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Water relation parameters in *Vitis vinifera* L in drought period. Effects of leaf age

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Summary — The aim of this study was to evaluate the influence of leaf age on physiological mechanims adopted by grape vines (*Vitis vinifera* L) in order to sustain a favourable growth and production under drought conditions. The relationships between the water, turgor, osmotic potentials and leaf water content were studied during leaf ontogeny using both the pressure/volume and the psychometric technique. The symplasmic water fraction of the total water content decreased with leaf age. The capability for osmoregulation was almost the same in immature and mature leaves but decreased in old leaves. The calculated volumetric modulus of elasticity increased rapidly with leaf maturity and seems to be the dominant strategy for adaptation to drought conditions.

leaf ontogeny / tissue elasticity / water relations / water stress

Résumé — Relations hydriques chez *Vitis vinifera* L soumise à la sécheresse. Effet de l'âge des feuilles. Le but de cette étude est d'évaluer l'influence de l'âge des feuilles sur les mécanismes physiologiques manifestés par la vigne (*Vitis vinifera* L) afin de maintenir une bonne production en condition de sécheresse. Les relations hydriques dans les feuilles ont été étudiées au cours de leur ontogenèse par l'établissement de courbes pression–volume et l'utilisation de la méthode psychométrique. L'eau symplasmique comme fonction du volume total de l'eau tissulaire diminue en fonction de l'âge des feuilles. La capacité d'osmorégulation paraît avoir les mêmes valeurs chez les feuilles adultes que chez les feuilles jeunes mais elle est fortement diminuée chez les feuilles âgées. Le module d'élasticité volumique calculé augmente rapidement avec l'âge et il semble être le mécanisme prépondérant de l'adaptation à la sécheresse chez la vigne.

contrainte hydrique / module d'élasticité / ontogenèse des feuilles / relations hydriques

INTRODUCTION

The majority of vines grown in Greece are not irrigated and thus they are usually exposed to conditions of water and temperature stress during the summer period. Summer drought is assumed to be the important limiting factor of plants growth in the Mediterranean region (Noitsakis and Tsiouvaras, 1990). The most obvious effect of even mild water stress is to reduce growth. Cell enlargement is particularly sensitive to water

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deficit (Hsiao et al; 1985; Schulze, 1986). There is evidence that cell expansion depends on turgor pressure *P* (rather than total water potential) and that the expansion rate is proportional to the excess of *P* over a threshold value (Jones, 1986). Therefore, the maintenance of *P* over a particular threshold seems essential for continuous plant growth. Despite the low water availability, the vegetative growth of the vines in Greece continued until late summer. Probably the immature still expanding leaves possess a physiological mechanism, based on leaf water relations, which helps them to maintain a positive cell pressure, large enough to sustain its enlargement. Turgor pressure can be maintained by two distinct strategies: i) by lowering the osmotic potential (π) through the production of osmotically active solutes or ii) by increasing the elasticity of cell walls. The first strategy leads to the conservation of water in the tissue, whereas the second strategy allows the maintenance of the same turgor pressure with less water. There is some evidence that the type and relative importance of these strategies vary among species or even within a species (Parker and Pallardy, 1985), as well as among leaves of different ages (Patakas et al, unpublished).

There are also several approaches to determine leaf water relations (Pearcy et al, 1991). A recent approach is to deduce the water relations from the analysis of the dependence of leaf water potential (ψ) on relative water content (RWC) using living tissue. This method – commonly referred as pressure/volume technique – was originally developed by Scholander et al (1964) and has subsequently been refined by Tyree and co-workers (Tyree and Karamanos, 1980; Tyree and Jarvis, 1982). It leads to a detailed description of changes in *P* with the water content of the tissue as well as the calculation of the bulk modulus of elasticity (ε) by the derivative of that function. The psychrometric technique is often used to determine the diurnal changes in tissue osmotic potential (Turner, 1981). Most studies on plant water relations have been performed in mature leaves; much less information exists about changes in the variables of water relations during leaf ontogeny.

The objectives of this study were to determine parameters of water relations in grapevines leaves using both the pressure/volume and the psychrometric techniques as well as to evaluate the dominant physiological strategy employed by the leaves of different ages in order to adjust to water stress and/or sustain their growth.

