*Journal of Soil Science and Plant Nutrition,* 2017, 17 (2), 385-397
RESEARCH ARTICLE

# Grapevine nutritional status and K concentration of must under future expected climatic conditions texturally different soils

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## Abstract

Nutrition is a relevant issue for winegrowers because it influences grapevine growth, berry composition, as well as must and wine quality. In this research, the following impacts on the nutritional status of cv. Tempranillo grapevines were evaluated: simulated 2100 expected $CO_2$, temperature (T) and relative humidity (RH) conditions (FCC; 700 µmol $CO_2$/mol air, 28/18°C day/night and 33/53% RH, day/night) vs. current $CO_2$, T and RH conditions (Curr; 390 µmol $CO_2$/mol air, 24/14°C and 45/65% RH); well-watered (WW) vs. future expected water deficit (WD); and three texturally different soils with different clay contents (41, 19 and 8%). FCC resulted in reduced concentrations in leaf blades of N and Ca at veraison and N and Zn at full maturity. WD resulted in higher leaf blade Na and Mn concentrations at veraison and maturity, respectively compared to WW. However, K concentrations in the leaves and must were higher for WW than WD. Higher concentrations of Ca and Mn were found in leaf blades of grapevines sampled at full maturity from more clayey soils. Even when nutrient inputs exceeded plant extractions, high soil clay content increased the K concentration in must and consequently, could affect wine quality in terms of acidity loss. However, future expected water stress will have the opposite effect, reducing the berry K uptake under high soil clay (41%) conditions.

**Keywords:** Climate change, leaf analysis, potassium, clay content, water deficit

## 1. Introduction

Nutrition is an important issue for wine grape growers since it impacts grapevine growth, fruit set, crop yield, berry composition and ultimately the quality of the must and wine. Grapevine nutrient needs are moderate, but unbalanced fertilization may lead to both undesired effects on wine quality, including a lack of acidity in wines made from grapes with an excessive potassium (K) supply, and to environmental impacts as a consequence of groundwater nitrate contamination (Romero *et al.*, 2010). Increasingly, consumers and legislators are demanding sustainable production practices with concomitant reduction in vineyard inputs and environmental impacts, putting pressure on winegrowers to better manage fertilizers. Plant analysis is a reliable method to assess grapevine nutritional status. Therefore, fertilizer recommendation programmes are very useful for fruit tree and vineyard management. However, there is still discussion about the most suitable sampling dates and the appropriate tissues to analyse (Benito *et al*., 2013). Despite the obvious influence of soil fertilization on plant growth and yield, knowledge and understanding about nutrient availability, the actual uptake of different fertilizers and how they are affecting grapevine physiology and productivity is surprisingly poor (Brataševec *et al*., 2013).

Monitored data and model simulations indicate that climatic conditions are changing, resulting in increased atmospheric $CO_2$ concentrations, higher temperatures and rates of evaporation, changes in precipitation patterns, with greater seasonal variability, and more frequent extreme weather events (IPCC, 2013). Thus, the impacts of global warming at regional scales are raising many questions for wine producers. Regarding grapevine nutrition, changes in temperature and evaporation are likely to affect the availability of soil water for grapevines, as well as the internal mechanisms of water movement through the grapevine, with consequences for nutrient uptake and mobilization (Proffitt and Campbell-Clause, 2012). Martins *et al.* (2014) reported that the influence of enhanced atmospheric $CO_2$ concentrations on plant productivity and growth has often been explored in combination with alterations in photosynthesis, respiration and carbon use and allocation (Zhu *et al*., 2012), whereas corresponding modifications in mineral composition (with the exception of nitrogen) have received much less attention (Thiec *et al*., 1995). Heavier and more frequent rainfall events could increase the incidence of water logging, leaching and erosion, thereby causing unfavourable conditions for nutrient uptake, as well as a loss of nutrients from the soil (Proffitt and Campbell-Clause, 2012). This, in turn, is likely to directly affect the ability of grapevines to extract nutrients from the soil and, therefore, to modify nutrient mobility within the grapevine (Proffitt and Campbell-Clause, 2012).

Potassium is by far the major cation related to berry ripeness (Mpelasoka *et al*., 2003), and its concentration in grapes is related to the must acidity. In a climate change scenario, this cation becomes very relevant since high temperatures could possibly increase K levels (Mira de Orduña, 2010). Potassium is involved in a variety of physiological processes, including enzyme activation in photosynthesis and respiration and the maintenance of cellular osmotic potential in plants (Salisbury and Ross, 1992). Potassium is mobile within the grapevine, and due to the relatively high requirements in vineyards, it is often applied as a fertilizer (Mosse *et al.*, 2013). High K additions may negatively impact overall grapevine health and wine quality (Mpelasoka *et al*., 2003). Mainly because K is predominantly implicated in the neutralization of tartaric and malic acids in the berries, thereby affecting the acid characteristics of the grapes (Esteban *et al*., 1999), thus increasing the expected negative effect of the lack of wine acidity caused by climatic change (Leibar *et al.*, 2016). Application of K to grapevines has also been found to influence anthocyanin levels and colour intensity (Mosse *et al*., 2013).

