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Bunch transpiration is involved in the hastening of grape berry ripening under elevated temperature and low relative humidity conditions

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ABSTRACT

The present study aimed: i) to evaluate the impact of the changes in temperature and relative humidity (RH), projected by the year 2100, on grape ripening, and ii) to assess if bunch transpiration is a key physiological process involved in the advancement in grape development under future climate conditions. Fruit-bearing cuttings of *Vitis vinifera* L. cv. 'Tempranillo' were grown, from fruit set to maturity, in glasshouses under two conditions: 24°C/14°C and 55%/70% RH (day/night) (T) *vs* 28°C/18°C and 43%/58% RH (T+4). To elucidate the role of bunch transpiration in grape development in a future climate scenario, the bunches of half of the plants in the T+4 glasshouse were sprayed with an antitranspirant (AT+4). T+4 increased bunch transpiration, hastened the ripening process, increasing the rate of total soluble solid (TSS) accumulation and malic acid degradation, and reduced the concentration of total anthocyanins. The application of antitranspirant partially alleviated the effects of combined high temperature and low RH on maturation times, through lower TSS accumulation rates. Berries in AT+4 had the lowest concentrations of anthocyanins and color, likely related to a reduction in light transmittance by the antitranspirant film and to higher anthocyanin degradation due to the longer exposure to elevated temperatures. The results show a negative impact of elevated temperature and low RH on grape composition. The increased bunch transpiration under these conditions played an important role in the changes observed in phenology and sugar accumulation.

1. Introduction

According to the sixth assessment report of the Intergovernmental Panel on Climate Change land temperatures have increased by 1.59°C since the pre-industrial period (IPCC) (IPCC, 2021). Regardless of the emission scenario considered (Shared Socioeconomic Pathways, SSPs), global surface temperature will continue to increase until, at least, mid-century. Compared to the 1850–1900 period, global surface temperature at the end of the 21st century is very likely to be higher by 1°C to 1.8°C under the very low greenhouse gas (GHG) emission scenario considered (SSP1-1.9) and by 3.3°C-5.7°C under the most pessimistic GHG emission scenario (SSP5-8.5). Additionally, the frequency and intensity of hot extreme events are also expected to increase in the coming decades (IPCC, 2021). Models that project greater warming also show a stronger reduction in relative humidity (RH) in some parts of the globe, such as the Mediterranean region (Giorgi and Lionello, 2008). Consequently, rural agrosystems may suffer the major impacts of these future environmental conditions, including shifts in production areas of food crops, as well as adaptation strategies.

As other food crops, grapevine (*V. vinifera* L.) growth and performance are highly dependent on environmental factors. In the last decades, warming has driven early grape ripening with a consistent response across different locations (Ruml et al., 2016; Webb et al., 2012). Future projections indicate shorter growing seasons, as a consequence of early onset of phenophases and shorter phase duration, for different grapevine varieties and locations (Alikadic et al., 2019; Fraga et al., 2016; Garcia de Cortazar Atauri et al., 2017). The combination of higher temperatures and advanced phenology are also expected to cause strong impact on berry composition, increasing sugars, reducing organic acids, and altering secondary metabolite composition, such as

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anthocyanins and aroma precursors (Arrizabalaga-Arriazu et al., 2020; Van Leeuwen and Destrac-Irvine, 2017).

Transpiration accounts for the majority of grape berry water outflow throughout fruit development. Grape berry water loss contributes to the accumulation of solutes and ripening of the fruit. Previous studies have reported that the forced reduction of berry transpiration through the direct application of antitranspirants to the bunch (i.e di-1-*p*-menthene or vaseline) decreased the °Brix accumulation rate and delayed maturity in different grapevine cultivars (Pascual et al., 2022; Rebucci et al., 1997; Zhang et al., 2017). It has been reported that the water loss through berry transpiration reduces berry turgor, which helps to maintain the gradient of water potential between the stem and the fruit, and promote the importing of assimilates into the fruit (Morandi et al., 2010; Rebucci et al., 1997). Furthermore, since grape berry ripening is concomitant with a surplus of phloem-imported water (Keller, 2015), it has been suggested that berry transpiration may facilitate normal ripening through the discharging of excess phloem-imported water (Zhang et al., 2017). As for other fresh fruits, berry transpiration is strongly affected by environmental factors, including ambient temperature and RH, which determine vapor pressure deficit (VPD) (Poni et al., 2001; Rebucci et al., 1997; Zhang and Keller, 2015). Consequently, changes in grape berry transpiration are expected under future environmental scenarios, which may affect grape berry development and final composition.

We hypothesized that an increase in temperature combined with a reduction in RH in the air, may have an impact on grape berry development and composition, bunch transpiration being a key physiological mechanism underlying advanced maturity. Therefore, our first objective was to evaluate the effects of the projected changes in temperature and relative humidity for 2100 (temperature increase of 4°C and a reduction in HR of 12%) on the evolution of bunch transpiration, berry development and composition. Also, we aimed to assess if bunch transpiration is a key physiological process involved in the ripening of grape berries when they undergo climate change conditions. For that, fruit transpiration was artificially reduced in half of the plants cultivated at elevated temperature and low RH conditions by spraying the bunches with an antitranspirant.

2. Material and methods

2.1. Plant material and experimental design

An experiment with fruit-bearing cuttings of *V. vinifera* L. cv. 'Tempranillo' was performed in glasshouses with controlled conditions. In winter, dormant cuttings were taken from a vineyard of the Estación de Viticultura y Enología de Navarra (EVENA, Government of Navarra). Fruit-bearing cuttings were produced according to Mullins (1966) with some modifications (Arrizabalaga et al., 2018). Briefly, three-node cuttings were rooted in a warm bed at 27°C, after the application of indole butyric acid (300 mg L^{-1}). The cuttings were maintained into a cool room (5°C) for four weeks. The rooted plants were transplanted to pots containing a peat:perlite (2:1 v/v) based substrate and transferred to the glasshouse. The plants grew until fruit set at 25°C/15°C and 50%/70% RH (day/night), with natural daylight supplemented with high-pressure metal halide lamps (OSRAM®, Munich, Germany), with 500 μmol m^{-2} s^{-1} of photosynthetically photon flux density at the inflorescence level. Only one flowering stem was allowed to develop on each plant. Vegetative growth was controlled until fruit set, by manual pruning, in order to maintain the plants with four leaves until fruit set. Irrigation was performed with the nutrient solution described in Ollat et al. (1998) alternated with water.

