# Growth and physiology of four *Vitis vinifera* L. cv. Tempranillo clones under future warming and water deficit regimes

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**Running title:** Response of Tempranillo clones to climate change

## Abstract

**Background and Aim:** The interactive effects of simulated 2100 environmental conditions (air temperature and $CO_2$ level) and water deficit on four clones of *Vitis vinifera* cv. Tempranillo were investigated.

**Methods and Results:** Fruit-bearing cuttings were subjected to: (i) two temperature/$CO_2$/RH regimes: climate change (CC) (28°C/18°C, 700 µmol/mol $CO_2$ and 33%/53% RH, day/night) vs current climatic conditions (CS) (24°C/14°C, 400 µmol/mol $CO_2$ and 45%/65% RH), combined with (ii) two water availabilities: well-watered (WW) vs water deficit (WD). Climate change increased net photosynthesis ($A_n$), transiently ameliorating the low carbon fixation rates under drought, but not the reduction in vegetative and reproductive growth. Current climate increased intrinsic water use efficiency ($A_n/g_s$), especially when combined with WD, but not the instantaneous water use efficiency ($A_n/T$). The clones exhibited differences in the ripening time, plant vigour and reproductive growth. Variability in the response of $A_n$, phenology and growth to the simulated conditions was observed among clones.

**Conclusions:** Differences in the length of the reproductive cycle conditioned, in part, the physiological response of the clones to the environmental factors.

**Significance of the Study:** The study improves our understanding of the interactive effects of climate change factors and provides insights into the response of different clones, as the basis for the adaptation of cultivars in their traditional growing regions.



***Keywords****: climate change, gas exchange, growth, phenology,* Vitis vinifera *L., water deficit*

# Introduction

According to the 5th assessment report of the Intergovernmental Panel on Climate Change (IPCC), precipitation patterns are expected to be altered, with likely more frequent drought events predicted for regions that are already arid (Intergovernmental Panel on Climate Change 2013). These changes will be a consequence of the likely increase in the atmospheric $CO_2$ concentration, among other greenhouse gases (GHG). The IPCC has determined the magnitude of these changes according to different emission scenarios called Representative Concentration Pathways (RCPs). According to these time-dependent projections of atmospheric GHG, the atmospheric $CO_2$ concentration may increase up to between 670 and 936 µmol/mol, according to the RCP 6.0 and RCP 8.5, respectively, by the end of this century (Moss et al. 2008, 2010), thus contributing to an expected increase in global mean temperature of 2.2 ± 0.5°C and 3.7 ± 0.7°C, respectively, according to the concentration-driven CMIP5 model simulations (Taylor et al. 2012, Intergovernmental Panel on Climate Change 2013). Recent models also report a decrease in near-surface land RH with climate change (Byrne and O'Gorman 2016). Therefore, even if rainfall does not decrease locally, water deficit experienced by crops will likely increase because of the impact of temperature and RH on evapotranspiration, especially in summer (van Leeuwen and Darriet 2016).

Grapevine (*Vitis vinifera* L.) is one of the most widespread crops worldwide (7.5 Mha in 2016) (Organisation Internationale de la Vigne et du Vin 2017). Europe represents the largest vineyard area in the world (39% of the world area), the main part being located in the Mediterranean region (Organisation Internationale de la Vigne et du Vin 2017). The region is considered to be vulnerable to the impacts of climate change, especially concerning water availability (Fraga et al. 2019). Grapevine development and grape ripening are sensitive to environmental factors. Under conditions of water deficit, stomata closure is one of the early grapevine responses in order to prevent hydraulic failure (Charrier et al. 2018), thus restricting water loss, but also C assimilation (Flexas et al. 1998, Chaves et al. 2010). Consequently, drought has been reported to decrease grapevine vigour and final fruit production (Chaves et al. 2007, Salazar-Parra et al. 2012, Kizildeniz et al. 2015), with variable effects on phenology, depending on its intensity (van Leeuwen et al. 2009). Increases in temperature and atmospheric $CO_2$ concentration have been reported to impact the physiology and development of many plant species (Wang et al. 2012, Gray and Brady 2016, Kacienè et al. 2017). In the case of grapevine, leaf photosynthetic activity ($A_n$) is stimulated under elevated $CO_2$ conditions during the first days of exposure, then experiencing in some cases an acclimation process resulting in down-regulated photosynthesis rates (Salazar-Parra et al. 2012, 2015). Elevated $CO_2$ also increased grape yield of cvs Sangiovese, Cabernet Sauvignon and Riesling (Bindi et al. 2001, Wohlfahrt et al. 2018), but this effect was not so marked in other experiments with Tempranillo and Touriga Franca (Moutinho-Pereira et al. 2009, Kizildeniz et al. 2015, 2018). Studies of elevated temperature have mainly focused on grape ripening and composition (Kuhn et al. 2014, Gouot et al. 2019). Warm temperature has been reported to accelerate grapevine phenology, and to increase photosynthesis performance (Greer and Weedon 2012, De Cortázar-Atauri et al.

2017), however, extreme heat events (temperature higher than 40°C), reduce stomatal conductance and photosynthesis rates, slowing down grape ripening (Greer and Weedon 2013). As a consequence, vegetative growth and yield are also expected to decrease (Medrano et al. 2003, Webb et al. 2009).

Plants will not experience climate change factors individually, but simultaneously. The combined impact of these factors is frequently non-additive (Gray and Brady 2016), and can be either greater or lower than expected on single-factor studies (synergistic and antagonistic, respectively). The number of studies, however, on the interactive effects of environmental factors on grapevine physiology is still relatively few. Combined high temperature and elevated $CO_2$ concentration have been reported to increase the photosynthetic capacity of grapevine, hastening grape development (Martínez-Lüscher et al. 2015b, 2016). The interactive effects among water deficit and heat temperature on grapevine physiology have been also investigated in short-term experiments, showing a more severe effect of high-temperature events on grapevines experiencing water stress (Edwards et al. 2011, Galat Giorgi et al. 2019). The studies on the physiological and growth response of grapevine to multiple stress factors associated with climate change ($CO_2$, temperature and water deficit) remain limited due to their complexity. Authors report that the beneficial effect of increased air $CO_2$ and elevated temperature on the photosynthetic performance and growth of Tempranillo was eliminated by water deficit (Leibar et al. 2015, Kizildeniz et al. 2018).

In order to reduce adverse climatic effects, some adaptation of future viticulture is needed. Within this context, the choice of adequate plant material is one of the most powerful tools. Grapevine plants are propagated vegetatively, and new features can appear spontaneously in a bud after accidental modifications in the DNA. These modifications include point mutations, large deletions, illegitimate recombinations or variable number of repeats in microsatellite sequences (Pelsy 2010). This emergence of genetic variability leads to clonal variation within a cultivar. The existing collections of clones can be explored to detect any phenotypic variation that could be useful in the adaptation of cultivars to climate change in their traditional growing region (Duchêne 2016), without changing wine typicity, and giving rise to plant material immediately accepted by the corresponding protection figure (Ibáñez et al. 2015).

Phenology is considered the first biological indicator of climate change, this trait being considered one of the main factors to be explored for cultivar adaptation (Duchêne et al. 2010). In this sense, the selection of late-ripening clones has been proposed as a strategy to mitigate the alterations of grape quality caused by high temperature during fruit ripening (van Leeuwen and Darriet 2016). Among the cultivars grown in the Mediterranean area, Tempranillo is one of the most internationally recognised, with the highest number of certified clones (Ibáñez et al. 2015). Variability among Tempranillo clones for phenological development has previously been described (Arrizabalaga et al. 2018). Few studies, however, have assessed the performance of different clones of the same cultivar to climate conditions expected by 2100 (Torres et al. 2015, 2016, Tortosa et al. 2019).

The purpose of our study was to evaluate the response of four Tempranillo clones, differing in their maturation times, to the combined effects of elevated $CO_2$, elevated temperature and reduced RH in plants exposed to two irrigation regimes. The hypothesis behind this study is that differences among clones in the phenological development may condition their physiological and growth response to future climate conditions. One of the strengths of this study lies in the assessment of three-way interactions among clones, T/$CO_2$/RH regimes, and water availability. Considering that it is not possible to extrapolate plant responses to combined environmental conditions from the response derived from a single condition (Rampino et al. 2012), the information obtained from multi-stress approaches is crucial to predict the impact of projected climate change on grapevines and to develop appropriate adaptation strategies.

## Material and methods

*Plant material: origin and development*

Dormant cuttings of four clones of *Vitis vinifera* L. cv. Tempranillo were used in this experiment: three certified clones (CL306, RJ43 and VN31) and one non-commercialised clone (1084). Clones CL306 and RJ43 are two Spanish clones widely distributed during the previous decade (Ibáñez et al. 2015) and considered as short and intermediate cycle variants (Yuste et al. 2001, Estación de Viticultura y Enología de Navarra 2009, Vicente Castro 2012). According to the agronomic characterisation, 5-year study, done by the Instituto Tecnológico Agrario de Castilla y León (Junta de Castilla y León, Valladolid, Spain), the CL306 clone has a budburst to maturity period 5 days shorter than the average of the Tempranillo (Tinta Toro) cultivar, as well as higher potential alcohol concentration (Yuste et al. 2001). In a recent study, RJ43 and CL306 showed a complete cycle (budburst–maturity) of 166 and 155 days, respectively, and a flowering–maturity period of 107 and 102 days [1-year study, Instituto de Ciencias de la Vid y del Vino (ICVV), Logroño, Spain]. The plant material of these two clones was provided by Estación de Viticultura y Enología de Navarra (EVENA, Olite, Spain). Vitis Navarra nursery (Larraga, Spain) supplied the VN31, a certified clone characterised (5-year field study, unpublished data, 2016). It is described as a long-phenological development clone, based on its lower potential alcohol concentration at harvest, compared with that of the average of Tempranillo genotypes assessed (García García 2014). The non-commercialised accession 1084 was provided by the Instituto de Ciencias de la Vid y del Vino (ICVV, Logroño, Spain) and it is classified as a long-phenological development clone. This clone showed a complete cycle of 172 days (14 days longer than the average for the cultivar) and a flowering-maturity period of 117 days (11 days longer than the average of the cultivar) (data of a 3-year field study by ICVV).