The characteristics of a particular soil have a large influence on the availability of water and the concentration of nutrients stored within it and, in turn, on the availability of nutrients for the grapevine. Grapevine development and yield are affected by soil water and nutrient storage and availability, and these latter, in turn, are influenced by soil depth and fertility, soil physical texture and structure, and soil chemistry, biology and organic matter content (van Leeuwen *et al*., 2004). Among all of the factors affecting soil nutrition, storage capacity and accessibility, soil texture, root depth and organic matter concentration deserve particular attention (Gonzalez-Dugo *et al.*, 2010). In this regard, soil texture is an important component because it determines the amount of water that a soil can hold when fully wet and the amount of water and nutrients potentially available for grapevine uptake. In fact, any deficiency or excess of water can play a determinant role in root function and tissue mineral concentrations, causing unbalanced grapevine growth and poor yield (Ghaffari and Ferchichi, 2011).

Taking into account the importance of mineral nutrition and nutrient balance on grapevine, and within a climate change context, it is of crucial importance to know how changes in environmental factors, such as atmospheric $CO_2$, temperature, relative humidity (RH), and water availability, can affect plant nutritional status and the K concentration of must, which is central to wine quality. Nevertheless, there are few studies that have evaluated the combined effect of elevated $CO_2$ concentrations, water availability and elevated temperature on grapevine nutrition. Because such studies are complex, difficult and expensive to execute under field conditions (Salazar-Parra *et al*., 2015). Even less frequent are studies where the impact of climate change on different soil types is evaluated. Therefore, the aim of this work was to evaluate the effect of the simulated year 2100 expected climate conditions: (1) a combination of elevated $CO_2$, elevated temperature and reduced RH and (2) low water availability in comparison with current conditions on the nutritional status *Vitis vinifera* L. of cv. Tempranillo grown in soils of different textures.

## 2. Materials and Methods

### *2.1. Potted plant establishment*

Dormant cuttings of *Vitis vinifera* L. of cv. Tempranillo clone RJ-43 were obtained in 2012 from an experimental vineyard at the Institute of Vine and Wine Sciences (Logroño, La Rioja, Spain). The cuttings were selected to obtain fruit-bearing cuttings, grapevine plants with one single cluster, in accordance with Leibar *et al.* (2015). Rooting was induced using indole butyric acid (September 2012) in a rock-wool heat-bed (27 °C) kept in a cool room (4 °C). One month later (October 2012), rooted cuttings were planted in 2 L plastic pots containing a mixture of perlite, vermiculite and peat (1:1:1, v/v/v) and were transferred to the first greenhouse. Only a single flowering stem was allowed to develop on each plant, the rest were removed. Growth conditions until fruit set were 24/14 °C and 60/70% RH (day/night) with natural daylight. A supplemental system of high-pressure sodium lamps (HQI-TS 400W/D Osram, Augsburg, Germany) was triggered when photosynthetic photon flux density (PPFD) dropped below 1000 μmol $m^{-2}$ $s^{-1}$ (14 h photoperiod). Humidity and temperature were controlled using M22W2HT4X transmitters (Rotronic Instrument Corp., Hauppauge, USA). The PPFD was monitored with a LI-190SZ quantum sensor (LI-COR, Lincoln, USA). Plants were irrigated with a nutrient solution proposed by Ollat *et al.* (1998). The composition of the nutrient solution was $KNO_3$ (2.5 mM),

$MgSO_4 \cdot 7H_2O$ (1.0 mM), $KH_2PO_4$ (1.0 mM), $Ca(NO_3)_2 \cdot 4H_2O$ (2,5 mM), $MnCl_2 \cdot 4H_2O$ (9.2 µM), $H_3BO_3$ (46.4 µM), $Na_2MoO_4$ (0.013 µM), $ZnSO_4 \cdot 7H_2O$ (2.40 µM), $CuSO_4$ (0.5 µM), Na Fe(III)-EDTA (45 µM).

### *2.2. Experimental design*

At fruit set, the plants were transferred to growth chamber–greenhouses (GCGs) and divided into 12 homogeneous groups of 8-11 plants that would result in the treatments described in the next paragraph. These plants were selected to have similar intergroup variability in terms of grape bunch size, avoiding changes in berry quality due to sink strength. The transplant process was made with extreme care, and no significant amount of roots was lost.