MATERIAL AND METHODS

Ten-year old field grown grape vines (*Vitis vinifera* L cv Roditis) grafted on 110 Richter (*V rupestris* x *V Berlandieri*) rootstocks nearby the experimental station of the University of Thessaloniki were used. Leaves were classified according to their age in the following four groups: 18-day old still expanding immature leaves (L_1); 35-day old fully expanded mature leaves (L_2); 80-day old fully expanded mature leaves (L_3); 120-day old fully expanded leaves without any signs of senescence (L_4). In order to estimate exactly the leaf age, leaf appearance was systematically recorded. Irrigation had not been applied and no rainfall was recorded for 5 weeks before and during the experimental period. Diurnal field data concerning water relations and gas exchange parameters were measured for the leaves of the different groups. The experiment was repeated four times during summer. No significant differences were obtained between measurements on different days and hence the following results represent measurements for one single day.

Predawn leaf water potential was measured on three mature leaves (L_2) using the psychometric technique. Measurements were made at 10-day intervals starting in May (fig 1).

Of the 90 leaves tagged for each leaf age, seven leaves were used to study diurnal changes in leaf conductance with water vapour (C_w). This variable was mesured approximately hourly from 0600 to 2000 h using a steady-state porometer (Li-1600).

Leaf water (ψ) as well as osmotic (π) potentials were measured hourly from 0600 to 1800 h on five leaves per age group using the psychrometric technique (Wilson et al, 1979). Three pairs of 6 mm discs were punched from each leaf. The first pair of discs was used for the determination of leaf water potential. The discs were placed within seconds in sample chambers which were kept in a controlled temperature at about 22 °C, shielded with a polystyrene box. Osmotic potential was measured on the second pair of discs obtained from each leaf. These discs were rapidly sealed in teflon tape and aluminium foil and frozen on dry ice. Then the sealed discs were

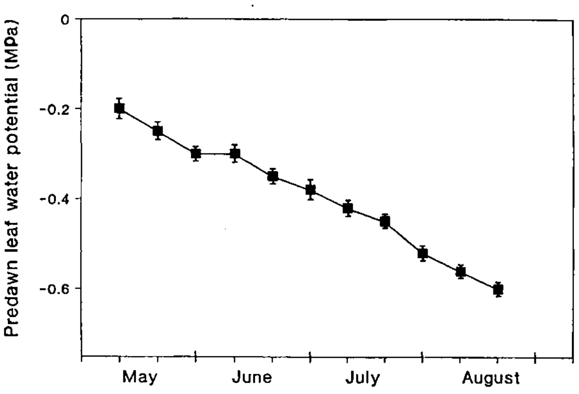

Fig 1. Seasonal changes in predawn leaf water potential in mature leaves. Bars indicate the standard error of the mean of three replicates.

thawed and placed in the chambers. Turgor potential was calculated as the difference between leaf water potential and osmotic potential.

Concomitant measurements of relative water content (RWC) were made on the third pair of discs obtained from the same leaf that was used for the determination of water potential components. The leaf discs were weighted quickly in a saturated atmosphere and their fresh weights (FW) were recorded. Afterwards, leaf discs were placed in distilled water for 10 h and the new weight was taken as turgid weight (TW). The discs were then dried at 80 °C and their dry weight (DW) was measured.

From FW, DW and TW, the relative water content (RWC) of leaves at different times of the day could be calculated by the formula:

$$\mathrm{RWC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100 \qquad \text{(Koide et al, 1991)}$$

Measurements of leaf water isotherms were made using the pressure chamber, which also enabled calculation of leaf turgor pressure (P) and the bulk modulus of elasticity (ε_{max}) (Koide et al, 1991). Five leaves per age group were hydrated to near maximum turgor by immersing their cut ends in distilled water, covering the leaves with black plastic bags and leaving them in the dark for 10 h. The water potential of the leaf when it was put into the pressure chamber was between –0.015 and –0.03 MPa. The pressure in the chamber was increased in steps of 0.2–0.3 MPa over the balance pressure (Koide et al, 1991).

Water potential, turgor pressure and osmotic potential (π) were all plotted against the relative symplasmic water loss (RSWL). This was calculated as loss of water expressed as percentage of the symplasmic water content at full turgor (W_0), ie,

$$\mathrm{RSWL} = \frac{W_0 - W}{W_0} \times 100$$

where W_0 is calculated from the intercept of the straight part of the pressure volume curve – relating $1/\psi$ vs RWC – with the abscissa (RWC) (Turner, 1981) and W is the actual weight of symplasmic water after pressurization.

The bulk modulus of elasticity (ε) was calculated from:

$$\varepsilon = \Delta P\ W_0 / \Delta W \qquad \text{(Turner, 1981)}$$

The value of ε obtained near maximum turgor (ε_{max}) was compared for leaves of different ages.

