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Three-year field response of drip-irrigated grapevine (*Vitis vinifera* L., cv. Tempranillo) to soil salinity

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Abstract

We evaluated the salinity tolerance of young Tempranillo grapevines over a three year's period, and the related effects on scion growth to leaf Na and Cl concentrations. Soil salinity, rootstock growth and leaf Na and Cl concentrations were measured in a drip-irrigated saline field. Salinity tolerance was determined using the slope (percent growth decline per unit increase in soil salinity) of the upper-boundary line fitted to the maximum growth-salinity observations. Based on a slope of 17.1% for the three years, Tempranillo was shown to be more sensitive to salinity than other reported varieties (slopes between 9.3% and 13.2%). The salinity tolerance of Tempranillo decreased significantly along the study period. Tempranillo excluded Cl and Na from the leaves more efficiently than other grape rootstock-scion combinations. Tempranillo was classified as moderately sensitive to salinity, and decreases in growth with increases in salinity were attributed to the osmotic effect rather than to specific ion toxicities.

Introduction

Soil salinity affects growth and yield in grapevine by osmotic and specific ion toxicities (Maas and Grattan 1999; Shani and Ben-Gal 2005; Stevens and Walker 2002). The osmotic effect on vine growth is proportional to the decrease in the osmotic potential of the soil solution, operates from low values of soil salinity, and reduces leaf water potential, transpiration and photosynthesis. The specific ion toxicity operates when the vines accumulate certain ions such as Chloride (Cl), Sodium (Na) and Boron (B) above levels that cause detrimental effects due to direct toxicities or nutritional-induced imbalances. In practical terms, since increases in salinity are normally linked to increases in some of the above mentioned toxic ions, the effects of the osmotic and specific ion stresses can not be generally separated.

Ion accumulation occurs largely in old leaves (Sinclair and Hoffmann 2003), produces marginal leaf necrosis (Maas and Grattan 1999) and decreases leaf area and growth (Fisarakis et al. 2001). Toxic levels of Na are uncommon in leaves because Na is not translocated in appreciable amounts from the roots to the leaves (Ehlig 1960). Hence, Cl is the principal toxic ion for grapevines growing under saline conditions. Thus, Bernstein et al. (1969) concluded that leaf Cl levels in excess of 300 mmol kg^{-1} were injurious in the five studied cultivars. Injury by Cl toxicity in grapevine varies depending on the ability for rootstocks to accumulate Cl and restrict its transport to the shoots (Downton 1977). Thus, the maximum permissible Cl in soil water without leaf injury varies between 60 and 80 meq l^{-1} depending on varieties and rootstocks (Maas and Grattan 1999).

Shani and Ben-Gal (2005) found that shoot tissue Na and Cl levels follow breakthrough-type curves, with low values of around 50 mmol Na kg^{-1} and 100 mmol Cl kg^{-1} at low irrigation electrical conductivity (EC) values (< 5 dS m^{-1}) and increases of up to 500 mmol Na kg^{-1} and 1100 mmol Cl kg^{-1} for EC values above 10 dS m^{-1}. However, the effects of leaf Na and Cl on growth have not been quantified in grapevine. Thus, Downton (1985) found in a glasshouse study that the relationship between decline in plant growth of Sultana grapevine and leaf Cl accumulation was not straightforward.

Grapevine has been classified on the basis of its shoot growth as moderately sensitive to soil salinity, with a threshold ECe (electrical conductivity of the soil saturation extract) of 1.5 dS m^{-1} and with a 9.6 percent growth decline per unit increase in ECe beyond the threshold (Maas and Hoffman 1977). However, these values should be taken with care because they were derived from short-term growth studies of potted vines in sand or solution culture rather than in long-term, field trials. Thus, Walker et al. (2002) calculated for field-grown own-rooted Sultana vines that the growth reduction per unit ECe increase above the threshold was similar (9.3%) to that reported by Maas and Hoffman (1977), but the threshold ECe was 73% higher (2.6 dS m^{-1}).

Prior et al. (1992a) found in a six-year trickle irrigated trail with own-rooted Sultana grapevines that growth losses for heavy soils were much greater than predicted by the Maas and Hoffman model and that the effect of salinity increased with time. The response of Sultana was well described by a generalized logistic equation, with a continuous decline in growth with increasing salinity. Although this equation does not give a threshold, they calculated that a 10% yield loss occurred at ECe values of around 1 dS m^{-1} at the end of winter (Prior et al. 1992b). Shani and Ben-Gal (2005) also found that growth and yield of grapevine declined continuously from very low values of soil salinity without a clear definition of a threshold value. These authors found a 13.2% decrease in biomass production and a 14.4% decrease in fruit yield per unit ECe increase, and classified grapevine as "moderately sensitive" where 50% loss is expected at an ECe value of about 4.5 dS m^{-1}.

To fill some of these knowledge gaps, we conducted a three-year field study to evaluate the response of rootstock diameter growth to soil salinity and leaf Na and Cl accumulation in young grapevines (*Vitis vinifera* L., cv. Tempranillo) grown in a drip-irrigated commercial vineyard. The Tempranillo variety was selected because it is grown in more than 61% of La Rioja Protected Denomination of Origin (PDO), one of the most renowned wine-producing areas in Spain.

The specific objectives of this study are (1) to quantify the salinity tolerance of the Tempranillo variety by calculating the growth decline per unit increase in soil salinity, (2) to ascertain changes in salinity tolerance along the study period, and (3) to determine the effect on growth of leaf Cl and Na concentrations.

Materials and methods

The 1.04 ha commercial vineyard was located in Calahorra (middle Ebro Valley, La Rioja, Spain; 42º 16' 45'' N, 1º 58' 53'' W). The soil in this field is medium in texture (loamy) and with salinity problems. The average water content at field capacity and saturation were, respectively, 16% and 35%. The climate of the area is characterized by a mean annual temperature of 13.5 ºC (mean monthly maximum of 29.3 ºC in July and minimum of 2.0 ºC in January), 399 mm of precipitation, and 1030 mm of reference evapotranspiration.

