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Impact of pre-veraison cluster treatments with nordihydroguaiaretic acid on the composition of verdejo grapes and wines

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A B S T R A C T

In warmer growing regions, climate change is imposing a general increment of the temperature to the vineyards, with a greater frequency of extreme phenomena such as heat waves and droughts. The global warming is advancing and accelerating the ripening process of the grapes, which produces wines with higher alcoholic degree, lower acidity and poorer sensory characteristics. The abscisic acid (ABA) accumulation in grapes plays a crucial role in the onset of ripening, so that applying ABA inhibitors as nordihydroguaiaretic acid (NDGA) could provide musts with better sugar/acidity balance as maturation process occurs later, under lower temperatures. In a field experiment, we studied the effects of pre-veraison NDGA treatments to clusters on the composition of Verdejo must and wine. Treatments consisting of three repeated applications with 100 mM NDGA each delayed the harvest date by 4 days versus untreated controls. Total acidity, tartaric acid concentration and total polyphenol index of the must increased in treated plants, at a constant level of total soluble solid content. Data from the analysis of volatile organic compounds indicated that NDGA could contribute to the coupling of technological and aromatic maturity of grapes, then improving the aromatic profile of wines by decreasing the concentrations of total acids. Our results demonstrate that NDGA applications are potentially useful to mitigate the negative effects of global warming on the quality of wines, by increasing the acidity and polyphenol content of the musts and improving the aromatic profile of the wines.

1. Introduction

Climate change is compromising the quality of the grapes and wines in different regions around the world (Cramer et al., 2018; Dejanovic et al., 2019). The gradual increase of temperature along with increasingly frequent and intense episodes of heat stress are a critical threshold for vegetative and fruit development of the grapevine (Mori et al., 2007; Poni et al., 2018), generating a reduction of the grape growing season (Moran et al., 2018), earlier flowering and veraison dates, an accelerated grape ripening which produces an increase in the accumulation of sugars; faster degradation of organic acids, an increase in pH and untypical aromatic profile, as well as a decoupling between technological and phenolic maturation (Palliotti et al., 2014). Therefore, this harvest anticipation in the calendar results in the production of wines with a higher alcoholic degree, lower acidity, and a deterioration of their organoleptic characteristics (Van Leeuwen & Destrac-Irvine, 2017).

The crop adaptation to global warming, in a short term, can be based on unconventional management techniques that lead to later ripening, in which the grapes would be to lower temperatures during day and a greater day-night thermal jump (Zheng et al., 2017), and thus produce wines with lower alcohol content, greater acidity, and a better aromatic profile. The application of plant growth regulators capable of interacting with fruit development process could be a useful tool to achieve this objective. Being abscisic acid (ABA) the main phytohormone controlling fruit ripening, ABA inhibitors as nordihydroguaiaretic acid (NDGA) could be potentially interesting products in this context.

NDGA inhibits the enzyme 9′-cis-epoxycarotenoid dioxygenase (NCED), which is an important regulatory enzyme in the ABA biosynthetic pathway (Han et al., 2004). Yu et al. (2020) have reported that NDGA downregulate the expression level of VvNCED1, a key gene for ABA synthesis, and decreased the expression level of VvCYP707A1 gene which is related to ABA degradation. Thunyamada et al. (2018) have demonstrated that the application of NDGA to the clusters before veraison in Shine Muscat grapes promoted the auxin signaling pathway and the activity of related genes with gibberellins, while suppressing the expression of genes for the synthesis of ethylene and ABA.

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Recently, it has been revealed the ability of NDGA to reduce the cracking (Yu et al., 2020) and postharvest abscission of table grapes (Zhu et al., 2022) by decreasing the production of ABA and ethylene. On the other hand, the ability of NDGA leaf treatments to delay grape ripening has also been reported (Saito et al., 2022). However, there are little information about the potential interest of NDGA treatment in field to improve grape composition under climate change condition. To our knowledge, its possible effects on wine quality have not been studied until now.

Besides being a crucial factor for controlling fruit ripening, ABA is involved in fundamental processes of plant development, such as the regulation of stomatal opening or the induction of responses to environmental stress (Ferrandino & Lovisolo, 2014). Therefore, it would be interesting to study the possible effects of NDGA treatment on physiological parameters, vigor and yield of the vines, which would have an indirect impact on grape and wine quality. The aim of this work was to evaluate the effects of the pre-veraison application of NDGA on agronomic parameters of the vineyard, harvesting date and composition of Verdejo white grapes, in order to mitigate the adverse effects of climate change on wine quality.

