Plant Soil (2010) 337:179–191
DOI 10.1007/s11104-010-0514-z

REGULAR ARTICLE

Effects of climate change scenarios on Tempranillo grapevine (*Vitis vinifera* L.) ripening: response to a combination of elevated CO_2 and temperature, and moderate drought

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Received: 14 May 2010 / Accepted: 26 July 2010 / Published online: 5 August 2010

Abstract Greenhouse experiments were conducted to investigate the impact of predicted climate change (elevated CO_2, 700 μmol CO_2 mol^{-1} air vs. ambient; elevated temperature, 28/18°C vs. 24/14°C, day/night; and partial irrigation, 40% of field capacity vs. well-irrigated) on grape berry quality characteristics during ripening. Grapevine (*Vitis vinifera* L. cv. Tempranillo) fruiting cuttings were used as experimental plant material. Climate change shortened the time between grape veraison and full maturity. At harvest time, many of the grape quality parameters determined were affected by the different grape maturity. The data were re-grouped according to total soluble solids to factor out changes due to the shortened time to maturity, and the effects on grape quality were then re-examined. Under current CO_2 and temperature conditions, partial irrigation decreased berry malic acid concentration and facilitated anthocyanins extractability. Elevated CO_2 and temperature decreased berry malic acid and total anthocyanins potential in well-irrigated plants and increased tonality index, irrespective of water availability. In partial irrigation conditions, elevated CO_2 and temperature hindered the anthocyanins extractability. In summary, results indicate that climate change (elevated CO_2, high temperature and partial irrigation) affects phenology and berry quality.

Keywords Anthocyanins · Climate change · Elevated CO_2 and temperature · Grape quality · Malic acid · Partial irrigation · Soluble solids · Tonality index · *Vitis vinifera*

Abbreviations

°Bx	°Brix
EA%	Cellular extractability
IPCC	Intergovernmental Panel on Climate Change
Mp%	Phenolic maturity of the grapes
RH	Relative humidity
Amb_WI	Ambient CO_2, ambient temperature and well irrigation
Amb_PI	Ambient CO_2, ambient temperature and partial irrigation

Responsible Editor: Peter J. Gregory.

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700 T_{+4}_WI	Elevated CO_2, elevated temperature and well irrigation
700 T_{+4}_PI	Elevated CO_2, elevated temperature and partial irrigation
VPD	Vapor pressure deficit

Introduction

Climate change is defined by the IPCC as any change in the state of the climate that persists for an extended period of time (IPCC 2007b). Changes in climate characteristics can be due to either natural variability and/or anthropogenic activities. Carbon dioxide (CO_2) is the most important anthropogenic greenhouse gas. The atmospheric CO_2 concentration has increased since pre-industrial period from 280 to 379 μmol mol^{-1} air (ppm) in the year 2005. It is expected that the values could increase to approximately 700 ppm at the end of the century (IPCC 2007a). Also, it is believed that climate change could increase plant water stress and the agricultural areas under drought, affecting crop production (IPCC 2007a). If greenhouse gases emissions continue at high levels, temperature is predicted to increase between 1.8 and 4.0°C (IPCC 2007b).

Grapevine growth is sensitive to different environmental factors, including temperature, water availability and CO_2. Furthermore, as in other C_3 plants, grapevine photosynthesis is CO_2-limited (Bindi et al. 1996; Mullins et al. 1992). Any increase of atmospheric CO_2 concentration could increase grapevine growth rate and yield (Bowes 1993; Rogers et al. 1994), resulting in a higher accumulation of vegetative biomass and fruits (Bindi et al. 1996). Nevertheless, the long-term photosynthetic response to CO_2 could be affected by acclimation processes, which decrease photosynthesis below its maximum potential (Erice et al. 2006; Jifon and Wolfe 2002; Long et al. 2004).

Information about effects of climate change on grapevine physiology is rather fragmentary. Some studies have documented effects of elevated CO_2 concentration, water availability and elevated temperature independently, but not in combination, because such studies in field conditions are complex, difficult and expensive to execute. Bindi et al. (2001) reported that elevated CO_2 stimulated grapevine yield without positive or negative effects on grape and wine qualities. In addition, this study determined that acids and sugars were positively affected during ripening, although these effects disappeared at harvest time, in line with other reports (Yamane et al. 2006). Other studies, focused on berries and wine quality, noted that sugar, total anthocyanins and tannins concentrations were not affected by the elevated CO_2 concentration (Gonçalves et al. 2009). Anthocyanin composition is an important grapevine quality factor, because it influences grape color. Many studies have shown that temperature affects the accumulation of these compounds (Cohen et al. 2008; Mazza and Miniati 1993; Shiraishi and Watanabe 1994; Yamane and Shibayama 2006). In regions where the temperature is higher, the anthocyanin accumulation is inhibited (Winkler et al. 1962) and contents were decreased in experiments with controlled high temperature (Kataoka et al. 1983; Kliewer 1970; Mori et al. 2007), but the mechanisms responsible for this inhibition have not been completely understood (Mori et al. 2007). Therefore, it is very likely that climate change-related temperature increase could affect anthocyanin accumulation. Temperature effects on malic acid concentrations have been widely observed (Koundouras et al. 2006), concentrations were shown to be higher in grapevines growing in cool rather than warm regions (Blouin and Guimberteau 2003; Lakso and Kliewer 1975). Water availability is the third important factor related to climate change. Grapevine yield and quality are influenced by water availability (Myburgh 2003). During berry growth (see below for details), water deficit restricts cell division, reducing berry size (Matthews and Anderson 1989; McCarthy 1997; Ojeda et al. 2002). Berry size can indirectly affect must phenolic content, including anthocyanins, possibly due to a modified skin surface-to-berry volume ratio (Koundouras et al. 2006; Ojeda et al. 2002; Roby and Matthews 2004; Singleton 1972). Also, it has been reported increased berry sugar content in response to water deficit (Antolín et al. 2006; Koundouras et al. 2006; Matthews et al. 1990; Ojeda et al. 2002). Effects of water stress on berry malic acid concentrations reported in the literature to date are rather contradictory, showing increases (López et al. 2007), decreases (De Souza et al. 2005; Intrigliolo and Castel 2009; Koundouras et al. 2006; Salon et al. 2005) or no change (Esteban et al. 1999) in response to drought. Crops are simultaneously exposed to different abiotic stress factors (Levitt 1980;

Mooney et al. 1991). Therefore, controlling CO_2 concentration, temperature, and water availability in greenhouse/growth chamber experiments and using fruiting cuttings technique (Mullins 1966; Ollat et al. 1998; Santa María 2004) could be a more viable approach than performing experiments under field conditions.