Once fruit set was complete, plants were divided into two groups and placed into two different glasshouses. One of the glasshouses was set at 24°C/14°C and 55%/70% RH day/night (T), control conditions, and the other one was set at 28°C/18°C and 43%/58% RH day/night (T+4), climate change scenario. The estimated VPDs during the day were 1.34 kPa and 2.15 kPa, T and T+4, respectively. The temperature values in the T+4 treatment were chosen in order to simulate the changes projected for the end of the 21st century, as per the SSP5-8.5 greenhouse emission scenario derived from the concentration-driven CMIP6 model simulations (IPCC, 2021). The RH values were chosen according to the predictions of the ENSEMBLES models (Max Planck Institute model, MPI-ECHAM5) as reported in Leibar et al. (2015). In order to study the involvement of bunch transpiration on ripening and composition under a future temperature and RH scenario, at the onset of veraison, half of the bunches in the T+4 treatment was sprayed with di-1-*p*-menthene ($C_{20}H_{34}$, VaporGard®, Miller Chemical & Fertilizer Co., Pennsylvania, USA) 10% diluted in water (AT+4). The antitranspirant was applied every 15 days to ensure a reduced berry transpiration rate during the whole ripening period. The number of plants per treatment was 38, both in T and T+4, and 13 in AT+4, thus giving a total number of biological replicates (considering one plant as a biological replicate) of between 5 and 11 depending on the phenological stage. All the plants grew until maturity, which was considered when the berry TSS content was about 23°Brix. The main shoot was allowed to grow up to about 12 leaves in order to maintain a balanced leaf area to fruit fresh mash ratio.

2.2. Influence of antitranspirant on light transmittance

To quantify the effect of di-1-*p*-menthene film on light transmittance, a quartz spectrophotometer cuvette was sprayed on one of the internal sides with the antitranspirant at the same concentration used in the experiment. The side of the cuvette was sprayed both once and twice, simulating cumulative applications received by the berries throughout the experiment. After each application, the cuvette was left to dry and the transmittance was measured with spectrophotometer UV/Vis (UVMini 1240, Shimadzu, Kyoto, Japan) from 280 nm to 1000 nm. The blank was done with a non-sprayed cuvette. The area under the transmittance curve for the range UV-B (280–310 nm), UV-A (310–400 nm), visible (400–700 nm) and infrared light (700–1000 nm) were calculated in order to estimate the percentage reduction in light transmittance produced by both one and two applications of the antitranspirant.

2.3. Phenological development, temperature and characteristics of the grape berries

The phenological development was evaluated for each plant individually, counting the number of days elapsed from fruit set to the onset of veraison (E-L 35), and from the onset of veraison to maturity (E-L 38). The onset of veraison was visually determined when the bunches had around 4 colored berries. Maturity was considered when the level of TSS in the must was about 23°Brix, which was established by sampling periodically two berries and measuring TSS with a refractometer (Abbe Digital 315RS, Zuzi, Beriain, Spain). When plants reached maturity, the temperature of berry surface was measured using an infrared thermometer (Fluke 568, Everett, USA). Measurements were done at midday in berries well exposed to uniform illumination within the glasshouses. In addition, 10 berries per bunch were sampled to calculate the berry fresh mass (FM), berry caliber using a calypter, and the relative skin mass by manually separating the skins.

2.4. Bunch transpiration

Bunch transpiration was measured at five phenological stages: pea size (E-L 31), chickpea size (E-L 32), onset of veraison (E-L 35), full veraison (all the berries in the bunch are colored) and maturity (E-L 38). The phenological stages were visually determined, except maturity, which was determined according to the TSS level as described in section 2.3. The stages were determined for each plant individually and each plant was measured when it reached the corresponding developmental stage. Bunch transpiration was measured with a device based on RH sensors from Vaisala described in Morales et al. (2022). Measurements

were done at the growth temperature and RH. For that, the transpiration chamber was placed into a plant growth chamber (Conviron, EF7H, Pembina, USA) set at either 24°C or 28°C (T and T+4/AT+4, respectively) and it was supplied with air previously conditioned at either 55% or 43% RH (T and T+4/AT+4 treatments, respectively). The rate of air flow to the transpiration chamber was 1 L min^{-1}. The number of bunches measured per treatment was between 5 and 11, depending on the phenological stage. Immediately after transpiration measurements, berry samples were taken from the top and middle portion of the bunch and immediately frozen at −80°C for biochemical analyses.

2.5. Cuticular wax extraction and quantification

At the onset of veraison and maturity, 15 berries per bunch (n = 5–9) were sampled in the treatments T and T+4. The berries were immediately weighted to determine their fresh mass (FM) and briefly rinsed in distilled water to remove dirt and dust. Wax extraction was performed according to Palliotti and Cartechini (2001) with some modifications. Grape berries were submerged for 30 s with gentle swirling in 15 mL of chloroform. Chloroform extracts were allowed to evaporate at ambient temperature until stable weight. Water-soluble exudates were partitioned from the waxes by dissolving in 5 mL of water and then 5 mL of chloroform. The water layer was removed with a pipette and the chloroform containing the waxes was dried as described previously. The wax content was determined using the weight by precision (0.1 mg) balance.

2.6. Total soluble solids (TSS), malic acid, pH and total acidity in the must

Twenty berries per plant (n = 5–11), sampled at pea size, chickpea size, onset of veraison, full veraison and maturity, were crushed to extract the juice and centrifuged at 4100 *g* for 10 min at 4°C. In the supernatant, TSS was measured using a digital refractometer, pH with a pH meter (MicropH, 2000; Crison Instruments, Barcelona, Spain), malic acid with an enzymatic method (Enzytec™ l-Malic Acid, Boerhinger Mannheim/R-Biopharm®) and tartaric acid with a colorimetric test (Tartaric acid Kit, BioSentec). Total acidity was analyzed at maturity by titrating 2 mL of extracts against NaOH 0.01 N according to the OIV (2016). The TSS accumulation rate and malic acid degradation rate were calculated dividing the difference between the respective values at veraison and maturity by the number of elapsed days between these two phases. Total sugar per berry mass was estimated from TSS according to the International Organization of Vine (OIV, 2016), in order to calculate the total sugar content per berry.