In May 2003, one-year old Tempranillo vines grafted on Paulsen-1103 rootstocks were trained to a "T" trellis at an intra-row distance of 1 m and an inter-row distance of 3 m. The metal wires close to the monitored vines were substituted by plastic wires, and the selected vines were at least one meter apart from the metal posts in order to avoid the influence of metals on the EM38 readings. The vineyard was irrigated by a single trickle line close to the vines with 2 l h^{-1} emitters located every 0.75 m. The EC of the irrigation water varied between 0.8 and 1.3 dS m^{-1} along the irrigated season. Irrigation management was established by the farmer with the restrictions imposed by La Rioja PDO (i.e., the last irrigation must be given 30 days before harvesting and no later than 15 August of each year). Although not measured, the amount of water applied was less than ETo due to these restrictions and as indicated by the low soil water contents given in Table 3. Fertilization (yearly applications of 60 kg ha^{-1} of phosphoric acid and 80 kg ha^{-1} of 33.5%-ammonium nitrate) and phytosanitary and herbicide treatments were also managed by the farmer.

Vine measurements

A total of 59 vines were selected in 10 August 2004 on the basis of its differential growth and their root zone ECa values. The final number of vines reported is 56, because three of the selected vines died along the 2004-2006 study period.

The growth of each vine was assessed by measuring the rootstock diameter with a digital calliper placed over a permanent-ink mark. A problem in this measurement was the partial detachment of the rootstock bark in some vines. For this reason, the bark was peeled at the beginning of each growing season. Eleven measurements were taken along the 2004-2006 years (three measurements in 2005 and four measurements in 2004 and 2005). The yearly absolute growth of the rootstock diameters (ΔRD) in each selected vine was obtained from the differences in rootstock diameters measured at the beginning (RD_i) and end (RD_f) of each growing season. The three-year absolute growth was calculated by summing-up each yearly growth.

Since the initial vigor of the transplanted vines could have an impact on its subsequent growth, we also calculated $\Delta_i RD$, the percent growth of the rootstock diameter relative to the initial rootstock diameter measured at the beginning of each growing season:

$$\Delta i RD = 100 \cdot \frac{\Delta RD}{RD i} \tag{1}$$

Some 20 apical leaves without chlorosis symptoms were sampled in August of 2004 and 2006 in each control vine. The leaves were carried to the lab in a refrigerator, washed three times with deionized water for a few seconds and dried in an oven at 70 ºC to a constant weight. The dried leaves were finely ground in a blender. Chloride (coulometric-amperometric titration; Cotlove 1963) and sodium (flame photometry using a continuous flow auto-analyzer) concentrations were determined on dilute nitric-acetic acid extracts, expressing the concentrations on a dry weight basis.

Salinity measurements

Soil salinity was measured in two ways: directly, through soil sampling and analysis, and indirectly, by means of the EM38 sensor.

The main advantages of the soil sampling approach are that the samples may be taken close to the vines and within the wetted bulb at various soil depths, and that soil salinity may be directly measured in soil extracts. The main disadvantages are that the frequency of sampling is necessarily low due to labor costs and soil disturbances and that soil sampling is punctual and subject to soil's spatial variability. An additional problem in this study is that the emitters were located every 0.75 m in the trickle line whereas the vines were planted every 1m, so that the samples taken close to the vines differed in their distances from the emitters.

The main advantages of the EM38 approach are that the ECa readings may be taken frequently without disturbing the soil, and that they explore a large soil volume. The main disadvantage is that these readings depend on several soil properties such as salinity, texture, water content, SAR, calcite and gypsum content, etc. (Rhoades et al. 1999; Corwin et al. 2006) so that the sensor must be calibrated against soil salinity in order to separate other soil factors from the salinity measurements. An additional disadvantage in this drip-irrigated field is that the EM38 sensor explores a soil volume larger than the wetted bulb that develops with this irrigation system.

Soil measurements

A total of 95 soil samples were collected along the 2004-2006 study period. Each sample was a composite of two sub-samples taken in the planting row at 0.25 m at both sides of the vine. Although soil sampling was performed for the 0-30 and 30-60 cm soil depths, the results are reported for the 0-60 cm average. The diameter of each soil core was 3.75 cm, and the total soil volume of the two sub-sampled cores was 1.32 dm^3. Basoi et al. (2003) indicates that most roots in high-frequency irrigated grapevine are located in the first 0.4 m soil depth.

The SP (saturation percentage), ECe (soil saturation extract) and EC_5 (1:5 soil:water extract) were measured in air-dried, grounded and sieved (< 2 mm) samples following USSL (1954). Na and Cl were measured in around half of the soil samples. The gravimetric water content (WC) was also measured following USSL (1954).

Since soil water content was variable in space and time, the soil solution electrical conductivity (ECss) was estimated to characterize the ground-truth salinity at which the vines were exposed:

$$\mathrm{ECss} = \frac{\mathrm{ECe \cdot SP}}{\mathrm{WC}} \qquad (2)$$

Although this estimation is an approximation because it assumes mass conservation and lack of interactions between the liquid and solid phases, the ECss approach may be more sensible than the ECe and EC_5 extracts because it takes into account the changes in soil salinity with changes in soil water content.

Electromagnetic measurements

A total of 708 ECa readings were taken along the 2004-2006 study period with the EM38 sensor placed on the ground in its horizontal dipole position next to each monitored vine. Soil temperatures were also measured each time at soil depths of 0.2 and 0.4 m with a digital soil thermometer in order to convert the ECa readings to a reference temperature of 25 ºC. The time-weighted average ECa (ECa*) for each control vine was obtained from these readings for each study year and for the 2004-2006 years.

The EM38 sensor was calibrated against ECe and EC_5 by obtaining the simple linear regressions of ECe and EC_5 on ECa. From these equations and the ECa* values, the time-weighted average root zone ECe (ECe*) and EC_5 (EC_5*) estimates were calculated for each control vine in each study year. The 2004-2006 ECe* and EC_5* were the mean of the three individual years.

