nufresh per 100 L of water was made on December 14, 2011 (berry size of ~5 mm diameter) using a 2000 L turbo-nebulizer (Fabrizio Levera, Chile). From the second application on (January 4, 2012), the dosage and spraying frequency were adjusted to 1.5 kg per 100 L of water at ~12-day intervals. The dosage adjustment was decided due to the incomplete kaolin-film formation after the first spraying. For the fruit netting treatment, the net was also installed on December 14, 2011 and removed at harvest (April 10, 2012).

2. Light reflectance and temperature

Absolute reflectance (350-2500 nm) was measured on the foliage and fruit (i.e. 30 cm above and at the fruit zone height, respectively) using a portable spectroradiometer (Fieldspec Jr. 3, ASD Inc., USA; equipment description is detailed by Lobos *et al.*, 2014) located at 1.5 and 0.2 m of distance, respectively (3472 and 61.73 cm² of area measured, respectively). The equipment was calibrated against a white reference panel (Spectralon, ASD Inc., USA) every 15 minutes (Garriga *et al.*, 2014). Here, absolute reflectance corresponds to the sum of (i) ultraviolet (UV): 350-399 nm, (ii) PAR: 400-700 nm, (iii) near-infrared 1 (NIR₁): 701-1400 nm, and (iv) near-infrared 2 (NIR₂): 1401-2500 nm. Measurements were taken on the west-facing side of the canopy between 13:00 and 15:00 h, with six measurements per experimental unit.

Similarly, fruit temperature was evaluated using a thermal imaging camera (FLIR i-40, Flir system Inc. OR, USA) positioned at ~0.4 m from the clusters. In the shaded treatment, the net was slightly moved to allow the camera to perform the measurements. Data processing was done with the software Flir Quick Report.

3. Plant water status & leaf gas exchange

Midday stem water potential (Ψ_s) was estimated from the leaf water potential measured with a pressure chamber (PMS Instrument Co., model 600, OR, USA), according to Scholander *et al.* (1965), between 12:00 to 14:00 h using two mature, healthy and fully expanded leaves per vine, selected from

the mid-upper part of the canopy. These leaves were covered for 2 hours with a plastic wrap and aluminum foil before the measurements, to allow leaf water potential equilibrate with stem water potential (Ortega-Farías *et al.*, 2004). Due to the time constraints involved in this analysis, two representative vines/replicate were marked and analyzed at any given measurement date (January 4th and 30th, February 21st, and March 27th), before the respective kaolin sprayings.

Stomatal conductance (g_s ; mol H₂O m⁻² s⁻¹), CO₂ assimilation rate (A ; mol m⁻² s⁻¹) and transpiration (E ; mmol H₂O m⁻² s⁻¹) were measured with an infrared gas analyzer (Li-6400 Li-Cor, Inc., NE, USA), at ambient conditions of light saturation (> 800 μ mol m⁻² s⁻¹), between 12:00 and 14:00 h, using the same plants as in the measurements of water potential. For the measurements in the control and kaolin-based treatments, two mature, healthy and fully exposed leaves per vine, per replicate, were chosen. In the Net treatment, the leaves were under the shade of the net. These evaluations were performed on January 30th, February 21st and March 27th.

4. Incidence and severity of berry dehydration

Incidence (I) and severity (S) of berry dehydration was evaluated on the west side of the canopy at harvest time. "I" was determined as the percentage of clusters with any type of cellular damage, whilst "S" was evaluated using the following scale: « 0 », clusters with no damage; « 1 », clusters with some damage (1-25 % dehydration); « 2 », moderate damage (26-50 % dehydration); « 3 », significant damage (51-75 % dehydration); and « 4 », severe damage (76-100 % dehydration). Damage severity was obtained from the sum of clusters with different damage levels out of the total clusters on each replication. Prior to these, 3 groups of 3 people each agreed on the categories and evaluated the treatments independently.

5. Chemical composition of berries

Two sets of ~15 kg of fruit/replicate were harvested, stored in iced coolers, and taken to the laboratory within 1 hour. One set of fruit was pressed through a lab-scale press. Approximately 1 L of juice was filtered and analyzed for juice density (g L⁻¹), pH, titratable acidity (g L⁻¹ of sulfuric acid), and free amino nitrogen (mg L⁻¹) (Bordeu and Scarpa, 2000). The second set of fruit was stored in polyethylene bags and frozen at -40°C for 30 days, until total polyphenol (absorbance at 280 nm) of the whole berry and

color of skin extracts (absorbance at 520 nm) were analyzed (Venencie *et al.*, 1997). In both cases, the measurements were expressed as absorbance units.

