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Influence of salinity on the transpiration efficiency of grafted and ungrafted grapevines.

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Introduction

An increase in water use efficiency (WUE = yield/irrigation+rainfall) is required for the expansion and long term sustainability of the Australian viticultural industries. Transpiration efficiency (W = dry matter produced/water transpired) is an important component of WUE and W has recently been shown to vary by up to 1.4-fold among grapevine varieties when grown under well-watered conditions (Gibberd *et al.,* 2001). Like many C_3 plants, such as wheat (Farquhar and Richards, 1984), sunflower (Virgona *et al*., 1990) and coffee (Meinzer *et al.,* 1990), a negative linear relationship has been demonstrated between W and carbon isotope discrimination (Δ) of grapevines. Variation in Δ among grapevines is predominately due to variation in stomatal conductance and to a lesser extent photosynthetic capacity (Gibberd *et al*., 2001). There is little known about the potential influence of abiotic stresses on grapevine W or Δ. This paper will focus on the influence of salinity since irrigation water is often saline.

Grapevine growth is sensitive to salinity (Maas and Hoffman, 1977) but both yield (Walker *et al.,* 1997) and grape/wine quality under saline conditions (Walker *et al*., 2000) are highly rootstock dependent. In general, grapevines exclude Na^+ and accumulate Cl^- (Downton, 1977; Downton, 1985). However, Cl^- uptake is highly dependent upon rootstock variety (Sauer, 1968) with, for example, Ramsey-rooted Sultana vines had 25 to 75% lower laminae Cl^- concentrations than own-rooted vines when exposed to a range of salinity treatments (Downton, 1985; Walker *et al.,* 1997). Salinity results in a reduction in leaf gas exchange of own-rooted grapevines, with a negative linear relationship between assimilation rate and laminae Cl^- concentration for Sultana grown with saline irrigation under glasshouse (Downton, 1977; Walker *et al*., 1981) and field (Walker *et al*., 1997) conditions. The decrease in assimilation was largely attributed to reduced stomatal and mesophyll conductance rather than a direct (toxic) effect of Na^+ or Cl^- on photosynthetic capacity (Downton, 1977; Walker *et al.,* 1981; Downton *et al.,* 1990). The influence of salinity on leaf gas exchange of grafted vines has not been reported in detail.

The aim of the experiment reported in this paper was to assess the transpiration efficiency, leaf gas exchange (under ambient and CO_2 saturated conditions) and carbon isotope discrimination of Sultana grafted to a Cl^--excluding rootstock (Ramsey) in comparison with own-rooted Sultana, when grown under a range of salinity levels in the glasshouse.

Materials and methods

Plant culture

Two year old vines of Sultana (*Vitis vinifera* var Sultana; syn Thompson seedless) on own roots or on Ramsey (*V. champini*) were transplanted into lysimeters containing 15L of a river sand:peatmoss:perlite:vermiculite (4:3:2:1 ratio) medium. Evaporation from the lysimeters was minimised by covering the soil surface with white polyethylene beads and a sheet of loosely-fitted white polyethylene film. The lysimeters were irrigated on a daily basis to excess of field capacity to maintain a leaching fraction of greater than 60% of applied irrigation. This minimised the accumulation of salt in the medium. Initial irrigation was with 0.2-strength nutrient solution (see Gibberd *et al.*, 2001 for details of nutrient solution) and the concentration of the nutrient solution was increased daily in 0.2-strength increments. Daily transpiration rate was assessed as irrigation volume – (drainage volume + soil evaporation). Soil evaporation was assessed as irrigation volume – drainage volume from a set of pots without plants.

Treatments

Four salinity treatments (1 = control, 20, 40 & 80 mol Cl^- m^{-3}) were applied 30 d after transplanting and maintained for a duration of 45 d. Salinity treatments were obtained by supplementing the irrigation solution with NaCl and $CaCl_2$ to maintain a Na^+:Ca^{2+} ratio of 15:1, and were imposed in daily increments of 20 mol Cl^- m^{-3}.

Initial total biomass was assessed at the start of the treatment period and a final harvest was performed after a further 45 d. Transpiration efficiency (W) was calculated as total biomass (dry weight) accumulated over the treatment period/total transpiration over the same period.

Gas exchange and leaf ion content

Gas exchange measurements were performed on 30 d old leaves using a CIRAS-1 (PPSystems, UK) infrared gas analysis unit and an automatic Parkinson leaf cuvette. Measurements were conducted under 'ambient' conditions (30 °C, 1200 μmol quanta m^{-2} s^{-1}, 360 ppm CO_2 and a relative humidity of 7 mbar) and 'CO_2-saturating' conditions which were similar to 'ambient' measurements except that the leaf was initially equilibrated in the chamber at 360 ppm CO_2 and then the CO_2 concentration was rapidly elevated to 1500 ppm. Measurements for the calculation of CO_2 saturated photosynthesis were taken at steady state. Gas exchange calculations were performed as described by von Caemmerer and Farquhar (1981).