Grape berry development involves a complex series of changes, which can be divided into 3 major phases. In Phase I, during initial berry growth, berry size increases along a sigmoidal growth curve due to cell division and subsequent cell expansion. Organic acids (mainly malic and tartaric acids), tannins, and hydroxycinnamates accumulate to peak levels. Phase II is characterized by a lag phase where cell expansion ceases, and sugars begin to accumulate. Veraison, the onset of ripening, marks the beginning of Phase III, in which berries undergo a second period of sigmoidal growth, due to additional mesocarp cell expansion, accumulation of anthocyanin pigments for berry color, volatile compounds for aroma, softening, sugar accumulation (mainly glucose and fructose), and a decline in organic acid accumulation. This is the rule (Bogs et al. 2005; de Freitas et al. 2000; Deluc et al. 2007; Downey et al. 2003; Hanlin and Downey 2009), but there are exceptions. For instance, although it is assumed that berry tannin synthesis is largely complete by veraison at the end of Phase II (Hanlin and Downey 2009), some works reported that grapes continue accumulating tannins until harvest (Esteban et al. 2001; Navarro et al. 2008). Therefore, although one cannot assume that grape quality is mainly determined post-veraison, ripening should be considered a key step in the grape final characteristics. Furthermore, the occurrence of abiotic stress factors during ripening may affect berry composition post-veraison. It is likely degradation processes during ripening, due to high temperatures (Mori et al. 2007), but effects of climate change on grape ripening have not been much explored.

Grapevine physiology is expected to respond to climate change during the whole period of growth. Significant phenological activities such as budburst, shoot extension, flowering and fruit set all occur in the period prior to veraison, and climate change is likely to impact on all of these and, subsequently, on grape quality. However, 2 out of the 3 main climate change-related stress factors, drought and increased temperature, occur during summer (July to September in Spain), and coincide with the period post-veraison (Phase III). Elevated CO_2, however, would occur throughout the growing period in a scenario of climate change, not just post-veraison. This means that greenhouse designs investigating ripening do not fully replicate one of the likely climate change conditions, i.e., the presence of elevated CO_2 during the whole growing season. Budburst and shoot extension will be promoted by climate change, increasing growth due to the high CO_2 and temperature (Bindi et al. 1996; Bowes 1993) that would lead to a decreased grape quality by vegetation excess. Grapevine growers would control it by green, summer pruning until it became ineffective or uneconomic. In the experimental designs aimed to investigate effects of climate change on grape quality, it is important to maintain a vegetative growth to grape mass ratio optimal for grape maturity as it is routinely made in field conditions (Jackson and Lombard 1993; Kliewer and Weaver 1971). Concerning flowering and fruit set, a grape mass per grapevine plant is desirable in all treatments, in order to avoid changes in quality due to either dilution by excessive grape growth or concentration because of reductions in grape size (Antolín et al. 2003; Ojeda et al. 2002; Roby et al. 2004). In line with all these considerations, grapes and wines from grapevines grown under elevated CO_2 from budburst, as a single climate change-related factor, have unaltered qualities (Bindi et al. 2001), including anthocyanins and tannins concentrations (Gonçalves et al. 2009). The latter suggests that the effects of CO_2 would be minor when compared to those of temperature and drought, and experimental designs should take into account this when choosing the timing for simultaneous application of CO_2, temperature and drought.

The aim of this work was to investigate effects of climate change on grape berry ripening in greenhouse experiments by combining elevated CO_2, high temperature and partial irrigation, using fruiting cuttings of *V. vinifera* cv. Tempranillo, the Spanish variety most internationally recognized. The beneficial effects of grapes and wine on human health have been widely documented (Renaud and de Lorgeril 1992), but climate change-related alterations in grape constituents might modify their properties.

Materials and methods

Experimental approach

Our experimental approach was based on all considerations regarding the expected effects of climate change in grapevine physiology, trying to minimize indirect effects on grape quality and focusing measurements on direct effects of elevated CO_2 and temperature, and moderate drought (see Introduction for further details).

Plant material and growth conditions

Dormant cuttings *of V. vinifera* L. cv. Tempranillo were obtained in January 2008 from an experimental vineyard of the Station of Viticulture and Enology of Navarra (Olite, Navarra, Spain). Cuttings were selected to get fruiting cuttings according to Mullins (1966) and modified by Ollat et al. (1998) and Santa María (2004). Rooting was made in a heat-bed (27°C) kept in a cool room (5°C). One month later, the cuttings were planted in 4-L plastic pots containing a mixture of peat and perlite (2:1: v/v) and transferred to a greenhouse. Only a single flowering stem was allowed to develop on each plant during growth. Growth conditions in the greenhouse were 26/15°C and 40/80% relative humidity (RH) (day/night) and a photoperiod of 15 h with natural daylight supplemented with high-pressure sodium lamps (SON-T Agro Phillips, Eindhoven, Netherlands), providing a minimum photosynthetic photon flux density (PPFD) of 350 $\mu mol m^{-2} s^{-1}$ at inflorescence level. Plants were irrigated until veraison with the nutrient solution proposed by Ollat et al. (1998).

Experimental design

At veraison (9 °Bx, approximately) plants were divided into four treatments (8 per treatment) and transferred to two greenhouses (Fig. 1). Treatments were a combination of two CO_2 levels (ambient, approximately 375 ppm or 700 ppm), two temperature regimes (24/14°C or 28/18°C, day/night) and two water availability conditions (well irrigated and partially irrigated). Therefore, treatments were as follows: i) treatment simulating climate change conditions (elevated CO_2, elevated temperature and partial irrigation; 700 T_{+4}_PI), ii) control treatment (ambient CO_2, ambient temperature and well-irrigated; Amb_WI), iii) treatment simulating climate change conditions in absence of drought (elevated CO_2, elevated temperature and well-irrigated; 700 T_{+4}_WI), and iv) control treatment in presence of partial irrigation (ambient CO_2, ambient temperature and partial irrigation; Amb_PI). One of the greenhouses was maintained at ambient CO_2, optimal temperature (24/14°C, day/night), 60% RH, and a photoperiod of 15 h with natural daylight supplemented with the high-pressure sodium lamps (Amb_WI and Amb_PI treatments). The other greenhouse was maintained at elevated CO_2 (700 ppm), elevated temperature (28/18°C, day/night), 60% RH, and a photoperiod of 15 h with natural daylight supplemented with the high-pressure sodium lamps (700 T_{+4}_WI and 700 T_{+4}_PI treatments). Vegetative growth was controlled by pruning, which was used to maintain in all treatments a leaf area to grape mass ratio optimal for berry ripening (between 10 and 15 leaves per plant, i.e., ca. 10 cm^2 of leaf area per gram of grape; Jackson and Lombard 1993; Kliewer and Weaver 1971). Pruning minimized any indirect effect of vegetative growth on grape quality, ensuring that changes observed were directly due to the effects of treatments on the ripening process. Also, plants transferred to these new greenhouses were selected to have similar grape bunch size, avoiding changes in berry quality due to either dilution by excessive grape growth or concentrations because of reductions in grape size. The experiment was repeated twice (from April to August and from May to September). Ripening time was made to coincide with summer months (August and September, respectively), because 2 out of the 3 factors investigated, temperature and drought, are at their maxima in the Spanish Tempranillo grapevines during ripening under field conditions. Since each experiment spent 5 months (from rooting to ripeness) (Fig. 1), we fixed the rooting of the cuttings 4–5 months before summer, and thus the period from veraison to ripeness would coincide with the summer months, as it occurs in field conditions.

We rooted 260 cuttings, 208 became fruiting cuttings, 138 had a good fruit set and 128 (4 treatments × 8 plants per treatment × 2 sampling dates × 2 experiments) were selected with homogeneous grape mass to carry out our two experiments.