An adapted protocol from Mullins and Rajasekaran (1981) was used to produce fruit-bearing cuttings, as described in Morales et al. (2016). Cuttings were treated with a solution of indole butyric acid (300 mg/L), kept for several weeks in a hot-bed at 27°C, and placed in a cold room at 5°C, until they developed roots. Then, cuttings were set in pots of 0.8 L with a mixture of 2:1 peat:sand (v/v) and transferred to growth chamber greenhouses (GCGs) (Morales et al. 2014) located at the University of Navarra. They grew up to fruitset at 25°C/15°C (day/night), air RH of 50% and natural light supplemented with high-pressure metal halide lamps (POWERSTAR HQI-TS 400W/D PRO, OSRAM, Augsburg, Germany) with a photosynthetic photon flux density of 500 µmol/($m^2 \cdot s$) at plant level for 15 h a day. Plants were irrigated with the nutritive solution described in Ollat et al. (1998). Vegetative growth was controlled by manual pruning, and a single flowering stem was allowed to develop on each plant in order to obtain a single berry bunch per plant. Plants were maintained with four leaves up to fruitset. At this moment, and for standardisation purposes, plants of each clone showing similar phenological development were transferred to 7 L pots with a mixture of 2:1 peat:sand (v/v).

*Experimental design*

At fruitset, plants of each of the four clones were transferred to growth chamber greenhouses (GCGs), and divided into four homogeneous groups. Plants were selected to have a similar number of berries. From fruitset to maturity (considered at 23°Brix), plants were subjected to two temperature, $CO_2$ concentration and RH (T/$CO_2$/RH) regimes: climate change conditions (CC: 28°C/18°C, day/night, 700 µmol/mol $CO_2$ and 33%/53% RH, day/night) vs current climatic conditions (CS: 24°C/14°C, 400 µmol/mol $CO_2$ and 45%/65% RH). Moreover, plants within each greenhouse were subjected to two water regimes: well-watered (WW) vs water deficit (WD, i.e. 60% of the irrigation received by the WW plants). The combination of clones, T/$CO_2$/RH regimes and water availability made a total of 16 treatments with eight replicates each.

The $CO_2$ and temperature conditions in the CC treatment (temperature 4ºC warmer and atmospheric $CO_2$ concentration 300 µmol/mol higher than in the CS treatment) approached the projections for the year 2100, as per the RCP 8.5 greenhouse emission scenario, derived from the concentration-driven CMIP5 model simulations (Taylor et al. 2012, Intergovernmental Panel for Climate Change 2013). Regarding RH, ENSEMBLES models (based on IPCC data), according to the Max Planck Institute model [MPI-ECHAM5 (Roeckner et al. 2003)], state that the RH for the summer period will likely be 12% lower at the end of the present century in the area of study (Navarra and La Rioja regions) (Leibar et al. 2015). For details about the motorisation and control of the environmental parameters within the GCGs, readers are referred to Morales et al. (2014). Irrigation regimes were designed following our previous experience with grapevine fruit-bearing cuttings (Kizildeniz et al. 2015, Leibar et al. 2015). The water deficit level was chosen to match conditions predicted for the end of the present century in the regions of Navarra and La Rioja by the model of the Max Planck Institute, that is likely 40% lower precipitation in the summer (Leibar et al. 2015). Soil water

sensors (EC-5 Soil Moisture Sensors, Decagon Devices, Pullman, WA, USA) were placed in the substrate to monitor soil water content. Well-watered plants were maintained at about 90% of the substrate field capacity (sensor value between 40–50%, $m^3$ $H_2O$/100 $m^3$ substrate), equivalent to 400–500 g $H_2O$/L substrate. In the water deficit treatment, plants were subjected to a drought that consisted of withholding irrigation until the soil moisture sensors reached a value of ca. 10% ($m^3$ $H_2O$/100 $m^3$), equivalent to 100 g $H_2O$/L substrate. Then, plants were irrigated with 60% of the volume received by the WW plants during the corresponding drought period. These periods of drought and re-watering were repeated successively throughout the experiment. In order to provide the same amount of nutrients to all the treatments, the WW plants were irrigated with a nutrient solution (Ollat et al. 1998) alternated with water, whereas WD plants were irrigated only with nutrient solution. The plants were drip irrigated, either with nutrient solution or water with, two drippers of 60 mL/min per pot. Pots had free drainage at the bottom.

*Pre-dawn leaf water potential*

Pre-dawn leaf water potential ($\Psi_{leaf}$) was measured at mid-veraison (half of the berries in the bunch had started to change colour) and 2 weeks after mid-veraison (56.0 ± 1.5 and 70.0 ± 1.5 days on average after fruitset, respectively), in young fully expanded leaves (three–five plants per clone and treatment), using a pressure chamber (SKYE SKPM 1400, Skye Instruments, Llandrindod, Wales) and according to the methodology described by Scholander et al. (1965).

*Phenological development*

The number of days between fruitset (E-L 27) and maturity (TSS of ca. 23°Brix, E-L 38) (Coombe 1995) was determined. Fruitset was assessed visually (young berries between 1–2 mm diameter). Maturity was established by sampling periodically two berries (from the top and middle portion of the bunch, which allocate the highest number of berries) and measuring the TSS in the must using a refractometer (Abbe Digital 315RS, Zuzi, Beriain, Spain). Every plant was assessed individually.

*Leaf gas exchange and chlorophyll content*

Net photosynthesis ($A_n$), transpiration ($T$) and stomatal conductance ($g_s$) were measured at mid-veraison and 2 weeks after mid-veraison in young fully expanded leaves (four–eight plants per treatment), using a portable photosynthesis system (LCi-SD with the PLUS5 compact light unit, ADC BioScientific, Hoddesdon, England). Measurements started 3 h after sunrise and extended over about 3 h. Temperature, $CO_2$ and RH conditions used in the measurement chamber corresponded to the respective growth conditions (400 µmol/mol $CO_2$, 24°C and 45% RH for CS

condition, and 700 µmol/mol $CO_2$, 28°C and 35% RH for CC condition). The photosynthetic photon flux density was set at 1200 µmol/($m^2 \cdot s$). With the values of gas exchange parameters measured, intrinsic and instantaneous water use efficiencies were calculated as $A_n/g_s$ ($WUE_i$) and $A_n/T$ ($WUE_{inst}$), respectively.

The concentration of chlorophyll was assessed in young, fully expanded leaves by non-destructive fluorescence measurements, using a multiparametric portable optical sensor (Multiplex_Research, FORCE-A, Orsay, France). Chlorophyll concentration is correlated to a parameter resulting from the ratio of far-red to red fluorescence (SFR_R index) (Gitelson et al. 1999).

*Plant growth and fruit production*

Leaf area was determined at mid-veraison, 2 weeks after mid-veraison and maturity, by measuring the shoot length, and using a regression model adapted for Tempranillo by Arrizabalaga et al. (2018). The model relates leaf area measured using a leaf area meter (LI-300 model; Li-COR Biosciences, Lincoln, NE, USA) (y) and total shoot length (x): leaf area ($dm^2$) = 13.859x + 200.33; $R^2$ = 0.9239 (x = shoot length). The vegetative production, expressed as dry matter mass, was measured at maturity by weighing the oven-dried leaves, stem and roots. The drying was done by placing the plant material in an oven at 80°C until constant mass. The fresh mass of individual berries was measured throughout the ripening process (mid-veraison, 1 week after mid-veraison, 2 weeks after mid-veraison and maturity). At maturity, the fresh bunch mass and the number of berries per bunch were determined.

*Statistical analysis*

The software used for the statistical analysis was R (version 3.5.1, Lucent Technologies, Murray Hill, NJ, USA). Data were first analysed using a three-way ANOVA (three factors: clone, T/$CO_2$/RH regime and water availability) in order to determine the effects of the treatments and their possible interactions. The Fisher's least significant difference (LSD) test was used as a post-hoc. Results were considered statistically significant at $P<0.05$. Results were also analysed using a principal component analysis (PCA).

# Results

*Pre-dawn leaf water potential*

Clones had similar $\Psi_{leaf}$ values ($P_{CL}$=0.243 at mid-veraison and $P_{CL}$=0.765 2 weeks after mid-veraison). Water deficit (WD) significantly reduced $\Psi_{leaf}$ compared with vines grown under WW conditions (Figure 1). A significant interaction between the T/$CO_2$/RH and irrigation regimes was observed at mid-veraison, when, under WD conditions, CS plants had lower $\Psi_{leaf}$ than the CC vines.

*Leaf gas exchange parameters and photosynthetic pigments*

The clones showed similar $A_n$, $T$ and $g_s$ values at mid-veraison, considering all the T/$CO_2$/RH and water regime situations altogether ($P_{CL}$ values of 0.295, 0.222 and 0.160 for $A_n$, $T$, and $g_s$, respectively). Nevertheless, under WW conditions, 1084 tended to have lower $A_n$, $T$ and, especially, $g_s$ values than the other clones (Figure 2a). Two weeks after mid-veraison, a significant interaction between clone and irrigation level was observed for $A_n$, $T$ and $g_s$. At this time, comparing the four clones under WW conditions, the 1084 accession showed significantly lower $A_n$ and $T$ compared with RJ43 and VN31 (CS conditions) and with VN31 (CC conditions), as well as the lowest $g_s$ values among the four clones (CS conditions). These differences, however, disappeared under WD conditions, which drastically reduced the values of gas exchange parameters. Considering the clones altogether, CC conditions enhanced significantly $A_n$ at mid-veraison, regardless of the water regime applied ($P_{T/CO2/RH}$=0.001, Figure 2b). Two weeks after mid-veraison, differences in the $A_n$ between CS and CC plants were maintained in WW plants, but these disappeared under WD (significant interaction between T/$CO_2$/RH and water availability). This effect was especially evident in VN31 and 1084 (Figure 2a). Stomatal conductance was reduced by CC conditions in WW plants, both at mid-veraison and 2 weeks later, but these differences disappeared under WD, with a significant interaction between T/$CO_2$/RH and water availability. The clones studied showed some variability in their gas exchange response to climate change both at mid-veraison and 2 weeks later, 1084 showing the greatest increase in $A_n$ and minor changes in $g_s$ between CC and CS compared with the other clones (Figure 2a).