Changes in symplasmic water content as a percentage of the total water content were also monitored in all leaves by calculating the symplasmic water fraction (SWF) (Pavlik, 1984) by:

$$\mathrm{SWF} = \frac{W_0}{\mathrm{TW} - \mathrm{DW}} \times 100$$

Data were analyzed using one-way analysis of variance and the LSD test (Snedecor and Cochran, 1980).

RESULTS

Predawn leaf water potential declined continuously from May to August (fig 1). The relationship between RWC and water potential in all ages of leaves is shown in figure 2. Higher values of RWC were found in the early morning (0600 h) and lower values in the early afternoon (1500 h). RWC as well as leaf water potential seemed to decrease in all leaves during the morning. Immature leaves (L_1) showed lower RWC values as measured at 0600 h than the mature and the old leaves (L_2, L_3, L_4) indicating

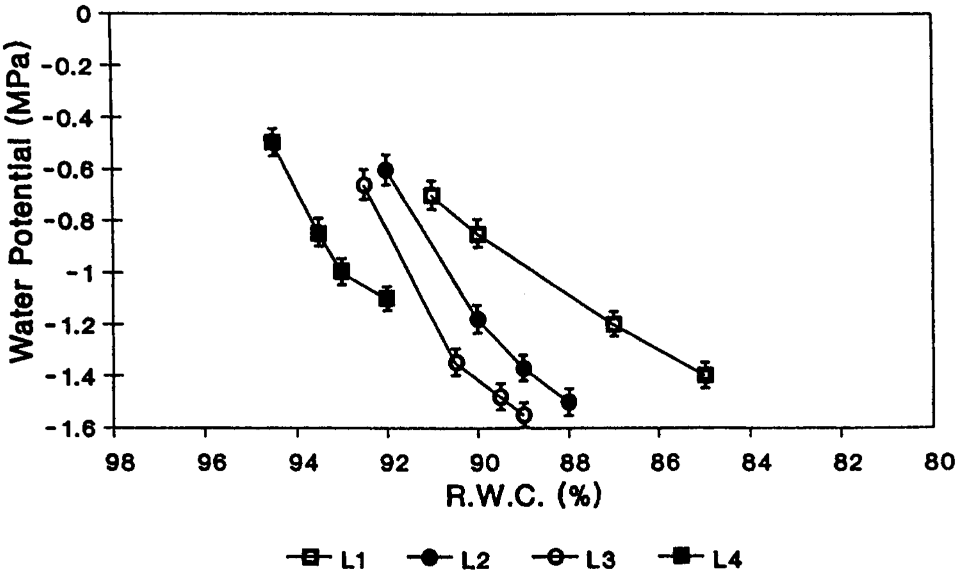

Fig 2. Diurnal changes in leaf water potential (ψ) in relation to relative water content (RWC) for leaves of different ages. Higher values of ψ corresponded to early morning (0600 h) and lower values to early evening (1500 h). Bars indicate the standard error of the mean of five replicates.

that these reached at a lower value of leaf hydration during the night. The decrease in RWC tended to be smaller in old leaves (L_4) and it was greater in immature leaves (L_1). Furthermore, immature leaves exhibited lower values of RWC for the same value of ψ. Leaf water potential at 0600 h was nearly the same in all leaves at about –0.6 MPa. It dropped to about –1.4 MPa in both mature and immature leaves and to –1.1 MPa in old leaves at 1500 h.

Porometer measurements indicated that old leaves (L_4) had lower stomatal conductance than mature (L_2, L_3) and immature leaves (L_1) regardless of the time of the day (fig 3a). The stomatal closure seemed to start at higher values of ψ (–0.85 MPa) in the old leaves than in the mature leaves (–1.3 MPa). Stomatal closure resulted in the reduction of transpiration in all leaves studied (fig 3b).

The symplasmic water fraction (SWF) as a percentage of the total water content, decreased with leaf age from 78% in the immature leaves (L_1) to approximately 62% in old leaves (L_4) (table I).

The analysis of water potential isotherms (fig 4) showed that statistically significant changes in both ψ and its components, P and π, occurred during leaf ontogeny. In particular, the osmotic potential at full turgor (ie, π_o, at symplasmic water loss = 0) decreased with the leaf age from –1.35 MPa in immature leaves (L_1) to –1.7 MPa in (L_4). Also the relative symplasmic water loss at incipient plasmolysis (ie, at $P = 0$) decreased during leaf development from 17% in immature leaves to 11.2% in old ones. Furthermore, the changes of dP vs dRSWL seemed to be lower in the immature than the mature and old leaves. Thus, for a 5% water loss the dP was 1.38–0.8 = 0.58 MPa in (L_1) in comparison to 1.5–0.64 = 0.86 MPa in (L_2), 1.6–0.59 = 1.01 MPa in (L_3) and 1.7–0.51 = 1.19 MPa in (L_4).