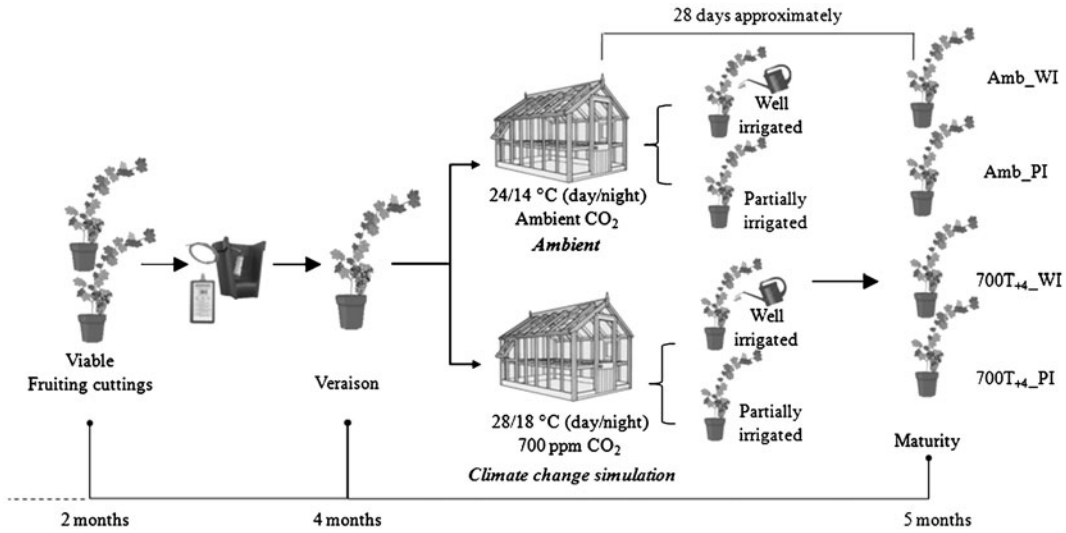

Fig. 1 Experimental design. Fruiting cuttings were developed, and a Water Mark device was placed into each pot. Plants grew until veraison in ambient conditions. Then, they were transferred into two greenhouses, climate change simulation and current ambient conditions. In each greenhouse, plants were divided into two groups, well-irrigated (WI) and partial irrigation (PI). Treatments were maintained until harvest time

Water treatments

In each greenhouse, plants were subjected to two different water availability regimes: well irrigated and partially irrigated (40% of field capacity). Soil water sensors (Watermark soil moisture sensor, Spectrum Technologies Inc., Illinois), placed into the pots, were used to control irrigation. Plants were irrigated, from veraison to ripeness, with half-strength Hoagland nutrient solution (Hoagland and Arnon 1950) or distilled water in order to receive the same amount of nutrients.

Grape bunch sampling

Plants were maintained under treatments until ripeness (approximately 28 days). The end of ripeness for cv. Tempranillo was defined at 21–23 °Bx. Berries (complete bunch) were harvested 14 days after beginning of treatments and at ripeness. At harvest times, the weight of the bunch and the number of berries were measured. Samples were frozen in liquid nitrogen and stored at −80°C until analysis.

Berry analyses

Ten samples of 25 berries by bunch from each treatment and harvest time were crushed without skins and seeds. Then, extracts were centrifuged, and the supernatants used for the following determinations: total soluble solids (°Bx) measured by a refractometer (Zuzi model nº 315, Digital ABBE); pH (pH-meter); total acidity determined by titrating 10 mL of extract against NaOH 0.1 N, and converted to a weighed quantity of tartaric acid; L-malic acid measured by an enzymatic method (Enzytec L-Malic Acid, Boehringer Manneheim/R-Biopharm), and tartaric acid by using the modified Rebelein's method (Rebelein 1973; Vidal and Blouin 1978).

Ten samples of 25 berries by bunch from each treatment and harvest time were grinded in blender to determine tannins by methyl cellulose-precipitable tannin assay (AWRI Standard Methods; www.crcv.com.au), phenolic maturity according to Glorie's method (Glories and Augustin 1993) and tonality index according to Sudraud's method (Sudraud 1958). Phenolic maturity and tonality index were determined after sample grinding and maceration during 4 h. For maceration, homogenate and buffer were mixed (1:1 by volume). Two different pH buffers were used. The first buffer was set at pH 3.2 (tartaric acid), and was used for extractable anthocyanins potential. It should be noted that this pH is comparable to that prevailing during the winemaking maceration step. The second buffer was set at pH 1 (HCl), and was used for total anthocyanins potential. Subsequently, the macerated

samples were centrifuged, and the supernatants were used for the following determinations. Phenolic richness was measured in the supernatant obtained after maceration at pH 3.2 (diluted 100 times with distilled water) at 280 nm (A_{280}). Contents of anthocyanins were determined in both supernatants according to Ribéreau-Gayon and Stonestreet (1965). Anthocyanins, extracted at pH 1.0 and pH 3.2 were measured, using absorbance at 520 nm. "Cellular extractability" (EA%=[(ApH1–ApH3.2)/ApH1] x 100) represents therefore the percentage of non-extracted anthocyanins at pH 3.2 over the maximum possible, extracted at pH 1. A decrease in EA% would indicate that anthocyanins present in grapes are more easily extracted, and an increase would indicate more difficulties for anthocyanins extraction. "Phenolic maturity of the grapes" (Mp%) was calculated as follows: $Mp\%=[(A_{280}-(ApH3.2\times 40)/1000)/A_{280}]\times 100$.

Tonality index was determined by the ratio between the measured absorbances at 420 and 520 nm of the grapes extracted at pH 3.2.

All absorbance readings were made in a Hitachi spectrophotometer (Model U-2001, Hitachi Instruments Inc., USA).

Statistical analysis

Data were first tested using a two-way ANOVA to determine the effects of the treatments and their possible interactions. When effects of treatments were statistically significant, differences among groups were tested with Least Significant Differences (LSD) *post-hoc* test. Results were considered statistically significant at $P<0.05$. Data are presented as means± standard error (SE). Each harvest (14 days and 28 days) was plotted independently. All these statistical analyses were carried out with the SPSS 15.0 statistical package for windows (SPSS inc., Chicago). Data that deviated±2 standard deviation from mean were not considered. All data were first plotted as a function of harvest time (data not shown, except those of °Bx; see Fig. 2). These results revealed a more advanced maturity (higher averaged °Bx values) in the climate change-related treatments. Therefore, with the aim of studying if climate change would have effects on berry quality independent on maturity level, samples were re-grouped to have similar °Bx values, and the different parameters were plotted as a function of °Bx. Ranges of °Bx chosen were from 16 to 19 (14 days after beginning of treatments), and from 20 to 23 (at ripeness).