2. Materials and methods

2.1. Study location and experimental design

The investigation was conducted in a Verdejo/110 Richter vineyard in full production, located in La Seca (Valladolid, Spain), within Rueda Designation of Origin (latitude 41°26′58.9″N 4°52′10.9″W; altitude 731 m). Row spacing is 3.0 m and vine spacing 1.5 m, giving a total of 2222 vines/ha. The vines are pruned in double Guyot and trained in a trellis system, with a load of about 35,000 buds/ha. The vineyard was drip-irrigated, receiving globally throughout the season an average water supply of around 30% of the reference evapotranspiration.

A field trial was carried out in 2022 to evaluate the effects of three successive applications of NDGA to the clusters at 45, 55 and 65 days after full bloom (DAFB), using a concentration of 100 μM of NDGA 97% (Thermo Scientific Chemicals, Basingstoke, UK) in each. This concentration is considered effective without toxicity (Lin et al., 2018). The experiment was performed in a randomized complete block design with three replications. The elementary plots were made up of 8 plants, leaving a border plant between each two plots.

A light defoliation was carried out manually in the cluster area in all plants before treatments. All treatments were applied with manual sprayers, on both sides of the trellis to full wetness. The solutions included 0.05% Agral® (Syngenta Agro, Madrid, Spain), a non-ionic surfactant. The control plants were sprayed with water plus Agral®.

2.2. Meteorological conditions

The temperature data recorded in the field trial are shown in Fig. 1. The year 2022 was much warmer than the 10-year-site average, showing higher mean temperatures from June to August (22.5 vs 21.3 °C). The highest temperature recorded in 2022 was 40.5 °C, in July. The number of days with maximum temperatures above 32 °C and 35 °C were 50 and 26, respectively.

The precipitation in 2022 (343 mm) was higher than the average of the last 10 years (296 mm). The value of cumulative rainfall from June to August was 26.7 mm in 2022.

2.3. Field data collection

The evolution of the technological maturity degree of the grapes was evaluated by random berry sampling, every week throughout the ripening process. Forty-eight berries were collected in each elemental plot and sampling date.

Vine yield and berry size were determined at harvest. Berry weight was obtained from a sample of 100 berries randomly collected from each elemental plot. Vigor was estimated as mean pruning weight.

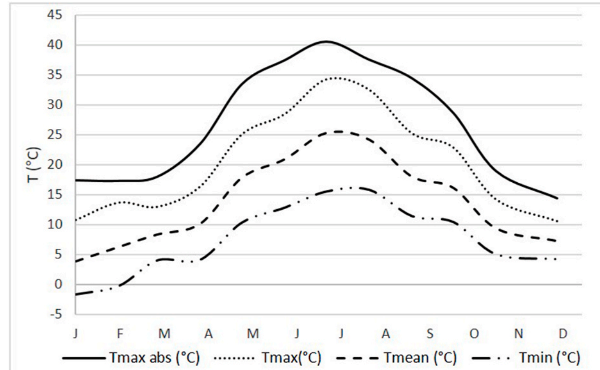

Fig. 1. Maximum absolute (Tmax abs) and monthly values of maximum (Tmax), average (Tmean) and minimum temperature (Tmin) registered in 2022. Data from meteorological station of Rueda, Valladolid, Spain (AEMET - VA103).

2.4. Winemaking process

The experimental treatments were harvested in stages, when the average soluble solids content of the must in each one of them reached a set level of 21.5°Brix.

A standard white winemaking process was used to produce wine from 20 kg of grapes, picked randomly from the harvest of each elementary plot, which were mechanically destemmed and then pressed using a pneumatic press (maximum pressure of 0.2 MPa). In each replicate, 7.5 L of must were taken, and potassium metabisulfite (Agrovin, Alcázar de San Juan, Ciudad Real, Spain) was added to them to establish the total sulfur at 40 mg/L. The musts were cleared for 24 h at 5 °C, and then racked for vinification. The alcoholic fermentation was induced with a commercial *Saccharomyces cerevisiae* yeast (Zymafkire Spark, Laffort, Bordeaux, France). Must fermentations were carried out in stainless steel tanks at a controlled temperature of 17 °C, and monitored by measuring must-wine density daily. The end of alcoholic fermentation was considered when the reducing sugar concentration was lower than 4 g/L. Free sulfur in wines was set at 40 mg/L once again, and wines were decanted at 4 °C for 48 h. After that, wines were stored for 30 days at 8 °C for tartaric stabilization in fully filled jars, without 'air space' to avoid oxidation. Before bottling, the free sulfur content of the wines was corrected until 40 mg/L and analyzed.