Clones did not differ systematically either in $WUE_i$ ($A_n/g_s$) or $WUE_{inst}$ ($A_n/T$), but 2 weeks after mid-veraison, plants under WD of CL306 and VN31 accessions showed higher $WUE_{inst}$ values (CS and CC, respectively) (Table 1). Water deficit significantly increased both $WUE_i$ and $WUE_{inst}$ at mid-veraison and 2 weeks after mid-veraison, regardless of the clone and the T/$CO_2$/RH regime. Climate change conditions significantly increased $WUE_i$, especially under WD (significant interaction observed 2 weeks after mid-veraison), but they did not affect $WUE_{inst}$.

The SFR_R index, parameter correlated with the leaf chlorophyll concentration, did not show significant differences among clones either at mid-veraison ($P_{CL}$=0.334) or 2 weeks after mid-veraison ($P_{CL}$=0.253) (data not shown). Water deficit significantly increased the chlorophyll index ($P_I$=0.001) from 2.05 ± 0.05 to 2.25 ± 0.03, WW and WD, respectively. Two weeks after mid-veraison, CC significantly increased the

SFR_R index compared with CS (2.09 ± 0.05 and 2.21 ± 0.03 relative units, CS and CC, respectively, $P_{T/CO2/RH}$=0.042), regardless of the clone and the irrigation regime.

*Phenological development*

Considering the clones altogether, CC conditions significantly shortened the elapsed time between fruitset and maturity (9 days on average), especially under WD (up to 13 days), with slight differences among clones (VN31 being less impacted under WW) (Figure 3a). A significant interaction was observed between clones and water availability, where WD significantly delayed maturity in RJ43, CL306 and VN31 clones, but not in the 1084 accession (Figure 3b). The elapsed time between fruitset and maturity was significantly different among clones, 1084 showing a grape developmental period 25.8 days longer on average than that of RJ43, CL306 and VN31 clones (Figure 3b).

*Total leaf area and vegetative growth*

A significant interaction between clone and irrigation level was observed for total leaf area measured at mid-veraison, 2 weeks after mid-veraison and at maturity, as leaf area was significantly reduced by WD throughout the experiment, with this reduction being more noticeable in the 1084 accession (Figure 4a). Clones showed significant differences in total leaf area at maturity in WW plants (higher in 1084), but these differences disappeared under WD conditions. The T/$CO_2$/RH regime also interacted with the irrigation level, and CC conditions significantly reduced leaf area at mid-veraison and 2 weeks after mid-veraison, compared with CS, only in WW plants (Figure 4b). No differences between T/$CO_2$/RH regimes were observed at maturity (Figure 4b).

Climate change conditions increased total leaf dry mass at maturity, when considering all the clones as a whole, but especially in VN31, but it did not affect either stem or root growth (Figure 5a,b). Clones showed significant differences in the final dry matter production of the different organs analysed, 1084 having the highest dry mass under WW conditions (Figure 5b). Water deficit reduced the final dry mass of all the organs, regardless of the T/$CO_2$/RH regime applied. There was a significant interaction between water deficit and clone for leaves, stem and total dry mass, with clone 1084 being the most negatively affected by drought (Figure 5b).

*Bunch characteristics*

No significant interactions among factors were observed for yield and grape characteristics (Table 2). The RJ43 accession had the highest bunch mass and number of berries per bunch, whereas 1084 had higher individual berry mass during the ripening period (significant differences with VN31, which had the lowest values). Climate change conditions did not affect either grape yield or yield components at maturity, although this

treatment had the lowest grape mass values at mid-veraison. The bunch mass, number of berries per bunch and individual berry mass were significantly reduced by WD. Even though there were no significant interactions between factors, the CL306 clone experienced the strongest reduction in bunch mass as a consequence of WD, especially under CC conditions (reduction of 63%), compared with the remainder of the clones (decreases of 24, 14 and 25% in RJ43, VN31 and 1084, respectively).

*Principal component analysis*

Phenology, gas exchange characteristics and vegetative and reproductive growth parameters were analysed by principal component analysis. The first two principal components (PC) explained more than 75% of the total variability (Figure 6). Differences between water availability were clearly observed along PC1, and they were mainly associated with lower values of vegetative growth (dry mass and total leaf area), leaf water potential, and gas exchange parameters ($A_n$, $T$ and $g_s$), as well as with higher *WUE* in the WD treatment. Under WW conditions, the 1084 accession was separated from the rest of the clones along PC2, regardless of the T/$CO_2$/RH regime, due to a longer fruitset to maturity period and a lower bunch size (fresh mass and number of berries). In contrast, these differences among clones were less evident under water deficit conditions.

# Discussion

*Response of leaf water potential and leaf gas exchange traits of Tempranillo clones to changes in the T/$CO_2$/RH conditions and water availability*

The water status of all the clones studied was affected in a similar manner by WD both at mid-veraison and maturity, droughted plants reaching lower $\Psi_{leaf}$ (more negative) than their respective WW counterparts. Values of $\Psi_{leaf}$ were in the range of previous experiments using fruit-bearing cuttings and similar water deficit levels (Leibar et al. 2015). Climate change conditions did not significantly affect $\Psi_{leaf}$ of WW plants, but in the case of WD, plants grown in CS conditions showed lower $\Psi_{leaf}$ compared with CC plants. It has been proposed that elevated $CO_2$ can improve water use efficiency under drought conditions, through the reduction of $g_s$ and, therefore, $T$ values (Tyree and Alexander 1993, Wullschleger et al. 2002). Even though CC conditions reduced $g_s$ under well-watered conditions, $g_s$ was similar in CC and CS treatments under water deficit. Also, leaf transpiration was even higher in CC (significant at mid-veraison), likely due to a higher vapour pressure deficit (VPD) in this treatment (VPD of 1.51 vs 1.19 kPa, CC and CS, respectively) associated with higher temperature and lower RH values. The higher $\Psi_{leaf}$ in CC/WD plants compared with CS/WD was not likely related to differences in root development between these treatments. Other factors, such as the improvement of whole plant water relations induced by elevated $CO_2$, through more effective water uptake (fine-root proliferation) or increased xylem conductivity (Wullschleger et al. 2002), are possible. Also, the differences in air temperature between CC and CS may be behind the differences observed in $\Psi_{leaf}$. In a recent study, Galat Giorgi et al. (2019) reported higher leaf water potential values in grapevine plants exposed

to elevated temperature (45/22°C) and water deficit, compared with their control temperature counterparts (35/20°C). The authors suggested that the control treatment may have kept their stomata open longer during the day, leading to a greater decrease in water potential.

Clone 1084 had the lowest $A_n$ values among the four clones studied. The 1084 plants grown under CS/WW conditions had a significantly lower $A_n$ and $g_s$ compared with other clones under the same T/$CO_2$/RH regime and water availability conditions, 2 weeks after mid-veraison (70.0 ± 1.5 days on average after fruitset, that is, from the beginning of the treatments). These differences were not associated with the concentration of leaf chlorophyll. Leaf photosynthesis in grapevine depends upon demand for assimilates and it is regulated by the source: sink relationship (Iland et al. 2011). Reduced stomatal conductance has been proposed as a regulatory mechanism in such relationships in different plant species, including grapevine (Quereix et al. 2001, Blanke 2009). The low fruit load (bunch mass) accompanied by a high leaf development of 1084 may explain such lower photosynthetic activity. Variability for $A_n$ among grapevine cultivars has been previously reported (Bota et al. 2001, Tomás et al. 2014, Greer 2018). Greer (2018) attributed, in part, such variability to differences in stomatal conductance, as in the present study, rather than to biochemical factors, such as RUBP carboxylation and regeneration. Potential mesophyll diffusion limitations may also explain these differences, although they remain largely unexplored in Tempranillo clones (Salazar-Parra et al. 2012).

The impact of water deficit on grapevine photosynthesis performance has been extensively studied (Flexas et al. 1998, Medrano et al. 2003, Chaves et al. 2010). In the present study, water availability was the factor that most affected gas exchange parameters, reducing $A_n$ values in all the clones studied, and overshadowed the differences among clones observed in WW plants. Such reduction in carbon assimilation was presumably related to a decrease in $CO_2$ availability when plants closed stomata to prevent water loss, as supported by the reduction in $g_s$. Under mild to moderate water deficits, stomata closure is among the earliest plant responses, restricting water loss and carbon fixation (Chaves et al. 2003).

Considering all the clones, CC conditions increased $A_n$ at mid-veraison, regardless of the water availability, thus partially compensating the impact of WD. Unfortunately, we cannot attribute this effect either to $CO_2$ or temperature, but it is likely that $CO_2$ was the main factor affecting photosynthetic rates under the present experimental conditions. Grapevine photosynthesis, as in other $C_3$ species, is limited by $CO_2$, and therefore high $CO_2$ has been reported to increase $A_n$ (Moutinho-Pereira et al. 2009, Salazar-Parra et al. 2015). Such increase has been recently associated with the up-regulation of Rubisco small chain and Rubisco activase proteins (Zhao et al. 2019). In addition, previous studies in different grapevine cultivars, grown in both natural and controlled environments, show that changes in temperature in the range of the present work (24–28°C) did not significantly affect grapevine photosynthesis (Greer 2018). Two weeks after mid-veraison, a significant interaction between T/$CO_2$/RH regime and irrigation regime was observed, and the positive effect of CC in WW plants was completely diminished by drought, in agreement with the studies of Leibar et al. (2015). Despite the absence of significant interaction between clone identity and T/$CO_2$/RH

regime, it is important to note that, under WW conditions the 1084 accession exhibited a more pronounced increase in $A_n$ in response to CC compared with the remaining clones studied, both at mid-veraison and 2 weeks later. These results may reflect some differences among clones in their photosynthetic response to the projected environmental conditions.