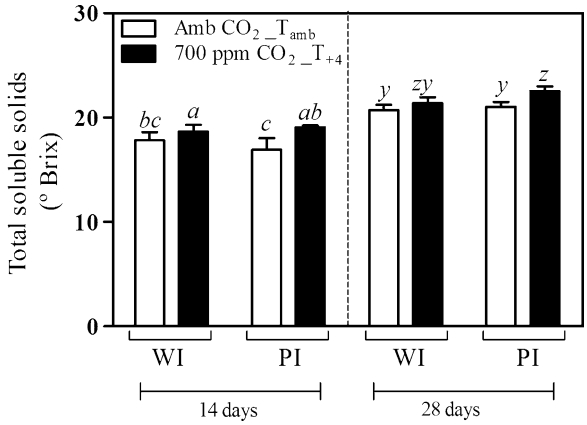

Fig. 2 Effect of CO_2, temperature and water availability on total soluble solids (°Bx) during ripening of *V. vinifera* cv. Tempranillo. Two harvests were performed at 14 and 28 days of treatment, and were plotted independently. Data (n=14–16, mean±S.E.). Different letters indicate significant differences between treatments ($P<0.05$) based on LSD test. WI, well-irrigated; PI, partial irrigation

Results

Total soluble solids (°Bx)

At 14 days of treatment, the combination of elevated CO_2 and temperature increased significantly the total soluble solids contents ($P<0.01$). Specifically, well-irrigated and partially irrigated berries had under elevated CO_2 and high temperature significantly higher total soluble solids contents with respect to ambient conditions ($P<0.05$ and $P<0.01$, respectively). Climate change simulation treatment (700 T_{+4}_PI) had 10% higher °Bx than control treatment (Amb_WI), but this was not significant ($P=0.058$) (Fig. 2). At 28 days of treatment, elevated CO_2 and high temperature increased significantly the °Bx on grapes ($P<0.01$). Under partial irrigation, berries subjected to elevated CO_2 and temperature had higher °Bx than ambient berries ($P<0.01$). Moreover, well-irrigated plants showed a tendency ($P<0.076$) to increase total soluble sugar contents under elevated CO_2 and high temperature with respect to ambient conditions. In addition, berries under simulated climate change treatment (700 T_{+4}_PI) had a significantly ($P<0.01$) 10% higher total soluble solids than control ones (Amb_WI) (Fig. 2).

In order to mitigate as much as possible the effects of maturation excess in the treatments of elevated CO_2 and elevated temperature, samples were divided according to similar °Bx (16–19 and 20–23 °Bx), as mentioned above. With this sampling re-grouping, we got samples of similar °Bx at harvest.

Berry weight

In the 16–19 and 20–23 °Bx groups (Fig. 3), no significant effects on berries weight (g of 100 units) between the different treatments were found.

pH

In the 16–19 °Bx group, no significant differences were found between treatments (Fig. 4a). In the 20–23 °Bx group, must pH was affected by elevated CO_2 and temperature ($P< 0.05$). In partially irrigated plants, pH was significantly higher ($P< 0.05$) under elevated CO_2 and high temperature with respect to ambient conditions. Climate change simulation treatment (700 T_{+4}_PI) showed a tendency to increase pH ($P= 0.068$) with respect to Amb_WI.

Total acidity

Elevated CO_2, high temperature and water stress did not affect total acidity (Fig. 4b). However, it should be noted that the simulation of climate change (with and without drought), in the 20–23 °Bx group, showed a slight, non-significant reduction of approximately 7% and 10% with respect to their controls, respectively (Fig. 4b).

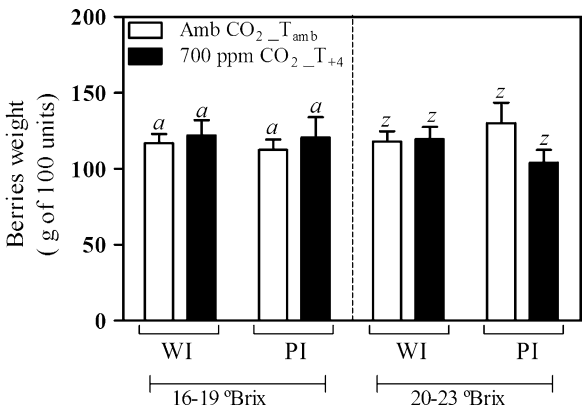

Fig. 3 Effect of CO_2, temperature and water availability on berries weight during ripening of *V. vinifera* cv. Tempranillo in the 16–19 and 20–23 °Bx groups. Groups were plotted independently. Data (n=8–10, mean±S.E.). Different letters indicate significant differences between treatments ($P< 0.05$) based on LSD test. WI, well-irrigated; PI, partial irrigation

Malic acid

In the 16–19 °Bx group, no effects of treatments on berry malic acid concentrations were observed. At ripeness (20–23 °Bx group), water availability affected malic acid concentration ($P< 0.05$). Moreover, malic acid concentration was affected by the interaction between the concentration of CO_2, temperature and water availability ($P< 0.05$). Under ambient conditions, malic acid concentration decreased significantly (51%) with partial irrigation ($P< 0.001$). In well irrigated plants, subjected to elevated CO_2 and temperature, malic acid concentration was significantly lower ($P< 0.01$) than in ambient conditions. Climate change simulation treatment (700 T_{+4}_PI) decreased significantly ($P< 0.01$) the malic acid concentration with respect to the control treatment (Amb_WI) (Fig. 5a).

Tartaric Acid

Tartaric acid levels in the 16–19 and 20–23 °Bx groups showed no significant differences between treatments (Fig. 5b).

Total anthocyanins (extracted at pH 1.0)

In the 16–19 °Bx group, the berries from the different treatments had similar total anthocyanin concentration (Fig. 6a). In the 20–23 °Bx group (Fig. 6a), the two-way ANOVA statistical analysis revealed that elevated CO_2 and elevated temperature as well as water availability induced no significant effects, whereas an interaction between them was observed ($P<0.05$). Specifically, total anthocyanin concentrations of the 700 T_{+4}_WI treatment were significantly lower with respect to Amb_WI treatment ($P< 0.05$). Climate change simulation treatment (700 T_{+4}_PI) did not show significant differences with respect to the control situation (Amb_WI).

Anthocyanin potential (extracted at pH 3.2)

In the 16–19 and 20–23 °Bx groups, no significant effects between treatments were observed on anthocyanins extracted at pH 3.2 (Fig. 6b).

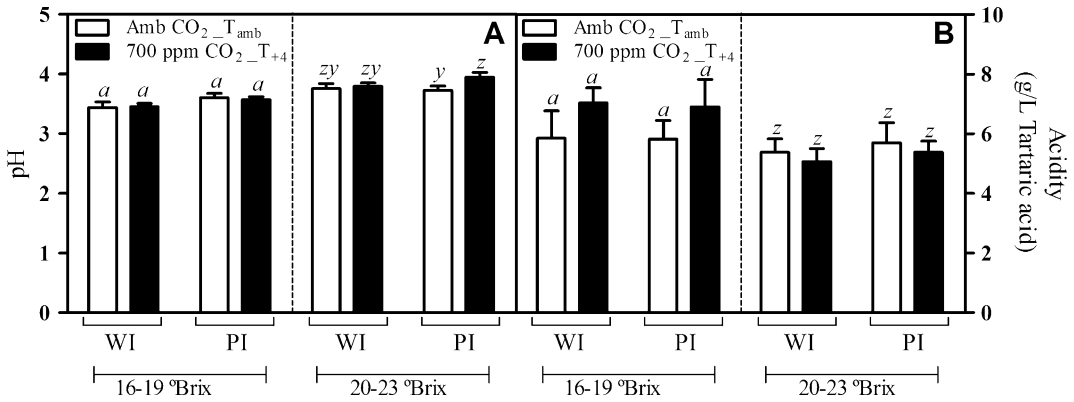

Fig. 4 Effect of CO_2, temperature and water availability on **a** pH and **b** total acidity during ripening of *V. vinifera* cv. Tempranillo in the 16–19 and 20–23 °Bx groups. Groups were plotted independently. Data (n=8–10, mean±S.E.). Different letters indicate significant differences between treatments ($P< 0.05$) based on LSD test. WI, well-irrigated; PI, partial irrigation

