Kaolin foliar-application improves the photosynthetic performance and fruit quality of Verdejo grapevines

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Abstract. Currently there is an urgent need to adapt vineyards to climate change in order to maintain and improve the quality of wines. In this context, it has been shown that the creation of a film of mineral particles, such as kaolin, on the vegetation can reduce stress caused by high temperatures in plants. The present study evaluated the effects of kaolin foliar-applications, from fruit set to veraison, on the physiological and agronomic behavior of a Verdejo white variety vineyard located in DOP Rueda (Spain). Compared to the controls, treated plants showed an increase in the photosynthetic performance, registering higher values of chlorophyll fluorescence parameters such as Fv/Fm, $\Phi PSII$ and ETR, and lower values of F0. Without affecting vigor and yield, grapes from treated vines produced musts with lower pH and phenolic content and greater color luminosity than those of untreated vines. The presented results suggest that kaolin treatments could be an effective tool to minimize the negative effects of climate change on the quality potential of white grape varieties grown in continental areas.

1 Introduction

Global warming has been affecting diverse viticulture regions around the world in the last years, causing severe summer stresses to the vines and therefore influencing negatively the grape quality [1,2]. Climate change have triggered a temperature increment, that more frequently exceeds 35°C, being a critical threshold for vegetative and fruit development of the vine [3,4]. The global warming generates an increase in sunburn damage, early dates on flowering and veraison and accelerated grape ripening, with an increase in the accumulation of sugars; a faster degradation of organic acids in grapes and an increase in pH, untypical aromatic profile, as well as a decoupling between technological and phenolic maturation [1]. Therefore, the harvest anticipation in the calendar produces wines with a higher alcoholic degree and lower acidity, causing negative consequences on their organoleptic characteristics [5].

Various techniques have been developed to adapt the vineyard to global warming challenges. The formation of mineral particles film such as kaolin in plants has been demonstrated to be a helpful and economic tool to reduce heat stress conditions during fruit development [6,7] and to increase drought tolerance in grapevines [8-10]. Kaolin has been used as an effective short-term climate change mitigation strategy by its property to reflects the ultraviolet and infrared radiation, which help to decrease

the leaf temperature, increase the photosynthetic efficiency by reducing photoinhibition [11], and improve the qualitative characteristics of the grapes [12-14]. Moreover, the kaolin film has exhibited protective properties against insect attacks [15,16].

Several studies have indicated that kaolin foliar application had no significant influence on soluble solids and total acidity of the must [6,12 17,18], whereas other authors reported an increased in grape maturity in treated plants in different climate conditions [19,20]. Additionally, an increase in the polyphenol and anthocyanin contents by the action of kaolin treatment has been reported in red grapes, without affecting vine yield [7,12,17,21].

White vine varieties are being highly affected by climate change due to their lower heat demands than red grapes varieties [22]. However, the effect of kaolin film on the composition of white grapes has been scarcely studied. On the other hand, it would be interesting to contrast the effects of kaolin film on grape quality in zones with a continental climate, where episodes of high temperature are not as frequent and intense as in warmer growing areas.

Therefore, the aim of this work was to evaluate the protective effect of kaolin treatment against the effects produced by climate change in Verdejo white grapevines

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growing in a continental climate, studying its influence on the photosynthetic performance and the quality potential of the fruits.

2 Material and methods

2.1 Experimental design

The experiment was carried out in 2021 in La Seca (Valladolid, Spain), within Rueda Designation of Origin. The vineyard corresponds to Verdejo variety, grafted on 110-Richter rootstock. Vines are conducted on double cordon, in a planting frame $3.0 \times 1.5 \text{ m}$ (2222 vines/ha), with a load of about 35,000 buds/ha. The vineyard was drip irrigated, receiving globally throughout the cycle an average water supply of around 30% of the reference evapotranspiration.

The experiment was performed in a randomized complete block design with four replications. The elementary plots were made up of 8 plants for kaolin treated (K) and control vines (C), leaving a border plant between each two plots. Before treatments, light defoliation was carried out manually in the cluster area in all plants. Kaolin treatments were performed three times between fruit set and veraison: 20, 42 and 63 days after full bloom (DAFB): June 29, July 20 and August 10. On each of these dates, all vegetation and clusters were treated with kaolin particles at a dose of 5% (w/v), using the commercial product Surround WG® (BASF Agricultural Solutions España). For the first application, the solution included 0.05% Agral® (Syngenta Agro, Madrid, Spain), a nonionic surfactant. The control plants were sprayed with water plus surfactant on the first date, and with water in the other two. All treatments were applied with a manual sprayer, on both sides of the trellis, to full wetness.