The dP change depends on the bulk modulus of elasticity (ε) of cell walls for a given symplasmic water loss. Under the same conditions, dP will be higher in cells with rigid cell walls (ie, higher value of ε) than in cells with more elastic cell walls because

$$dP = \varepsilon \frac{dV}{V}$$

where dV/V is the relative change in volume.

The ε value at full turgor (ε_{max}) increased significantly with leaf age from about 12.7 MPa in the immature leaves to about 22.5 and 25 MPa in (L_2) and (L_3), respectively (table I). Old leaves (L_4) showed even higher ε_{max} values of 28 MPa.

Relationships among leaf water potential at the incipient plasmolysis (ψ_{ip}), minimum diurnal leaf water potential (ψ_{min}) as measured at 1500 h and leaf age show that the (ψ_{min}) tended to diverge from (ψ_{ip}) as leaves matured (fig 5). This suggests

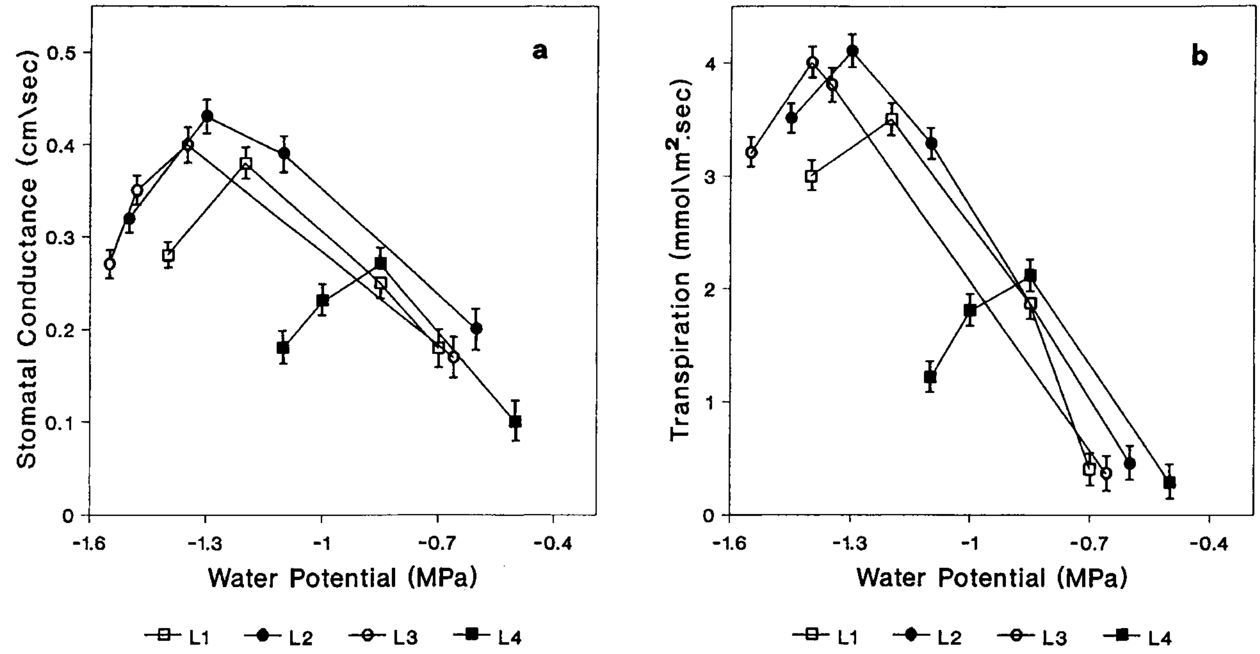

Fig 3. Diurnal changes in stomatal conductance (a) and transpiration rate (b) in relation to leaf water potential in leaves of different ages. Higher values of ψ corresponded to early morning (0600 h) and lower values to early evening (1500 h). Bars indicate the standard error of the mean of seven replicates.

Table I. The values of symplasmic water fraction (%), relative symplasmic water loss (%) and bulk modulus of elasticity (ε_{max}) in different leaf ages. Different letters within columns indicate significant differences at the 5% level between leaves ages.