Data analysis

The salinity tolerance of Tempranillo was quantified by the percent growth decline per unit increase in soil salinity (referred as slope). Salinity tolerance was not defined on the basis of a threshold salinity because, as in other studies discussed in the introduction section, it was not identified in this trial. In this respect, it should be noted that the minimum ECe values measured in this work were higher than the threshold ECe reported by Maas and Hoffman (1977).

Due to the variable and uncontrolled stresses to which crops are typically exposed in trials performed in fields agronomically managed by the farmer and the above mentioned difficulties found in some vine and soil measurements, data scattering was substantial and the classical Maas and Hoffman growth response model could not be properly fitted to the observations. We used the "boundary-line" analysis, first presented by Webb (1972), that facilitates isolation of single-factor yield responses from data in which yields are affected by multiple factors (Shatar and McBratney 2004). In our study, the upper boundary line represents the maximum value of ΔRD or Δ_iRD that can be observed at a particular value of soil salinity. This upper boundary will represent the limiting response to soil salinity, and variates that fall below the boundary will represent those sites where stress factors other than salinity limit growth (Milne at al. 2006). We fitted the maximum ΔRD and Δ_iRD observations to soil salinity using the eye-fitting upper-envelope approach that we have previously validated with other quantitative statistical analysis (Aragüés et al. 2004) and applied in the study of the field response of olive to soil salinity (Aragüés et al. 2005).

Using the boundary line analysis, ΔRD and Δ_iRD for the pooled 2004-2006 years were fitted to ECe, EC_5 and ECss (soil salinity approach) and to ECa*, ECe* and EC_5* (EM38 approach). Yearly fittings were also obtained with the EM38 approach but not with the soil salinity approach due to its insufficient number of observations. In all cases, the slopes of the upper boundary lines determined the salinity tolerance of Tempranillo for the given period. Similarly, ΔRD and Δ_iRD for years 2004 and 2006 were fitted to the 2004 and 2006 average leaf Na and Cl concentrations using the upper boundary approach.

Results

Vine measurements

Table 1 shows the basic statistics of the growth variables (ΔRD and Δ_iRD) for the 56 control vines and the three years examined. The vines had a ΔRD in 2004 (7.8 mm) that doubled that in 2005 (3.7 mm) and 2006 (3.6 mm), whereas the coefficients of variation (CV) of the means were high and similar in the three years (35 to 39%). These high CVs were due to the selection of the 56 vines on the basis of its differential growth along the maximum ECa interval measured in the field. The cumulative mean ΔRD for the 2004-2006 period was 15.1 mm, with a maximum of 27.6 mm and a minimum of 4.5 mm.

Table 2 shows the basic statistics of leaf Cl and Na concentrations measured in 2004 and 2006. The mean Na in 2006 was two times higher than in 2004, whereas the mean Cl in 2006 was almost four times higher than in 2004. However, if two leaf Cl outliers higher than 100 mmol kg^{-1} are deleted in 2006, the mean Cl in 2006 (9.6 mmol kg^{-1}) was only 2.5 times higher than in 2004. The CVs in 2006 were considerably higher than in 2004 showing that the variability in soil salinity along the study years had an impact on the differential accumulation of these ions in the monitored vines.

Salinity measurements

Table 3 shows the basic statistics of measured ECe, EC_5, SP, WC and estimated ECss for the three years examined. The mean ECe for the pooled 2004-2006 years was 4.1 dS m^{-1} and increased by 39% during the study period. The maximum and minimum ECe values also increased along 2004-2006, whereas the CVs were high and relatively constant (26 to 29%). The EC_5 gave similar results, but their CVs were considerably higher than those for ECe. These higher CVs were attributed to the presence of gypsum in some of the soil samples that were gypsum-saturated in the saturation extract but not in the 1:5 soil:water extract. The WC was relatively low (mean WC = 11.5%) and variable (CV = 19%), with very low minimum values that reflect the already mentioned differences in the distances between the emitters and the points of soil sampling, and the fact that La Rioja PDO does not permits to irrigate the vines after 15 August of each year.

The mean ECss, estimated from ECe, SP and WC, was 15 dS m^{-1} for the 2004-2006 years and increased by 20% during the study period. In contrast to the mean ECe, the mean ECss in 2006 was lower than in 2005 due to the higher WC in 2006 than in 2005 (Table 3).

Table 4 shows the ECa-ECe and ECa-EC_5 calibrations obtained in each year and for the pooled 2004-2006 years. The number of sampling dates and soil samples are also indicated. The coefficients of determination (R^2) of these regressions are significant at $P < 0.001$. Since these regressions were different among years, the yearly ECe and EC_5 estimates were obtained from each yearly equation.

Table 5 shows the number of ECa readings taken along each year in the selected vines. From these readings, the time-weighted average ECa (ECa*) was calculated for each control vine in each year. The yearly mean ECa* remained constant during the study period, and the CVs varied between 26 and 30%. From the ECa* readings and the calibration equations obtained in each year (Table 4), the corresponding ECe* and EC_5* estimates were calculated. ECe* and EC_5* consistently increased along the study period, resembling the ECe and EC_5 increases shown in Table 3. However, the mean ECe* and EC_5* were higher than the corresponding ECe and EC_5, mainly due to increases in the minimum ECe* and EC_5* over those for ECe and EC_5.