Within the context of the IPCC predictions for a decrease in water availability, improved crop water use efficiency has become a priority in basic and applied research in recent years (Tortosa et al. 2019). Although clonal variability of the $WUE_i$ has been reported for cv. Tempranillo (Tortosa et al. 2016, 2019), considering all the T/$CO_2$/RH and irrigation regimes, we did not observe significant differences in $WUE_i$ and $WUE_{inst}$ among the clones considered in the present work. The results agree with Tortosa et al. (2016, 2019), who also did not observe differences among RJ43, VN31 and 1084 either under field or pot conditions. Focusing on drought conditions, however, CL306 and VN31 showed higher values of $WUE_{inst}$ than the other accessions (CS and CC, respectively) 2 weeks after veraison. The result suggests that under long-term water deficit, and depending on the T/$CO_2$/RH regime, these Tempranillo accessions may exhibit improved WUE.

Considering all the clones as a whole, both WD and CC significantly increased $WUE_i$, which was associated with a drop in $g_s$ values in the first case, and with a higher photosynthetic capacity and lower $g_s$, in the second case. The improvement in $WUE_i$ was especially remarkable when these two environmental conditions were combined (CC/WD), suggesting that, in a future environment with high $CO_2$ and elevated temperature, grapevine $WUE_i$ may be improved under drought conditions. When leaf transpiration, however, was used to calculate $WUE_{inst}$, the gain in water use efficiency of plants under CC conditions disappeared. That is because the reduced $g_s$ observed under CC was not accompanied by low transpiration, likely due to the higher vapour pressure deficit under these conditions. Therefore, the increase in water vapour concentration difference between leaf and air, as a consequence of the projected air temperature and RH conditions, would largely offset the potential gain in WUE produced by elevated $CO_2$ (Kaminski et al. 2014). These results highlight the importance of studying the combined effect of $CO_2$ with other climate change factors (e.g. changes in temperature or precipitation), which may modulate the photosynthetic and water use efficiency response of plants to $CO_2$. In addition, the $WUE_{inst}$ appears to be a more suitable parameter to estimate the photosynthetic water use efficiency under environmental conditions that modify vapour pressure deficit.

*Phenological response of Tempranillo clones to changes in the T/$CO_2$/RH conditions and water availability*

Differences in the timing of maturity among Tempranillo clones have been reported in previous studies, the 1084 accession being characterised as having a long phenological development (fruitset–maturity period 22.7 days longer than the average of the clones assessed) (Arrizabalaga et al. 2018). Such behaviour, however, could not be completely explained in the present study by a lower photosynthetic activity, although the differences observed may have partially contributed to the delayed maturity in 1084. Grapevine phenology is greatly influenced by temperature

(Duchêne 2016), especially the early phenological events. For later phenological events, however, the level of complexity increases and other environmental factors may also influence the timing of phenophases (Martínez-Lüscher et al. 2016). In the present study, water availability was the factor that most strongly affected grape development. The severe reduction in $A_n$ and total leaf area in WD plants probably limited carbon availability, thus slowing down grape ripening in these treatments. Whereas mild water deficit has been proven to enhance ripening, severe water deficit has been reported to reduce carbon fixation, and consequently, to impair berry ripening (Chaves et al. 2010, Martínez-Lüscher et al. 2015a). In addition, the interaction between clone and irrigation reveals a differential response of the studied genotypes to water deficit, 1084 being the least responsive clone. This result may be explained by the lower difference in the $A_n$ between WW and WD conditions observed in this accession. In contrast to WD, CC advanced grape maturity when all the clones were taken into consideration, and compensated for the delaying effect due to drought. This is in accordance with previous studies under controlled and semi-controlled conditions (Leibar et al. 2015, Martínez-Lüscher et al. 2016). From mid-veraison onwards, the majority of photoassimilates is directed to berry maturation (Lebon et al. 2008). Therefore, the advancement of ripening in plants grown under CC conditions indicates faster sugar accumulation in grapes, associated with the higher photosynthetic rates measured in CC compared with CS (both in WW and WD at mid-veraison, and in WW 2 weeks later).

*Vegetative and reproductive response of Tempranillo clones to changes in the T/$CO_2$/RH conditions and water availability*

The higher photosynthetic rates of plants grown under CC conditions were associated with increased leaf dry mass (only under WW conditions), with minor effects on reproductive growth. Kizildeniz et al. (2018) presented similar results in two Tempranillo cultivars, with a greater effect of elevated $CO_2$ on vegetative than on reproductive growth. In contrast, previous studies have reported a positive effect of elevated $CO_2$ on bunch size (Bindi et al. 2001, Goncalves et al. 2009, Wohlfahrt et al. 2018), especially after consecutive years of $CO_2$ enrichment treatment. The lack of $CO_2$ effect on bunch mass and number of berries in the present study may be related to the fact that initiation of inflorescence primordia takes place in the previous season, and in our case, plants were not exposed to elevated $CO_2$ during this process (Wohlfahrt et al. 2018).

Water deficit was the most limiting factor for plant growth and yield. Its impact on C assimilation, previously discussed, was clearly reflected at a plant level, reducing leaf area and dry matter production of all the vegetative organs analysed, regardless of the clone and the T/$CO_2$/RH regime. Roots, however, were less affected than the above-ground part, as was also reported by Kizildeniz et al. (2015, 2018). The significant interaction between clone and irrigation regime reveals a certain degree of intra-cultivar diversity in the growth response of Tempranillo to WD, 1084 being one of the accessions more negatively affected. Probably, its longer cycle, and consequently, longer exposure to WD contributed to exacerbate the differences between WW and WD plants at maturity for this clone.

Water deficit also reduced reproductive growth. In a 10-year study of the effect of water availability on two Spanish grapevine cultivars, Medrano et al. (2003) concluded that there was a close link between water availability and grape yield, through water stress effects on photosynthesis. The reduction in bunch mass in the present study was explained by a lower berry size, but also to a reduced number of berries per bunch, thus suggesting a loss of berries produced by a severe water deficit. The reduction in average berry mass for WD plants was already evident at mid-veraison (56.0 ± 1.5 days on average after fruitset), and differences between the WW and WD treatments were maintained constant thereafter until maturity, thus reflecting a higher sensitivity of berry growth to water limitations imposed before mid-veraison. Similarly, McCarthy (1997) and Roby and Matthews (2008) reported that berry size is more sensitive to water deficit before mid-veraison, whereas water deficit after mid-veraison had only minor effects on berry mass at maturity.

This study was performed under controlled conditions. Consequently, we cannot directly extrapolate these results to natural conditions, where grapevine plants can be exposed to other abiotic and biotic factors. This is the first step, however, to explore the impact of multi-stresses on grapevine physiology prior to validation under field conditions.

## Conclusion

Simulated temperature, $CO_2$, and RH conditions expected with CC by the end of the century, under controlled environment, hastened grape phenological development and increased leaf biomass of the four clones of Tempranillo studied, and was associated with an increase in their photosynthetic rate. Such effects, however, were overshadowed by water deficit, which was the factor that most strongly affected gas exchange, vegetative and reproductive growth. Climate change increased $WUE_i$, especially when combined with drought. It did not modify $WUE_{int}$, however, probably due to the higher vapour pressure deficit induced by the environmental conditions in the CC treatment. Although the studied clones showed, in general, a similar behaviour under the simulated CC conditions, some degree of variability in their response to changes in the T/$CO_2$/RH regime was observed for $A_n$ and $g_s$, as well as in the response to water deficit for gas exchange parameters, phenology and vegetative and reproductive growth. The differences among clones observed in terms of phenological development appeared to condition the impact of the environmental conditions on vegetative growth, especially that of water deficit.

## Acknowledgements

This work was supported by the Ministerio de Economía y Competitividad of Spain [AGL2014-56075-C2-1-R]; Fundación Universitaria de Navarra [PIUNA 2018] and Asociación de Amigos de la Universidad de Navarra (grant to Marta Arrizabalaga-Arriazu). Special thanks to Ms Mónica Oyarzun, Mr Amadeo Urdiain and Dr Hector Santesteban for their excellent technical assistance and Dr Enrique García-Escudero, Dr José Miguel Martínez-Zapater, Ms Edurne Baroja (ICVV), Mr José Félix Cibrain (EVENA) and Mr Rafael García (Vitis Navarra) for the selection of clones and for providing the plant material to do the experiments.