2.7. Phenolic maturity

Fifteen berries per plant (n = 8–9) sampled at maturity were ground in a batch ball mill (Retsch Mixer Mill MM 200, Haan, Germany). The homogenate was divided into two tubes in equal and known volumes. The two homogenates were macerated for 4 h at 4°C with a solution of either HCl (pH 1) or tartaric acid (pH 3.2). Extracts were centrifuged at 4100 *g* for 10 min at 4°C. Total and extractable anthocyanins were analyzed using the SO_2 bleaching method (Ribéreau-Gayon and Stonestreet, 1965) in the extracts macerated at pH 1 and pH 3.2, respectively. Anthocyanin extractability (AE) was calculated according to Nadal (2010). Total polyphenol index (TPI) was determined in the extract macerated at pH 3.2 by measuring the absorbance at 280 nm (Ribéreau-Gayon and Stonestreet, 1965). The TPI and the concentration of extractable anthocyanin were used to calculate the seed maturity index (SM) (Nadal, 2010). Color intensity was determined in the extract macerated at pH 3.2, as the sum of 420 nm, 520 nm and 620 nm absorbance, and tonality was calculated as the absorbance ratio at 420 nm to that at 520 nm (Glories, 1984; Glories and Augustin, 1993).

2.8. Statistical analysis

The statistical analysis of the data was performed with the RStudio V. 4.1.1. program. Significant differences between treatments (T and T+4) in the parameters analyzed at pea size, chickpea and onset of veraison were determined by a student-t test. A one-way ANOVA test, followed by the post hoc Fisher's least significant difference test ($P < 0.05$), was used to detect significant differences among the treatments T, T+4 and AT+4 in those parameters analyzed at both full veraison and maturity. A multivariate analysis - principal component analysis (PCA) was also performed with data obtained at maturity.

3. Results

3.1. Influence of antitranspirant on light transmittance

Light transmittance was reduced after the application of both one and two layers of antitranspirant compared with the control without antitranspirant (Fig. S1A). With the application of two layers of antitranspirant, the area under the transmittance curve was significantly lower for the different wavelength ranges, dropping by 20%, 15.5%, 13% and 11.5%, in the UV-B, UV-A, visible and infrared ranges, respectively (Fig. S1B).

3.2. Bunch transpiration and cuticular wax

Bunch transpiration decreased throughout berry development from pea size stage to maturity in all the treatments (Fig. 1A and B). The treatment with elevated temperature and low relative humidity (T+4) significantly increased the loss of water at the five stages studied, especially in the green stages (pea, chickpea and onset of veraison). The application of antitranspirant to the bunches developed under T+4 conditions (treatment AT+4) significantly reduced bunch transpiration, both at full veraison and maturity, to levels comparable to those of treatment T (Fig. 1B). The wax content per gram of berry fresh mass decreased from the onset of veraison (0.807 ± 0.093 and 0.747 ± 0.039 mg g^{-1} FM, T and T+4, respectively), to maturity (0.442 ± 0.016 and 0.440 ± 0.022 mg g^{-1} FM, T and T+4, respectively). No significant differences on the wax load were observed between T and T+4 at any of the two stages analyzed ($P > 0.05$).

3.3. Phenological development and grape berry characteristics

Elevated temperature (T+4) significantly reduced the elapsed time between fruit set and veraison and between veraison and maturity in 4.1 and 10 days, respectively, with respect to T (Table 1). The application of the antitranspirant under conditions of elevated temperature and low RH (AT+4) slowed down berry ripening compared with T+4. The number of days elapsed between veraison and maturity in AT+4 did not statistically differ from T. Berry caliber and berry fresh mass were reduced in the treatment T+4 compared with T and AT+4 (significant differences only for berry caliber) (Table 1). The treatments T+4 and AT+4 did not significantly modified the relative skin mass of the berries and bunch mass. Berry temperature in the treatments with elevated temperature, T+4 and AT+4, was on average 6.0°C higher than in T (Table 1). The application of the antitranspirant did not significantly increased the temperature with respect to the non-sprayed berries.

3.4. Evolution of total soluble solids (TSS), malic and tartaric acids, and pH

T+4 hastened the accumulation of TSS in the berries (Fig. 2A). Plants in T+4 reached maturity (TSS around 23°Brix) 15 days earlier on average, compared to those grown in T. Analyzing each phenological stage, the concentration of TSS of berries developed under T+4 at chickpea, full veraison and maturity were significantly higher than those