Grapevine growth - soil salinity relationships

The 2004-2006 absolute (ΔRD) and relative (Δ_iRD) rootstock diameter growths were in general poorly related to soil salinity. Nevertheless, an upper boundary line fitting the maximum growth responses to each soil salinity value could be delineated (Figs. 1 and 2). The best results were obtained with ECss (estimated soil solution EC), where the upper-boundary line fitted reasonably well the maximum ΔRD (Fig. 1) and Δ_iRD (Fig. 2) observations (i.e., only the maximums ΔRD = 27.6 mm and Δ_iRD = 278% were positioned above the upper-boundary line). In contrast, the poorest relationships were obtained with ECa* (time-weighted EM38 readings), where three maximum ΔRD and Δ_iRD observations sited above the upper-boundary line. These results indicate that ECss is the most sensible soil salinity variable in relation to growth, whereas the ECa readings are less reliable because the EM38 senses soil volumes inside and outside the wetted bulbs by the emitters, besides the effect of other soil parameters on the ECa readings (Rhoades et al. 1999). Figs. 1 and 2 also show that Δ_iRD did not generally improve the fittings over ΔRD, suggesting that the initial vigor of the vines did not significantly affected these relationships.

Grapevine growth - leaf Na and Cl relationships

The 2004-2006 absolute (ΔRD) and relative (Δ_iRD) rootstock diameter growths were poorly related to the average 2004 and 2006 leaf Na and Cl concentrations (Fig. 3). Relatively consistent upper-boundary lines for leaf Na could only be delineated if three (with ΔRD) and four (with Δ_iRD) observations were discarded. The reliability of the upper-boundary lines for leaf Cl was even poor since, besides the two already discarded outliers with values above 100 mmol kg^{-1}, five (with ΔRD) and six (with Δ_iRD) observations were outside the lines (Fig. 3).

Discussion

Vine measurements

The six-fold difference between the maximum and minimum ΔRD (Table 1) reflects to a significant degree the differential salinity stresses at which the vines were exposed. Although a high growth in the first 2004 year after planting of the vines in 2003 and decreasing growths thereafter is typical in vines and other woody crops, soil salinity could also have an impact on these declining growths. Thus, the maximum ΔRD that reflects the growth in low or non-saline conditions, decreased by 39% in 2005 and 46% in 2006 from the maximum 2004 growth. In contrast, the mean ΔRD that reflects the growth for the average soil salinity, decreased by 53% in 2005 and 54% in 2006, suggesting that this higher decline in growth was also due to soil salinity. Similar results were obtained using Δ_iRD (Table 1).

Leaf Cl and Na concentrations measured in 2004 and 2006 (Table 2) were lower than those found in previous works. Francois and Clark (1979) measured average leaf Na and Cl concentrations of 12 and 11 mmol kg^{-1} in three grape cultivars irrigated with demineralized water in a two year trial, as compared with minimum leaf Na (< 1.9 mmol kg^{-1}) and leaf Cl (< 4.5 mmol kg^{-1}) concentrations measured in our work. Fisarakis et al. (2001) found average leaf Na and Cl levels of 90 and 280 mmol kg^{-1} in one year old Sultana vines grafted on six rootstocks and subject at 50 mM NaCl for a period of 60 days, and Shani and Ben-Gal (2005) found in a five year trial shoot tissue Na and Cl levels of around 50 mmol Na kg^{-1} and 100 mmol Cl kg^{-1} for irrigation EC values below 5 dS m^{-1} and 500 mmol Na kg^{-1} and 1100 mmol Cl kg^{-1} for EC values above 10 dS m^{-1}, as compared with maximum leaf Na (< 26 mmol kg^{-1}) and leaf Cl (< 85 mmol kg^{-1} if the two outliers are deleted) concentrations measured in this work. These results indicate that the Tempranillo variety grafted on Paulsen-1103 was able to exclude Na and Cl from the leaves more efficiently than other cultivar-rootstock combinations reported in the literature.

Mean Na and Cl concentrations measured in the soil saturation extract were, respectively, 12.9 and 7.7 meq l^{-1} in 2004 and 17.8 and 13.0 meq l^{-1} in 2006. Thus the Na/Cl ratio in the soil was 1.7 in 2004 and 1.4 in 2006. In contrast, the leaf Na/leaf Cl ratio was 0.6 in 2004 and 0.3 in 2006. The lower leaf than soil Na/Cl ratios indicate that the vines excluded Na more effectively than Cl with the result that leaf Na was lower than leaf Cl in spite of the higher Na than Cl concentrations in the soil. Similar results have been found in vines (Ehlig, 1960) and other woody crops such as olive (Aragüés et al., 2005).

Salinity measurements

The coefficients of determination of the ECa-ECe and ECa-EC_5 calibrations shown in Table 4 were lower than those found in other studies using flood and sprinkler irrigation systems (Isla et al., 2003). The reasons for these low R^2 values are that WC is low and variable (Table 3) and that gypsum is present in some soil samples. Thus, a multiple linear regression of ECa on soil salinity and WC increased R^2 to values close or above 0.7 in all years. Likewise, if the soil samples were grouped by the presence/absence of gypsum, R^2 increased above 0.8.

Another reason for the low R^2 values in Table 4 is that the volume measured by the EM38 is larger than the wetted volume by the emitters, so that the sensor detects areas close to the emitters that are at or near saturation as well as areas outside the wetted volume that are dry. This is an important constraint for using electromagnetic measurements in drip-irrigated systems with localized and relatively small wetted volumes, since the ECa lectures are considerably affected by WC (Rhoades et al., 1999). Yet, the EM38 sensor was used in this study because it allows estimating the temporal variability of soil salinity without disturbing the soil.

Although the ECe* and EC_5* estimates shown in Table 5 depict better the temporal variability of soil salinity than ECe and EC_5, they should be taken with care due to the relatively low coefficients of determination of the calibration equations from which they were derived. In addition, the CVs for ECe* and EC^*_5 (Table 5) were considerably lower that those for ECe and EC_5 (Table 3), a limitation in its use for obtaining the salinity tolerance of crops.