Plants were subjected from fruit set to maturity (defined at 21-23 Brix) to the following conditions: i) two glasshouse conditions (GC), combining two $CO_2$ concentrations, two temperatures (T) and two RH regimes. Simulated year 2100 expected climate conditions (FCC; 700 µmol $CO_2$/mol air (ppm) $CO_2$, 28/18 °C, day/night and 33/53% RH, day/night) vs. current climate conditions (Curr; 390 ppm $CO_2$, 24/14 °C and 45/65% RH). ii) Three soil textures: 41 (high), 19 (medium) and 8% (low) of soil clay contents. And iii) two water availabilities: well-watered (WW) vs. expected future water deficit (WD: 60% of the water applied to the WW plants). As in potted plant establishment, the above-mentioned supplemental light system of high-pressure sodium lamps was used, and the photoperiod was 15 h. Thus, 12 treatments were applied in a factorial design (2 glasshouse conditions x 3 soil textures x 2 water availabilities) as previously reported (Leibar *et al*. 2015). To prevent an edge effect, plants were rotated once a week within the corresponding greenhouse. Vegetative growth was not pruning-controlled. The $CO_2$, T and RH conditions projected by the IPCC (2007) for the period from 2070-2100 were simulated in two different GCGs, with increases in the $CO_2$ concentration and temperature of up to 700 ppm and 4 °C, respectively. For the RH, ENSEMBLES models (based on IPCC data), based on the Max Planck Institute model (MPI-ECHAM5; Roeckner *et al.*, 2003), state that the RH for the summer period will be 12% lower (change signal) at the end of the present century in the study area (i.e., in the grid or region of interest, "Denominación de Origen Calificada Rioja, DOCa Rioja").

Soils with a high red clay content are typical in DOCa Rioja (Barrios, 1994). To study the influence of three contrasting soil textures on grape yield and quality, a typical clay soil from La Rioja with high pH and low organic matter content was collected. It was mixed with sand to obtain three different soil textures: 41% clay (soil), 19% clay (soil and sand 1:1, v/v) and 8% clay (soil and sand 1:3, v/v) and put into 6 L plastic pots. Analysis by X-ray diffraction was carried out on the same soil type in the area where the sample was taken and showed that approximately 69% of the clay was illite, 10% chlorite, 12% kaolinite and 9% of one interlayer of vermiculite / smectite. The container capacity of each soil was measured as described by Leibar *et al.* (2015). The properties of the different soil:sand mixtures are shown in Table 1. The K soil content was slightly low according to the reference contents proposed by the regional extension service in the more clayey soil and was very low in the less clayey soils. The soil P content was low in the three soils, and the Mg soil content was low for sandy soils and high for the more clayey ones (Table 1).

Plants under WW conditions were maintained at container capacity (20-35% of soil water content), where soil water content was maintained above 20%. This represented daily inputs of approximately 500-800 mL of water or nutrient solution. The same amount of water was applied to all plants in order to observe the water availability of each soil type. WD plants received

60% of the water applied to those of WW. This water deficit level was chosen based on the model from the Max Planck Institute, which establishes that, in the summer period, precipitations will be 40% lower in DOCa Rioja at the end of the present century. Soil water sensors (EC-5 volumetric water content sensor, Decagon Devices, Washington) were placed into the pots to control irrigation. Irrigation was done from fruit set to ripeness, with a half-strength Hoagland nutrient solution or distilled water in order to provide the same amount of nutrients to all treatments.

**Table 1**. Physico-chemical properties of the three texturally different soils from DOCa Rioja used in the experiment.

| Physico-chemical soil properties | High clay | Medium clay | Low clay |
|---|---|---|---|
| Sand (%)[a] | 8 | 58 | 86 |
| Silt (%)[a] | 51 | 23 | 6 |
| Clay (%)[a] | 41 | 19 | 8 |
| Texture[a] | Silty clay | Sandy loam | Loamy sand |
| Bulk density (g cm$^{-3}$) | 1.230 | 1.325 | 1.410 |
| pH[b] | 8.84 | 9.21 | 9.39 |
| Organic matter (%)[a] | <0.01 | <0.01 | <0.01 |
| Carbonates (%)[a] | 70.18 | 19.39 | 6.32 |
| Active lime (%) | 10.17 | 4.61 | 0.00 |
| N (%)[c] | 0.03 | 0.01 | 0.01 |
| P (mg kg$^{-1}$)[a] | 1.83 | 1.39 | 1.27 |
| Mg (meq 100 g$^{-1}$)[a] | 5.26 | 2.72 | 1.15 |
| K (mg kg$^{-1}$)[a] | 138 | 67 | 29 |
| Na (meq 100 g$^{-1}$)[a] | 0.11 | bld | bld |
| Ca (meq 100 g$^{-1}$)[a] | 20.51 | 18.55 | 15.50 |
| CEC (meq 100 g$^{-1}$)[a] | 12.75 | 6.21 | 2.43 |