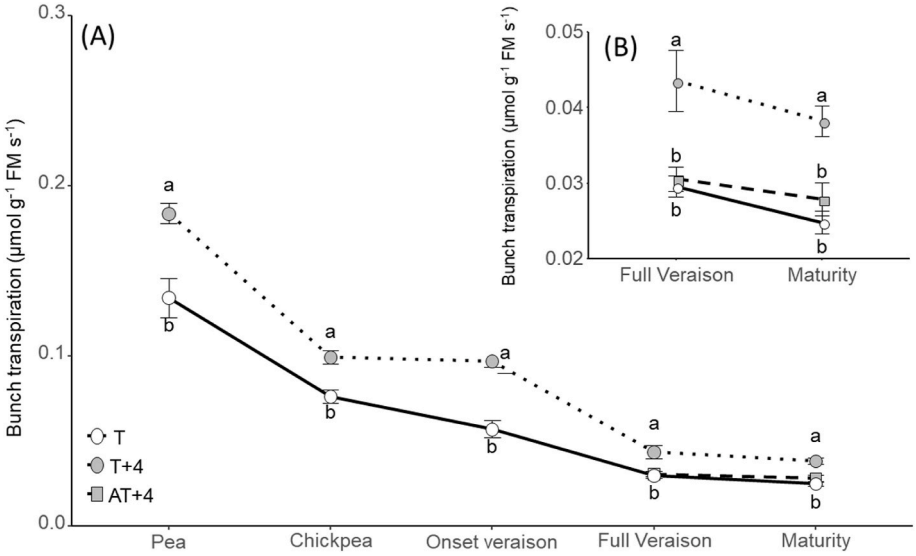


Fig. 1. Evolution of bunch transpiration (A) and detail of bunch transpiration at full veraison and maturity (B) in *V. vinifera* L. cv. 'Tempranillo' plants grown at 24°C/14°C and 55%/70% RH day/night (T), 28°C/18°C and 43%/58% RH day/night (T+4) and 28°C/18°C and 43%/58% RH day/night with antitranspirant (AT+4). Data are means ± standard errors (n = 5–11). Means with letters in common within the same phenological stage are not significantly different according to Fisher's LSD-test ($P > 0.05$).

Table 1
Phenological development, grape berry and bunch characteristics and berry temperature of *V. vinifera* L. cv 'Tempranillo' plants grown at 24 °C/14 °C and 55%/70% RH day/night (T), 28°C/18°C and 43%/58% RH day/night (T+4) and 28°C/18°C and 43%/58% RH day/night with antitranspirant (AT+4). Data are means ± standard errors (n = 5–11). Means with letters in common within each parameter are not significantly different according to Fisher's LSD-test ($P > 0.05$).

	T	T+4	AT+4
Fruit set - Veraison (days)	46.1 ± 1.2 a	42.0 ± 0.7 b	–
Veraison - Maturity (days)	44.6 ± 2.2 a	34.6 ± 2.3 b	38.2 ± 2.9 ab
Caliber (mm)	11.3 ± 0.5 a	10.0 ± 0.3 b	11.4 ± 0.4 a
Berry fresh mass (g FW berry^{-1})	1.21 ± 0.09	0.96 ± 0.07	1.22 ± 0.11
Relative skin mass (% Berry FM)	15.8 ± 0.7	14.6 ± 1.0	15.3 ± 1.9
Bunch mass (g)	170.4 ± 16.4	150.8 ± 6.1	142.9 ± 14.2
Berry temperature (°C)	25.6 ± 0.2	31.5 ± 0.3	31.8 ± 0.2

of T berries at the corresponding stages, except at the onset of veraison (Fig. 2E). Berries in the AT+4 treatment had significantly lower TSS levels than T and T+4 at full veraison and lower than T+4 at maturity. The pH values of the must increased from the onset of veraison to maturity, especially in those berries grown under T+4 and AT+4 (Fig. 2B–F). Malic acid concentration increased up to the onset of veraison, this increase being faster in T+4 than in T (Fig. 2C). From the onset of veraison onwards, the concentration of malic acid decreased in all the treatments, reaching the minimum values at maturity. Such decrease was, however, less pronounced in the treatment T and the concentration of malic acid in T+4 was significantly lower than in T at the stage of full veraison (Fig. 2G). The concentration of tartaric acid decreased during the green phases of berry development, maintaining almost constant values during the ripening period (Fig. 2D). In general, no significant differences in tartaric acid were observed among treatments within the phenological stages studied, except at the onset of veraison, when berries developed in T+4 had significantly higher tartaric acid levels than those grown in T (Fig. 2H).

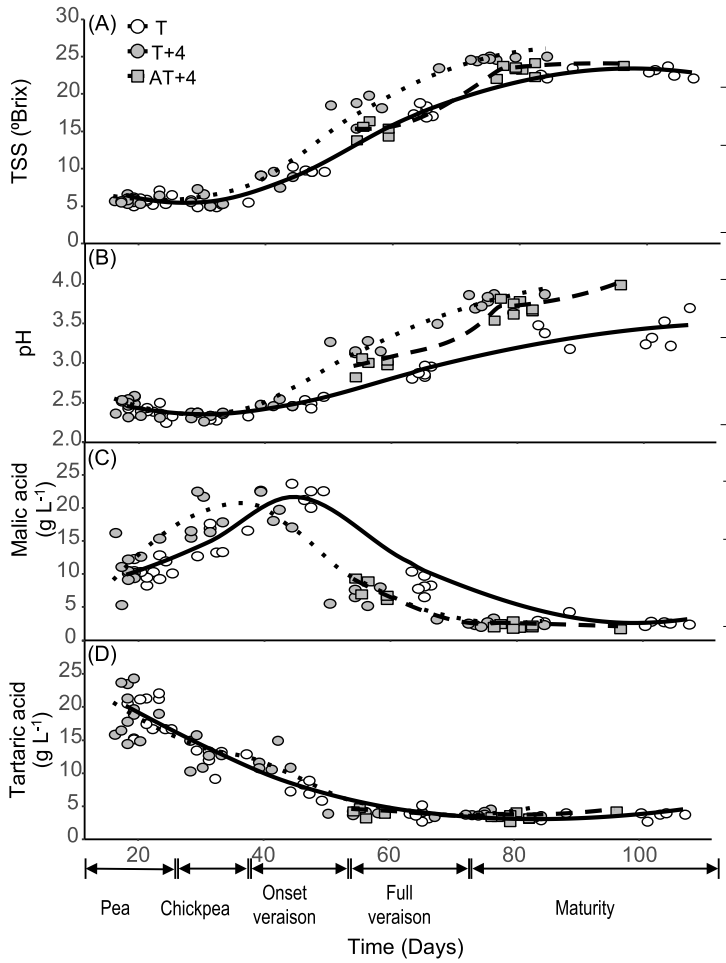
3.5. Technological and phenolic maturity parameters

Titratable acidity was significantly lower in the treatments T+4 and AT+4 compared with T (Table 2). Consequently, berries grown under T+4 and AT+4 had pH values significantly higher than those in the T treatment. The TSS accumulation rate and the degradation rates of both malic and tartaric acid were higher in T+4, followed by AT+4 and T (Table 2). T+4 significantly reduced the concentration of total anthocyanins and the anthocyanin extractability compared with T (Table 2). T+4 did not significantly modified the concentration of extractable anthocyanins, the seed maturity index (SM), color intensity and the total polyphenol index (TPI), but led to a significantly higher tonality in the must compared with T. The application of antitranspirant under simulated future temperature and RH conditions significantly reduced the concentration of total and extractable anthocyanins with respect to both T and T+4 (Table 2). The extractability of anthocyanins in AT+4 berries was similar to that in T+4 and significantly lower than in T. Grape berries of the plants in the AT+4 treatment had higher SM and tonality values, as well as lower color intensity, when compared with both T and T+4. TPI values did not differed significantly among treatments (Table 2).