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**Table 1.** Effect of current climatic conditions and climate change conditions combined with two irrigation regimes on the intrinsic water use efficiency and instantaneous water use efficiency of four *Vitis vinifera* cv. Tempranillo clones.

| Clone/treatment | | | $WUE_i$ (µmol $CO_2$ /mol $H_2O$) | | $WUE_{inst}$ (µmol $CO_2$ /mmol $H_2O$) | |
|---|---|---|---|---|---|---|
| | | | Mid-veraison | 2 Weeks after mid-veraison | Mid-veraison | 2 Weeks after mid-veraison |
| RJ43 | | | 104.0±12.2a | 130.2±14.4a | 4.93±0.40a | 5.16±0.24a |
| CL306 | | | 119.5±14.6a | 123.2±16.8a | 4.85±0.28a | 4.93±0.39a |
| VN31 | | | 104.5±9.9a | 109.7±10.3a | 4.50±0.38a | 5.42±0.37a |
| 1084 | | | 115.2±11.7a | 123.4±13.3a | 4.50±0.25a | 4.63±0.24a |
| CS | | | 78.5±5.8b | 83.5±5.9b | 4.52±0.24a | 4.81±0.20a |
| CC | | | 140.6±8.4a | 165.5±9.7a | 4.86±0.23a | 5.27±0.24a |
| WW | | | 87.5±5.2b | 94.0±5.8b | 4.15±0.11b | 4.60±0.13b |
| WD | | | 147.2±10.9a | 157.2±11.7a | 5.45±0.34a | 5.49±0.28a |
| RJ43 | CS | WW | 62.9±4.4fg | 64.0±8.5d | 4.21±0.17bcde | 4.73±0.34bcd |
| | | WD | 107.7±18.4bcde | 96.8±18.7c | 6.25±1.21a | 5.64±0.56bc |
| | CC | WW | 132.9±12.4cde | 130.4±11.1bc | 4.35±0.44bcde | 4.78±0.23bcd |
| | | WD | 158.1±30.6abc | 224.2±28.2a | 5.58±1.35abc | 5.54±0.70bc |
| CL306 | CS | WW | 50.7±2.9fg | 63.0±11.1d | 3.91±0.26cde | 4.47±0.28bcd |
| | | WD | 115.8±15.8bcde | 99.3±17.0cd | 4.90±0.87abcde | 6.23±1.15ab |
| | CC | WW | 133.2±9.1bcd | 134.7±21.0bc | 4.92±0.13abcde | 4.87±0.65bcd |
| | | WD | 200.4±45.9a | 235.0±39.0a | 5.84±0.71ab | 4.51±0.97bcd |
| VN31 | CS | WW | 55.6±5.2g | 58.9±2.7d | 3.55±0.23e | 4.12±0.30cd |
| | | WD | 118.1±10.8bcd | 126.8±25.2c | 5.37±1.07abcd | 5.25±0.53bcd |
| | CC | WW | 111.2±9.4def | 141.6±9.2c | 3.83±0.30de | 5.16±0.37bcd |
| | | WD | 161.4±44.3ab | 231.3±42.1ab | 5.98±1.09ab | 7.42±1.20a |
| 1084 | CS | WW | 43.4±2.9efg | 50.8±5.0d | 3.75±0.31de | 3.85±0.48d |
| | | WD | 129.2±17.1cdef | 115.7±8.4cd | 5.37±0.60abcde | 4.66±0.57bcd |
| | CC | WW | 105.9±7.5bcd | 114.6±9.4c | 4.65±0.35abcde | 4.88±0.20bcd |
| | | WD | 165.8±14.5abc | 186.4±30.1a | 4.68±0.71abcde | 5.11±0.50bcd |
| $P_{(CL)}$ | | | n.s. | n.s. | n.s. | n.s. |
| $P_{(T/CO2/RH)}$ | | | *** | *** | n.s. | n.s. |
| $P_{(I)}$ | | | *** | *** | *** | ** |
| $P_{(CL \times T/CO2/RH)}$ | | | n.s. | n.s. | n.s. | n.s. |
| $P_{(CL \times I)}$ | | | n.s. | n.s. | n.s. | n.s. |
| $P_{(T/CO2/RH \times I)}$ | | | n.s. | * | n.s. | n.s. |

| | | | | |
|---|---|---|---|---|
| $P_{(CL \times T/CO2/RH \times I)}$ | n.s. | n.s. | n.s. | n.s. |

Results (values are means ± SE) are shown according to clone identity ($n$ = 21-32), T/$CO_2$/RH condition ($n$ = 52-60), irrigation regime ($n$ = 39-61) and the three factors together ($n$ = 3-8). Means with letters in common within the same parameter, stage and factor (clone, T/$CO_2$/RH, irrigation regime, or their interaction) are not significantly different according to LSD test ($P$ > 0.05). Probability values ($P$) for the main effects of clone, $P_{(CL)}$; T/$CO_2$/RH regime $P_{(T/CO2/RH)}$; irrigation regime, $P_{(I)}$; and their interactions, $P_{(CL \times T/CO2/RH)}$, $P_{(CL \times I)}$, $P_{(T/CO2/RH \times I)}$ and $P_{(CL \times T/CO2/RH \times I)}$. ***, $P$ < 0.001; **, $P$ < 0.01; *, $P$ < 0.05; n.s., not significant. CS, current climatic conditions (T/$CO_2$/RH), 24°C/14°C, 400 µmol/mol and 45%/65 % RH; CC, climate change conditions (T/$CO_2$/RH) CC 28°C/18°C, 700 µmol/mol and 33%/53% RH; WW, well-watered; WD, water deficit at mid-veraison and 2 weeks after mid-veraison; $WUE_i$, intrinsic water use efficiency; $WUE_{inst}$, instantaneous water use efficiency.

**Table 2.** Effect of current climatic conditions and climate change conditions combined with two irrigation regimes on the bunch mass, number of berries per bunch and individual berry mass (throughout berry development) of four *Vitis vinifera* cv. Tempranillo clones.

| Clone/treatment | | | Bunch mass (g FM) | No. of berries/bunch | Mass of individual berries (g FM) | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | Mid-veraison | 1 Week after mid-veraison | 2 Weeks after mid-veraison | Maturity |
| RJ43 | | | 154.5±12.10a | 152.9±9.9a | 0.85±0.04b | 1.02±0.05ab | 1.16±0.04a | 0.95±0.04ab |
| CL306 | | | 98.0±11.29b | 103.3±10.6b | 0.76±0.04bc | 0.97±0.04b | 1.11±0.04ab | 0.99±0.05a |
| VN31 | | | 98.3±8.94b | 114.2±9.0b | 0.74±0.04c | 0.92±0.04b | 1.02±0.04b | 0.85±0.04b |
| 1084 | | | 97.0±8.89b | 91.2±6.4b | 1.02±0.05a | 1.10±0.05a | 1.19±0.05a | 0.99±0.06a |
| CS | | | 114.5±8.30a | 116.8±7.0a | 0.93±0.03a | 1.04±0.03a | 1.15±0.03a | 0.93±0.03a |
| CC | | | 110.3±7.53a | 115.6±7.1a | 0.76±0.03b | 0.97±0.03a | 1.09±0.03a | 0.96±0.03a |
| WW | | | 133.8±8.36a | 125.8±6.9a | 0.94±0.03a | 1.14±0.03a | 1.24±0.03a | 1.01±0.04a |
| WD | | | 91.1±6.40b | 106.1±7.0b | 0.74±0.03b | 0.86±0.03b | 1.00±0.03b | 0.87±0.03b |
| RJ43 | CS | WW | 184.9±39.5a | 159.8±28.6ab | 0.99±0.10bc | 1.21±0.11ab | 1.28±0.09abc | 1.03±0.11ab |
| | | WD | 126.2±17.8bcd | 130.1±21.0abc | 0.90±0.05bcd | 0.94±0.04cdef | 1.13±0.03abcde | 0.93±0.05abc |
| | CC | WW | 174.6±12.6ab | 158.5±11.6ab | 0.88±0.03bcde | 1.14±0.06abc | 1.30±0.06ab | 1.06±0.07ab |
| | | WD | 132.2±13.6abc | 163.3±15.5a | 0.63±0.07fg | 0.76±0.06f | 0.93±0.06efg | 0.77±0.07c |
| CL306 | CS | WW | 129.2±17.4abcd | 112.0±15.5bcd | 1.00±0.04bc | 1.14±0.03abc | 1.29±0.06abc | 1.17±0.13a |
| | | WD | 95.0±16.8cdef | 106.1±23.4cd | 0.73±0.07defg | 0.89±0.05def | 1.08±0.07cdefg | 0.87±0.07bc |
| | CC | WW | 122.5±26.4bcde | 113.1±24.1bcd | 0.77±0.06cdef | 1.07±0.08abcd | 1.19±0.07abcd | 1.01±0.08abc |
| | | WD | 45.3±16.9f | 73.4±21.9d | 0.53±0.05g | 0.78±0.08ef | 0.89±0.09fg | 0.85±0.09bc |
| VN31 | CS | WW | 117.3±17.5cde | 134.4±17.7abc | 0.92±0.04bcd | 1.06±0.07abcd | 1.13±0.03abcde | 0.87±0.05bc |
| | | WD | 74.8±9.4def | 92.9±12.7cd | 0.73±0.07defg | 0.80±0.07ef | 0.94±0.07efg | 0.75±0.04c |
| | CC | WW | 108.3±20.7cde | 114.9±21.6abcd | 0.68±0.10efg | 1.02±0.10bcd | 1.14±0.10abcde | 0.93±0.12abc |
| | | WD | 92.8±21.1cdef | 114.9±19.2abcd | 0.62±0.07fg | 0.79±0.05ef | 0.87±0.06g | 0.86±0.04bc |
| 1084 | CS | WW | 116.8±18.8cde | 113.8±12.9bcd | 1.23±0.10a | 1.27±0.10a | 1.33±0.10a | 0.92±0.12bc |
| | | WD | 71.4±17.3ef | 78.7±11.8d | 0.90±0.09bcde | 0.93±0.13cdef | 1.00±0.10defg | 0.89±0.10bc |
| | CC | WW | 114.3±17.6cde | 96.4±11.1cd | 1.06±0.12ab | 1.22±0.08ab | 1.29±0.07abc | 1.10±0.12ab |
| | | WD | 85.5±15.1cdef | 74.5±12.3d | 0.88±0.07bcde | 0.98±0.07cde | 1.11±0.11bcdef | 1.04±0.11ab |

Results (values are means ± SE) are shown according to clone identity ($n$ = 26–32), T/$CO_2$/RH condition ($n$ = 59-62), irrigation regime ($n$ = 58-62) and the three factors together ($n$ = 5–8); means with letters in common within the same parameter, stage and factor (clone, temperature and $CO_2$, irrigation regime, or their interaction) are not significantly different according to LSD test ($P > 0.05$). All probability values for the interactions of factors [$P_{(CL \times T/CO2/RH)}$, $P_{(CLxI)}$, $P_{(T/CO2/RH \times I)}$ and $P_{(CL \times T/CO2/RH \times I)}$] were statistically not significant ($P > 0.05$). CS, current climatic conditions (T/$CO_2$/RH), 24°C/14°C, 400 µmol/mol and 45%/65 % RH; CC, climate change conditions (T/$CO_2$/RH) CC 28°C/18°C, 700 µmol/mol and 33%/53% RH; FM, fresh mass; WW, well-watered; WD, water deficit at mid-veraison and 2 weeks after mid-veraison.