[a] Texture by pipette method, organic matter, carbonates, P, Mg, K, Na, Ca, CEC (MAPA, 1994); [b] pH (1:2.5 soil:water); [c] Total N (dry combustion using an LECO TruSpec®13 CHNs); bld = below limit of detection

### *2.3. Measurements*

Leaf blade samples opposite from the cluster were collected in all of the plants, as stated by Benito *et al*. (2013), at veraison and maturity. Samples were dried at 70 °C in a forced-air oven for 48 h. Blades from every two plants within the same treatment were pooled in order to have 4-6 biological replicates per treatment; therefore, two plants made one biological replicate. Samples of 0.5 g were taken and the K, Mg, Na, Ca, Mn, Fe and Zn concentrations were determined by flame atomic absorption spectrometry (SpectrAA-200, Varian, Australia) following digestion with a mixture of $HNO_3$/$HClO_4$. Nitrogen was measured using dry combustion in an elemental analyser (TruSpec CN, Leco, St. Joseph, Michigan) from a 0.1 g sample.

All of the plants were harvested individually at maturity, that is, when the berries reached 21-23 Brix. The leaves were counted, and the total (leaves, shoots and roots) dry weight was calculated after drying in an oven.

To analyse the K concentration of must, 50 berries per plant were randomly collected. Berries from two different plants within the same treatment (8-11 plants) were pooled in order to have 4-6 biological replicates (two plants made one biological replicate), making 100 berries per sample. Berries were weighed and ground in a blender (Omni Mixer). Part of the extract (10 ml) was centrifuged at 4.302 g for 10 min and diluted 10 times. Then, it was used to determine the K concentration by flame atomic absorption spectrometry (AAS, Varian) following a similar digestion procedure as the leaf samples.

### *2.4. Statistical analysis*

Statistical analysis was carried out with R software (R Development Core Team, Vienna, Austria).

The data were analysed with a 3-way analysis of variance to investigate the effects of glasshouse condition, soil texture, water availability and their interactions. The results were considered statistically significant at $p < 0.05$. The Tukey HSD test was used as a post hoc technique when the effects of treatments were statistically significant. When there was any interaction between factors, further analysis of variance was required, separating factors into levels. Data are presented as the means ± standard error (SE).

## 3. Results

The FCC leaf blades tended to have lower nutrient concentration than those of Curr, although significant differences were only observed for N and Ca at veraison (Table 2) and N and Zn at full maturity (Table 3). WD leaf blades had generally higher concentrations of most of the nutrients compared to those of WW, even though significant differences were only found for Na at veraison (Table 2) and Mn at full maturity (Table 3). However, the K concentration was 14.29% higher in leaf blades at full maturity in the WW than WD treatments (Tables 3). Soil texture did not affect plant nutrition at veraison (Table 2). Nevertheless, at full maturity, plants grown under more clayey soils had a higher Ca and Mn concentration in their blades than those grown under the other two soil textures (Table 3). There was no significant interaction among factors, with the exception of Mn and Fe blade concentration at veraison (Table 2), where glasshouse conditions (GC) and water availability interacted.

**Table 2**. Average concentrations and standard errors of macronutrients and micronutrients in grapevine cv. Tempranillo leaf blades at veraison, grown under two glasshouse conditions (GC): current conditions (Curr) and future expected conditions (FCC); two water availabilities (WA): well-watered (WW) and future expected water deficit (WD); and three soil textures (ST): 41 (high), 19 (medium) and 8% (low) clay contents. Significance codes: ns = no significance differences, *** $p< 0.001$, ** $0.001 \leq p < 0.01$, * $0.01\leq p <0.05$. Capital letters A and B indicate significant differences ($p<0.05$) between FCC and Curr. Small letters a and b indicate significant differences ($p<0.05$) between WW and WD. INT = interaction between GC and WA.