Grapevine growth - soil salinity relationships

The slopes of the upper-boundary lines using ECe and ECe* for the 2004-2006 study years were, respectively, 17.0% and 17.3% for ΔRD and 18.2% and 14.5% for Δ_iRD (Table 6). The similar slopes of the ΔRD-ECe and ΔRD-ECe* upper-boundary lines gives consistency to these results. The mean slope (17.1%) is around 80% higher then those given by Maas and Hoffman (1977) (9.6%) and Walker et al. (2002) (9.3%), and

30% higher then the slope reported by Shani and Ben-Gal (2005) (13.2%). These comparisons indicate that Tempranillo is more sensitive to salinity than other grapevines reported in the literature.

The average ECe_{50} (ECe producing 50% of the maximum growths shown in the upper-boundary lines) calculated for the ΔRD-ECe, ΔRD-ECe*, Δ_iRD-ECe and Δ_iRD-ECe* relationships was 6.55 dS m^{-1} (standard error = 0.07 dS m^{-1}), ranking the Tempranillo variety as moderately sensitive to soil salinity. This value agrees with the ECe_{50} of 6.38 dS m^{-1} calculated by Steppuhn et al. (2005) using a declining, sigmoid-shaped, modified compound-discount function.

The yearly slopes of the upper boundary lines were calculated with the EM38 approach (ECa*, ECe* and EC_5* soil salinity variables), since the number of the yearly observations with the soil salinity approach was insufficient for this analysis. Table 6 shows that the slopes increased in all cases along the study period. For example, the slopes of the ΔRD-ECa* upper-boundary lines were 125% in 2004, 130% in 2005 and 252% in 2006. Thus, the salinity tolerance of Tempranillo decreased along the study period and, in particular, in the last 2006 year when the slopes increased significantly over those for 2004 and 2005. Prior et al. (1992a) also found in own-rooted Sultana grapevines that the effect of salinity increased along their six-year trickle irrigated trail. This decrease in salinity tolerance with time of exposure to salts has been also found in other woody crops such as olive (Aragüés et al., 2005). Although, as shown later, leaf Na and Cl concentrations were below those reported as toxic in grapevine, mean leaf Cl in 2006 (14.9 mmol kg^{-1}) was almost four times higher than mean leaf Cl in 2004 (3.9 mmol kg^{-1}) (Table 2), an increasing trend that agreed with the lower salinity tolerance in 2006 than in 2004.

Grapevine growth - leaf Na and Cl relationships

Although inverse relationships between Tempranillo growth and leaf Na and Cl could be visualized through the boundary line analysis (Fig. 3), these relations were not reliable taking into account the number of observations located outside the upper-boundary lines. Based on ΔRD, the slopes of these lines were 13.2% for Na and 6.8% for Cl (i.e., the growth decline per mmol kg^{-1} increase was almost twice for Na than for Cl). The lack of consistent relationships between grapevine growth and leaf Na and Cl accumulation suggest that these ions were not toxic to the Tempranillo variety. Previous findings showed that leaf Cl levels injurious to grapevines exceeded 300 mmol kg^{-1} (Bernstein et al, 1969), in contrast to the low leaf Cl concentrations shown in Table 2. Maas and Grattan (1999) pointed out that leaf injury in vines was relevant only for Cl concentrations in soil water above 60 meq l^{-1}. Our mean soil solution Cl concentrations were much lower than this threshold concentration (around 28 meq l^{-1} in 2004 and 47 meq l^{-1} in 2006) and no apparent leaf injury and necrosis were observed in the field. Thus, decreases in growth for Tempranillo grafted on Paulsen-1103 were attributed to an osmotic effect rather then to specific ion toxicities.

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References

Aragüés R, Puy J, Isidoro D (2004) Vegetative growth response of young olive trees (Olea europaea L., cv. Arbequina) to soil salinity and waterlogging. Plant Soil 258:68-80.

Aragüés R, Puy J, Royo A, Espada JL (2005) Three-year field response of young olive trees (*Olea europaea* L., cv. Arbequina) to soil salinity: Trunk growth and leaf ion accumulation. Plant Soil 271:265-273.

Bassoi LH, Hopmans JW, Jorge LAdC, Alencar CMd, Silva JAMe (2003) Grapevine root distribution in drip and microsprinkler irrigation. Sci Agric. Doi:10.1590/s0103-90162003000200024

Bernstein L, Ehlig CF, Clark RA (1969) Effect of grape rootstocks on chloride accumulation in leaves. J Amer Soc Hort Sci. Doi:10.1007/s11104-004-2695-9

Consejo Regulador de la Denominación de Origen Calificada RIOJA. http://www.riojawine.com.

Cotlove E (1963) Determination of true chloride content of biological fluids and tissues. II. Analysis by simple nonisotopic methods. Anal Chem 35:101–105.

Corwin DL, Lesch SM, Oster JD, Kaffka SR (2006) Monitoring management-induced spatio-temporal changes in soil quality throough soil sampling directed by apparent electrical conductivity. Geoderma 131:369-387.

Downton WJS (1977) Influence of Rootstocks on Accumulation of Chloride, Sodium and Potassium in Grapevines. Aust J Agric Res. Doi:10.1071/AR9770879

Downton WJS (1985) Growth and mineral composition of the sultana grapevine as influenced by salinity and rootstock. Aust J Agric Res. Doi:10.1071/AR9850425

Ehlig CF (1960) Effects of salinity on four varieties of table grapes grown in sand culture. J Amer Soc Hort Sci 76:323-331

Fisarakis I, Chartzoulakis K, Stavrakas D (2001) Response of Sultana vines (v. vinifera L.) on six rootstoks to NaCl salinity exposure and recovery. Agr Water Manage. Doi:10.1016/S0378-3774(01)00115-9

Francois LE, Clark RA (1979) Accumulation of Na and Cl in leaves of sprinkler-irrigated grapes. J Amer Soc Hort Sci 104:11-13

Isla R, Aragüés R, Royo A (2003) Spatial variability of salt-affected soils in the middle Ebro valley (Spain) and implications in plant breeding for increased productivity. Euphytica. Doi:10.1023/B:EUPH.0000004988.25257.91

Maas EV, Hoffman GJ (1977) Crop salt tolerance-current assessment. J Irrig Drain 103 (IR2):115-134