Elevated temperature, both without and with the application of the antitranspirant (T+4 and AT+4, respectively), decreased the titratable acidity:TSS and the malic acid:TSS ratios in mature berries (Fig. 3A–B). The total anthocyanins:TSS ratio decreased in T+4 and, especially, in AT+4, when compared with T. The extractable anthocyanins:TSS ratio was significantly lower in the treatment AT+4 than in T and T+4 (Fig. 3C–D).

3.6. Principal component analysis

In order to identify the most responsive variables to the treatment with combined elevated temperature and low RH, as well as to the application of the antitranspirant under these conditions, a principal component analysis (PCA) of bunch transpiration, phenology, technological and phenolic maturity parameters was performed. PC1 and PC2 covered about 32% and 20% of total variability, respectively (Fig. 4). The treatments of elevated temperature and low RH, regardless of the application of antitranspirant (T+4 and AT+4), were clearly separated from the control treatment (T) in the score plot through PC1 (Fig. 4A). Such distinction was mainly associated with a shorter fruit set-veraison period, higher pH, as well as lower acidity and total anthocyanin levels in the treatments T+4 and AT+4 (Fig. 4B). T+4 and AT+4 were also separated through PC2. The differences between these two treatments



(E)

Treatment	TSS (°Brix)				
	Pea	Chickpea	Onset veraison	Full Veraison	Maturity
T	6.13 ± 0.19	5.31 ± 0.11 b	10.1 ± 0.14 a	18.1 ± 0.31a	23.1 ± 0.21b
T+4	6.15 ± 0.12	6.23 ± 0.35 a	9.14 ± 0.35 b	18.4 ± 0.74 a	24.9 ± 0.16 a
AT+4				15.5± 0.45 b	23.6 ± 0.26 b

(F)

Treatment	pH				
	Pea	Chickpea	Onset veraison	Full Veraison	Maturity
T	2.42 ± 0.02	2.36 ± 0.02	2.53 ± 0.03	2.92 ±0.03 b	3.41 ± 0.06 b
T+4	2.50 ± 0.03	2.37 ± 0.02	2.51 ± 0.02	3.22 ± 0.03 a	3.78 ± 0.04 a
AT+4				3.02 ± 0.04 b	3.76 ±0.05 a

(G)

Treatment	Malic acid (g L^{-1})				
	Pea	Chickpea	Onset veraison	Full Veraison	Maturity
T	10.9 ± 0.4	15.5 ± 0.9 b	22.4 ± 0.62	8.87 ± 0.57 a	3.21 ± 0.22
T+4	11.4 ± 0.8	18.8 ± 1.2 a	20.4 ± 1.12	6.97 ± 0.56 b	2.98 ± 0.14
AT+4				8.03 ± 0.64 ab	2.53 ± 0.14

(H)

Treatment	Tartaric acid (g L^{-1})				
	Pea	Chickpea	Onset veraison	Full Veraison	Maturity
T	18.9 ± 0.8	12.7 ± 0.7	7.82 ± 0.53b	4.14 ± 0.34	3.92 ± 0.18
T+4	19.5 ± 1.0	12.3 ± 0.9	12.1 ± 0.81a	4.37 ± 0.10	4.13 ± 0.12
AT+4				4.50 ± 0.26	3.89 ± 0.19

Fig. 2. Evolution of total soluble solids (TSS, A and E), pH (B and F), malic acid (C and G) and tartaric acid (D and H) in grape berries of *V. vinifera* L. cv 'Tempranillo' plants grown at 24 °C/14 °C and 55%/70% RH day/night (T), 28°C/18°C and 43%/58% RH day/night (T+4) and 28°C/18°C and 43%/58% RH day/night with antitranspirant (AT+4). In the charts, points represent individual samples taken throughout grape development. Tables represent means ± standard errors (n = 5–11) for each phenological stage. Means with letters in common within the same parameter and phenological stage are not significantly different according to Fisher's LSD-test ($P > 0.05$).

were related to increased bunch transpiration, higher sugar accumulation and malic acid degradation rates, and increased extractable anthocyanins, as well as lower tonality and SM in T+4 compared with AT+4. The loading plot also reveals a negative correlation between bunch transpiration and the length of the ripening period ($R^2 = -0.55$, $P = 0.005$), as well as a positive relationship between bunch transpiration and the sugar accumulation rate ($R^2 = 0.67$ and $P < 0.001$).

4. Discussion

Fresh fruits continuously release water vapor into the surrounding atmosphere through transpiration (Léchaudel et al., 2013). Grape berries have low stomatal density on the berry surface (less than one stomata per mm^2) (Blanke and Leyhe, 1988). Furthermore, stomata become partially or completely covered by epicuticular waxes as grapes ripen, thus becoming unfunctional (Dimopoulos et al., 2020). Due to this lack of stomatal regulation of transpiration, the water loss in berries mainly occurs through the cuticle and it varies significantly according to environmental conditions such as temperature and relative humidity (RH), which determine the vapor pressure deficit (VPD) (Zhang and Keller, 2015). In the present study, bunch transpiration was significantly higher in the treatment T+4 (Fig. 1). This treatment had a higher VPD compared with the treatment T, due to a higher temperature and lower RH in the air. Differences in transpiration were more pronounced in the green stages (pea size, chickpea size and onset of veraison) than in the ripening period, which suggests a progressive adaptation of grape berries to elevated VPD conditions (Rebucci et al., 1997). The application of the antitranspirant (AT+4) effectively reduced the transpiration rates under high temperature and low RH conditions to values similar to those measured in T.