Cellular extractability (EA%)

In the 16–19 °Bx group (Fig. 6c), the two-way ANOVA analysis showed that water availability had significant effects on EA% ($P< 0.05$). In ambient conditions, however, partially irrigated plants showed only a tendency ($P= 0.074$) to decrease the cellular extractability with respect to well-irrigated plants. In the 20–23 °Bx group (Fig. 6c), the two-way ANOVA analysis showed no effects for the combination of elevated CO_2 and elevated temperature, and water availability, showing however an interaction ($P< 0.01$) between them. This interaction was reflected in a significant decrease (23–26%) of EA% in the Amb_PI with respect to Amb_WI and 700 T_{+4}_PI treatments ($P< 0.01$). The Amb_PI treatment exhibited lower (18%; $P= 0.052$) EA% than 700 T_{+4}_WI. Climate change simulation treatment (700 T_{+4}_PI) showed no significant differences on EA% with respect to the control one (Amb_WI).

Phenolic maturity of the grapes (Mp%), phenolic richness and tannins

No significant differences were observed in these parameters in any of the two groups (16–19 and 20–23 °Bx) in response to the treatments imposed (Fig. 7a, b and 8). It should be noted, however, that tannin levels tended to decrease with moderate drought treatments, especially in the 16–19 °Bx group ($P= 0.055$) (Fig. 8).

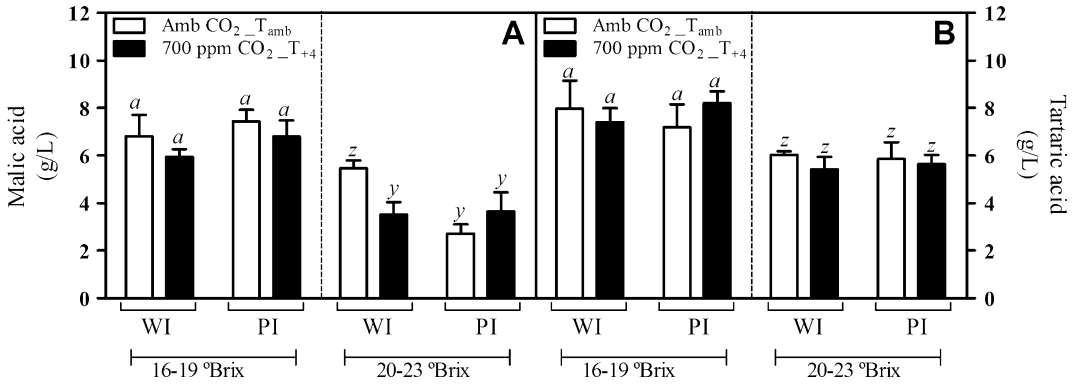

Fig. 5 Effect of CO_2, temperature and water availability on **a** malic acid and **b** tartaric acid during ripening of *V. vinifera* cv. Tempranillo in the 16–19 and 20–23 °Bx groups. Groups were plotted independently. Data (n=8–10, mean±S.E.). Different letters indicate significant differences between treatments ($P< 0.05$) based on LSD test. WI, well-irrigated; PI, partial irrigation

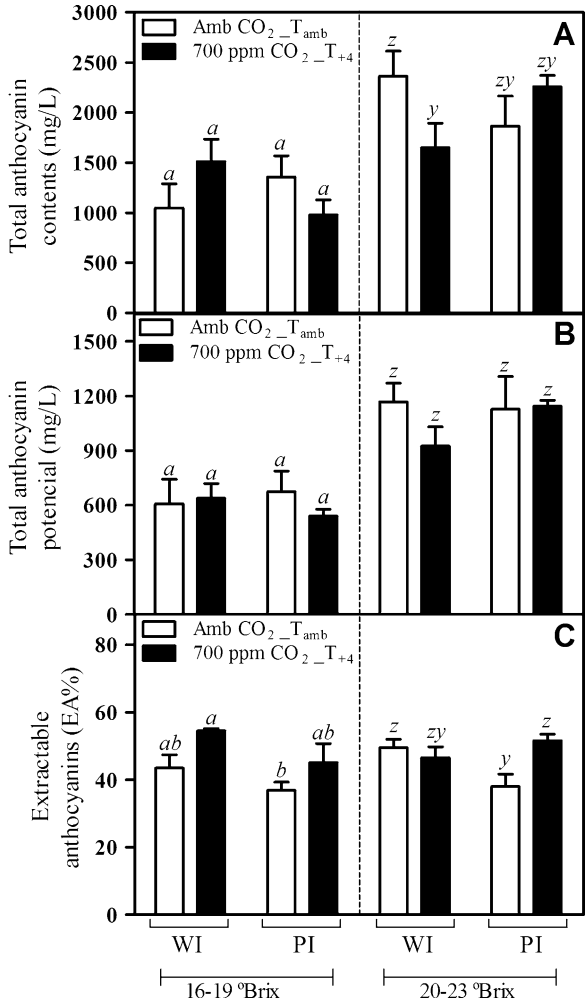

Fig. 6 Effect of CO_2, temperature and water availability on **a** anthocyanins extracted at pH 1.0, **b** anthocyanins extracted at pH 3.2 and **c** cellular extractability (EA%) during ripening of *V. vinifera* cv. Tempranillo in the 16–19 and 20–23 °Bx groups. Groups were plotted independently. Data (n=8–10, mean±S.E.). Different letters indicate significant differences between treatments ($P< 0.05$) based on LSD test. WI, well-irrigated; PI, partial irrigation

Tonality

In the 16–19 °Bx group (Fig. 9), the two-way ANOVA analysis showed no effects due to treatments. However, the simulation of climate change treatment (700 T_{+4}_PI) slightly increased tonality index with respect to Amb_WI, although this change was not statistically significant ($P= 0.083$). In the 20–23 °Bx (Fig. 9), elevated CO_2 and temperature, and water availability induced significant effects on the tonality ($P< 0.001$ and $P< 0.01$, respectively). Under elevated CO_2 and temperature conditions, tonality index was increased in both well-irrigated and partially irrigated plants ($P< 0.01$ in both cases) when compared to the current CO_2 and temperature. Plants subjected to ambient conditions showed a lower tonality index under partial irrigation ($P< 0.05$). Climate change simulation treatment (700 T_{+4}_PI) had no significant differences with respect to Amb_WI.

Discussion

Grapevine is a worldwide economically important crop. Any future global climate change trend might influence grapevine growth and development, consequently affecting wine quality. The final aim of the research project we are now developing is to gain knowledge on the responses of the grapevine to climate change scenarios both from a physiological and grape quality points of view. Towards this aim, we selected two phenological states from which applying the elevated CO_2 treatment: fruit set (experiments are now in progress) and veraison (this work). Fruit set and veraison are the two main critical steps in the phenological development of the grapes, when stress factors associated to climate change may act affecting grape quality. In the former, the elevated CO_2 may influence all phases of berry growth and accumulation of grape constituents, whereas in the latter effects are restricted to Phase (III) where our data indicate that in the cv. Tempranillo there is no further growth (see Fig. 3). By comparing the results of elevated CO_2 (simultaneously to elevated temperature and moderate drought) acting either from fruit set or from veraison, we will understand better how climate change scenarios influence grape growth and their effects on quality. Quality effects observed with the application of elevated CO_2 from veraison (this work) will be taken as a reference where there is no further grape growth during the CO_2 application. It should be also noted that the other stress factors (temperature and drought) are maximal in summer, just from veraison.