Leaf age	*Symplasmic water fraction (%)*	*Relative symplasmic water loss (%)*	ε_{max}
L1	78^a	17^a	12.7^a
L2	69^b	14.5^b	22.5^b
L3	66^b	12^c	25^b
L4	62^c	11.2^c	28^c
LSD (*a* = 0.05)	3.21	1.43	2.72

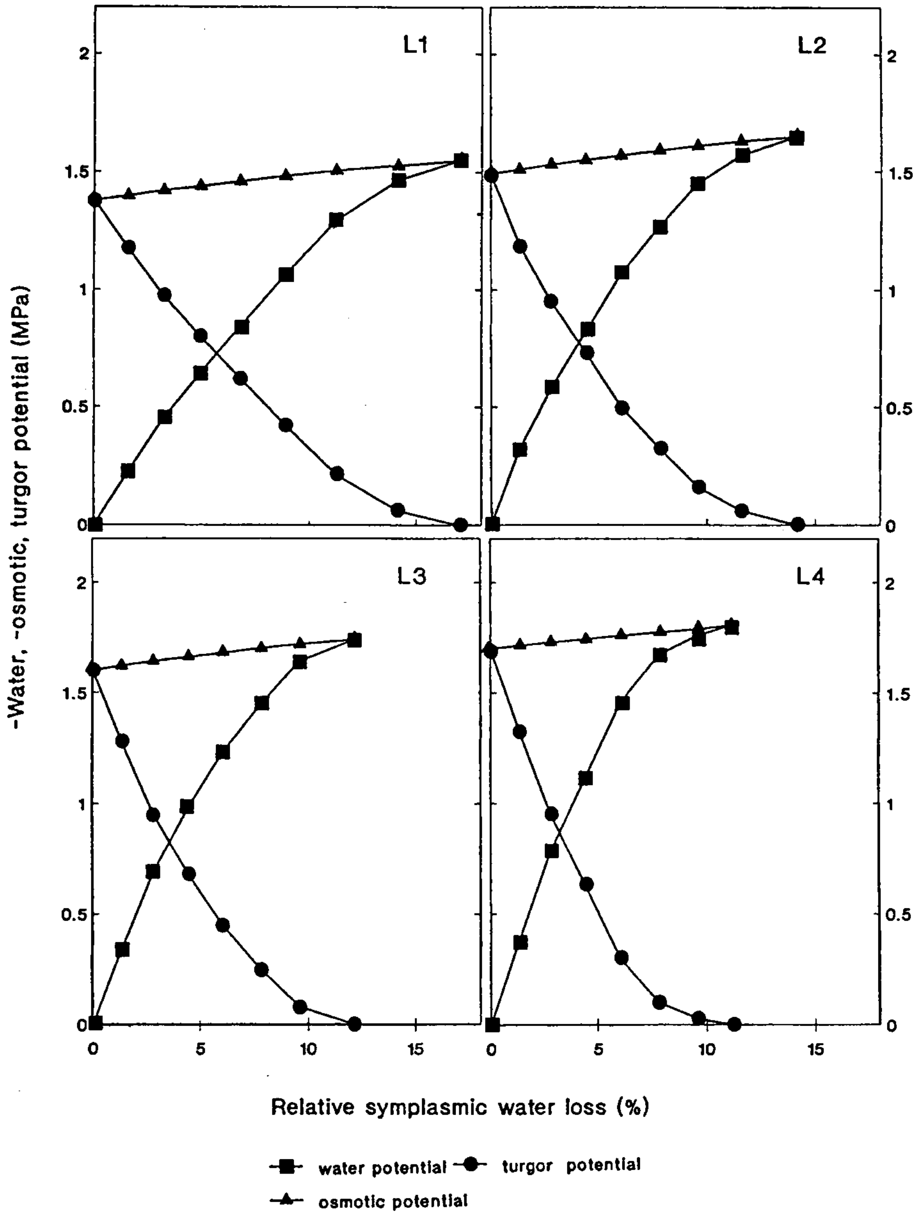

Fig 4. Water potential isotherms relating relative symplasmic water loss to water, osmotic and turgor potentials on leaves of different ages.