Waxes are the principal cuticle component, and prevent grape berry from dehydration. The wax load per berry FM at maturity was lower than at the onset of veraison. Previous studies have also reported a peak in the amount of total cuticular waxes around veraison and, thereafter, a decrease with berry growth, especially in the level of triterpenoids (Dimopoulos et al., 2020; Pensec et al., 2014). Such decrease has been recently associated with a down-regulation of related biosynthetic genes (Dimopoulos et al., 2020). The observed developmental changes in the cuticular wax load were not, however, associated with an increase in bunch transpiration throughout fruit development (Fig. 1). The decrease in transpiration observed at maturity in all the treatments assayed may be, therefore, a consequence of chemical modifications in cuticular wax composition as suggested by Dimopoulos et al. (2020) and Leide et al. (2007). Furthermore, the accumulation of skin flavonoid compounds (i.e. flavonols and anthocyanins) in the polymer matrix of the cuticle throughout the ripening period, has been reported to modulate cuticular water permeability in tomato fruits (Luque et al., 1995). Temperature and RH have been shown to affect cuticle deposition (Trivedi et al., 2019). However, in the present study, we did not observe a significant impact of these factors on the cuticular wax load, which may be due to

Table 2
TSS accumulation rate, malic and tartaric acid degradation rates, technological and phenolic maturity parameters of grape berries of *V. vinifera* L. cv 'Tempranillo' plants at 24 °C/14 °C and 55%/70% RH day/night (T), 28°C/18°C and 43%/58% RH day/night (T+4) and 28°C/18°C and 43%/58% RH day/night with antitranspirant (AT+4). Data are means ± standard errors (n = 5–11). Means with letters in common within each parameter are not significantly different according to Fisher's LSD-test ($P > 0.05$). AE, anthocyanin extractability; SM, seed maturity index; TPI, total polyphenol index; AU, arbitrary units.

	T	T+4	AT+4
Titratable acidity (g L^{-1})	5.45 ± 0.29 a	4.19 ± 0.25 b	4.19 ± 0.26 b
pH	3.41 ± 0.06 b	3.78 ± 0.04 a	3.76 ± 0.05 a
TSS accumulation rate (°Brix day^{-1})	0.28 ± 0.02 c	0.50 ± 0.03 a	0.39 ± 0.03 b
Malic acid degradation rate (g L^{-1} day^{-1})	0.41 ± 0.02 b	0.55 ± 0.03 a	0.48 ± 0.0 ab
Tartaric acid degradation rate (g L^{-1} day^{-1})	0.09 ± 0.01 b	0.25 ± 0.02 a	0.22 ± 0.02 a
Total anthocyanins (mg L^{-1})	756.7 ± 43.1 a	521.1 ± 48.7 b	364.2 ± 29.0 c
Extractable anthocyanins (mg L^{-1})	255.8 ± 17.4 a	258.8 ± 14.7 a	195.2 ± 12.2 b
AE (%)	65.6 ± 2.63 a	47.4 ± 4.81 b	45.2 ± 3.68 b
SM (AU)	69.4 ± 2.4 b	72.5 ± 1.5 ab	77.4 ± 1.7 a
Color intensity (AU)	3.83 ± 0.33 a	3.76 ± 0.24 a	2.66 ± 0.17 b
TPI	34.3 ± 2.6	37.7 ± 1.1	35.6 ± 3.0
Tonality (AU)	0.41 ± 0.01 c	0.46 ± 0.01 b	0.50 ± 0.02 a

the moderate increase of temperature applied in the T+4 treatment. VanderWeide et al. (2022), who evaluated the effect of one or two heatwave episodes on the berry cuticular wax profile of cv. Gewürztraminer, did not observe significant differences, at mid ripening, in total waxes. Only those grapes exposed to a double heatwave increased the wax content at maturity compared with the control ones, thus indicating that wax deposition respond mainly to cumulative effects of extreme temperatures rather than to isolated events. Trivedi et al. (2022) studied the impact of an increase in temperature (18°C *vs* 12°C) on wax deposition of bilberry fruits. In their study, wax load increased with temperature only in the northern clones of this plant species, thus suggesting a higher sensitivity of these clones. The absence of differences in the wax load observed between T and T+4 may also indicate a limited responsiveness of cv. 'Tempranillo' to the moderate increase in temperature assayed in the present study.

Temperature has noticeable effects on grape berry development and on the timing of the phenological stages (Arrizabalaga-Arriazu et al., 2020; Arrizabalaga et al., 2018; Cohen et al., 2012). In the present study, the treatment T+4 reduced the elapsed time between fruit set and the onset of veraison, and, especially, between the onset of veraison and maturity, which was related to a faster accumulation of TSS in the berries (Fig. 2A and Table 2). Soluble sugar accumulation in fleshy fruits, including grapevine, results from the complex interplay of three main processes: sugar import, sugar metabolism and water dilution. From mid-veraison onwards, most of photoassimilates are directed to berry ripening (Lebon et al., 2008), therefore berry sugar import strongly depends on leaf photosynthetic activity. A recent study under similar experimental conditions reports an increase in net photosynthesis of 'Tempranillo' fruit-bearing cuttings grown at 28°C compared with those grown at 24°C, such increase being concomitant with a higher concentration of glucose and fructose in the fruit and a reduction of TSS in the leaves (Goicoechea et al., 2023). Although photosynthesis was not measured in the present work, we cannot rule out an increased translocation of photoassimilates from the leaves to the fruits in T+4, which may have contributed to the faster ripening observed in this treatment. In addition, the higher transpiration rates measured in the berries under T+4 may have produced an imbalance between water loss and phloem influx, thus concentrating carbon compounds in the fruit and increasing the gradient between the fruit and the stem, thus promoting the importing of assimilates into the berry (Fahey and Rogiers, 2019; Rebucci et al., 1997; Zhang et al., 2017). Interestingly, when the