6. Statistical analyses

Analysis of variance (ANOVA) and Tukey's test ($p \leq 0.05$) for multiple pairwise comparisons was done using SAS (SAS Institute Inc., Cary, NC, USA). The percentages of incidence and severity of berry dehydration were transformed by the arcsine square-root function.

RESULTS

1. Light reflectance and temperature

As expected, the Net treatment showed lower absolute reflectance than the control and kaolin treatments, either for fruit and foliage measurements. In the last measurement date, when accumulation of kaolin film was noticeable, the fruit under kaolin showed higher reflectance than the control (Table 2). In the case of foliage, the kaolin treated plants had between 26 to 155 % more UV and PAR reflectance throughout the season than the control plants (data not shown). Compared

to fruit reflectance, these differences were noticeable earlier in the season (January 30, 2012), probably due to the surface characteristics of leaves versus clusters (e.g. exposed surface, waxiness, and rugosity).

Berry temperatures were consistently and significantly lower in the Net treatment compared to the control and Kaolin treatments (Table 3). Shading reduced berry temperature on average by $\sim 3.8^\circ\text{C}$ compared to the control. Berry temperatures in the kaolin treatments were lower than the control on two of the four measurement dates.

2. Plant water status & leaf gas exchange

Two Ψ_s measurements (January 30th and February 21st) showed significant differences ($p < 0.05$) between Net and control (Table 4) with lower (more negative) values for Net, but no differences were observed between the kaolin and control treatments on any date. Similarly, there were no statistical differences among treatments regarding stomatal conductance (g_s), assimilation (A) and transpiration measurements (E) in the three measurements performed (Table 4).

Table 2. Absolute reflectance by area of the spectra_x, measured at fruit at midd

Region of the spectra	Jan 04, 2012	Jan 30, 2012	Feb 21, 2012	Mar 27, 2012
UV				
Control	1.8 a ^z	1.3 b	2.8 a	5.0 b
Kaolin ^y	1.7 a	1.5 a	2.9 a	6.1 a
Net	0.9 b	0.7 c	1.0 b	2.0 c
PAR				
Control	18.3 a	10.4 a	15.5 b	28.8 b
Kaolin	17.3 a	10.4 a	20.0 a	45.4 a
Net	9.2 b	6.3 b	5.4 c	12.1 c
NIR ₁				
Control	219.5 a	136.7 a	133.7 a	213.5 b
Kaolin	212.8 a	123.3 a	142.7 a	238.7 a
Net	141.9 b	91.6 b	74.8 b	127.3 c
NIR ₂				
Control	31.6 a	42.2 a	19.3 a	36.5 b
Kaolin	35.8 a	23.3 b	24.2 a	59.9 a
Net	12.6 b	19.6 b	6.3 b	24.4 c

^xSum of the proportions (from 0: no reflectance to 1: 100% reflectance) of the spectra for each wavelength between UV= 350-399 nm; PAR= 400-700 nm; NIR₁= 701-1400 nm; NIR₂= 1401-2500 nm.

^yFirst application made on December 14, 2011.

^zReflectance followed by the same letter by column represents no statistical differences (Tukey's test, $p \leq 0.05$). All p values were lower than 0.0001.

Table 3. Effects of vine netting and Kaolin-based sprayings on fruit temperature (°C)

Treatment	Jan 04, 2012	Jan 30, 2012	Feb 21, 2012	Mar 27, 2012
Control	41.7 a ^z	41.3 a	40.8 a	41.7 a
Kaolin ^y	41.2 a	38.9 b	40.1 a	40.9 b
Net	37.4 b	37.8 c	37.2 b	34.4 c

^zMeasurements made by infrared thermography between 14:00 and 16:00 h.

^yFirst application made on December 14, 2011 ; ^zTemperatures on the same column followed by different letters are statistically different (Tukey's test, $p \leq 0.05$). All p values were lower than 0.0001.