2.2 Meteorological conditions

Meteorological conditions recorded in study area during 2021, as well as the temperature average values in the last 10 years are shown in Table 1. The mean annual temperature of 2021 was similar than the average of last 10 years, being 2021 a rainier year. June and July 2021 registered lower monthly mean and maximum temperatures than the average of the last 10 years, but higher in August, which was the hottest month in 2021.

Critical maximum temperatures in 2021 were recorded on 11, 17, 20 and 21 July, reaching values between 34 and 35 °C, while absolute maximum temperatures were around 35.5 and 38.6 °C from 12 to 15 August. However, it was notable that during 2021 the days with extreme temperatures were very few, registering 20 days with temperatures above 32 °C and only 6 days with temperatures above 35°C during July and August.

Of the four days in which the leaf temperature was measured during the study (July 8 and 30; August 16 and 31), it was observed that the hottest days were on July 30 (52 DAFB) and on August 31 (84 DAFB), without reaching maximum temperatures higher than 32°C on both days.

2.3 Field data collection

Water status measurements of the plants, as stem water potential, were tested at 30, 51, 63 and 84 DAFB and photosynthetic activity was checked at 30, 52, 69 and 84 DAFB. Stem water potentials were measured between 11 and 13 hours (solar time) in adult leaves, on the shaded side of the trellis, previously covered with aluminum bags for 1.5 hours before measurement, using a Scholander-type pressure chamber (Solfranc Technologies SL, Spain).

Table 1. Monthly values of maximum absolute (Tmax abs), maximum (T max), average (Tavg), minimum absolute (Tmin abs), minimum temperature (Tmin) (°C) and precipitation (P, in mm), registered in the meteorological station of Rueda, Spain (VA103).

	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
2021												
Tmax abs	18.2	17.4	25.8	22.6	31.1	32.6	35.5	38.6	33.6	26.9	15.7	16
Tmax	7.0	13	15.7	16.9	21.8	26.5	29.7	31.1	24.4	20.8	10.8	11.2
Tavg	2.6	8.3	8.8	10.9	14.8	18.8	21.6	22.1	17.7	13.2	5.26	6.4
Tmin	-1.0	4.3	2.5	5.7	7.9	11.7	13.2	13.2	12.1	6.7	0.7	2.7
Tmin abs	-9.1	-1.6	-2.3	-1.1	3.4	8.4	7.6	8.0	6.2	0.8	-2.0	-3.0
Р	11.2	51.6	5.8	61.8	19.1	57.9	0.8	4.1	47.2	31.7	45.6	30.8
2010-2020												
Tmax abs	14.1	17.1	21.2	24.9	29.5	35.5	36.3	36.4	33.1	27.14	19.9	15.1
Tmax	8.3	10.8	13.0	17.5	22.0	26.8	30.8	30.3	26.2	20.3	12.5	8.8
Tavg	3.8	5.1	8.0	11.3	15.1	19.3	22.5	22.0	18.2	13.2	7.6	4.4
Tmin	0.2	0.1	3.5	5.4	8.2	11.7	14.0	13.7	11.0	7.1	3.5	0.7
Tmin abs	-5.8	-4.8	-3.0	-0.3	2.5	5.5	8.1	8.1	5.5	0.2	-2.9	-5.7
Р	29.9	21.1	36.0	41.4	22.1	17.2	6.8	4.2	18.0	34.4	35.1	30.0

Net assimilation (μ mol CO₂/m²/s), leaf and ambient temperature (°C), and chlorophyll fluorescence parameters were determined with a LI-Cor 6400 portable infrared gas analyzer (IRGA) equipped with a 6400-40 leaf chamber pulse width modulation fluorometer (Li-Cor, Inc. Lincoln, Nebr., USA). The fluorescence parameters measured were: minimum fluorescence (F_0) , efficiency (**PSII**) and maximum efficiency of photosystem II (F_v/F_m), apparent electron transport rate (ETR) and photochemical (qP) and non-photochemical (qN) quenching. Photosynthesis measurements were taken between 11 and 13 hour (solar time) on the interveinal space of the right main lobe of exposed leaves of the middle zone of the shoot, on a sample of two leaves in each elemental plot. The airflow rate through the leaf chamber was kept at 500 µmol/s.