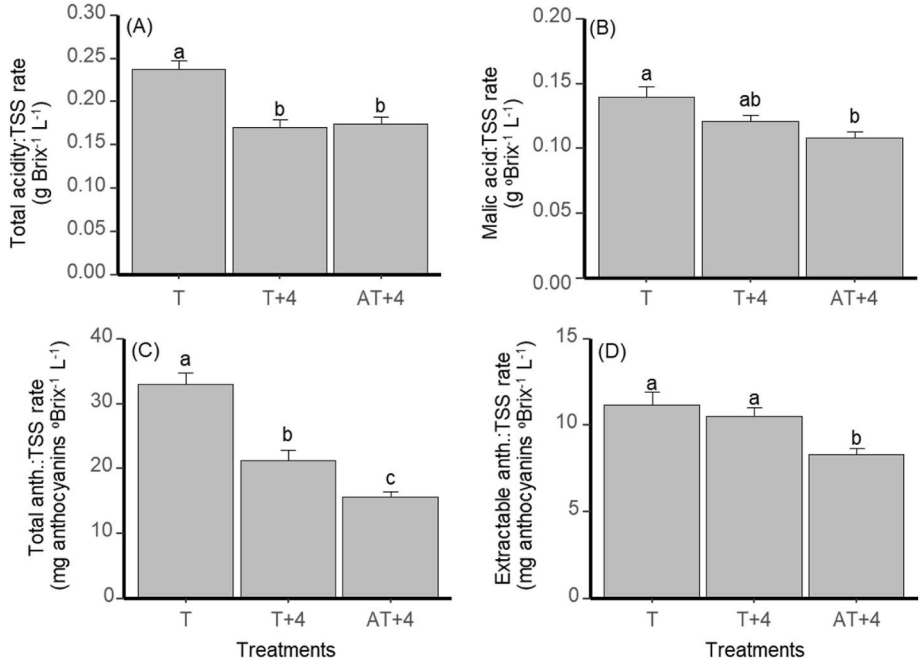


Fig. 3. Total acidity:TSS (A), malic acid:TSS (B), total anthocyanins:TSS (C), and extractable anthocyanins:TSS (D) ratios in mature grape berries of *V. vinifera* L. cv 'Tempranillo' plants grown at 24°C/14°C and 55%/70% RH day/night (T), 28°C/18°C and 43%/58% RH day/night (T+4) and 28°C/18°C and 43%/58% RH day/night with antitranspirant (AT+4). Columns are means ± standard errors (n = 8–9). Means with letters in common within each parameter are not significantly different according to Fisher's LSD-test ($P > 0.05$).

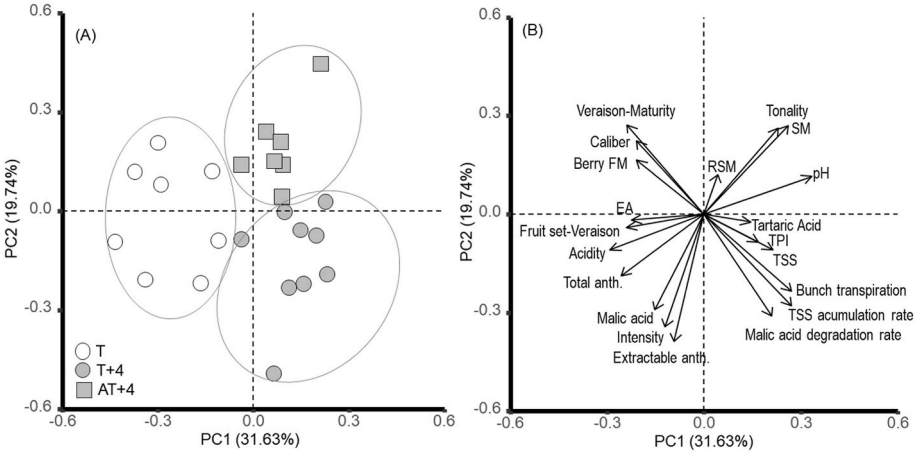


Fig. 4. Principal component analysis of phenological development and grape technological and phenolic maturity: score (A) and loading plot (B). Anth., anthocyanins; EA, extractability of anthocyanins; TPI, total polyphenol index; RSM, relative skin mass; TSS, total soluble solids; SM, seed maturity; and FM, fresh mass.

transpiration of berries was artificially reduced with the application of the antitranspirant under elevated temperature and low RH conditions, a slowdown in the accumulation of TSS in AT+4 plants was observed in comparison with T+4. Although berry size in AT+4 was larger than in T+4, we can discard a dilution effect in AT+4 berries, since the accumulation rate of sugars expressed on a berry basis was also lower compared with T+4 (Fig. S2). Then, assuming a similar photosynthetic activity in T+4 and AT+4 plants, both the reduction in the sugar accumulation rate with the antitranspirant and the positive correlation between both parameters observed in the PCA suggest that water loss from grape berries had a relevant contribution to the accelerated sugar import observed under elevated temperature and low RH conditions, thus confirming our initial hypothesis.

Malic acid constitutes about half the total acidity of grape berries, thus representing one of the most significant influences on the acidity and pH of the must. Berries accumulate malate until they undergo a metabolic shift at veraison, when malic acid is used as a carbon source for respiration and gluconeogenesis during ripening. The reduction of malic acid concentration in response to heating has been related to a higher degradation, associated with a higher malate export rate from the vacuole to the cytoplasm and increased expression and activity of enzymes involved in malate catabolism, rather than reduced synthesis pre-veraison (Carbonell-Bejerano et al., 2013; Sweetman et al., 2014). Similarly, in the present study, elevated temperature led to a higher malic acid concentration at pre-veraison stages, pea and chickpea, but induced faster degradation during ripening (Fig. 2C–G). This resulted in a more pronounced increase in pH, as well as lower acidity and malic acid values (not significant differences in the latter) at maturity, and consequently the imbalance between titratable acidity and TSS (Fig. 2 and Fig. 3B). Tartaric acid, however, is less sensitive than malic acid to environmental conditions during ripening, which explains the absence of significant differences in the tartaric levels between T and T+4 at maturity (Fig. 2D–H). Regarding the application of the antitranspirant, although the results suggest a slight reduction in the degradation rate of malic acid in AT+4 compared with T+4, thus bringing it closer to T plants (Table 2), the final concentration of malic acid in AT+4 tended to be lower than in T+4 (Fig. 2C–G). The lengthening of the ripening period with the application of the antitranspirant and, consequently, the longer exposure to elevated temperatures of berries grown under AT+4, likely contributed to higher malate degradation in this treatment.