2.5. Basic composition of musts and wines

To analyze the composition of the musts throughout the maturation monitoring, berries were crushed with a Create® low-pressure blender (Woods & Go Design, S.L, Valencia, Spain). Total soluble solid content (TSS), pH and total titratable acidity (TA) of the must were determined in each sampling date.

At harvest, the must from 100 berries randomly collected in each elementary plot was used to determine TSS, pH, TA, malic and tartaric acid concentrations, yeast assimilable nitrogen (YAN), potassium content and total polyphenol index (TPI).

Wine basic composition was determined by measuring alcoholic grade, pH, TA and volatile acidity. All must and wine analysis were performed according to the OIV methods (OIV, 2020).

Chromatic characteristics of musts and wines were evaluated using a UV/VIS spectrophotometer (V-530, Jasco Corp., Tokyo, Japan). The CIELAB parameters (L*, a*, b*, C*, H*) were recorded using the D65 illuminant as reference (CIE, 1986).

2.6. Antioxidant activity and volatile composition of wines

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay was used to measure the free radical-scavenging capacity of the wines using the method described by Rivero-Pérez et al. (2007).

Headspace-solid-phase microextraction-gas chromatography–mass spectrometry (HS-SPME-GC-MS) was carried out to determine the concentration of volatile compounds (VOCS) in wines (Sánchez et al., 2022). A CombiPal RSI 120 autosampler (CTC Analytics AG, Zwingen, Switzerland) connected with a 7890 A gas chromatograph (Agilent Technologies, Santa Clara, USA) and a 5977-mass selective detector (Agilent Technologies) were used for the quantification of wine VOCS. The extraction of wine VOCS was carried out by the methodology described by Massera et al. (2012) with slight modifications. A 20-mL vial was filled with 5 mL of wine saturated with 3 g of NaCl (Panreac Química SLU, Castellar del Vallès, Barcelona, Spain) and 50 μL of methyl nonanoate (0.059 mg/L) and 50 μL of methyl nonanoate (0.059 mg/L) as internal standard from Merck KGaA (Darmstadt, Germany). The vial was sealed with a magnetic screw cap provided with a PTFE/silicone septum and heated at 40 °C for 15 min with agitation (250 rpm). Extraction of VOCS was performed in the headspace vial at 40 °C for 30 min with agitation (250 rpm) using the fiber 50/30 μm DVB/CAR/PDMS (Supelco, Inc., Bellefonte, USA), previously preconditioned at 270 °C for 15 min. After extraction, the fiber was introduced into the injector of GC (250 °C) to desorb the volatiles for 15 min.

The GC conditions were as follows: injector temperature, 250 °C; injection mode, splitless (1 min); capillary column, HP-Innowax column (60 m, 0.250 mm, 0.5 μm) (J &W Scientific, Folsom, CA, USA); oven temperature, 40 °C held for 5 min, then increased to 230 °C at a rate of 2.5 °C/min and then maintained at this temperature for 20 min; carrier gas, helium at a constant pressure of 22.4 psi and a flow of 1.2 mL/min. MS was operated under the SCAN mode and the mass range studied was from 30 to 200 m/z. The identification was performed by comparing GC mass spectra with pure standards and with spectra from the NIST08 y Wiley 7 libraries. Quantification was carried out using the internal standard quantification method with standards as was described by Sánchez et al. (2022). Samples were analyzed in triplicated.

2.7. Statistical analysis

Data were subjected to factorial analysis of variance (ANOVA) to evaluate the effects of the NDGA treatments and the block influence on different variables studied. Means of treated and control plants were compared using Student's t-test. An acceptance level of $p = 0.05$ was considered.

The statistical analysis was performed with version 9.2 of the SAS software package (SAS Institute Inc., Cary NC, USA).

3. Results

Berry weight, pruning weight and vine yield were not influenced by experimental treatments, obtaining average values of 183.1 g/100 berries, 1.04 kg/vine and 6.9 kg/vine, respectively.

The NDGA treatments applied significantly delayed the harvest date by four days longer than controls (97 vs 101 DAFB, $p < 0.05$). TSS of the must at 81 DAFB were in treated plants 1.3°Brix lower than in untreated ones, but these differences were not observed at the harvest date of controls (Fig. 2).