One of the main effects of climate change on grape and wine quality could be through effects on vegetative growth that occurs before veraison. However, this is the case only if growers allow plant to grow freely before veraison. Growers usually control this excess of vegetation growth by green, summer

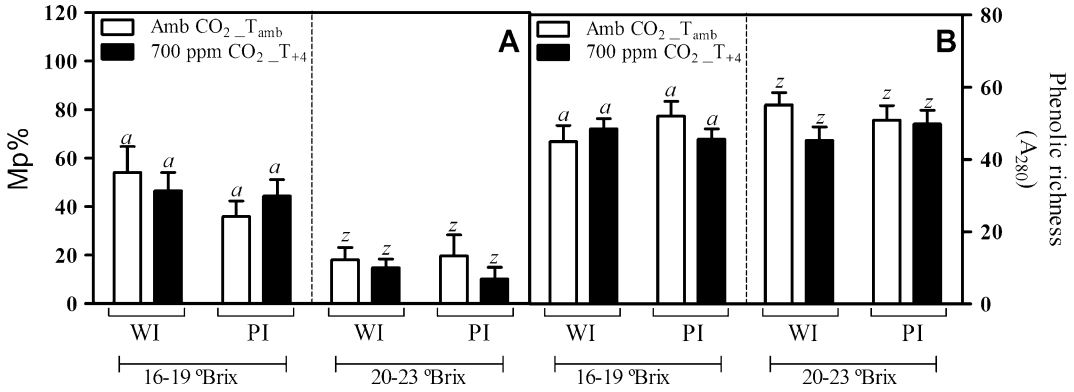

Fig. 7 Effect of CO_2, temperature and water availability on **a** phenolic maturity of the grapes (Mp%) and **b** phenolic richness during ripening of *V. vinifera* cv. Tempranillo in the 16–19 and 20–23 °Bx groups. Groups were plotted independently. Data (n=8–10, mean±S.E.). Different letters indicate significant differences between treatments ($P<0.05$) based on LSD test. WI, well-irrigated; PI, partial irrigation

pruning, minimizing undesirable effects on quality, until it becomes ineffective or uneconomic. In a similar way, we pruned our plants in order to maintain always vegetation to grape mass ratios optimal for grape maturation, simulating a common practice carried out under field conditions.

Grape quality is highly dependent on berry size and grape bunch size, the former because changes in size may concentrate (if size is reduced) or dilute (if size is increased) berry constituents (Antolín et al. 2003; Esteban et al. 2001; Koundouras et al. 2006; Ojeda et al. 2002), and the latter because grape bunch size influences shading and temperature pattern, and it is well known the light and temperature dependence of several grape constituents (Blouin and Guimberteau 2003; Kataoka et al. 1983; Kliewer 1970; Koundouras et al. 2006; Lakso and Kliewer 1975; Mori et al. 2005, 2007). We therefore selected plants with a grape mass per grapevine plant homogeneous in all treatments, when they were subjected to the climate change scenario.

Grape quality characteristics were therefore evaluated in greenhouse experiments simulating climate change conditions with elevated CO_2, elevated temperature and partial irrigation acting simultaneously during the ripening process. When compared to current ambient conditions, berries grown under climate change conditions had higher total soluble solids at harvest time,

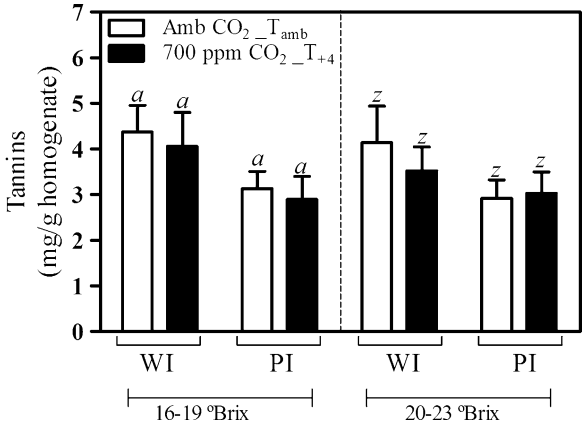

Fig. 8 Effect of CO_2, temperature and water availability on tannins during ripening of *V. vinifera* cv. Tempranillo in the 16–19 and 20–23 °Bx groups. Groups were plotted independently. Data (n=8–10, mean±S.E.). Different letters indicate significant differences between treatments ($P<0.05$) based on LSD test. WI, well-irrigated; PI, partial irrigation

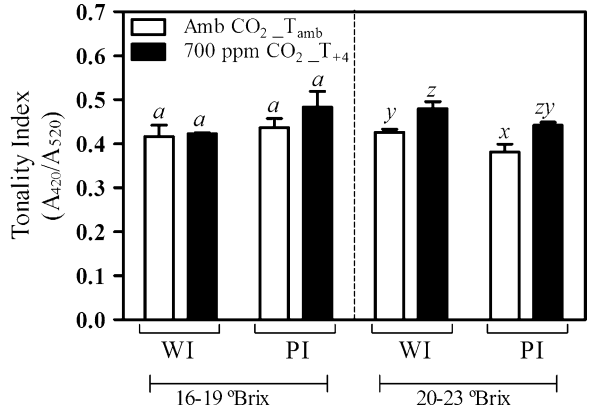

Fig. 9 Effect of CO_2, temperature and water availability on tonality index during ripening of *V. vinifera* cv. Tempranillo in the 16–19 and 20–23 °Bx groups. Groups were plotted independently. Data (n=8–10, mean±S.E.). Different letters indicate significant differences between treatments ($P<0.05$) based on LSD test. WI, well-irrigated; PI, partial irrigation

suggesting earlier grape harvest time in the future. Other effects indicative of maturation excess were higher grape color intensity (measured as the sum of absorbances at 420, 520 and 620 nm in the must), an increase in the anthocyanins extractability index (i.e., more difficulties for anthocyanins extraction), a decreased malic acid concentration and a pH increase (data not shown). If effects of grapevine phenological stage were as much as possible mitigated by regrouping grapes having similar total soluble solids, berry quality parameters, measured in this work, showed some important findings that can be summarized as follows: (i) under current CO_2 and temperature conditions, partial irrigation markedly decreased grape malic acid concentrations, (ii) partial irrigation increased the amount of anthocyanins extracted over the maximum possible under current CO_2 and temperature conditions, but decreased it under elevated CO_2 and temperature conditions, (iii) in absence of drought, elevated CO_2 and temperature decreased grape malic acid and total anthocyanins concentrations, (iv) either in presence or in absence of drought, elevated CO_2 and temperature increased tonality index that clearly modifies wine characteristics if future scenarios of climate change are confirmed (Bindi et al. (2001), on the contrary, reported no differences in color tonality and color intensity in wines obtained from grapevines subjected to elevated CO_2 concentrations with respect to controls), and (v) no effects on total anthocyanins, tannins, phenolic maturity of the grapes and richness, and on phenolic maturity of the grapes suggest that simulated climate change (when elevated CO_2, elevated temperature, and partial irrigation interact) would not influence wine quality from a phenolic composition and wine astringent characteristics point of view. These latter results are in line with others showing no differences on tannins (Gonçalves et al. 2009) and on berries and wine polyphenols (Bindi et al. 2001) when exposed to elevated CO_2. On the contrary, high diurnal temperature was shown to lead to higher tannins and flavors synthesis (Mori et al. 2005). Also, phenolic maturity of the grapes (Mp%) during ripening might be related to tannins extraction in seeds, and with the astringent character of the wine (Segade et al. 2008). Our data show no changes on this parameter, fact that could imply that under climate change conditions the wine astringent characteristics would not be affected.