## Figure legends

**Figure 1.** Pre-dawn leaf water potential at mid-veraison and 2 weeks after mid-veraison of four *Vitis vinifera* cv. Tempranillo clones, RJ43, CL306, VN31 and 1084, grown under two T/$CO_2$/RH conditions: CS, current climatic conditions (24°C/14°C, 400 µmol/mol and 45%/65% RH); and CC, climate change conditions (28°C/18°C, 700 µmol/mol and 33%/53% RH), combined with two irrigation regimes: WW, well-watered (□); and WD, water deficit (■). Results (values are means ± SE) are represented according to the T/$CO_2$/RH and irrigation regimes ($n$ = 14–19). Means with letters in common within the same stage are not significantly different according to LSD test ($P$ > 0.05). Probability values ($P$) for the main effects of clone, $P_{(CL)}$ mid-veraison (MV), n.s., 2 weeks after mid-veraison (2WAMV), n.s..; T/$CO_2$/RH regime, $P_{(T/CO2/RH)}$ MV, n.s., 2WAMV, n.s.; irrigation regime, $P_{(I)}$ MV, ***, 2WAMV, ***; and their interactions, $P_{(CL\ x\ T/CO2/RH}$) MV, n.s., 2WAMV, n.s., $P_{(CL\ x\ I)}$ MV, n.s., 2WAMV, n.s., and $P_{(T/CO2/RH\ x\ I)}$ MV, **, 2WAMV, n.s.;***, $P$ < 0.001; **, $P$ < 0.01; n.s., not significant. Interaction of all factors $P_{(CL\ x\ T/CO2/RH\ x\ I)}$ was statistically not significant ($P$ > 0.05).

**Figure 2.** Net photosynthesis ($A_n$), transpiration ($T$) and stomatal conductance ($g_s$) of the four *Vitis vinifera* cv. Tempranillo clones, RJ43, CL306, VN31 and 1084, grown under two T/$CO_2$/RH conditions: CS, current climatic conditions (24°C/14°C, 400 µmol/mol, and 45%/65% RH) and CC, climate change conditions (28°C/18°C, 700 µmol/mol and 33%/53% RH), combined with two irrigation regimes: WW, (□) well-watered; and WD (■) water deficit, at mid-veraison and 2 weeks after mid-veraison. Results (values are means ± SE) are represented according to (a) the clones, T/$CO_2$/RH and irrigation regimes ($n$ = 5–8) and (b) to T/$CO_2$/RH and irrigation regimes, considering the clones altogether ($n$ = 18–31). Means with letters in common within the same parameter and stage are not significantly different according to LSD test ($P$ > 0.05). Probability values ($P$) for the main effects of clone, $P_{(CL)}$; T/$CO_2$/RH regime, $P_{(T/CO2/RH)}$; irrigation regime, $P_{(I)}$; and their interactions, $P_{(CL\ x\ T/CO2/RH)}$, $P_{(CL\ x\ I)}$ and $P_{(T/CO2/RH\ x\ I)}$; ***, $P$ < 0.001; **, $P$ < 0.01; *, $P$ < 0.05; n.s., not significant. Interaction of all factors $P_{(CL\ x\ T/CO2/RH\ x\ I)}$ was statistically not significant ($P$ > 0.05) in all the cases.

**Figure 3.** Number of days between fruitset and maturity (ca. 23°Brix) of the four *Vitis vinifera* cv. Tempranillo clones, RJ43, CL306, VN31 and 1084, grown under two T/$CO_2$/RH conditions: CC, (□) current climatic conditions (24°C/14°C, 400 µmol/mol and 45%/65% RH) and CC, (■) climate change (28°C/18°C, 700 µmol/mol and 33%/53% RH), combined with two irrigation regimes: WW, well-watered; and WD water deficit. Data (values are means ± SE) are presented according to: (a) the T/$CO_2$/RH and irrigation regimes considering the clones altogether ($n$ = 28–31) and (b) the clones, T/$CO_2$/RH and irrigation regimes ($n$ = 6–8). Means with letters in common within the same chart, (a) or (b), are not significantly different according to LSD test ($P$ > 0.05). Probability values ($P$) for the main effects of clone, $P_{(CL)}$, ***; T/$CO_2$/RH regime, $P_{(T/CO2/RH),\ ***}$; irrigation regime, $P_{(I),\ ***}$; and their interactions, $P_{(CL\ x\ T/CO2/RH),}$ n.s. and $P_{(CL\ x\ I),\ **}$ and $P_{(T/CO2/RH\ x\ I),}$ n.s.; ***, $P$ < 0.001; **, $P$ < 0.01; n.s., not

significant. Interaction of all factors $P_{(CL \times T/CO2/RH \times I)}$ was statistically not significant ($P > 0.05$).

**Figure 4.** Total leaf area at mid-veraison, 2 weeks after mid-veraison and maturity of the *Vitis vinifera* cv. Tempranillo clones, RJ43 (Δ,▲), CL306 (□,■), VN31 (∇,▼) and 1084 (○,●), grown under two T/$CO_2$/RH conditions: CS, current climatic conditions (24°C/14°C, 400 μmol/mol and 45%/65% RH); and CC, climate change (28°C/18°C, 700 μmol/mol and 33%/53% RH), combined with two irrigation regimes: WW, well-watered (Δ, □,∇,○): and WD, water deficit (▲,■,▼, ●). Data (values are means ± SE) are represented according to (b) the clones, and irrigation regimes ($n$ = 14–16) and to (b) the T/$CO_2$/RH and irrigation regimes ($n$ = 31). Means with letters in common within the same stage in chart (b) are not significantly different according to LSD test ($P > 0.05$). Probability values ($P$) for the main effects of clone, $P_{(CL)}$ mid-veraison (MV), n.s., 2 weeks after mid-veraison (2WAMV), n.s., maturity (M), ***; T/$CO_2$/RH regime, $P_{(T/CO2/RH)}$ MV, n.s., 2WAMV, *, M, n.s.; irrigation regime, $P_{(I)}$ MV, ***, 2WAMV, ***, M, ***; and their interactions, $P_{(CL \times T/CO2/RH)}$ MV, n.s., 2WAMV, n.s., M, n.s., $P_{(CL \times I)}$ MV, **, 2WAMV, **, M, *** and $P_{(T/CO2/RH \times I)}$ MV, **, 2WAMV, ***, M, n.s.;***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., not significant. Interaction of all factors $P_{(CL \times T/CO2/RH \times I)}$ was statistically not significant ($P > 0.05$).

**Figure 5.** Leaf, root, stem and total dry mass of the *Vitis vinifera* cv. Tempranillo clones, RJ43, CL306, VN31 and 1084, grown under two T/$CO_2$/RH conditions: CS, current climatic conditions (24°C/14°C, 400 μmol/mol and 45%/65% RH); CC, and climate change (28°C/18°C, 700 μmol/mol and 33%/53% RH), combined with two irrigation regimes: WW, well-watered (□); and WD, water deficit (■). Data (values are means ± SE) are represented according to (a) the T/$CO_2$/RH and irrigation regimes ($n$ = 28–31) and (b) the clones, T/$CO_2$/RH and irrigation regimes ($n$ = 5–8). Means with letters in common within the same chart, (a) or (b) and organ are not significantly different according to LSD test ($P > 0.05$). Probability values ($P$) for the main effects of clone, $P_{(CL)}$; T/$CO_2$/RH regime, $P_{(T/CO2/RH)}$; irrigation regime, $P_{(I)}$; and their interactions, $P_{(CL \times T/CO2/RH)}$ and $P_{(CL \times I)}$ and $P_{(T/CO2/RH \times I)}$; ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; n.s., not significant. Interaction of all factors $P_{(CL \times T/CO2/RH \times I)}$ was statistically not significant ($P > 0.05$).

**Figure 6.** Principal component analysis of pre-dawn water potential, phenology, gas exchange and dry mass production parameters: (a) score and (b) loading plot. CS, current climatic conditions (24°C/14°C, 400 μmol/mol and 45%/65% RH); CC, climate change conditions (28°C/18°C, 700 μmol/mol and 33%/53% RH); WW, well-watered (○); WD, water deficit (●). FM, fresh mass; DM, dry mass; $A_n$, net photosynthesis; $T$, leaf transpiration; $g_s$, stomatal conductance; $WUE_i$, intrinsic water use efficiency; $WUE_{inst}$, instantaneous water use efficiency; $\Psi_{leaf}$, pre-dawn leaf water potential; fruitset–maturity, number of days between fruitset and maturity.