Among all the must composition parameters studied, only YAN was affected by the block factor ($F = 44.7$, $p < 0.01$), probably due to the nitrogen assimilation of plants would be modified by soil variability interblocks. The concentrations of tartaric acid and TA at harvest, for the same TSS, were significantly higher in treated plants than in controls, whereas malic acid concentration did not show significant differences (Table 1). TPI of the must obtained from the treated plants increased by 19% compared to the untreated plants. YAN in the treated plants was lower than in controls. Regarding the color components of the must, NDGA treatment increased b × and C* coordinates.

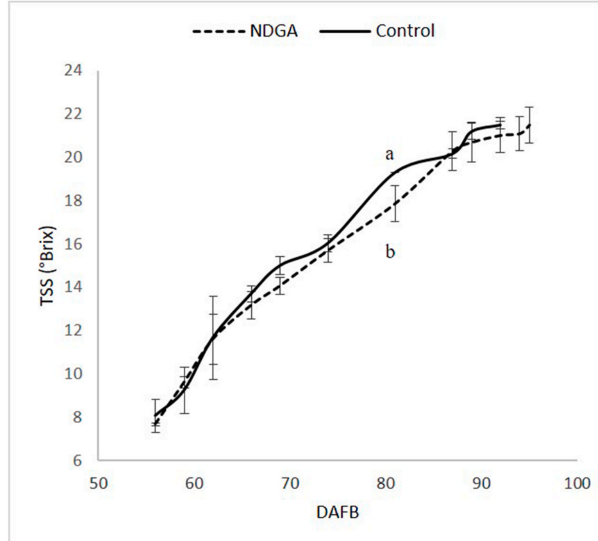

Fig. 2. Evolution of total soluble solid content of the must throughout grape ripening in vines treated with nordihydroguaiaretic acid (NDGA) and control vines. Error bars show the standard deviation of three blocks replicates. Different letters mean significant differences ($p < 0.05$).

Table 1
Mean values of must composition parameters recorded in controls and plants treated with nordihydroguaiaretic acid (NDGA).

Parameters	Treatment		Significance
	Control	NDGA	
Soluble solid content (°Brix)	21.90 ± 0.40	22.40 ± 0.83	ns
pH	3.46 ± 0.11	3.45 ± 0.04	ns
Titratable acidity (g/L)	3.64 ± 0.27	4.53 ± 0.17	**
Tartaric acid concentration (g/L)	1.12 ± 0.24	1.68 ± 0.11	*
Malic acid concentration (g/L)	1.37 ± 0.29	1.19 ± 0.49	ns
Total polyphenol index	8.90 ± 0.11	11.11 ± 0.67	**
Yeast assimilable nitrogen (mg/L)	166.23 ± 10.52	141.40 ± 15.72	*
Potassium content (mg/L)	1010.70 ± 110.72	1071.71 ± 133.49	ns
Color parameters			
L*	90.8 ± 1.1	90.9 ± 1.0	ns
a*	−0.31 ± 0.04	−0.30 ± 0.06	ns
b*	3.34 ± 0.62	4.64 ± 0.29	*
C*	3.36 ± 0.62	4.65 ± 0.29	*
H* (°)	94.67 ± 2.81	93.79 ± 1.02	ns

*Significant $p < 0.05$; **Significant $p < 0.01$; ns = not significant.

Despite the differences found in the musts mentioned above, the basic parameters of the wines were not different depending on the experimental treatments (data not shown). DPPH index did not show significant differences either and did not correlate with TPI ($r = 0.41$, $p > 0.05$).

With respect to aromatic profile, forty-three different VOCS were identified in wines, comprised of an aldehyde, 18 esters, 9 alcohols, 6 acids, 5 acetates, 3 ketones and 1 phenol. Among them, the compounds ethyl-octanoate, ethyl-dodecanoate, ethyl-decanoate, ethyl hexadecanoate, 3-methylbutan-1-ol, 2-phenylethanol and ethyl dec-9-enoate had the highest concentrations. Total volatile concentrations of wines did not show significant differences between control and treated plants, recording 308.0 mg/L and 301.6 mg/L, respectively ($p > 0.05$). As Table 2 shows, few differences in the wine aromatic profile were

Table 2
Mean values of the concentrations of volatile organic compounds in wines (mg/L) from controls and plants treated with nordihydroguaiaretic acid (NDGA).