Environmental conditions of the greenhouses used are not common in the field under Mediterranean conditions. Conditions were 26/15°C and 40/80% RH (day/night) until veraison (VPD=2.01/0.34 kPa day/night). From veraison to ripeness, conditions were 24/14°C and 60% RH (ambient; VPD=1.19/0.64 kPa day/night) versus 28/18°C and 60% RH (climate change scenario; VPD=1.51/0.82 kPa day/night). Experiments are now in progress in different greenhouses (Temperature Gradient Greenhouse, TGG) where natural changes in temperature and RH are tracked. In these TGG in a typical summer day at midday, temperature could reach 28–30°C and 40% RH (VPD=2.26–2.54 kPa), decreasing to 14–15°C and increasing to 75–80% RH (VPD=0.32–0.43 kPa) during the night (our unpublished results). Therefore, differences are not so large until veraison but become important from veraison to ripeness, which were imposed by the establishment of treatments conditions. Grapevine development in higher VPDs, i.e., in more stressful conditions, may influence grape quality in the TGG experiments, and results, which will be presented in a separate report, may differ from those reported in this work.

Acknowledgements Financial support from Spanish Ministry of Science and Innovation (BFU2008-01405/BFI), Fundación Universitaria de Navarra (Plan de Investigación de la Universidad de Navarra) and Caja Navarra is gratefully acknowledged. Fermín Morales wishes to thank Gobierno de Aragón (A03 research group) for financial support. Carolina Salazar-Parra was the recipient of a grant from Asociación de Amigos de la Universidad de Navarra. Authors wish to thank A. Urdiain and M. Oyarzun for excellent technical assistance, and Station of Viticulture and Enology of Navarra (Olite, Navarra, Spain) for dormant cuttings supply.

References

Antolín MC, Baigorri H, De Luis I, Aguirrezabal F, Geny L, Broquedis M, Sánchez-Díaz M (2003) ABA during reproductive development in non-irrigated grapevines (*Vitis vinifera* L. cv. Tempranillo). Aust J Grape Wine Res 9:169–176

Antolín MC, Ayari M, Sánchez-Díaz M (2006) Effects of partial rootzone drying on yield, ripening and berry ABA in potted Tempranillo grapevines with split roots. Aust J Grape Wine Res 12:13–20

Bindi M, Fibbi L, Gozzini B, Orlandini S, Miglietta F (1996) Modelling the impact of future climate scenarios on yield and yield variability of grapevine. Clim Res 7:213–224

Bindi M, Fibbi L, Miglietta F (2001) Free Air CO_2 Enrichment (FACE) of grapevine (*Vitis vinifera* L.): II. Growth and quality of grape and wine in response to elevated CO_2 concentrations. Eur J Agron 14:145–155

Blouin J, Guimberteau G (2003) Maduración. In Maduracion y madurez de la uva. Mundi-Prensa Libros, pp. 32–40

Bogs J, Downey MO, Harvey JS, Ashton AR, Tanner GJ, Robinson SP (2005) Proanthocyanidin synthesis and expression of genes encoding leucoanthocyanidin reductase and anthocyanidin reductase in developing grape berries and grapevine leaves. Plant Physiol 139:652–663

Bowes G (1993) Facing the inevitable—plants and increasing atmospheric CO_2. Annu Rev Plant Physiol Plant Mol Biol 44:309–332

Cohen SD, Tarara JM, Kennedy JA (2008) Assessing the impact of temperature on grape phenolic metabolism. Anal Chim Acta 621:57–67

de Freitas VAP, Glories Y, Monique A (2000) Developmental changes of procyanidins in grapes of red *Vitis vinifera* varieties and their composition in respective wines. Am J Enol Vitic 51:397–403

De Souza CR, Maroco JP, Dos Santos TP, Rodrigues ML, Lopes CM, Pereira JS, Chaves MM (2005) Grape berry metabolism in field-grown grapevines exposed to different irrigation strategies. Vitis 44:103–109

Deluc LG, Grimplet J, Wheatley MD, Tillett RL, Quilici DR, Osborne C, Schooley DA, Schlauch KA, Cushman JC, Cramer GR (2007) Transcriptomic and metabolite analyses of Cabernet Sauvignon grape berry development. BMC Genomics 8:429

Downey MO, Harvey JS, Robinson SP (2003) Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. Aust J Grape Wine Res 9:15–27

Erice G, Irigoyen JJ, Pérez P, Martínez-Carrasco R, Sánchez-Díaz M (2006) Effect of elevated CO_2, temperature and drought on photosynthesis of nodulated alfalfa during a cutting regrowth cycle. Physiol Plant 126:458–468

Esteban MA, Villanueva MJ, Lissarrague JR (1999) Effect of irrigation on changes in berry composition of Tempranillo during maturation. Sugars, organic acids, and mineral elements. Am J Enol Vitic 50:418–434

Esteban MA, Villanueva MJ, Lissarrague JR (2001) Effect of irrigation on changes in the anthocyanin composition of the skin of cv Tempranillo (*Vitis vinifera* L.) grape berries during ripening. J Sci Food Agric 81:409–420

Glories Y, Augustin M (1993) Maturité phénolique du raisin, consèquences technologiques: applications aux millésimes 1991 et 1992. Actes du Colloque "Journée technique du CIVB" 21 Janvier, Bordeaux, 56

Gonçalves B, Falco V, Moutinho-Pereira J, Bacelar E, Peixoto F, Correia C (2009) Effects of elevated CO_2 on grapevine (*Vitis vinifera* L.): volatile composition, phenolic content, and in vitro antioxidant activity of red wine. J Agric Food Chem 57:265–273

Hanlin RL, Downey MO (2009) Condensed tannin accumulation and composition in skin of Shiraz and Cabernet Sauvignon grapes during berry development. Am J Enol Vitic 60:13–23

Hoagland DR, Arnon DI (1950) The water-culture method for growing plants without soil. California Agricultural Experiment Station. Circular 347:1–32

Intrigliolo DS, Castel JR (2009) Response of *Vitis vinifera* cv. 'Tempranillo' to partial rootzone drying in the field: water relations, growth, yield and fruit and wine quality. Agric Water Manag 96:282–292

IPCC (2007a) Climate change and its impacts in the near and long term under different scenarios. In: Core Writing Team, Pachauri RK, Reisinger A (eds) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, pp 44–54