Figure 1

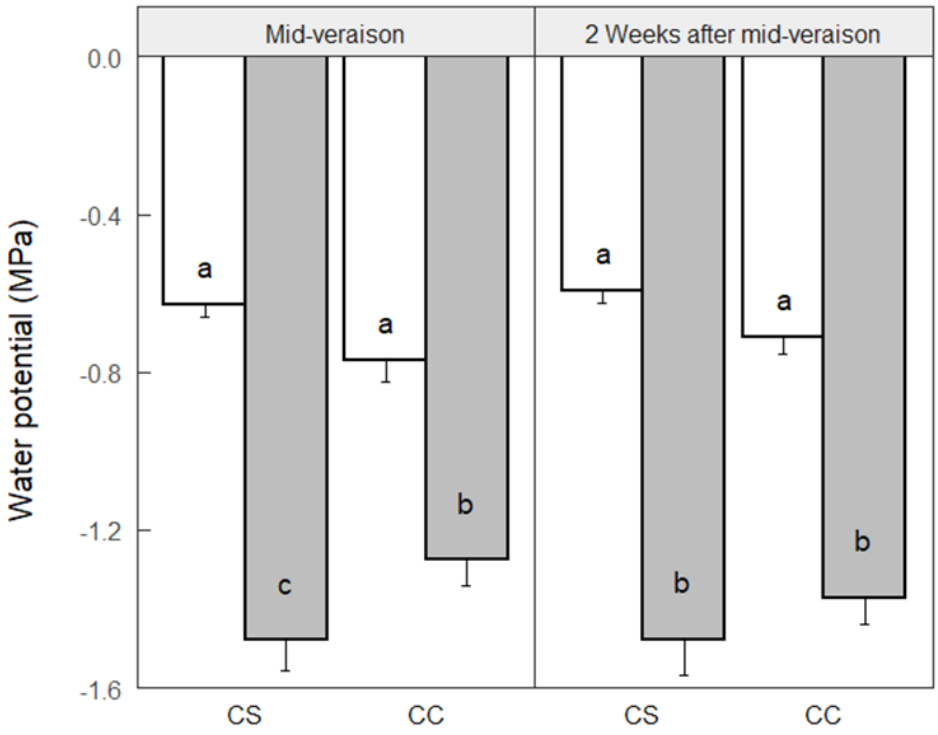

Mid-veraison
2 Weeks after mid-veraison
Water potential (MPa)
0.0
-0.4
-0.8
-1.2
-1.6
a
c
a
b
a
b
a
b
CS
CC
CS
CC

Figure 2

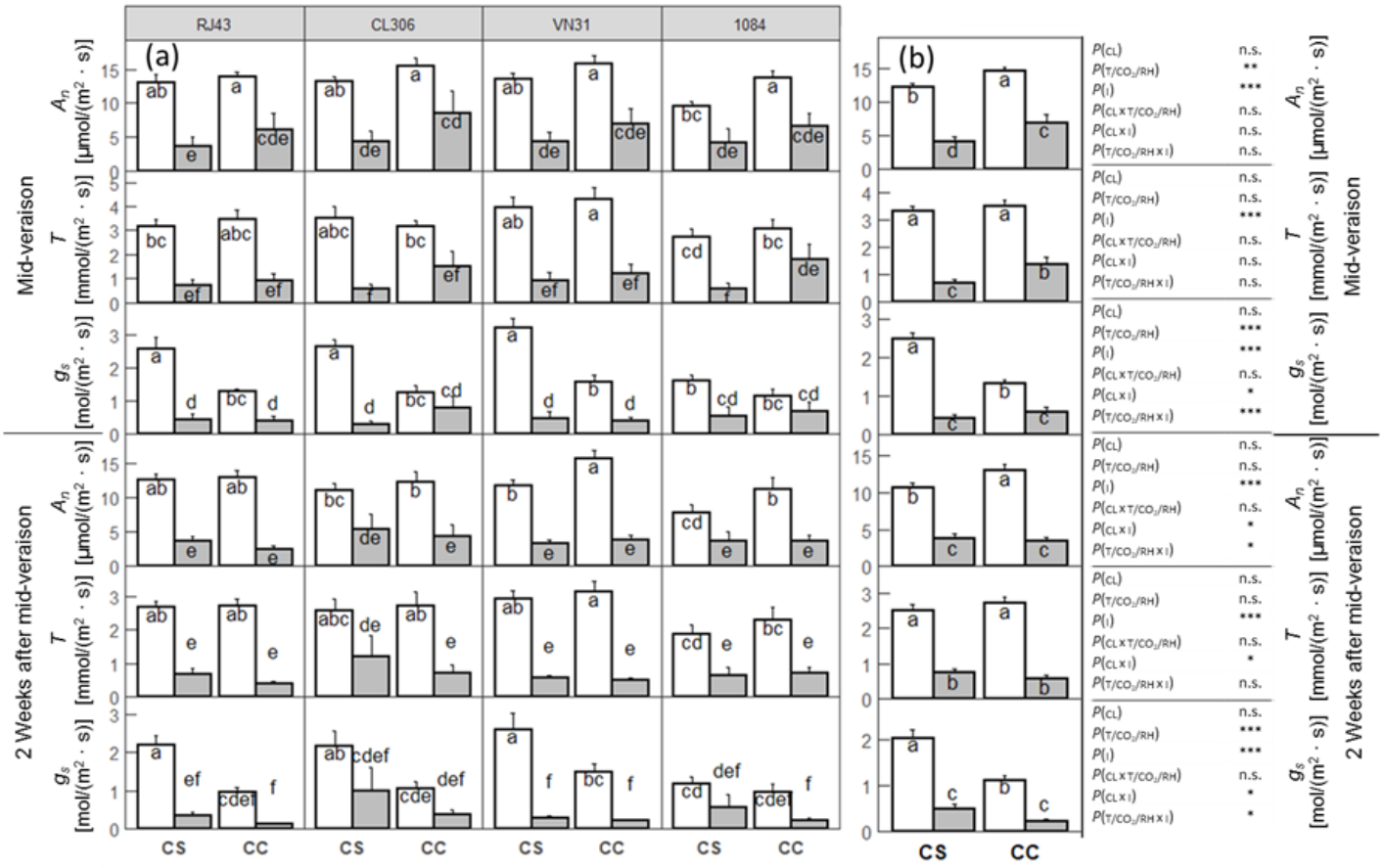

RJ43
CL306
VN31
1084
(a)
(b)
Mid-veraison
2 Weeks after mid-veraison
$A_n$ [µmol/(m² · s)]
$T$ [mmol/(m² · s)]
$g_s$ [mol/(m² · s)]
CS
CC
P(CL) n.s.
P(T/CO2/RH) **
P(I) ***
P(CL x T/CO2/RH) n.s.
P(CL x I) n.s.
P(T/CO2/RH x I) n.s.
P(CL) n.s.
P(T/CO2/RH) n.s.
P(I) ***
P(CL x T/CO2/RH) n.s.
P(CL x I) n.s.
P(T/CO2/RH x I) n.s.
P(CL) n.s.
P(T/CO2/RH) ***
P(I) ***
P(CL x T/CO2/RH) n.s.
P(CL x I) *
P(T/CO2/RH x I) ***
P(CL) n.s.
P(T/CO2/RH) n.s.
P(I) ***
P(CL x T/CO2/RH) n.s.
P(CL x I) *
P(T/CO2/RH x I) *
P(CL) n.s.
P(T/CO2/RH) n.s.
P(I) ***
P(CL x T/CO2/RH) n.s.
P(CL x I) *
P(T/CO2/RH x I) n.s.
P(CL) n.s.
P(T/CO2/RH) ***
P(I) ***
P(CL x T/CO2/RH) n.s.
P(CL x I) *
P(T/CO2/RH x I) *

Figure 3

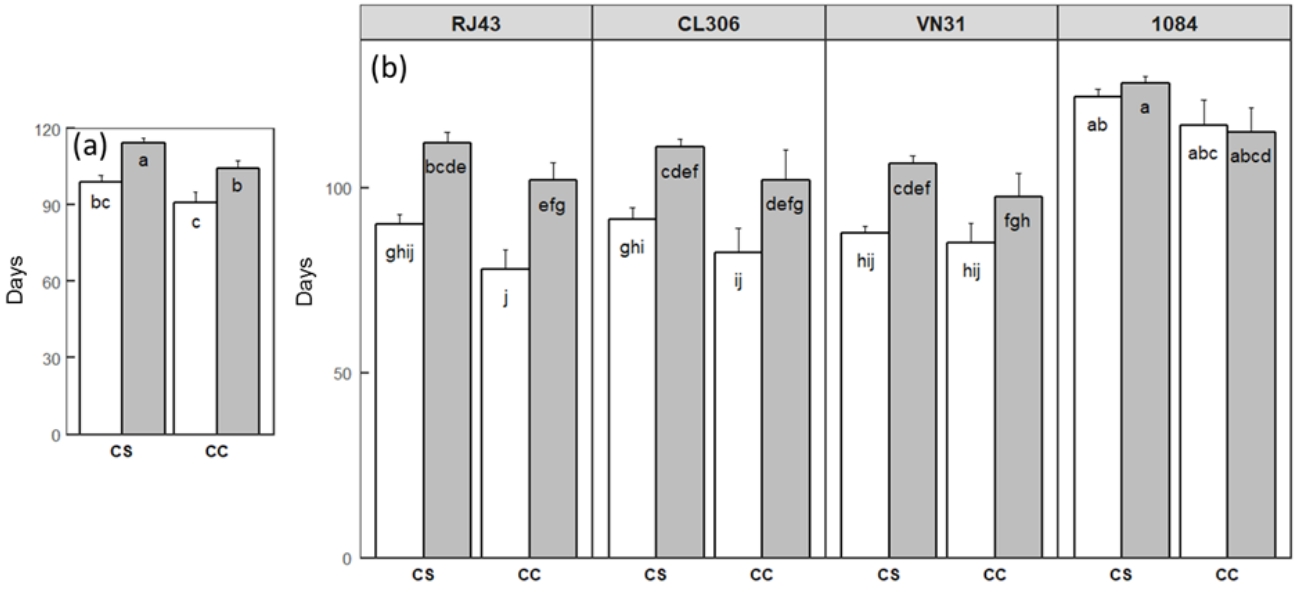

(a)
Days
120
90
60
30
0
bc
a
c
b
CS
CC
(b)
RJ43
CL306
VN31
1084
100
50
ghij
bcde
j
efg
ghi
cdef
ij
defg
hij
cdef
hij
fgh
ab
a
abc
abcd