The total production per plant was determined at harvest. 100 berry weight was obtained from a sample randomly collected from each elemental plot. Vigour was estimated as mean pruning weight.

2.4 Grape composition analysis

The harvest of the trial was carried out when the average value of the total soluble solids content of the must samples reached 21.3 °Brix. The must obtained from 100 berry samples in each elementary plot was used to determine the total soluble solid content (°Brix), pH, titratable acidity, yeast assimilable nitrogen, potassium concentration, total polyphenol index and color parameters (CIELAB) according to OIV methods [23].

2.5 Statistical analysis

Student's t-test were applied to evaluate the effects of the treatment with kaolin on different variables studied. Data analysis was performed with version 9.2 of the SAS software package (SAS Institute Inc., Cary NC, USA).

3 Results and Discussion

3.1 Leaf temperature

Figure 1 shows the differences between leaf and air temperature at midday registered in treated and untreated vines, during four different growth stages.

Leaves of treated plants had always lower temperature than control ones, showing significant differences at 52 and 84 DAFB (the warmest days on which it was measured). The effect of kaolin treatment to reduce the canopy temperature have been previously reported [11,24,25]. The lower leaf temperature obtained in treated plants is related to the solar reflection effect of kaolin particles, which reduces the risk of leaf and fruit damage from sunburn and high temperatures [9]. This temperature drop shows a reduced leaf stress by the decrease in H_2O_2 content and catalase activity in the leaves [26].

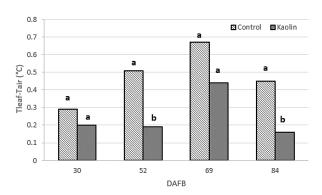


Figure 1. Differences between mean leaf and air temperatures (Tleaf-Tair), measured around midday in Verdejo grapevines, treated with kaolin and without treatment, at four different days after full boom (DAFB).

3.2 Water status and photosynthetic performance

Similar to previous findings [8,20], the values of stem water potential measured in treated and untreated plants did not show significant differences (average values from -0.84 to -0.86 MPa) in all test dates, indicating that kaolin film did not influence the vine water status.

The net CO₂ assimilation of treated plants at 52 and 84 DAFB tended to be lower than control plants but with no significant differences at p<0.05 (data not shown). It has been reported that the effect of kaolin film on the photosynthesis is variable, decreasing net photosynthetic rate under optimal hydration and when kaolin is used in environments with low irradiance [27,28]. However, kaolin is able to increase net assimilation in water limited environments, with high temperatures or salinity [10]. The values obtained from the meteorological station at the study site showed that July had very few hot days, without reaching maximum temperatures above the optimum (see Sect. 2.2).

Significant differences found were between experimental treatments for the leaf chlorophyll fluorescence parameters (Table 2). The plants treated with kaolin had higher values of F_v/F_m, ΦPSII, qP and ETR, and lower rates of F_0 compared with the control ones, in agreement with the results of Dinis et al. [30] in white grapes cv. Cerceal. The increases in the efficiency of PSII and photochemical quenching occurred in treated plants would be due to the protective effect of the kaolin film, which decrease the susceptibility to photoinhibition [25,29]. The decrease in F0 observed in treated plants showed that the photoprotective capacity of the leaves was not exceeded and photoinhibitory damage in the PSII had prevented [31]. Higher values of qN were observed at 69 and 84 DAFB in treated vines compared with control vines (Table 2). This means that the dissipation of excess energy produced by heat is greater in treated plants, with the aim to avoid photosynthetic damage by oxidation [32].

Table 2. Chlorophyll fluorescence parameters measured at noon in different days after full bloom (DAFB) in leaves of kaolin treated and control grapevines.

Treatment	DAFB	Fo	F _v /F _m	ΦPSII	ETR	qP	qN
Control	30	473.7a	0.34a	0.15a	98.5a	0.43a	1.53a
Kaolin		448.7a	0.39a	0.15a	95.3a	0.42a	1.55a
Control	52	444.7a	0.40b	0.13b	77.5b	0.33b	1.69a
Kaolin		342.2b	0.44a	0.16a	100.7a	0.37a	1.76a
Control	69	440.6a	0.38a	0.17b	106.8b	0.45a	1.55b
Kaolin		407.54a	0.39a	0.19a	122.4a	0.48a	1.67a
Control	84	434.9 a	0.35b	0.15 b	96.8b	0.43b	1.54b
Kaolin		378.3b	0.37a	0.17 a	113.3a	0.48a	1.59a
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Parameters: basal fluorescence (F₀), maximum quantum efficiency of photosystem II (F_v/F_m), effective PSII efficiency (Φ PSII), photochemical quenching (qP), electron transport rate (ETR, µmol e- m⁻² s⁻¹), and non-photochemical quenching (qN). Different letters indicate significant differences between means within the same the day (p < 0.05).