Table 4. Effects of vine netting and Kaolin-based sprayings on stem water potential (Ψ_s), stomatal conductance (gs), CO₂ assimilation (A), and transpiration (E) of Cabernet-Sauvignon

Treatment	Jan 04, 2012	Jan 30, 2012	Feb 21, 2012	Mar 27, 2012
Stem water potential (Ψ_s) (MPa)				
Control	-0.59 ^z	-0.62 a	-0.67 a	-0.68
Kaolin	-0,6	-0.69 ab	-0.75 ab	-0.68
Net	-0,67	-0.74 b	-0.78 b	-0.68
Stomatal conductance (gs) ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$)				
Control	-	0,17	0,18	0,13
Kaolin	-	0,15	0,14	0,11
Net	-	0,18	0,17	0,1
CO ₂ assimilation rate (A) ($\mu\text{mol m}^{-2} \text{s}^{-1}$)				
Control	-	6,29	9,57	5,59
Kaolin	-	5,19	8,55	5,14
Net	-	4,3	7,7	4,35
Transpiration (E) ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)				
Control	-	6,78	5,03	4,94
Kaolin	-	6,28	4,3	4,14
Net	-	4,94	4,19	3,62

^zAverages on the same column followed by different letters are statistically different (Tukey's test, $p \leq 0.05$). All significant p values were lower than 0.05.

3. Incidence and severity of berry dehydration

The lowest damage incidence rate was observed in the Net treatment (with an average severity level of 20.7 %), followed by the kaolin (44.3 % on average) and the control treatment (56.7 % on average). Clusters with severity level 0 were always more under Net (79.3 %), followed by kaolin (55.7 %) and control (43.3 %). Among severity levels 2 to 4, the Net treatment yielded the lowest frequency values. Concerning kaolin effects, among severity levels 1 to 4, only in level 3 did the kaolin yield a lower frequency value than the control (Figure 1).

4. Chemical composition of grape berries

All values obtained were not statistically different ($p < 0.05$) among the treatments for any of the analyses performed (Table 5). Soluble solids at the time of harvest were around 24.5 Brix. Regarding fruit acidity, the Kaolin treatment showed the highest pH and the lowest titratable acidity; however, no statistical differences among treatments were detected. Similarly, the variability observed in free amino nitrogen and phenolic measurements precluded finding significant differences.

DISCUSSION

The lack of differences in light reflectance and fruit temperature between the kaolin and control treatments at the early measurement dates could be indicative of the need to increase the kaolin-based product concentration or the frequency of spraying. It has been reported that the kaolin film produces an increase in light reflectance, primarily ultraviolet and infrared from the surface of apple fruit (Glenn *et al.*, 2002), a situation that was also noticed in this study, in which the kaolin treatment produced significantly higher UV, PAR and NIR values compared to the control, at the latest measurement date, possibly due to a thicker kaolin film at the end of the season. Also, the fruit or foliage of kaolin treated vines in our experiment had a reduced fruit temperature, as was the case in apple trees, accompanied by an increased stomatal conductance in the leaves (Glenn *et al.*, 2010), which was not observed, however, in our conditions. This lack of response from the stomata could be partially explained by a mild to moderate water stress condition as indicated by *gs* values recorded in this study (Cifre *et al.*, 2005). On the other hand, the use of shading nets produced a reduction in plant (data not shown) and fruit temperature as previously reported elsewhere for blueberries (Lobos *et al.*, 2012 and 2013). Likewise, a lower incidence and severity of berry damage was observed as compared to both kaolin and control plants.

The combination of ultraviolet and temperature has been proposed as the main factor inducing sunburn (Glenn *et al.*, 2002). This might be a likely explanation for the lower incidence and severity of fruit damage observed in the Net and Kaolin treatments as compared to the control. Moreover, given the higher incidence and severity of berry damage observed in the Kaolin vs. the Net treatment, it is possible that an insufficient coverage with kaolin after the first spraying led to more