Maas EV, Grattan SR (1999) Crop growths as affected by salinity. In: Skaggs RW and van Schilfgaarde (Eds.) Agricultural drainage, Agronomy 38, Amer Soc Agron, Madison, Wisconsin, pp 55-110

Milne AE, Ferguson RB, Lark RM (2006) Estimating a boundary line model for a biological response by maximum likelihood. Ann Appl Biol. Doi:10.1111/j.1744-7348.2006.00086.x

Prior LD, Grieve AM, Cullis BR (1992a) Sodium chloride and soil texture interactions in irrigated field grown sultana grapevines. I. Yield and fruit quality. Aust J Agric Res. Doi:10.1071/AR9921051

Prior LD, Grieve AM and Cullis BR (1992b) Sodium chloride and soil texture interactions in irrigated field grown sultana grapevines. II. Plant mineral content, growth and physiology. Aust J Agric Res. Doi:10.1071/AR9921067

Rhoades JD, Chanduvi F, Lesch S (1999) Soil salinity assessment. Methods and interpretation of electrical conductivity measurements. FAO Irrigation and Drainage Paper nº 57. Rome

Shani U, Ben-Gal A (2005) Long-term response of grapevines to salinity: Osmotic effects and ion toxicity. Am J Enol Viticult 56:148-154

Shatar TM, McBratney AB (2004) Boundary-line analysis of field-scale yield response to soil properties. J Agr Sci 142:553-560

Sinclair C, Hoffmann AA (2003) Monitoring salt stress in grapevines: are measures of plant trait variability useful?. J Appl Ecol. Doi:10.1046/j.1365-2664.2003.00843.x

Stevens RM, Walker RR (2002) Response of grapevines to irrigation-induced saline-sodic soil conditions. Aust J Exp Agr 42:323-331

USSL (1954) Diagnosis and improvement of saline and alkali soils. United States Salinity Laboratory, United Sates Department of Agriculture Handbook No. 60

Walker RR, Blackmore DH, Clingeleffer PR, Correll RL (2002) Rootstock effects on salt tolerance of irrigated field-grown grapevines (Vitis vinifera L. cv. Sultana).: 1. Yield and vigour inter-relationships. Aust J Grape Wine R. Doi:10.1111/j.1755-0238.2002.tb00206.x

Webb RA (1972) Use of the boundary line in the analysis of biological data. J Hortic Sci 47:309-319.

Table 1. Basic statistics of the growth in rootstock diameter: absolute values (ΔRD) and relative values over the yearly initial rootstock diameters (Δ_iRD) for the 2004-2006 study years.

	Year			
	2004	2005	2006	2004-2006
ΔRD (mm)				
Mean	7.8	3.7	3.6	15.1
CV (%)	35	37	39	29
Maximum	14.3	8.7	7.7	27.6
Minimum	1.6	1.0	1.0	4.5
Δ_iRD (%)				
Mean	73.2	21.9	18.0	143
CV (%)	34	43	35	29
Maximum	144	66.8	31.7	278
Minimum	17.6	6.3	5.9	49.4

Table 2. Basic statistics of leaf Cl and Na concentrations for the 2004 and 2006 study years.

	Cl (mmol kg^{-1})		Na (mmol kg^{-1})	
Year	2004	2006	2004	2006
No. Vines	49	49	52	52
Mean	3.9	14.9	2.4	4.9
CV (%)	29	184	41	72
Maximum	8.4	165	7.5	25
Minimum	2.4	4.4	1.0	1.8

Table 3. Number of vines with soil salinity measurements, number of sampling dates, number of soil samples and basic statistics of ECe, EC_5, SP, WC and estimated ECss averaged for the 0-60 cm soil depth for the 2004-2006 study years.

	Year			
	2004	2005	2006	2004-2006
No. Vines	33	15	25	73 (42*)
No. Sampling dates	3	1	4	8
No. Soil samples	46	15	34	95
ECe (dS m^{-1})				
Mean	3.6	4.2	5.0	4.1
Coefficient of variation (%)	26	26	29	29
Maximum	6.9	6.8	7.7	7.2
Minimum	2.3	2.8	3.0	2.4
EC_5 (dS m^{-1})				
Mean	0.65	0.80	0.86	0.73
Coefficient of variation (%)	42	36	36	39
Maximum	1.44	1.37	1.74	1.62
Minimum	0.32	0.44	0.42	0.39
SP (%)				
Mean	39.0	39.7	39.2	39.0
Coefficient of variation (%)	4.2	3.8	3.8	4.1
Maximum	42.0	41.5	41.5	42.0
Minimum	34.8	36.5	36.7	34.8
WC (%)**				
Mean	11.4	10.5	12.3	11.5
Coefficient of variation (%)	20	22	16	19
Maximum	16.1	16.1	17.3	17.3
Minimum	7.6	6.9	9.5	7.6
Estimated ECss (dS m^{-1})				
Mean	13.3	16.6	16.0	15.0
Coefficient of variation (%)	28	32	24	26
Maximum	23.4	29.5	25.6	25.6
Minimum	6.7	10.4	11.4	6.7

* Number of different vines sampled along 2004-2006.

** In 2004, WC was measured only in 25 out of the 46 soil samples

Table 4. Calibration of the EM38 sensor: Number of sampling dates, number of soil samples and calibration equations of ECa against ECe and EC_5 averaged for the 0-60 cm soil depth for the 2004-2006 study years.

	Year			
	2004	2005	2006	2004-2006
No. Sampling dates	4	3	4	11
No. Soil samples	68	25	33	126
ECe = a ECa + b				
a	4.1	4.1	4.7	3.7
b	0.7	1.7	1.8	1.6
R^2	0.700	0.500	0.505	0.433
EC_5 = a ECa + b				
a	1.09	1.01	1.06	0.95
b	-0.15	0.18	0.16	0.09
R^2	0.723	0.422	0.475	0.487

Table 5. Basic statistics of ECa* (time-weighted average ECa measured in the control vines) and ECe* and EC_5* (time-weighted ECe and EC_5 estimated from ECa* and the calibration equations shown in Table 2) for the 2004-2006 study years.