IPCC (2007b) Observed changes in climate and their effects. In: Core Writing Team, Pachauri RK, Reisinger A (eds) Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. IPCC, Geneva, pp 30–33

Jackson D, Lombard P (1993) Environmental and management practices affecting grape composition and wine quality—A review. Am J Enol Vitic 44:409–430

Jifon J, Wolfe D (2002) Photosynthetic acclimation to elevated CO_2 in *Phaseolus vulgaris* L. is altered by growth response to nitrogen supply. Glob Chang Biol 8:1018–1027

Kataoka I, Kubo Y, Sugiura A, Tomana T (1983) Effects of temperature, cluster shading and some growth regulators on L-phenylalanine ammonia-lyase activity and anthocyanin accumulation in black grapes. Mem Coll Agric Kyoto Univ 124:35–44

Kliewer W (1970) Effect of day temperature and light intensity on coloration of *Vitis vinifera* L. grapes. J Am Soc Hortic Sci 95:693–697

Kliewer WM, Weaver RJ (1971) Effect of crop level and leaf area on growth, composition and coloration of Tokay grapes. Am J Enol Vitic 22:172

Koundouras S, Marinos V, Gkoulioti A, Kotseridis Y, van Leeuwen C (2006) Influence of vineyard location and vine water status on fruit maturation of nonirrigated cv. Agiorgitiko (*Vitis vinifera* L.). Effect on wine phenolic and aroma components. J Agric Food Chem 54:5077–5086

Lakso AN, Kliewer WM (1975) The influence of temperature on malic acid metabolism in grape berries: I. enzyme responses. Plant Physiol 56:370–372

Levitt J (1980) Responses of plants to environmental stresses. Academic

Long SP, Ainsworth EA, Rogers A, Ort DR (2004) Rising atmospheric carbon dioxide: plants face the future. Annu Rev Plant Biol 55:591–628

López MI, Sánchez MT, Díaz A, Ramírez P, Morales J (2007) Influence of a deficit irrigation regime during ripening on berry composition in grapevines (*Vitis vinifera* L.) grown in semi-arid areas. Int J Food Sci Nutr 58:491–507

Matthews MA, Anderson MM (1989) Reproductive development in grape (*Vitis vinifera* L.): responses to seasonal water deficits. Am J Enol Vitic 40:52–60

Matthews M, Ishii R, Anderson M, O'mahony M (1990) Dependence of wine sensory attributes on vine water status. J Sci Food Agric 51:321–335

Mazza G, Miniati E (1993) Anthocyanins in fruit, vegetables, and grains. CRC, Boca Raton

McCarthy MG (1997) The effect of transient water deficit on berry development of cv. Shiraz (*Vitis vinifera* L.). Aust J Grape Wine Res 3:102–108

Mooney H, Winner WE, Pell EJ (1991) Responses of plants to multiple stresses. Academic

Mori K, Sugaya S, Gemma H (2005) Decreased anthocyanin biosynthesis in grape berries grown under elevated night temperature condition. Sci Hortic 105:319–330

Mori K, Goto-Yamamoto N, Kitayama M, Hashizume H (2007) Loss of anthocyanins in red-wine grape under high temperature. J Exp Bot 58:1935–1945

Mullins MG (1966) Test-plant for investigations of the physiology of fruiting in *Vitis vinifera* L. Nature 209:419–420

Mullins MG, Bouquet A, Williams LE (1992) Developmental physiology: the vegetative grapevine. In: Biology of the grapevine. Cambridge University Press, New York, pp. 80–111

Myburgh PA (2003) Responses of *Vitis vinifera* L. cv. Sultanina to water deficits during various pre- and post-harvest phases under semi-arid conditions. S Afr J Enol Vitic 24:25–33

Navarro S, Leon M, Roca-Perez L, Boluda R, Garcia-Ferriz L, Perez-Bermudez P, Gavidia I (2008) Characterisation of Bobal and Crujidera grape cultivars, in comparison with Tempranillo and Cabernet Sauvignon: evolution of leaf macronutrients and berry composition during grape ripening. Food Chem 108:182–190

Ojeda H, Andary C, Kraeva E, Carbonneau A, Deloire A (2002) Influence of pre- and postveraison water deficit on synthesis and concentration of skin phenolic compounds during berry growth of *Vitis vinifera* cv. Shiraz. Am J Enol Vitic 53:261–267

Ollat N, Geny L, Soyer J (1998) Les boutures fructiferes de vigne: validation d'un modele d'etude du developpement de la physiologie de la vigne. I. Caracteristiques de l'appareil vegetatif. J Int Sci Vigne Vin 32:1–9

Rebelein H (1973) Rapid quantitative determination of tartaric acid. Chem Mikrobiol Technol Lebensm 2:33–38

Renaud S, de Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339:1523–1526

Ribéreau-Gayon J, Stonestreet E (1965) Le dosage des anthocyanes dans le vin rouge. Bull Soc Chim 9:2649–2652

Roby G, Matthews MA (2004) Relative proportions of seed, skin and flesh, in ripe berries from Cabernet Sauvignon grapevines grown in a vineyard either well irrigated or under water deficit. Aust J Grape Wine Res 10:74–82

Roby G, Harbetson JF, Adams DA, Matthews MA (2004) Berry size and vine water deficits as factors in winegrape composition: anthocyanins and tannins. Aust J Grape Wine Res 10:100–107

Rogers HH, Runion GB, Krupa SV (1994) Plant-responses to atmospheric CO_2 enrichment with emphasis on roots and the rhizosphere. Environ Pollut 83:155–189

Salon JL, Chirivella C, Castel JR (2005) Response of cv. Bobal to timing of deficit irrigation in Requena, Spain: water relations, yield, and wine quality. Am J Enol Vitic 56:1–8

Santa María E (2004) Incidencia de *Botrytis cinera* en relación con diferentes aspectos fisiológicos de la vid. PhD Thesis Dissertation, University of Navarra, Spain.

Segade SR, Vázquez ES, Losada ED (2008) Influence of ripeness grade on accumulation and extractability of grape skin anthocyanins in different cultivars. J Food Compos Anal 21:599–607

Shiraishi S, Watanabe Y (1994) Anthocyanin pigments in the grape skins of cultivars (*Vitis* spp.). Sci Bull Fac Agric Kyushu Univ 48:255–262

Singleton V (1972) Effects on red wine quality of removing juice before fermentation to simulate variation in berry size. Am J Enol Vitic 43:63–70

Sudraud P (1958) Interpretation des courbes d'absorption des vins rouges. Ann Technol Agric 7:203–208

Vidal M, Blouin J (1978) Dosage colorimetrique rapide de l'acide tartrique dans les moûts et les vins. Rev Fr Oenologie 16:39–46

Winkler AJ, Cook JA, Kliewer WM, Lider LA (1962) Development and composition of grapes. In: General viticulture. University of California Press, Berkeley, pp. 138–196

Yamane T, Shibayama K (2006) Effects of changes in the sensitivity to temperature on skin coloration in 'Aki Queen' grape berries. J Jpn Soc Hortic Sci 75:458–462

Yamane T, Jeong ST, Goto-Yamamoto N, Koshita Y, Kobayashi S (2006) Effects of temperature on anthocyanin biosynthesis in grape berry skins. Am J Enol Vitic 57:54–59