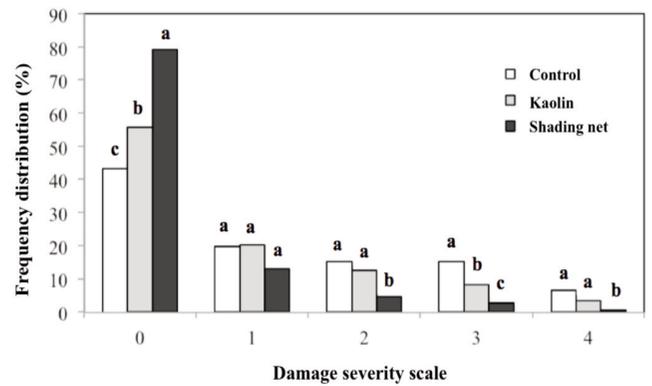


Figure 1. Fruit dehydration severity levels. Bars with the same letters by severity level represent no statistical differences (Tukey's test, $p \leq 0.05$).

dehydrated berries compared to Net plants. In other studies conducted in vineyards, but aiming at different objectives, higher kaolin doses of up to 60 g L^{-1} with a wetting of 950 L ha^{-1} have been used (Glenn *et al.*, 2010; Shellie and Glenn, 2008). In these cases, the effects of kaolin treatments varied depending on the vine water status. For instance, well irrigated vines had lower canopy temperature, increased leaf water potential and reduced *gs*, and presented slight fruit compositional variations depending on the grape cultivar analyzed.

With regards to plant water status and leaf gas exchange, all Ψ_s values were within weak water restriction (Sibille *et al.*, 2007). In this case, two mid-season measuring dates showed statistical differences, and only between Net and control treatments. Similar to a prior report conducted in blueberries (Lobos *et al.*, 2009 and 2012), shading under black nets (50 % shade) produced slightly lower (more negative) Ψ_s values compared with no-shaded plants, accompanied with a higher specific leaf area as to improve mesophyll CO_2

Table 5. Effects of vine netting and Kaolin-based sprayings on the juice chemical composition, the concentration of total phenolics, and the color at 520 nm of Cabernet-Sauvignon grapes

Treatment	Density g L^{-1}	pH	Titrateable acidity $\text{g L}^{-1} \text{ Eq. Sulfuric acid}$	FAN ^x mg L^{-1}	Total phenolics (Abs. 280 nm) AU ^y	Color (Abs. 520 nm) AU
Control	1103.00 ^z	3,55	4,16	300,53	0,2003	0,1107
Kaolin	1105	3,62	3,93	307,74	0,1993	0,1323
Net	1103,66	3,55	4,07	301,63	0,178	0,1117

^xFAN: Free amino nitrogen

^yAU: Absorbance units

^zAll p values were not significant.

diffusion. Therefore, when water restriction is present, more negative stem water potential values could be found under shaded treatments.

Also, the *A* and *E* results are in agreement with those by Glenn *et al.* (1999 and 2010) and Kerns and Wright (2000), in which no changes in photosynthesis after kaolin treatments were observed. Moreover, Morandi *et al.* (2011) and Otero *et al.* (2011) did not find changes in transpiration when shading nets were used in apple and citrus. Similar to Lobos *et al.* (2012), our results indicated that shading nets (50 % shade) produce no changes in *A* values. Other studies have reported that treatments with kaolin do not reduce photosynthesis and plant growth, but lessen the adverse effects of water stress (Glenn *et al.*, 1999; Kerns and Wright, 2000), and the photo-inhibition caused by intense solar radiation and high vapor pressure deficit in warmer areas (Lo Verde *et al.*, 2011).

The results of chemical composition were in agreement with those by Glenn *et al.* (2010), who did not observe differences in soluble solids, pH and titratable acidity of berries with and without kaolin applications on Merlot and Viognier. Despite the lack of compositional changes, sunburnt, dehydrated or shrivel fruit can have detrimental effects on fruit yield, they can cause fruit rejection, and they can adversely affect wine quality through a lower aroma potential (Bonada *et al.*, 2013; Mira de Orduña, 2010).

CONCLUSION

Both of the treatments tested (i.e. kaolin-based and fruit zone netting treatments) were able to moderate the effects of excessive radiation and temperature on fruit, without affecting the physiology and fruit composition variables measured. This result is particularly relevant in areas in which the effects of climate change have produced increments of radiation and mean temperatures during the growing season. The results also indicated the importance of the appropriate timing and - in the case of kaolin - product's dosage for higher efficiency. Further studies are required to evaluate the effects of kaolin-based particle films or fruit zone netting treatments under different growing conditions, as well as the effects of residual kaolin on wine quality.

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