	Year			
	2004	2005	2006	2004-2006
ECa*				
No. ECa readings	295	177	236	708
Mean	0.68	0.67	0.68	0.68
Coefficient of variation (%)	26	29	30	28
Maximum	1.21	1.30	1.36	1.29
Minimum	0.46	0.46	0.45	0.46
ECe*				
Mean	3.5	5.2	5.9	4.9
Coefficient of variation (%)	21	15	17	17
Maximum	5.7	7.8	9.3	7.6
Minimum	2.6	4.3	4.9	3.9
EC_5*				
Mean	0.59	0.86	0.88	0.78
Coefficient of variation (%)	33	23	24	26
Maximum	1.17	1.49	1.60	1.42
Minimum	0.35	0.64	0.63	0.55

Table 6. Salinity tolerance of Tempranillo grapevine: percent growth decline per unit increase in soil salinity for the pooled 2004-2006 study years based on the soil salinity approach and for the pooled 2004-2006 years and the individual years based on the EM38 approach. ΔRD = absolute growth of rootstock diameter for the given period. Δ_iRD = percent growth of rootstock diameter relative to the initial rootstock diameter for the given period.

	Year							
	2004-2006		2004		2005		2006	
	ΔRD	Δ_iRD	ΔRD	Δ_iRD	ΔRD	Δ_iRD	ΔRD	Δ_iRD
Soil salinity approach								
ECe (dS m^{-1})	17.3	18.2						
EC$_5$ (dS m^{-1})	51.7	90.3						
ECss (dS m^{-1})	5.0	4.6						
EM38 approach								
ECa* (dS m^{-1})	74.7	63.9	125	107	130	136	252	178
ECe* (dS m^{-1})	17.0	14.5	30.1	26.6	31.9	31.0	50.3	37.2
EC$_5$* (dS m^{-1})	70.3	59.5	111	100	138	130	237	182

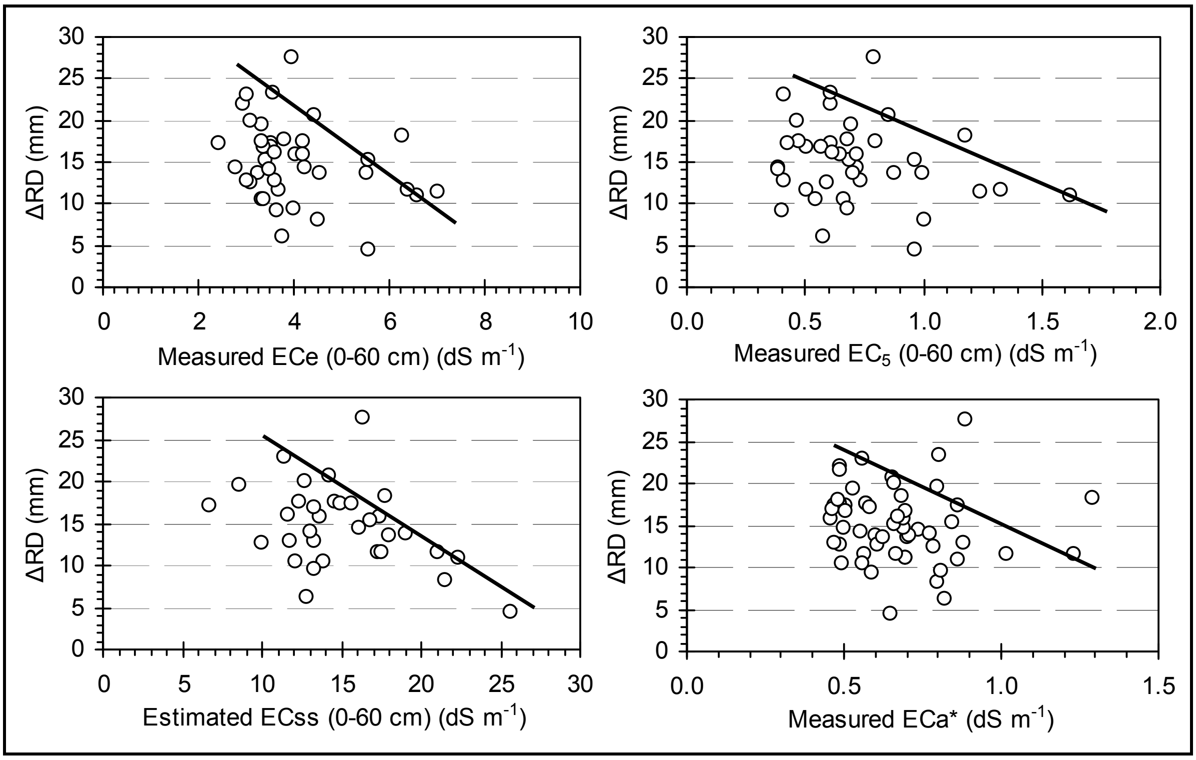

Fig. 1. Relationships between the 2004-2006 rootstock diameter growth (ΔRD) and the average 2004-2006 ECe, EC_5, ECss and ECa* soil salinity values. The solid lines represent the eye-fitting upper boundary line