Group/Compound	Treatment		Significance
	Control	NDGA	
Aldehydes			
Benzaldehyde	0.18 ± 0.04	0.18 ± 0.03	ns
Acids			
Total acids	10.76 ± 1.78	9.92 ± 2.05	**
Dodecanoic acid	0.31 ± 0.07	0.31 ± 0.16	ns
Dec-9-enoic acid	0.51 ± 0.19	0.29 ± 0.05	ns
Decanoic acid	3.15 ± 0.53	3.37 ± 0.57	ns
2-methylpropanoic acid	0.08 ± 0.02	0.06 ± 0.01	ns
Nonanoic acid	0.04 ± 0.01	0.05 ± 0.02	ns
Octanoic acid	6.65 ± 1.16	5.84 ± 1.62	ns
Acetates			
Total acetates	4.62 ± 2.02	3.61 ± 1.61	ns
Ethyl acetate	1.58 ± 0.89	1.15 ± 0.61	ns
Heptyl acetate	0.13 ± 0.11	0.08 ± 0.05	ns
Hexyl acetate	0.29 ± 0.07	0.19 ± 0.05	ns
Ethyl 2-phenylacetate	0.06 ± 0.05	0.04 ± 0.01	ns
3-methylbutyl acetate	4.19 ± 1.53	3.33 ± 1.88	ns
Alcohols			
Total alcohols	43.93 ± 10.26	40.37 ± 9.49	ns
3-methylpentan-1-ol	0.07 ± 0.01	0.08 ± 0.00	ns
3-methylbutan-1-ol	23.09 ± 1.32	20.92 ± 1.43	ns
3-methylsulfanylpropan-1-ol	0.16 ± 0.03	0.13 ± 0.01	ns
Propan-1-ol	0.25 ± 0.02	0.16 ± 0.04	*
2-methylpropan-1-ol	0.99 ± 0.18	0.71 ± 0.14	ns
Undecan-2-ol	0.03 ± 0.01	0.07 ± 0.02	ns
2-phenyl ethanol	19.32 ± 1.48	18.31 ± 1.17	ns
C6-alcohols			
Total C6-alcohols	0.66 ± 0.15	0.65 ± 0.08	ns
Hexan-1-ol	0.44 ± 0.14	0.38 ± 0.07	ns
Heptan-1-ol	0.22 ± 0.15	0.26 ± 0.10	ns
Ethyl esters			
Total ethyl esters	93.29 ± 10.16	87.18 ± 8.18	ns
Ethyl acetate	1.58 ± 0.89	1.15 ± 0.61	ns
Ethyl butanoate	0.20 ± 0.15	0.19 ± 0.09	ns
Ethyl heptanoate	0.28 ± 0.17	0.22 ± 0.10	ns
Ethyl octanoate	37.82 ± 14.17	30.50 ± 1.71	ns
Ethyl dodecanoate	7.56 ± 4.49	9.02 ± 3.47	ns
Ethyl nonanoate	0.09 ± 0.05	0.124 ± 0.07	ns
Ethyl hexanoate	7.24 ± 3.53	6.77 ± 2.10	ns
Ethyl decanoate	21.56 ± 11.24	23.81 ± 3.87	ns
Ethyl octadecanoate	0.30 ± 0.16	0.37 ± 0.16	ns
Ethyl hexadecanoate	3.66 ± 2.80	5.11 ± 2.84	ns
Ethyl dec-9-enoate	11.90 ± 4.89	8.71 ± 0.45	ns
Ethyl benzoate	0.07 ± 0.01	0.06 ± 0.01	*
Ethyl 4-hydroxybutanoate	0.29 ± 0.19	0.18 ± 0.04	ns
Ethyl 3-hydroxyoctanoate	0.04 ± 0.01	0.03 ± 0.03	ns
Ethyl tetradecanoate	0.33 ± 0.37	0.54 ± 0.34	ns
Ethyl pentadecanoate	0.05 ± 0.05	0.08 ± 0.04	ns
Ethyl (E)-hexadec-9-enoate	0.22 ± 0.11	0.32 ± 0.17	ns
Other esters			
Total other esters	0.36 ± 0.06	0.47 ± 0.10	ns
3-methylbutyl octanoate	0.14 ± 0.07	0.16 ± 0.08	ns
3-methylbutyl decanoate	0.22 ± 0.15	0.31 ± 0.15	ns
Ketones			
Total ketones	0.20 ± 0.01	0.23 ± 0.02	ns
Nonan-2-one	0.07 ± 0.03	0.10 ± 0.05	ns
4-hydroxybutan-2-one	0.07 ± 0.04	0.07 ± 0.01	ns
Undecan-2-one	0.05 ± 0.00	0.05 ± 0.05	ns
Phenols			
Phenol	0.03 ± 0.01	0.03 ± 0.01	ns

*Significant $p < 0.05$; **Significant $p < 0.01$; ns = not significant.

observed between experimental treatments. A lower value of the total volatile acid concentration than control was observed in NDGA treatments, as well as decreases in the concentrations of propan-1-ol and ethyl benzoate.