Leaf disks (six disks per sample with a total area of 1.8 cm^2) were taken from the area of laminae where gas exchange measurements were performed, for determination of specific leaf weight, Cl^- content and Na^+ content. For each set of samples a fresh weight was determined then dry weights were determined after the leaf disks were dried at 80 °C for 2 d. Specific leaf area was calculated as leaf area/dry weight. Leaf-tissue water content was determined as the fresh weight minus the dry weight. Leaf Cl^- and Na^+ concentrations were determined as described by Walker *et al.* (1997).

Carbon isotope discrimination

At harvest, laminae of the 5 newest, expanding leaves were separated, dried at 80 °C for 2 d and then finely ground (<100 μm). The ^{13}C:^{12}C ratio of the leaf dry matter was determined by ratio mass spectrometry against a working standard of known carbon isotope composition relative to Pee Dee Belemnite. Carbon isotope discrimination was calculated as described by Farquhar and Richards (1984) assuming a ^{13}C:^{12}C ratio of CO_2 in air equal to 7.6‰. Leaf nitrogen content was determined by GC-MS elemental analysis of the same gas samples used for carbon isotope analysis.

Results

Irrigation with saline nutrient solution resulted in an increase in leaf Na^+ concentration of both Ramsey-rooted and own-rooted Sultana, with no significant difference between grafted and ungrafted vines (Fig. 1A). Leaf Cl^- concentration also increased with the salinity of the nutrient solution. However, unlike Na^+, the leaf Cl^- concentration of own-rooted vines was 2- to 4-fold greater than Ramsey-rooted vines (Fig 1.B).
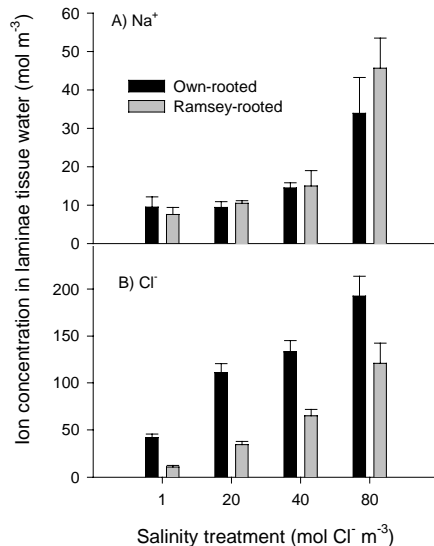

Fig. 1. Concentration of A) Na^+ and B) Cl^- in 30 d old leaves of own-rooted and Ramsey-rooted Sultana grapevines grown in containers irrigated with nutrient solution containing 1 to 80 mol Cl^- m^{-3}. Data are means (n=6) $\pm$ se.

Growth of Sultana was reduced by salinity (Fig 2A). However, the magnitude of the growth reduction was greater for the own-rooted than Ramsey-rooted vines. For example, at a salinity level of 40 mol Cl^- m^{-3}, growth of Ramsey-rooted vines was reduced by 12% compared to growth of controls. This contrasts with a 55% reduction in growth of own-rooted vines (Fig. 2A). Transpiration efficiency (W) of Ramsey-rooted vines was 1.3-fold higher than the ungrafted vines under control conditions and increased by 1.25-fold at 40 and 80 mol Cl^- m^{-3}. On the other hand, W of own-rooted vines was decreased by 31%. Thus, at 40 and 80 mol Cl^- m^{-3}, W of the Ramsey-rooted vines was 2-fold higher than own-rooted vines (Fig. 2B).

Photosynthesis, stomatal conductance and transpiration were decreased by saline irrigation for both Ramsey-rooted and own-rooted vines with no significant difference between grafted and ungrafted vines. Stomatal conductance (G) and transpiration (T) were more sensitive at 20 and 40 mol Cl^- m^{-3} than photosynthesis (Pn), and at 80 mol Cl^- m^{-3} Pn was reduced by 15% compared to a 30% reduction in G (Table 1). There was a small increase in the Pn/G ratio, but the T/G ratio also increased. Thus the ratio of Pn/T was not significantly influenced by salinity.