| Veraison | N | K | Ca | Mg | Na | Fe | Mn | Zn |
|---|---|---|---|---|---|---|---|---|
| | % | % | % | % | mg $kg^{-1}$ | mg $kg^{-1}$ | mg $kg^{-1}$ | mg $kg^{-1}$ |
| Glasshouse conditions (GC) | | | | | | | | |
| Curr | 2.77A ± 0.06 | 0.71 ± 0.04 | 2.41A ± 0.09 | 0.42 ± 0.02 | 107.8 ± 6.6 | 58.1 ± 2.1 | 123.4 ± 6.4 | 33.8 ± 2.6 |
| FCC | 2.47B ± 0.07 | 0.70 ± 0.03 | 2.13B ± 0.09 | 0.39 ± 0.02 | 101.8 ± 6.5 | 52.1 ± 2.4 | 107.0 ± 6.4 | 27.6 ± 2.5 |
| | ** | ns | * | ns | ns | INT | INT | ns |
| Water availability (WA) | | | | | | | | |
| WW | 2.57 ± 0.06 | 0.72 ± 0.03 | 2.17 ± 0.10 | 0.39 ± 0.01 | 86.6b ± 4.9 | 51.6 ± 2.5 | 107.0 ± 7.1 | 28.8 ± 2.8 |
| WD | 2.70 ± 0.08 | 0.69 ± 0.03 | 2.38 ± 0.08 | 0.42 ± 0.03 | 126.6a ± 5.8 | 59.2 ± 1.7 | 124.8 ± 5.1 | 32.8 ± 2.1 |
| | ns | ns | ns | ns | *** | INT | INT | ns |
| Soil texture (ST) | | | | | | | | |
| High (41%) clay | 2.62 ± 0.11 | 0.71 ± 0.03 | 2.27 ± 0.14 | 0.41 ± 0.03 | 109.2 ± 9.1 | 56.1 ± 3.8 | 112.9 ± 8.8 | 34.0 ± 4.1 |
| Medium (19%) clay | 2.66 ± 0.06 | 0.68 ± 0.04 | 2.30 ± 0.09 | 0.39 ± 0.02 | 103.4 ± 7.5 | 52.8 ± 2.0 | 117.7 ± 8.1 | 28.1 ± 2.5 |
| Low (8%) clay | 2.59 ± 0.07 | 0.72 ± 0.04 | 2.23 ± 0.11 | 0.41 ± 0.01 | 101.7 ± 7.6 | 56.3 ± 2.5 | 114.5 ± 7.5 | 30.0 ± 2.7 |
| | ns | ns | ns | ns | ns | ns | ns | ns |
| GC x WA | ns | ns | ns | ns | ns | ** | * | ns |
| GC x ST | ns | ns | ns | ns | ns | ns | ns | ns |
| WA x ST | ns | ns | ns | ns | ns | ns | ns | ns |
| GC x WA x ST | ns | ns | ns | ns | ns | ns | ns | ns |

FCC significantly reduced the concentration of Mn and Fe, but only for WW (Figure 1). Nutrient leaf concentrations were similar when comparing veraison and full maturity values. Sodium was the only element decreasing slightly its concentration at the end of the cycle.

**Table 3**. Average concentrations and standard errors of macronutrients and micronutrients in grapevine cv. Tempranillo leaf blades at full maturity, grown under two glasshouse conditions (GC): current conditions (Curr) and future expected conditions (FCC); two water availabilities (WA): well-watered (WW) and future expected water deficit (WD); and three soil textures (ST): 41 (high), 19 (medium) and 8% (low) clay contents. Significance codes: ns = no significance differences, *** $p< 0.001$, ** $0.001 \le p < 0.01$, * $0.01\le p <0.05$. Capital letters A and B indicate significant differences ($p<0.05$) between FCC and Curr. Small letters a and b indicate significant differences ($p<0.05$) between WW and WD. Capital letters Y and Z within the same parameter indicate significant differences ($p < 0.05$) among different soil textures.