3.3 Vigor and yield

The application of kaolin had no significant influence on yield (average values of 4.35 kg/vine) and pruning weight (average values 1.25 kg/vine). Previous reports have also shown that the foliar reflective film did not modify the yield and vigor of the vineyard [8,17,21].

3.4 Grape composition

The soluble solids, yeast assimilable nitrogen and potassium concentrations of the must were not affected by kaolin foliar treatment (Table 3). The values of titratable acidity in must from treated plants tended to be higher than in controls, but without significant differences at p<0.05. It was observed that kaolin treatment decreased the pH values in comparison with control plants. The protective film might have decreased the temperature of the grapes and tended to reduce the respiratory breakdown of acidity in grapes. Recent studies showed the same tendency of pH in Touriga Franca [33] and Cerceal grapes [30] treated with kaolin. Obtaining musts with lower pH is very interesting to produce balanced wines in the context of climate change.

The total polyphenol index was 16% higher in grapes from kaolin treated plants than controls (Table 3). Various authors [7,30,34] have reported an increase of the anthocyanin and phenolic concentrations in the berries at the end of the ripening associated to kaolin treatments. According to Dinis et al. [13], the improvement in the phenolic concentration could be due to kaolin treated plants adapt better to excessive solar radiation at reducing water loss through the decrease in leaf and fruit tissue temperature [34]. This improvement has been also related to the kaolin influence of stimulating phenylpropanoids and flavonoid-flavanol pathway at molecular level [14, 33]. The polyphenol content is an essential indicator of grape quality [35] because it provides important compounds which contribute greatly to the organoleptic properties of the wine.

Most of the CIELAB parameters did not show significant differences between musts analyzed (Table 3). Nevertheless, it was notable that the must from treated vines showed higher values of lightness than control plants.

 Table 3. Mean values of must composition parameters

 obtained in vines treated with kaolin and without treatment.

Parameters	Treatment			
Must composition	Control	Kaolin		
°Brix	21.3a	21.3a		
pH	3.43a	3.40b		
Titratable acidity (g/L)	6.15a	6.24a		
Total Polyphenol index	15.5a	13.01b		
Assimilable Nitrogen (mg/L)	180.3a	176.4a		
Potassium content (mg/L)	1715.4a	1162a		
CIELAB color parameters				
L*	88.2b	91.5a		
a*	0.32a	0.29a		
b*	9.34a	14.93a		
С	9.35a	7.94a		
h	88.01a	88.02a		

Different letters represent significant differences between treated and untreated plants(p<0.05).

The preliminary results obtained in this work showed that kaolin treatment applied triggered an improvement in the plant physiology at increasing the efficiency of photosystem II. Moreover, kaolin film had positive influence in the composition of white Verdejo grapes under the conditions of the trial, at obtaining berries with high phenolic compounds, and musts with lower pH and greater color luminosity. The kaolin treatment could contribute to improving the quality of white wine grapes in the context of climate change, even in continental areas where high temperature stress is limited.

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References

- A. Palliotti, S. Tombesi, O. Silvestroni, V. Lanari, M. Gatti and S. Poni. Sci. Hort. **178**, 43-54 (2014)
- D. Santillán, A. Iglesias, I. La Jeunesse, L. Garrote, V. Sotes. Sci. Total Environ. 657, 830-852 (2019)
- K. Mori, N. Goto-Yamamoto, M. Kitayama, K. Hashizume. J Exp Biol. 58, 1935-1945 (2007)
- 4. S. Poni, M. Gatti, M, A. Palliotti, Z. Dai, E. Duchene, T.T. Truong, G. Ferrara, A.M.S.