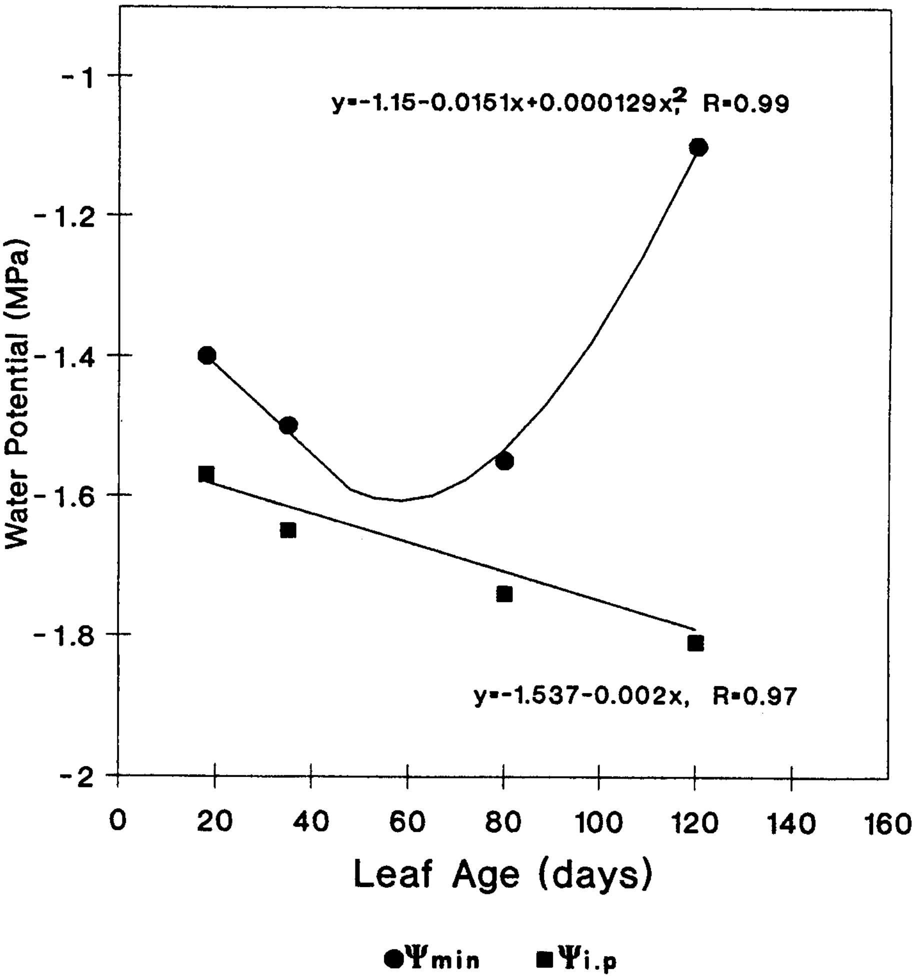

Fig 5. Changes in leaf water potential at the incipient plasmolysis (ψ_{ip}) and minimum diurnal leaf water potential as measured at 1500 h (ψ_{min}) in relation to leaf age.

that in older leaves turgor pressure is maintained at a higher level than in immature leaves. In fact, it can be seen (figs 4 and 5) that in the old leaves (L_4), turgor pressure was maintained at a higher level (0.7 MPa) than in the immature leaves (0.1 MPa).

The results obtained from the psychometric technique (fig 6) compared to those from the pressure/volume technique (fig 4) indicated a greater decrease in osmotic potential (π) for the same changes in ψ. Furthermore, the diurnal decrease of π in relation to water loss tended to be greater in immature and mature leaves than in the old leaves (fig 6). A more meaningful description of osmotic change requires a distinction between the changes due to removal of water from the tissue symplasm and those due to active accumulation of solutes. The theoretical equation relating osmotic potential (π) and water volume V_w is:

$$\pi = RTn_s/(V_w)$$

where (n_s) is the number of moles of solutes. If there is no osmotic adjustment n_s remains constant. Comparing the first and last point of π in figure 6 and the corresponding point of RWC the net increase of solutes in leaf cells can be calculated (Noitsakis and Tsiouvaras, 1990). From table II it can be seen that the calculated net increase of solutes in cells was significantly higher in the mature and immature leaves than in the old leaves.

DISCUSSION

Approximately 31–34% of the water in mature leaves is located outside the plasmalemma. The values reported for a number of species using the pressure/volume technique have all been in the range 5–45% (Vos and Oyartzun, 1988; Andersen et al, 1991). Values of the apoplasmic and of the symplasmic fraction seem to be highly dependent on leaf maturity. Mature and old leaves showed significantly lower values compared to immature leaves both in the symplasmic water fraction and in the fraction of water extracted to reach turgor loss point (table I), suggesting that water moved