| Full maturity | N<br>% | K<br>% | Ca<br>% | Mg<br>% | Na<br>mg kg$^{-1}$ | Fe<br>mg kg$^{-1}$ | Mn<br>mg kg$^{-1}$ | Zn<br>mg kg$^{-1}$ |
|---|---|---|---|---|---|---|---|---|
| Glasshouse conditions (GC) | | | | | | | | |
| Curr | 2.60A ± 0.07 | 0.73 ± 0.04 | 2.44 ± 0.09 | 0.38 ± 0.03 | 83.4 ± 4.2 | 62.6 ± 3.0 | 110.3 ± 6.2 | 47.3A ± 3.6 |
| FCC | 2.25B ± 0.05 | 0.65 ± 0.03 | 2.55 ± 0.10 | 0.43 ± 0.03 | 83.5 ± 5.4 | 55.51± 2.2 | 103.7 ± 5.7 | 28.6B ± 1.7 |
| | *** | ns | ns | ns | ns | ns | ns | *** |
| Water availability (WA) | | | | | | | | |
| WW | 2.40 ± 0.07 | 0.74a ± 0.03 | 2.42 ± 0.10 | 0.41 ± 0.03 | 77.2 ± 4.3 | 57.3 ± 2.7 | 93.2b ± 5.9 | 37.3 ± 3.3 |
| WD | 2.44 ± 0.07 | 0.63b ± 0.04 | 2.58 ± 0.09 | 0.41 ± 0.03 | 90.3 ± 5.2 | 60.6 ± 2.6 | 121.5a ± 4.8 | 37.9 ± 3.1 |
| | ns | * | ns | ns | ns | ns | *** | ns |
| Soil texture (ST) | | | | | | | | |
| High (41%) clay | 2.44 ± 0.11 | 0.69 ± 0.03 | 2.69Y ± 0.14 | 0.43 ± 0.05 | 83.9 ± 4.6 | 64.1 ± 3.2 | 117.0Y ± 7.7 | 40.1 ± 3.5 |
| Medium (19%) clay | 2.52 ± 0.08 | 0.71 ± 0.04 | 2.45YZ ± 0.10 | 0.41 ± 0.03 | 89.0 ± 5.2 | 56.8 ± 2.1 | 111.4YZ ± 7.5 | 40.5 ± 4.3 |
| Low (8%) clay | 2.30 ± 0.07 | 0.68 ± 0.05 | 2.35Z ± 0.09 | 0.38 ± 0.03 | 77.5 ± 7.3 | 56.1 ± 3.9 | 92.7Z ± 5.8 | 32.4 ± 3.9 |
| | ns | ns | * | ns | ns | ns | * | ns |
| GC x WA | ns | ns | ns | ns | ns | ns | ns | ns |
| GC x ST | ns | ns | ns | ns | ns | ns | ns | ns |
| WA x ST | ns | ns | ns | ns | ns | ns | ns | ns |
| GC x WA x ST | ns | ns | ns | ns | ns | ns | ns | ns |

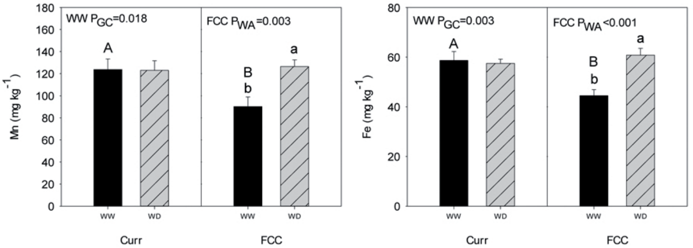

**Figure 1**. Glasshouse conditions (GC) and water availability (WA) factor interactions for leaf Mn and Fe concentrations at veraison in grapevine cv. Tempranillo. Different capital letters indicate significant differences ($p<0.05$) between the future expected conditions (FCC) and current conditions (Curr) treatments within each water availability treatment (WW $_{\mathrm{PGC}}$). Different small letters indicate significant differences ($p<0.05$) between the well-watered (WW) and future expected water deficit WD within each glasshouse condition (FCC $_{\mathrm{PWA}}$).

Regarding the K concentration of must, there was no significant difference between FCC and Curr (Table 4). The K concentration was 6.19% higher for must from the WW than the WD treatments (Table 4). Berries from plants grown under the more clayey soils had 7.56% and 3.89% higher K concentration in the must than berries from plants grown under the other soil textures (Table 4).

**Table 4.** Mean and standard error values of the K concentration in the must of cv. Tempranillo grapevines grown under two glasshouse conditions (GC): current conditions (Curr) and future expected conditions (FCC); two water availabilities (WA): well-watered (WW) and future expected water deficit (WD); and three soil textures (ST): 41 (high), 19 (medium) and 8% (low) clay contents. Significance codes: ns = no significant differences, *** $p<0.001$, ** $0.001 \leq p < 0.01$, * $0.01 \leq p < 0.05$. Different letters indicate significant differences ($p<0.05$) among levels of the applied factor.

| | K (g kg$^{-1}$) |
|---|---|
| GC | |
| Curr | 2.37 ± 0.04 |
| FCC | 2.29 ± 0.03 |
| | ns |
| WA | |
| WW | 2.40 ± 0.04 a |
| WD | 2.26 ± 0.03 b |
| | ** |
| ST | |
| High (41%) clay | 2.42 ± 0.05 a |
| Medium (19%) clay | 2.33 ± 0.05 ab |
| Low (8%) clay | 2.25 ± 0.03 b |
| | * |
| GC x WA | ns |
| GC x ST | ns |
| WA x ST | ns |
| GC x WA x ST | ns |

Furthermore, no significant differences in the leaf dry weight or in the dry weight of other organs (shoots and roots) were observed in plants grown under different GC and soil textures (Table 5). However, well-watered plants had 69.55% higher leaf dry weights and 20.67% higher total dry weights than plants subjected to water stress (Table 5).