4. Discussion

Without modifying vigor and yield of the vines, the applications of NDGA allowed the harvesting date (must reached 21.5°Brix) to be delayed by 4 days. This delay was probably caused by the action of NDGA inhibiting ABA biosynthesis (Lin et al., 2018) and was enough, as discussed below, to generate positive changes in the must composition for the production of quality wines in growing conditions under high temperatures.

Some authors have reported that NDGA is capable of inhibiting the accumulation of sugars in grapes before veraison or during ripening when applied to clusters (Lin et al., 2018; Yu et al., 2020). Otherwise, Saito et al. (2022) indicated that NDGA applied on leaves could decrease the accumulation of sucrose in berry peel but without affecting the sugar metabolism in the flesh.

The results obtained in the present study show that TA of the must at harvest, for a constant level of TSS, tended to increase by the NDGA treatments. The concentration of acids in grape berries, especially in the case of malic acid, is highly sensitive to temperature changes (Liu et al., 2006). The rise in temperature during the ripening process can provoke an accelerated respiration of acids, which leads to a decrease in TA and an increase in pH (Rienth et al., 2014; Ruffner et al., 1976). Our results suggest that NDGA treatments might reduce the degradation of the tartaric acid throughout maturation, which would contribute to improving the potential quality of the musts in the context of climate change, by producing more balanced and fresher wines (Payan et al., 2023).

The increase of TPI was another notable change in the composition of the must from treated plants. This increase, for a constant TSS, reflects a more balanced maturation of the grapes, by reducing the decoupling between technological and phenolic maturity that occurs under high-temperature conditions (Palliotti et al., 2014). Phenolic compounds play a crucial role in white wines due to their vulnerability to oxidation and their contribution to color stability (Vilar-Bustillo et al., 2023). The higher TPI would be related to the greater chromaticity detected in the must from treated plants, with an increase in yellow tones in relation to blue ones (Gómez-Míguez et al., 2007).

The showed increases of TA and TPI in the musts from treated plants suggest that NDGA application could be useful to improve the potential quality of the grapes in vineyards under summer stress conditions. Additionally, the delay in maturation could be interesting to improve vintage management in areas where the harvest maturity window of certain cultivars is concentrated in time.

To our knowledge, this is the first time the effects of NDGA treatments on the wine aromatic profile have been studied. The wine volatile composition showed some significant changes associated with the application of the product. Thus, the plants treated with NDGA gave wines with lower concentration of total acids than untreated plants. Acids contribute to the aromatic complexity of wines, although they can present unpleasant odorants of butter and cheese at high concentrations.

This result could be associated with a higher degree of aromatic maturity in treated grapes. Previous studies (Kalua & Boss, 2010; Moreno-Olivares et al., 2020; Sánchez-Palomo et al., 2007; Sun et al., 2011) have reported a higher concentration of esters, C6-alcohols and acids in grapes with a lower degree of technological maturity. Given that in the present study the grapes were harvested with the same TSS, our results suggest that NDGA could contribute to the coupling of technological and aromatic maturity in ripening conditions under high temperature.

Regarding specific VOCs, it is interesting to remark the decrease in the concentration of propan-1-ol in wines from treated plants, an unpleasant alcohol that adds pungent notes (Moreno-Olivares et al., 2020; Zhang et al., 2015). However, NDGA treatment reduced the concentration of ethyl benzoate, which provide fruity notes (Lu et al., 2022).

5. Conclusions

Our results demonstrate that NDGA pre-veraison treatments applied to the clusters are potentially useful to mitigate the negative effects of global warming on the quality of wines. Without altering the vigor and yield of the vines, NDGA would be able to induce a more balanced grape maturity, increasing the acidity and total polyphenol index in the must at a constant level of TSS, and improving the aromatic profile of the wine. Further research is needed to better understanding of the effects of NDGA by studying different doses of the product in several growing seasons.

CRediT authorship contribution statement

Marie Azuara: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **María Rosa González:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **José Manuel Rodríguez-Nogales:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis. **Pedro Martín:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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