Salinity resulted in a decline in Δ (4.6‰ at 80 mol Cl^- m^{-3}) indicating that the intercellular CO_2 concentration (C_i) was greatly reduced by the salinity treatment. This is consistent with a reduction in stomatal conductance limiting CO_2 supply for photosynthesis. Furthermore, on a leaf area basis, there was only an 11% reduction in the CO_2-saturated rate of photosynthesis (Pn_{sat}) at a salinity treatment of 80 mol Cl^- m^{-3} (Table 2). However, salinity also resulted in a 32 to 43% decrease in specific leaf area and, as the %N on a dry matter basis was unchanged (data not given), there was a 1.45 to 1.9-fold increase in specific leaf nitrogen. Thus, while the reduction in Pn_{sat} at 80 mol Cl^- m^{-3} was small (15%) when expressed on a leaf area basis, there was a large reduction in Pn_{sat} when expressed on a leaf N basis (33 to 54% for Ramsey-rooted and own-rooted vines, respectively).
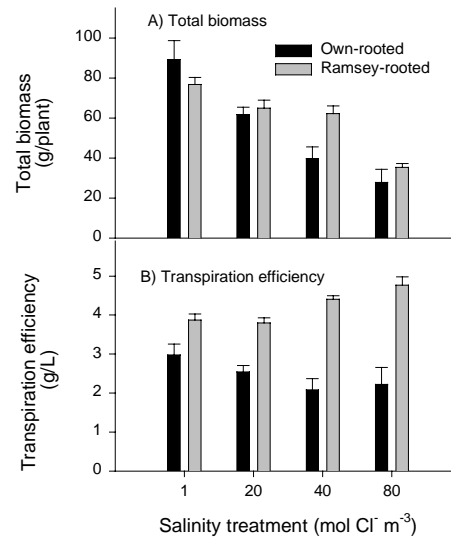
Fig. 2. A) Total biomass and B) transpiration efficiency of own-rooted and Ramsey-rooted Sultana grapevines grown in containers irrigated with nutrient solution containing 1 to 80 mol Cl⁻ m⁻³. Data are means (n=6) ± se.

Table 1. Leaf gas exchange characteristics of own-rooted or Ramsey-rooted Sultana vines grown in containers irrigated with nutrient solution containing 1 to 80 mol Cl⁻ m⁻³. Data are means (n=6) ± se. Measurements were performed on 30 d old leaves at 30 °C, 1200 μmol quanta m^{-2} s^{-1}, 360 ppm CO_2 and a relative humidity of 7 mbar.

Treatment	Pn $\mu mol\ m^{-2}\ s^{-1}$	G $mmol\ m^{-2}\ s^{-1}$	T $mmol\ m^{-2}\ s^{-1}$	Pn/T	Pn/G	T/G
Own-rooted						
1	18.3±0.52	375±29	6.6±0.3	2.72	0.050	0.018
20	17.4±0.54	354±29	6.4±0.3	2.64	0.049	0.019
40	17.1±0.51	306±21	5.9±0.2	2.79	0.053	0.020
80	15.8±0.52	265±16	5.3±0.2	2.83	0.055	0.021
Ramsey–rooted						
1	16.9±0.52	327±17	6.1±0.2	2.69	0.049	0.019
20	16.8±0.62	304±13	5.9±0.1	2.70	0.052	0.020
40	16.3±0.68	287±10	5.8±0.2	2.60	0.054	0.020
80	14.1±0.59	222±14	5.0±0.2	2.64	0.062	0.023

Discussion

This study has demonstrated that the whole-plant transpiration efficiency (W) of Sultana vines grown under saline conditions is highly dependent on root genotype. Salinity resulted in an increase in W of the Ramsey-rooted vines, but a decrease in the W of the own-rooted vines (Fig. 2). However, the differences in W between the Ramsey-rooted and own-rooted vines were not reflected by significant differences in the short-term measurements of the efficiency of leaf gas exchange as determined by the ratio of assimilation to transpiration (Pn/T). Nor was the difference in W between grafted and ungrafted vines reflected by a substantial difference in Δ, a more integrative measure of leaf gas exchange. Ramsey-rooted vines tended to have lower values of Δ at each salinity level than the own-rooted vines, but the difference

in Δ was too small to explain the 2-fold difference in W at the higher salinity levels. Thus it appears that factors other than leaf gas exchange were responsible for the difference in response of W to salinity between grafted and ungrafted vines. A possible explanation is that, under saline conditions, a higher proportion of assimilated carbon was lost in respiration by own-rooted vines than by Ramsey-rooted vines (Farquhar and Richards, 1984). Such a difference in respiratory costs under saline conditions may be related to the greater ability of Ramsey roots to slow Cl^- transport to the leaves (Fig. 1B) as proposed by Munns $et\ al.$ (1995)

Table 2. CO_2 saturated photosynthesis (Pn_{sat}), carbon isotope discrimination (Δ), specific leaf area (SLA), specific leaf nitrogen content (SLN) and the ratio of CO_2 saturated photosynthesis to leaf nitrogen content (Pn_{sat}/N) of 30 d old leaves of own-rooted and Ramsey-rooted Sultana grapevines grown in containers irrigated with nutrient solution containing 1 to 80 mol Cl^- m^{-3}. Data are means (n=6) $\pm$ se.