**Table 5**. Averages of total leaf dry weight and total weight and standard errors of cv. Tempranillo grapevines at full maturity, grown under two glasshouse conditions (GC): current conditions (Curr) and future expected conditions (FCC); two water availabilities (WA): well-watered (WW) and future expected water deficit (WD); and three soil textures (ST): 41 (high), 19 (medium) and 8% (low) clay contents. Significance codes: ns = no significance differences, *** $p< 0.001$, ** $0.001 \leq p < 0.01$, * $0.01\leq p <0.05$. Different small letters indicate significant differences (p<0.05) between WW and WD.

| | Total leaf dry weight (g) | Total weight (g) |
|---|---|---|
| GC | | |
| Curr | 17.70 ± 1.01 | 116.02 ± 3.10 |
| FCC | 16.13 ± 0.87 | 113.20 ± 3.22 |
| | ns | ns |
| WA | | |
| WW | 21.16a ± 0.93 | 124.99a ± 3.27 |
| WD | 12.48b ± 0.49 | 103.59b ± 2.23 |
| | *** | *** |
| ST | | |
| High (41%) clay | 16.66 ± 1.35 | 114.62 ± 3.91 |
| Medium (19%) clay | 16.92 ± 1.11 | 114.50 ± 3.51 |
| Low (8%) clay | 17.20 ± 1.06 | 114.60 ± 4.22 |
| | ns | ns |
| GC x WA | ns | ns |
| GC x ST | ns | ns |
| WA x ST | ns | ns |
| GC x WA x ST | ns | ns |

## 4. Discussion

Potassium, Mg and Mn concentrations of leaf blades sampled at veraison were within the normal range for cv. Tempranillo for DOCa Rioja (K 0.65-0.97%, Mg 0.36-0.51% and Mn 69-119 mg kg$^{-1}$) (García-Escudero et al., 2013). Lower values were observed for Ca and Fe (Ca < 2.6% and Fe < 112 mg kg$^{-1}$) and higher values were found for Zn and N (Zn > 24 mg kg$^{-1}$ and N > 2.5%). Nutrient values at maturity were also very similar to those found by García-Escudero *et al*. (2013).

The lower nutrient concentration in leaf blades under FCC plants (in particular, N and Ca at veraison and N and Zn at full maturity) was associated with a greater number of leaves on these plants (29.53 ± 1.62) in comparison to those plants grown under Curr (22.49 ± 1.30) (Leibar *et al.*, 2015). Greater leaf biomasses usually resulted in a dilution of nutrients and, therefore, lower nutrient concentrations (Ata-Ul-Karim *et al*., 2014). In our case, this effect could be discarded since there were no differences in biomass in the FCC plants. Martins *et al*. (2014) found that leaf mineral nutrient concentrations are frequently observed to decline in the biomass in response to exposure to enhanced atmospheric $CO_2$ concentrations. This has been largely attributed to higher growth rates but also to increased amounts of non-structural carbohydrates (mainly starch) or to lower leaf transpiration rates (Thiec *et al*., 1995). As reported by Leibar *et al*. (2015), at veraison there were no differences in stomatal conductance, but at maturity, water-stressed plants grown under FCC (0.63 ± 0.10 mmol $H_2O$ m$^{-2}$ s$^{-1}$) had lower leaf transpiration compared to plants grown under Curr (1.22 ± 0.17 mmol $H_2O$ m$^{-2}$ s$^{-1}$). Among well-watered plants, there were no differences between FCC and Curr in leaf transpiration (Leibar *et al.*, 2015). Therefore, the lower Ca and Zn concentrations under FCC might be explained by a higher transpiration rate only when plants were grown under a water deficit. The reduced leaf N concentration of FCC plants, at veraison and full maturity, is in line with Salazar-Parra *et al*. (2015) and Leibar *et al*. (2015), who reported lower N concentrations in plants grown under elevated $CO_2$ as evidence of photosynthetic acclimation (down-regulation of assimilation) to elevated $CO_2$.

The higher nutrient concentrations of leaf blades in the water-stressed plants (significant differences only for Na at veraison and Mn at full maturity) could be because the well-watered plants had higher leaf dry weight and higher total dry weight than the plants subjected to water stress. This caused a dilution effect and, consequently, nutrients were more concentrated in plants grown under WD (Ata-Ul-Karim *et al*., 2014). On the other hand, Wang *et al*. (2008) suggested that the capacity to maintain Na in plant tissues under drought is part of the water stress avoidance mechanism. In contrast, the K concentration was higher in leaf blades and must at full maturity in the WW than WD treatment. Similarly, Dundon and Smart (1984) stated that K availability and uptake is reduced under conditions of limited soil water. In this regard, Esteban *et al*. (1999) reported that the absorption of K may be limited in dry soils. Klein *et al*. (2000) reported an increase of K in leaf blades under irrigation, which indicates a general increase in grapevine K status. Therefore, an increase in the root uptake of K under irrigated conditions may account for elevated grapevine and berry K accumulation (Mpelasoka *et al*., 2003), which was observed in this experiment. Irrigation enhances the dissolution of K from clay particles and its movement in the soil solution, which facilitates its supply to roots and a higher plant uptake (Klein *et al*., 2000). In addition, Rühl *et al*. (1992) stated that a water deficit might reduce mineral absorption because of lower root activity and root growth, as Leibar *et al*. (2016) observed for root growth in this experiment.