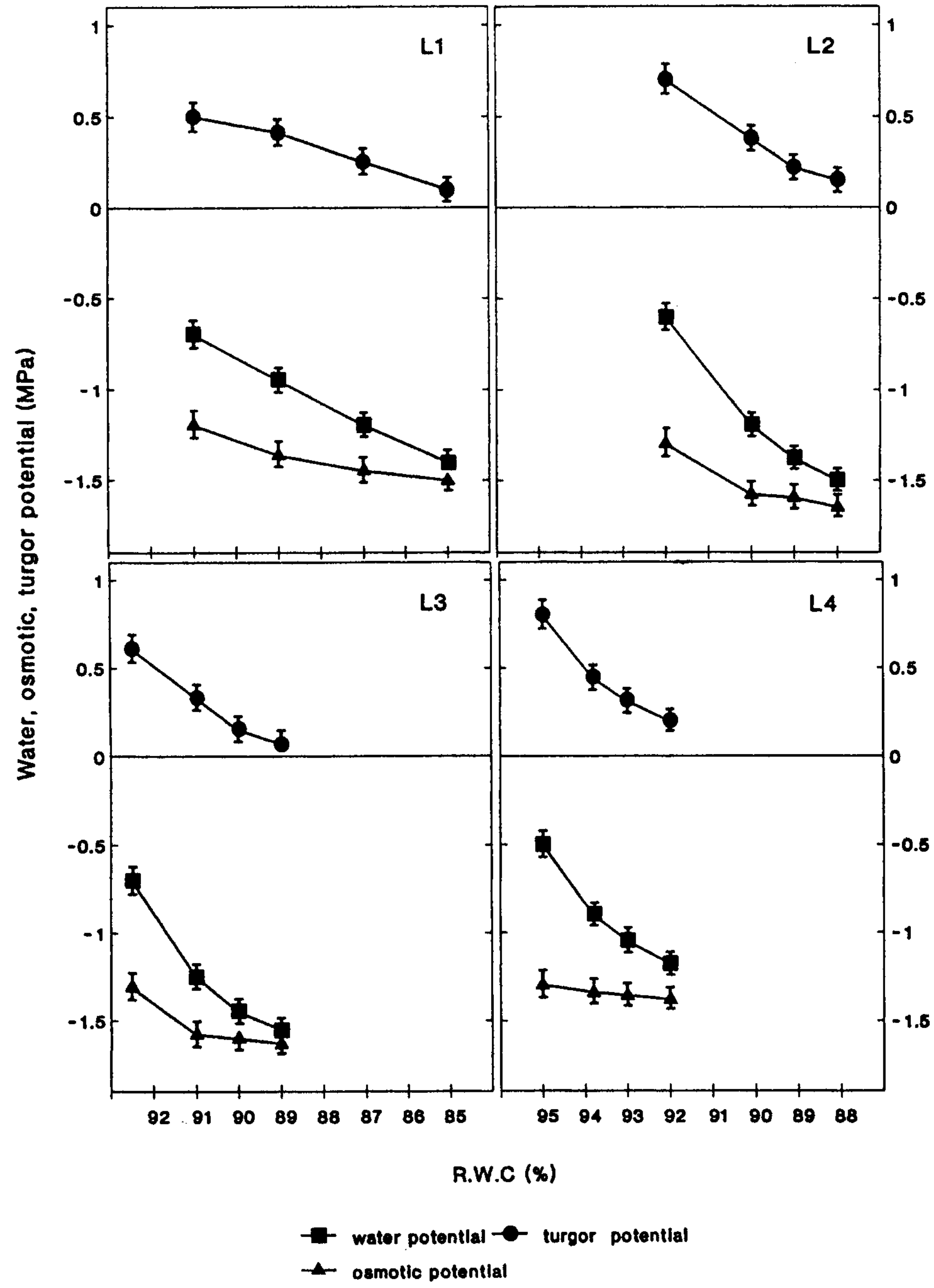

Fig 6. Diurnal changes in leaf water potential components in relation to relative water content (RWC) in leaves of different ages. Bars indicate the standard error of the mean of five replicates.

Table II. Estimation of net increase of solutes in cells of different leaf ages during the day.

Leaf age	*First point*		*Last point*		*Net solute increase (%)*
	π	*RWC*	π	*RWC*	
L1	-1.2	91	-1.5	85	14.4
L2	-1.3	92	-1.65	88	17.8
L3	-1.31	92.5	-1.63	89	16.5
L4	-1.3	95	-1.38	92	2.7 **