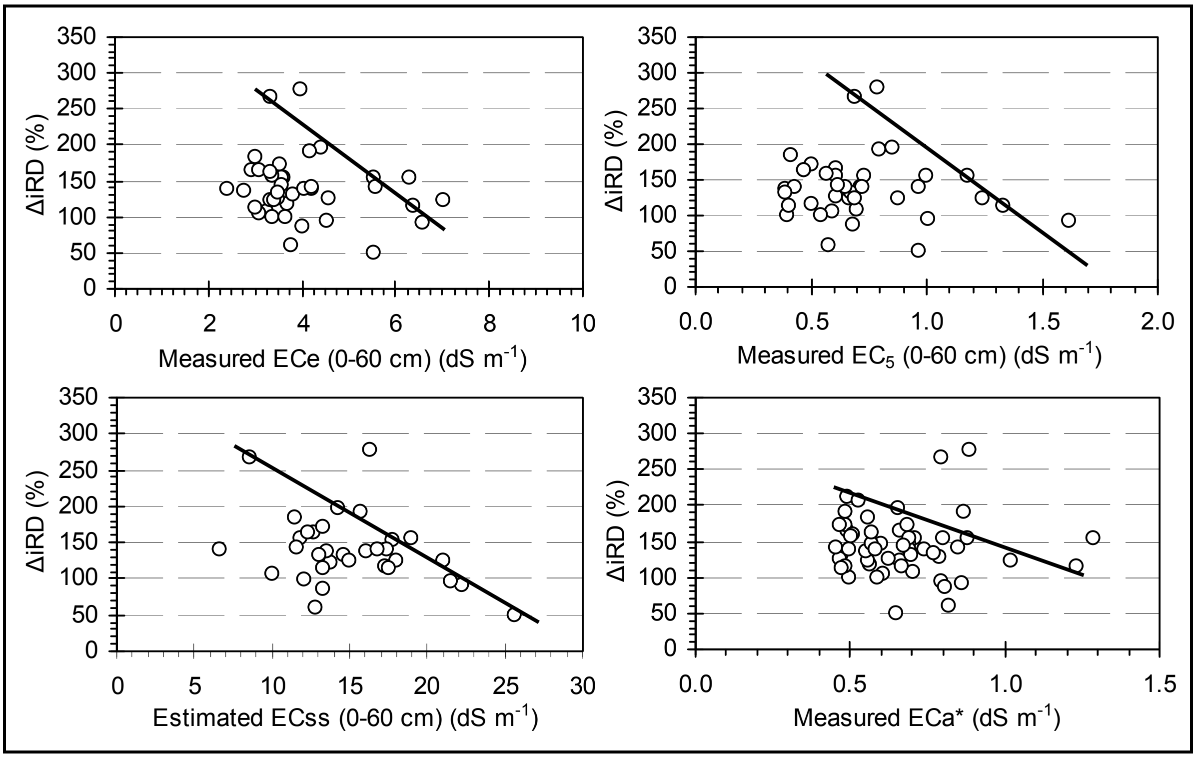

Fig. 2. Relationships between the 2004-2006 percent rootstock diameter growth relative to the initial rootstock diameter (Δ_iRD) and the average 2004-2006 ECe, EC_5, ECss and ECa* soil salinity values. The solid lines represent the eye-fitting upper boundary line

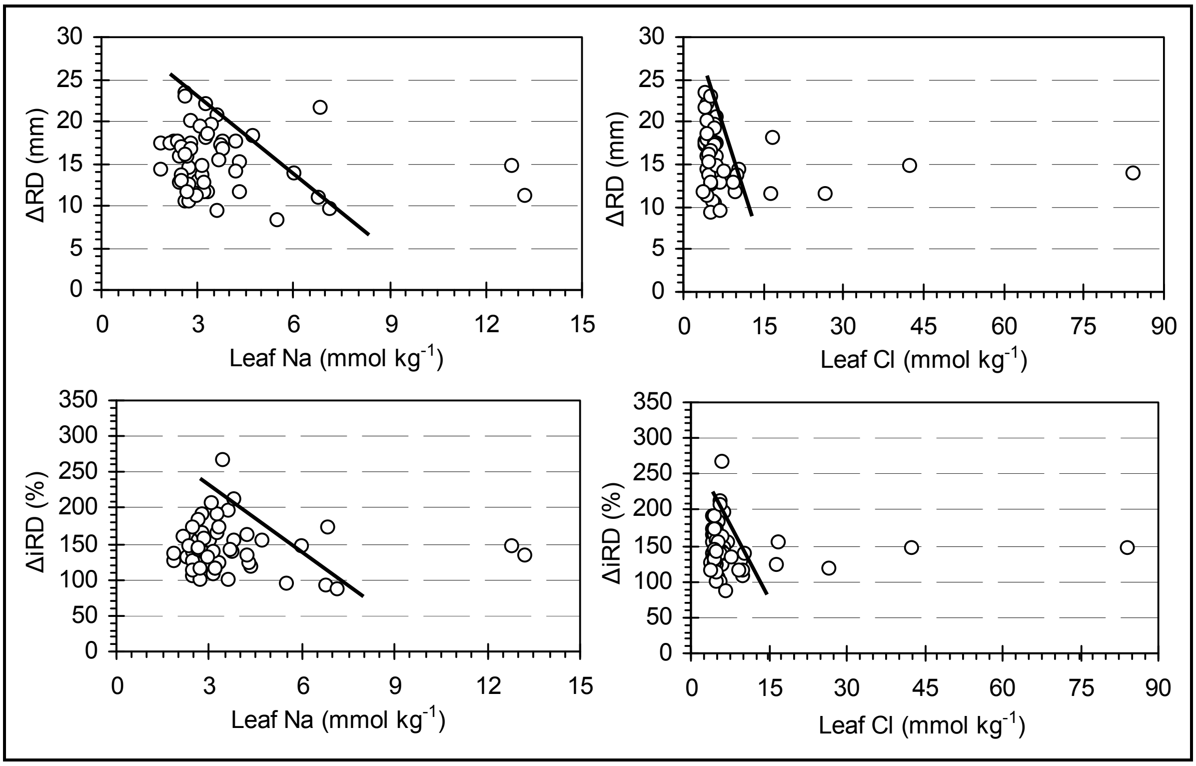

Fig. 3. Relationships between the 2004-2006 rootstock diameter growth (ΔRD) and percent rootstock diameter growth relative to the initial rootstock diameter (Δ_iRD), and the average 2004 and 2006 leaf Na and Cl concentrations. The solid lines represent the eye-fitting upper boundary line