The finding of an absence of a soil texture effect at veraison is in agreement with Shange and Conradie (2012), who did not observe a consistent pattern of soil parent materials influence on leaf blade nutritional status. The higher leaf Ca and Mn concentrations at full maturity in grapevines grown in more clayey soil may be related to the higher initial nutrient content and cation exchange capacity (CEC) of these soils, as clay particles can hold nutrients due to their high CEC (Pal and Marschner, 2016). In our study, sandy soils had higher pH (9.39) than clayey soils (8.84); similarly, Pearson and Goheen (1988) and Rengel (2015) stated that Mn deficiency symptoms were more likely to occur in alkaline sandy soils, as Mn availability decreases in high pH soils. However, in our case, Mn values were within normal ranges, even high in all treatments, and no visual symptoms of Mn deficiency were observed. Although the K concentration in leaf blades did not exhibit significant differences among different soil textures, berries from plants grown under more clayey soils had higher K concentration in the must than berries from plants grown under other soil textures. This indicates that grape berries are a strong sink for K, particularly during ripening, as observed by Mpelasoka *et al*. (2003). Even if the three soils received the same high K input (206.94 g), and this amount was much higher than total K leaf biomass + must extraction (0.12 + 0.56 g), the higher exchangeable K content, higher CEC and the predominance of 2:1 clay minerals as illites may explain the higher K concentration in the must. The ability to fix or release K from 2:1 clay minerals led to authors such as Barré *et al*. (2008) to postulate that they behave as a K reservoir in soils. Esteban *et al.* (1999) asserted that the K concentration in must mainly comes from root absorption, which is determined by the level of assimilable K in the soil. The capability of our soil to provide K was so high that the K concentration of the must was higher in plants grown in the clayey soil, even when the root system was less developed (Leibar *et al.*, 2016). A positive correlation has been found between the K concentration and pH of grape berry juice (Mpelasoka *et al*., 2003), high pH adversely affecting the stability of the wine. It is well known how climate change (higher T and higher $CO_2$ concentration) will worsen must and wine acidity (Jones *et al*., 2005; Leibar *et al*., 2016); and soils with medium-high K content could contribute to this pH increase. Nevertheless, long-term field experiments under climate change conditions would be necessary to extract more definitive conclusions. The effects could be more or less pronounced when grapevines grow under these conditions for several years. As an example it is largely stated that plants acclimated its photosynthesis rate to high $CO_2$ atmospheric concentration as it was reported at this experiment (Leibar *et al*., 2015).

## 5. Conclusions

The elevated $CO_2$ and temperature and reduced RH expected in climate change predictions resulted in N concentration reductions in leaf blades regardless of their phenological stage. Future expected water deficits significantly altered grapevine nutritional status, thereby leading to higher concentrations of Na at veraison and Mn at maturity compared to well-watered plants. The higher aerial biomass was probably the cause of this dilution effect in the treatment with no water deficit. The exception was K, which showed lower concentrations in the leaves and in the must under the water deficit treatment. Soil texture influenced concentrations of Ca and Mn in the leaves and K in the must, as they were higher for grapevines in more clayey soils. Even when K input from fertilization was much higher than plant K extraction, a greater availability of soil K could increase the K concentration in plant tissues and must. Thus, special care should be taken with soils that have a great ability to supply K, because this property is difficult to change. This increased K concentration of must could be a problem in terms of wine acidity loss, especially since one of the adverse effects of climate change (elevated $CO_2$ and T) will also be lower must acidity. However, it is worth mentioning that future expected reduced water availability would cause a decrease in the K concentration of must.

**Abbreviations:** T = temperature; RH = relative humidity; FCC= simulated year 2100 expected $CO_2$, T and RH conditions; Curr = current $CO_2$, T and RH conditions; WA = water availability; WW = well watered; WD = future expected water deficit; ST = soil texture; CEC - cation exchange capacity.

## Acknowledgements

We thank the Department of Economic Development and Competitiveness of the Basque Government and Aragón Government (A03 research group) for financial support. Urtzi Leibar was the recipient of a grant from “Fundación Cándido Iturriaga y María Doñabeitia”. The authors would like to thank A. Urdiain and M. Oyarzun (Universidad de Navarra) for their excellent technical assistance and the Institute of Vine and Wine Sciences (Logroño, La Rioja, Spain) for supplying the dormant cuttings.

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