Anthocyanins are colored compounds synthesized in the epidermal and hypodermal cells of the grape berry skin and accumulated in the cell vacuoles from veraison onwards. These pigments give color to red grapes and wines. Elevated temperature has been widely reported to produce negative effects on anthocyanin accumulation, especially when hot conditions are applied around veraison (Gouot et al., 2019). In the present study, a decrease in total anthocyanins was observed in the berries ripened under T+4, thus producing a clear imbalance between total anthocyanins and TSS at maturity (Table 2 and Fig. 3C). The repression of the major anthocyanin biosynthesis regulators and genes, such as *VviMYBA1* and *VviUFGT*, as well as the promotion of anthocyanin degradation, possibly via the increased activity of peroxidases, have been proposed as the main causes of reduced anthocyanin accumulation in grape berries ripened under high temperature (Rienth et al., 2021). Interestingly, the higher extractability of anthocyanins of the berries in the T+4 treatment offset the lower total anthocyanin concentration, which resulted in extractable anthocyanins and color intensity levels comparable to those of T plants, as well as on a more balanced extractable anthocyanin:TSS ratio (Table 2 and Fig. 3D). Regarding the impact of the antitranspirant, the reduction in both total and extractable anthocyanins, as well as in color intensity, even below the levels observed in T+4, reveals an additive effect of the antitranspirant to that caused by warm temperatures (Table 2). Tissue cooling is one of the main functions of transpiration. Therefore, it may be hypothesized that the lower transpiration in AT+4 berries may have reduced their ability to cool themselves, thus intensifying the deleterious effect of T+4 on anthocyanin accumulation. In addition, the film layer formed by the antitranspirant over the berries may reduce the reflectance from the wax platelets, thus reinforcing their potential overheating (Fahey and Rogiers, 2019). However, the temperature of berries in the AT+4 treatment was not significantly different from that of T+4 (Table 1). Similarly, a recent study with cv. Merlot reveals only a slight increase of 0.9°C in fruits sprayed with di-1-*p*-menthene relative to the untreated control (Fahey and Rogiers, 2019). Alternatively, anthocyanin biosynthesis is regulated by both light intensity and quality, especially ultraviolet and blue light (Jaakola, 2013). Radiation modulates the transcriptomic accumulation of anthocyanin biosynthetic transcription factors and genes such as *VviMYBAa*, *VviMYBA1* and *VviUFGT* (Rienth et al., 2021). The significant reduction in light transmittance produced by the antitranspirant film, particularly at low wavelengths, may explain, at least partially, the lower anthocyanin concentration of berries in the AT+4 treatment compared to T+4 (Fig. S1). In addition, as a consequence of the lengthening of ripening period (Table 1), berries under AT+4 had a longer exposure to elevated temperatures compared with T+4, which may have also contributed to a higher degradation of anthocyanins. The reduction in the concentration of anthocyanins with the antitranspirant agrees with previous studies in different grapevine cultivars that report a delayed coloration of berries after the application of di-1-*p*-menthene both directly to the bunches and to the entire canopy (Brillante et al., 2016; Palliotti et al., 2013; Zhang

et al., 2017). In contrast, other authors have reported either no effect or even an increase in the concentration of anthocyanins with the application of the same product (Di Vaio et al., 2019; Palliotti et al., 2010; Pascual et al., 2022). In some of these studies, however, the application of antitranspirant led to a reduced berry size, not observed in the present study, which may have produced a concentration effect of anthocyanins.

The increase of the SM index in the berries treated with the antitranspirant indicates a higher contribution of the seed tannins to the wine phenolic richness, and suggests an increased potential green astringency of the wines obtained from the sprayed berries (Pérez-Álvarez et al., 2021) (Table 2). Such effect of the antitranspirant under elevated temperature should be carefully considered in a context in which climate change is already causing a decompensation among the maturity of the pulp, skin and seeds, which is reflected in a greater presence of seed tannins in wine (Mira de Orduña, 2010). The significant increase in the tonality index observed with the application of the antitranspirant was due to an increase in yellow tones and a reduction in blue, and may be related to changes in the anthocyanin profile (Downey et al., 2006).

5. Conclusion

In the present study, a 4°C increase in temperature combined with a 12% reduction in RH (T+4) increased bunch transpiration, advanced maturity and altered grape berry composition (higher TSS and pH, as well as lower acidity and total anthocyanins). The artificial reduction of bunch transpiration under these environmental conditions partially attenuated the effects produced by warmer and drier air conditions on the sugar accumulation rate and, consequently, on the ripening time. The application of the antitranspirant, however, had a negative impact on both anthocyanins and color properties of the must. This was likely due to a lower biosynthesis and a higher degradation of these compounds, as a consequence of the reduction in light transmittance and the longer exposure to elevated temperatures in this treatment, respectively.

These physiological and biochemical findings unlock the door to further transcriptomic experiments that will provide information to explain the underlying mechanisms involved in some of the changes observed in the present study as a consequence of the application of the antitranspirant or the change in environmental conditions (i.e. reduction in the concentration of grape anthocyanins or acidity). Target transcription factors and genes for future experiments include *VviMYBAa*, *VviMYBA1* and *VviUFGT,* which have been shown to be repressed by temperature and/or modulated by radiation.

This is the first study evaluating the impact of the antitranspirant application under high temperature conditions. The results suggest that some of the changes on grape berry development expected under future climate scenarios, especially those related to sugar accumulation and ripening time, can be attributed to an increased bunch transpiration under the projected environmental conditions.

Author contributions

Conceptualization: FM and IP; Methodology and investigation: AC, FM, IP; Data curation and statistical analysis: AC; Writing, review and editing: AC, FM and IP; Funding acquisition and project administration: FM and IP.

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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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2023.108258.

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