Figure 4

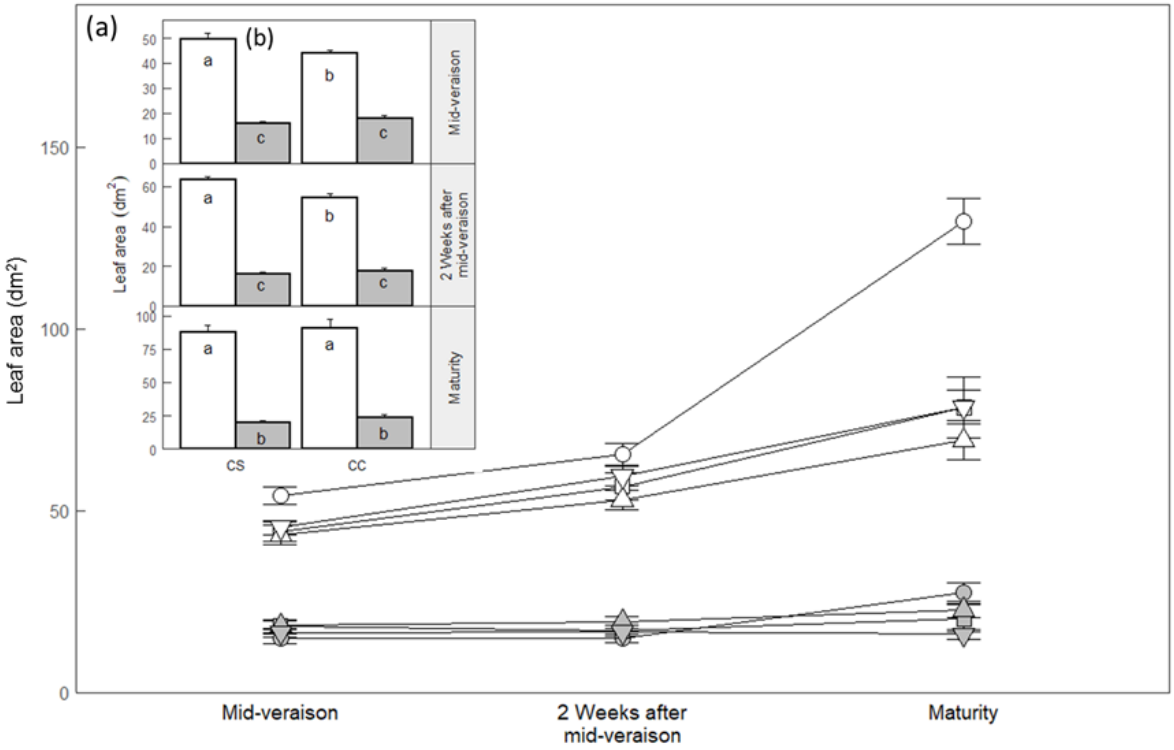
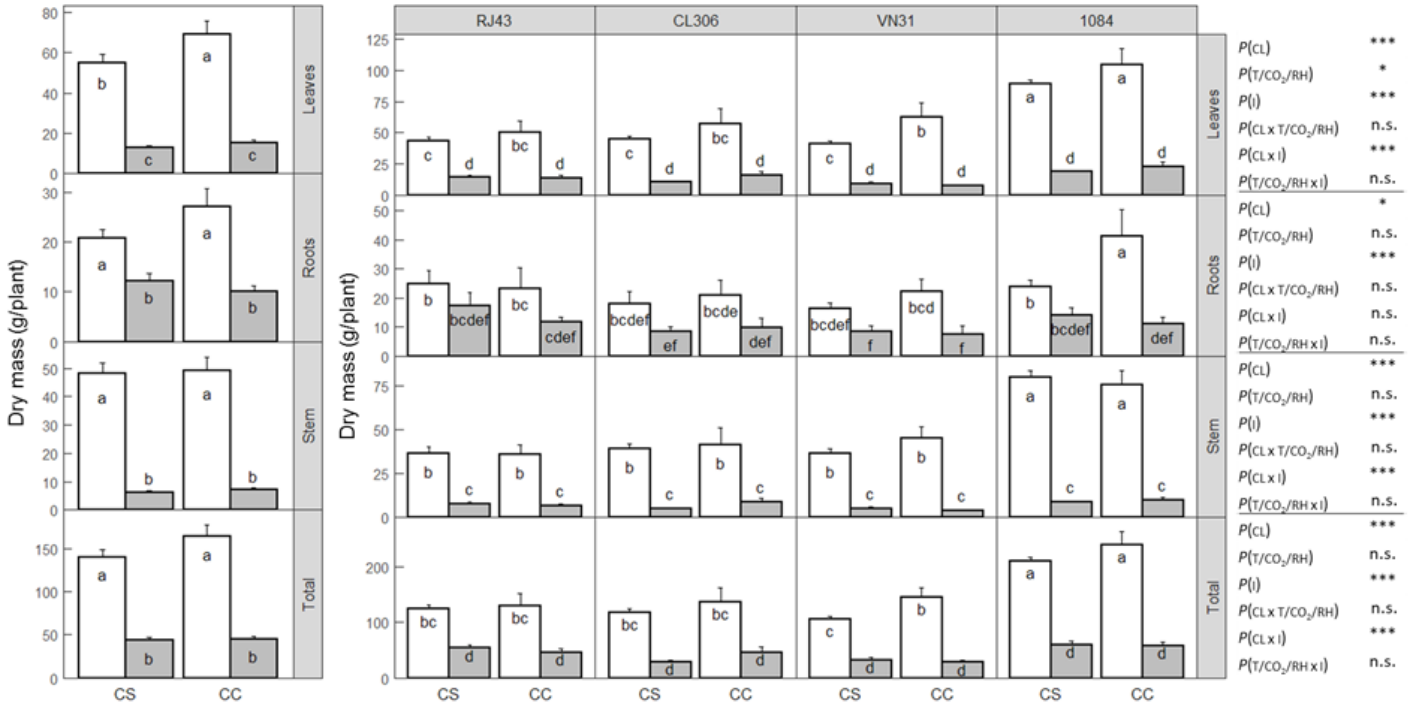

(a)
(b)
Leaf area (dm²)
Mid-veraison
2 Weeks after mid-veraison
Maturity
cs
cc
0
50
100
150
Mid-veraison
2 Weeks after mid-veraison
Maturity

Figure 5


Dry mass (g/plant)
Leaves
Roots
Stem
Total
CS
CC
RJ43
CL306
VN31
1084
P(CL) ***
P(T/CO2/RH) *
P(I) ***
P(CL x T/CO2/RH) n.s.
P(CL x I) ***
P(T/CO2/RH x I) n.s.
P(CL) *
P(T/CO2/RH) n.s.
P(I) ***
P(CL x T/CO2/RH) n.s.
P(CL x I) n.s.
P(T/CO2/RH x I) n.s.
P(CL) ***
P(T/CO2/RH) n.s.
P(I) ***
P(CL x T/CO2/RH) n.s.
P(CL x I) ***
P(T/CO2/RH x I) n.s.
P(CL) ***
P(T/CO2/RH) n.s.
P(I) ***
P(CL x T/CO2/RH) n.s.
P(CL x I) ***
P(T/CO2/RH x I) n.s.

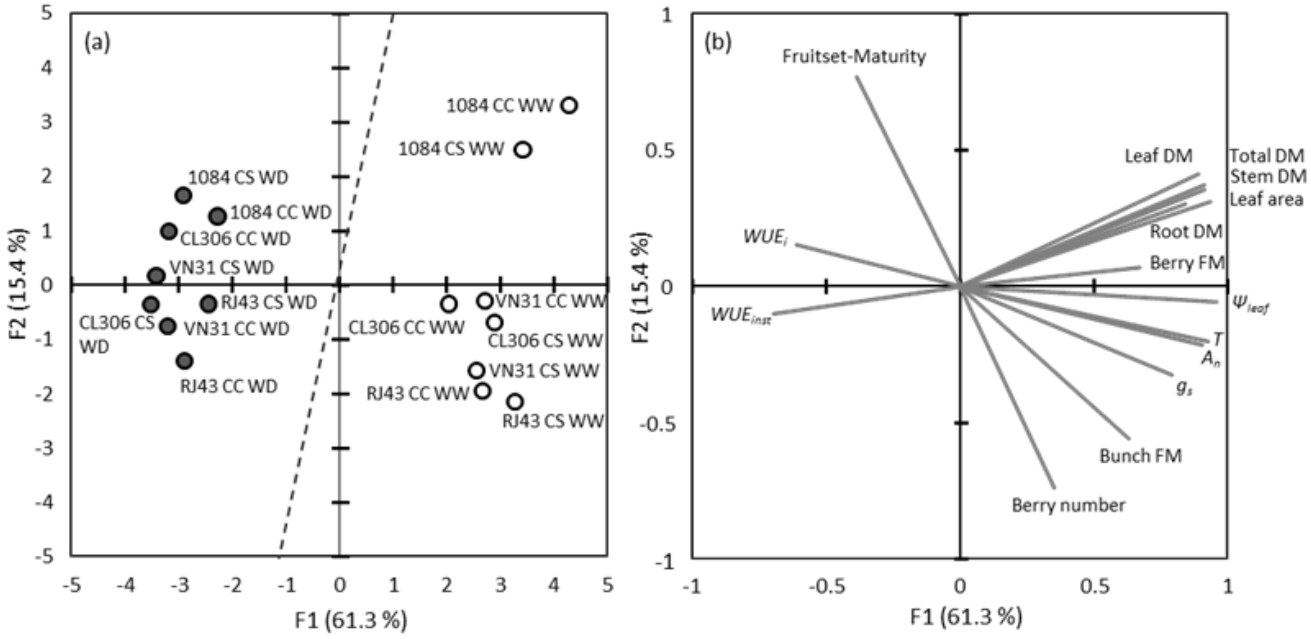

(a)
1084 CC WW
1084 CS WW
1084 CS WD
1084 CC WD
CL306 CC WD
VN31 CS WD
RJ43 CS WD
CL306 CS WD
VN31 CC WD
RJ43 CC WD
CL306 CC WW
VN31 CC WW
CL306 CS WW
VN31 CS WW
RJ43 CC WW
RJ43 CS WW
F2 (15.4 %)
F1 (61.3 %)
(b)
Fruitset-Maturity
Leaf DM
Total DM
Stem DM
Leaf area
Root DM
Berry FM
WUE_i
WUE_inst
ψ_leaf
T
A_n
g_s
Bunch FM
Berry number
F2 (15.4 %)
F1 (61.3 %)

Figure 6