Matarrese, A. Gallotta, A. Bellincontro, F. Mencarelli, S. Tombesi. Sci. Hort. **234**, 445-462 (2018)

- 5. C. Van Leeuwen and A. Destrac-Irvine. Oeno-one **51**, 147-154 (2017)
- L. Brillante, N. Belfiore, F. Gaiotti, L. Lovat, L. Sansone, S. Poni, and D. Tomasi. PLoS ONE 11, 6 (2016)
- 7. D. Kok, and E. Bal. Erwerbs-Obstbau 60, 39-45 (2018)
- 8. D.M. Glenn. J. Am. Soc. Hortic. Sci. 135, 25-32 (2010)
- 9. D.M. Glenn. HortScience. 47, 710-711 (2012)
- C. Brito, L.T. Dinis, J. Moutinho-Pereira, C. Correia. Sci. Hortic. 250, 310-316 (2019)
- A. Conde, A. Neves, R. Breia, D. Pimentel, L.T. Dinis, S. Bernardo, C.M. Correia, A. Cunha, H. Geros, J. Moutinho-Pereira. J. Plant Physiol. 223, 47-56 (2018)
- 12. K.C. Shellie. Am. J. Enol. Viti. 66, 348-356 (2015)
- T. Dinis, H. Ferreira, G. Pinto, S. Bernardo, C.M. C orreia, J. Moutinho-Pereira.Photosynthetica 54, 47-55 (2016a)
- A. Conde, D. Pimentel, A. Neves, L.T. Dinis, S. Bernardo, C.M Correia, H. Ceros, Moutinho-Pereira, J. Front. Plant Sci. 7, 7 (2016b)
- 15. F. Tacoli, E. Cargnus, F.K. Moosavi, P. Zandigiacomo, F. Pavan. J Pest. **92**, 465-475 (2019)
- S. Tombesi, A. Nardini, T. Frioni, M. Soccolini, C. Zadra, D. Farinelli, S. Poni, A. Palliotti. Sci Rep. 5, 12449 (2015)
- 17. Shellie, K.C. and B.A. King. Am. J. Enol. Vit. 64, 214-222 (2013b)
- T. Frioni, S. Saracino, C. Squeri, S. Tombesi, A. Palliotti, P. Sabbatini, E. Mugnanini, S. Poni. Plant Physiol. 242, 153020 (2019)
- Y. Wang, T. Xue, X. Han, L. Guan, L. Zhang. Hort. Sci. 55, 1987-2000 (2020)
- K.C. Shellie and Glenn D.M. HortScience 43,1392-1397 (2008)

- 21. K.C. Shellie and B.A. King. Am J Enology and Viticulture 64, 223-230 (2013a)
- 22. L.T. Dinis, C.M. Correia, H.F. Ferreira, B. Gonçalves, I. Gonçalves, J.F. Coutinho, M.I. Ferreira, A.C. Malheiro, J. Moutinho-Pereira. Sci. Hortic. **175**, 128-138 (2014)
- 23. OIV. Compendium of international methods of wine and must analysis. International Organisation of Vine and Wine, Paris, France (2020)
- 24. A. Garrido, J. Serôdio, R. De Vos, A. Conde, A. Cunha. Agronomy **9**, 6852019 (2019)
- 25. E. Cataldo, M. Fucile, G.B. Mattii. Agriculture 12, 491 (2022)
- 26. P. Paciello, F. Mencarelli, A. Palliotti, B. Ceccantoni, C. Thibon, P. Darriet, M. Pasquini, A. Bellincontro. J. Sci. Food Agric. 97 (2017)
- V. Cantore, B. Pace, R. Albrizio. Environ. Exp.Bot. 66, 279-288 (2009)
- F. Boari, A. Donadio, M.I. Schiattone, V. Cantore. Agric. Water Manag. 47, 154-162 (2015)
- L.T Dinis, S. Bernardo, A. Luzio, G. Pinto, M. Meijon, M. Pinto-Marijuan, A. Cotado, C. Correia, J. J. Moutinho-Pereira. Plant Physiol. 220, 181-192 (2018)
- L.T. Dinis, S. Bernardo, C. Matos, A. Malheiro, R. Flores, S. Alves, C. Costa, S. Rocha, C. Correia, A. Luzio, J. Moutinho-Pereira. Agronomy 10, 1422 (2020)
- F. Valladares, R.W. Pearcy. Plant Ce. Environ. 20, 25-36 (1997)
- 32. N.R. Baker. Annu. Rev. Plant Biol. **59**, 89-113 (2008)
- R.K. Singh, J. Afonso, M. Nogueira, A.A. Oliveira, F. Cosme, V. Falco. Biology 9, 58 (2020)
- E. Khaleghi, K. Arzani, N. Moallemi, M. Barzegar. Food Chem. 166, 35-41 (2015)
- L.J. Mills, J.C. Ferguson, M. Keller. Amer. J. Enol. Viticult. 57, 194-200 (2006)