Treatment	Pn_{sat} $\mu molm^{-2}s^{-1}$	$\Delta\ ^{13}C$	SLA $(cm^2\ g^{-1})$	SLN $(mg\ N\ cm^{-2})$	Pn_{sat}/N $\mu mol\ g^{-1}N\ s^{-1}$
Own-rooted					
1	33.4±1.4	20.8±0.22	128±4.9	0.33±0.021	10.1
20	32.3±0.9	18.9±0.16	95±8.2	0.49±0.042	6.6
40	31.0±0.8	17.9±0.19	91±9.2	0.52±0.061	6.0
80	29.8±1.6	16.1±0.27	74±3.2	0.63±0.033	4.7
Ramsey–rooted					
1	31.3±1.3	20.0±0.16	105±14.9	0.40±0.037	7.8
20	33.2±0.9	18.6±0.16	97±13.5	0.48±0.065	6.9
40	32.4±1.4	17.1±0.28	80±7.4	0.54±0.051	6.0
80	28.4±1.5	15.4±0.08	72±5.7	0.58±0.038	4.9

Under well-watered, non-saline conditions, Gibberd $et\ al.$ (2001) observed a negative linear relationship between Δ and W of grapevines. Assuming the same relationship between Δ and W derived by Gibberd $et\ al.$ (2001), for the Ramsey-rooted vines in the present study the magnitude of the decrease in Δ under saline conditions (4.6‰ at 80 mol Cl^- m^{-3}) should have been reflected in a 1.45-fold increase in W. The observed increase in W was considerably less than this (1.25-fold). This may reflect enhanced respiration losses for Ramsey-rooted vines under saline conditions compared with non-saline conditions. Alternatively it may be that the Δ values measured under the saline conditions of this experiment were influenced by decreased discrimination associated with a reduction in mesophyll conductance. Salinity can result in an increase in specific leaf weight (as shown in this experiment), leaf thickness (for example, Downton $et\ al.,$ 1985) and mesophyll resistance (for an example with Sultana see Downton, 1977). This has been demonstrated to result in a decrease in Δ for salt treated cotton (Brugnoli and Lauteri, 1991).

Leaf Cl^- concentration of the Ramsey-rooted vines was at least 50% less than own-rooted vines across the range of salinity treatments. This is consistent with the previous findings of Downton (1985), Walker $et\ al.$ (1981) and Walker $et\ al.$ (1997). While salinity resulted in a reduction in G, Pn and T, there was only a small difference between the Ramsey-rooted and own-rooted vines in these parameters despite large differences in leaf Cl^- concentration. The poor relationship between leaf Cl^- and Pn rate suggests that other factors than Cl^- may reduce G, which in turn limits CO_2 supply for Pn and reduces C_i. For example, a root chemical

signal (such as Abscisic acid) or a hydraulically mediated process my be involved. Further evidence of this is the large reduction in Δ (allowing for a potential influence of mesophyll resistance on Δ) and the comparatively small (15%) reduction in Pn_{sat}. However, this does not rule out an involvement of Cl^- in the reduction of photosynthetic capacity as it is important to note that salinity also resulted in a large reduction in specific leaf area and an increase in specific leaf nitrogen such that Pn_{sat} per unit of leaf N declined by as much as 54%. Hence, while the influence of salinity on Pn expressed on a leaf area basis appeared small and primarily mediated by G, there was also a large reduction in photosynthetic capacity per unit of leaf nitrogen and this was closely correlated ($R^2 = 0.93$) with leaf Cl^- concentration (data not shown). It is unclear if this is due to increased partitioning of leaf N to alternative N-pools such as non-protein amino acids for osmotic or electrochemical adjustment or if it is due to a reduction in the efficiency of the photosynthetic apparatus *per se*.

We conclude that, in this experiment, W of Sultana under saline conditions was dependent on root genotype. However there was little effect of root genotype on leaf gas exchange or Δ. As such, it is likely that the decreased W of the own-rooted vines was the result of larger respiratory losses of carbon from own-rooted than Ramsey-rooted vines. This requires further investigation.

Acknowledgments

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