** Indicates significant differences in net solute increase between leaves age at 1% level.

out from the symplasm into the apoplasm, ie the cell wall, as leaves matured. Such a change in the allocation of water increases the concentration of solutes in the symplasm and therefore results in a decrease in π even without changes in the net solutes amount (Nunes et al, 1989). This suggestion explains the lower values in ψ and π at full turgor and along the whole range of tissue dehydration to turgor loss point obtained in mature and old leaves (fig 4). Similar results were reported by Lakso et al (1984) and Salleo and Lo Gullo (1990). Of greater interest are the measured diurnal changes in π which were quite large in mature and immature leaves (fig 6). This contrasts with the small changes predicted by any pressure/volume measurement, presumably due to the higher rates in tissue dehydration. Reduction in the degree of osmoregulation in relation to the increase in the rate of tissue dehydration has also been reported by Turner and Jones 1980. On the other hand the psychrometric technique is known to be subjected to errors owing to the dilution of the apoplasmic with the symplasmic fraction resulting in an overestimation of osmotic potential (Koide et al, 1991). This disadvantage can be overcome with the estimation of the net solute increase in cells. This can be calculated using the data provided by the psychrometric technique (Hsiao et al, 1985; Noitsakis and Tsiouvaras, 1990). Net solute increase refers to the active solute accumulation, which determines the degree of osmoregulation in leaf cells. From our results it can be seen that the diurnal reduction in osmotic potential was partly due to active accumulation of solutes (table II). Similar results concerning short term osmotic regulation have also been reported by Turner and Jones (1980). The active accumulation of solutes in old leaves was very low. These results indicated that grapevines generally are capable of osmoregulation but that this capacity is reduced in old leaves. Such regulation in immature leaves contributes to the maintenance of mid-day growth rates (Bressan et al, 1990). An identical process is apparently occurring in mature leaves.

Older leaves (L_4) showed higher RWC values compared to mature and immature leaves, which can be attributed to the significantly lower transpiration rates due to their lower stomatal conductance (fig 3a and b). Similar results were reported for various plant species (Dufrene and Saugier, 1993). The reduction in stomatal conductance with leaf age has generally been attributed to either an insufficient functioning of stomatal apparatus relating to age, and/or to a change in stomatal physiology (Sandanam et al, 1981). In our results leaf conductance and transpiration rate decreased rapidly from mature to old leaves. This rapid reduction might be attributed to the fact that the age-dependent reduction in stomatal conductance could be accelerated under water stress conditions (Thomas and Stoddart, 1980). In addition, stomatal closure started at higher values of ψ in old leaves restricting even more water losses. Therefore, it could be assumed that in old leaves, water saving owing to very low transpiration rates seems to contribute to the turgor maintenance under water stress conditions.

It will also be expected that the higher RWC values in mature leaves (L_2, L_3) compared to immature leaves (L_1) (fig 2) were due to their ability to better control their water balance by low stomatal conductance and consequently by low transpiration. However, mature leaves exhibited higher stomatal conductance and hence higher transpiration rates than immature leaves (fig 3a and b). Higher transpiration rates in mature leaves resulted in a lower leaf water potential in the early afternoon, and this response might be explained by the greater changes in $d\psi$ due probably to the greater change in dP and/or $d\pi$. In most leaves, about 80% of the water potential changes between full turgor and incipient plasmolysis have been attributed to a change in P (Cheung et al, 1975). Since the capacity for osmoregulation was almost the same in mature and immature leaves (table II), the greater changes in $d\psi$ in mature leaves could be attributed to the higher changes in dP in relation to water loss. Assuming that the elasticity of cell walls determines the rate of change in turgor pressure in relation to water loss, the higher values of ε in mature leaves are responsible for the greater changes in dP in relation to relative symplasmic water loss. Bulk modulus of elasticity (ε) and thus cell wall rigidity, increased almost 2-fold from an intermediate stage of leaf development (L_1) to leaf maturity (L_2, L_3). This considerable increase of ε in relation to leaf maturity could be interpreted as a physiological mechanism conferring on mature vine leaves the capability to change rapidly their water potential (high $d\psi$) in response to small leaf water losses (low dRWC). The high values of ε, observed in the mature leaves of vines, mean a more rigid cell wall and consequently easy loss of turgor under water stress conditions. At this stage of leaf ontogeny, when the leaf expansion has been completed, this physiological mechanism might be considered as more efficient to face the typical short-term water stress conditions occurring in the Mediterranean region through the large diurnal changes in air temperature and because of poor soil water availability. In addition, the drastic decrease in ψ in mature leaves could also help to maintain water uptake from drying soils without undergoing a large tissue water deficit. These characteristics of water relations of mature leaves should favour production under drought conditions (Abrams and Menges, 1992).

Conversely, immature vine leaves are characterized by more elastic cell walls. Lower ε in immature leaves resulted in the maintenance of a positive cell turgor at lower values of leaf water content than in mature leaves. The maintenance of turgor in still expanding leaves is very important for plant growth (Boyer, 1988; Matsuda and Rayan, 1990). It seems, that the higher cell wall elasticity of immature leaves is an effective strategy that enables the vines to maintain a positive pressure in cells, large enough to sustain enlargement and thus plant growth under mild water stress conditions.

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