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Adaptation of leaves of *Vitis vinifera* L. to seasonal drought as affected by leaf age

by

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S u m m a r y : The aim of this study was to evaluate the water, turgor, osmotic potentials and leaf water content during leaf ontogeny using both the pressure volume and the psychrometric techniques. The symplasmic water fraction of the total water content decreased with leaf age from 78 % in immature leaves to 62 % in old leaves. The capability for osmoregulation was almost the same in immature and mature leaves but decreased in old leaves. The volumetric modulus of elasticity (ε) was lower in immature leaves (12.7 MPa) and increased in mature (22.5 MPa) and old leaves (28 MPa). The high elasticity of young leaves seems to be an important strategy for adaptation to drought.

K e y w o r d s : leaf ontogeny, osmoregulation, tissue elasticity, water relations, water stress.

Introduction

The majority of vines grown in Mediterranean countries are not irrigated and thus they are usually exposed to conditions of water stress in summer. The most obvious effect even of mild water stress is reduced growth. Cell enlargement which is particularly sensitive to water deficits (HSIAO *et al.* 1985; SCHULZE 1986) depends on turgor pressure (P) and the expansion rate was shown to be proportional to the extent of P over a threshold value (JONES 1986). Turgor pressure can be maintained by two strategies: 1) lowering of the osmotic potential (Π) by osmotically active solutes or 2) increase of the elasticity of cell walls (for grapevines: DÜRING 1984; SCHULTZ and MATTHEWS 1993; DÜRING and DRY 1995). 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 with physiological leaf age (PATAKAS, unpubl.).

The objectives of this study were to use both the pressure volume and the psychrometric technique to evaluate adaptational processes of leaves during the season to drought stress.

Material and methods

Ten-year-old field-grown grapevines (*Vitis vinifera* L. cv. Roditis) grafted on 110 Richter (*V. rupestris* x *V. berlandieri*) rootstocks near the experimental station of the University of Thessaloniki were used. According to their age leaves were classified in 4 groups: 18-d-old still expanding young leaves (L_1); 35-d-old fully expanded, mature leaves (L_2); 80-d-old mature leaves (L_3); 120-d-old leaves without any signs of senescence (L_4). Irrigation had not been applied and no rainfall was recorded 5 weeks be-fore and during the experiment. The experiment was repeated 4 x in August 1994. No significant differences between measurements on single days were obtained.

Predawn leaf water potential of 3 mature leaves (L_2) was measured using the psychrometric technique (WILSON *et al.* 1979). Measurements were made at 10-d-intervals starting in May 1994.

Diurnal changes of leaf conductance to water vapour (C_w) were determined on 7 leaves per age group. C_w was measured approximately hourly from 06.00 to 20.00 h using a steady state porometer (Li-1600, Li-Cor, Nebraska, USA).

Leaf water potential (ψ) as well as the osmotic potential (Π) were measured hourly from 06.00 to 18.00 h on 5 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 the leaf water potential while the second was used for measurements of the osmotic potential (KOIDE *et al.*1991). Turgor potential was calculated as the difference between ψ and Π.

Concomitant measurements of the relative water content (RWC) were made on the third pair of discs obtained from the same leaf that was used for the determination of the water potential components. After determination of fresh weight (FW), leaf discs were placed in distilled water for 10 h and the turgid weight (TW) was measured. The discs were then dried at 80 °C for 24 h and their dry weight (DW) was recorded. According to KOIDE *et al.* (1991) RWC of leaves was calculated by the formula:

$$RWC = \frac{FW - DW}{TW - DW} \times 100 \qquad (1)$$

The net solute increase in leaves of different age groups was calculated relating Π to water volume (Vw) (HSIAO *et al.* 1985):

$$\Pi = RTn_s/(Vw) \qquad (2)$$

where n_s is the number of moles of solutes, R the gas constant and T the absolute temperature.

Changes in water potential components in relation to a relative lowering of symplasmic water (RLSW) were de-

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termined on 5 leaves per age group using the pressure chamber (SCHOLANDER *et al.* 1964; CHEUNG *et al.* 1975). RLSW was calculated as water loss expressed as percentage of the symplasmic water content at full turgor (W_o), i.e.

$$RLSW = \frac{W_o - W}{W_o} \times 100 \quad (3)$$

where W_o is calculated from the intercept of the straight part of the pressure-volume curve with the abscissa (TYREE and KARAMANOS 1980) and W is the actual weight of symplasmic water after pressurization.

The bulk modulus of elasticity (ε) was calculated according to TURNER (1981):

$$\varepsilon = \Delta P \Delta W_o / \Delta W \quad (4)$$

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

Changes in the symplasmic water fraction (SWF) as a percentage of the total water content were calculated according to PAVLIK (1984):

$$SWF = \frac{W_o}{TW - DW} \times 100 \quad (5)$$

Results

Predawn ψ values declined continuously from -0.25 MPa in May to -0.55 MPa in August. Diurnal changes of ψ *vs.* RWC for all leaf ages are shown in Fig. 1. Young leaves (L_1) presented lower RWC values at 0600 h than mature and old leaves (L_2, L_3, L_4). The decrease in RWC tended to be smaller in old leaves (L_4) compared to young leaves (L_1). Furthermore young leaves exhibited lower values of RWC at the same value of ψ. In all leaves the ψ value at 06.00 h (-0.6 MPa) was similar to that at 15.00 h; it decreased to about -1.4 MPa in both mature and young leaves and to -1.1 MPa in old leaves.

Old leaves (L_4) had a lower stomatal conductance than mature (L_2, L_3) and young leaves (L_1) regardless of the time of day (Fig. 2). Stomatal closure seemed to start at higher values of ψ (-0.85 MPa) in old leaves compared to mature leaves (-1.3 MPa).

The symplasmic water fraction (SWF) decreased with leaf age from ca. 78 % in young leaves (L_1) to ca. 62 % in old leaves (L_4) (Table).

Significant changes in ψ and its components, P and Π, were observed during leaf ontogeny (Fig. 3). In particular, Π at full turgor (Π_o) decreased with leaf age from -1.35 MPa in young leaves (L_1) to -1.5 MPa in L_2, -1.6 MPa in L_3 and to -1.7 MPa in L_4 leaves. Also the RLSW values at incipient plasmolysis (i.e. at P=0) decreased during leaf ontogeny from 17 % in young leaves to 11.2 % in old leaves indicating more rigid cell walls. Furthermore, changes of dP *vs.* dRLSW seem to be lower in young compared to mature and old leaves. Thus, for a 5 % water loss dP was 0.58 MPa in L_1 leaves compared to 0.86 MPa in L_2, 1.01 MPa in L_3 and 1.19 MPa in L_4 leaves.

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

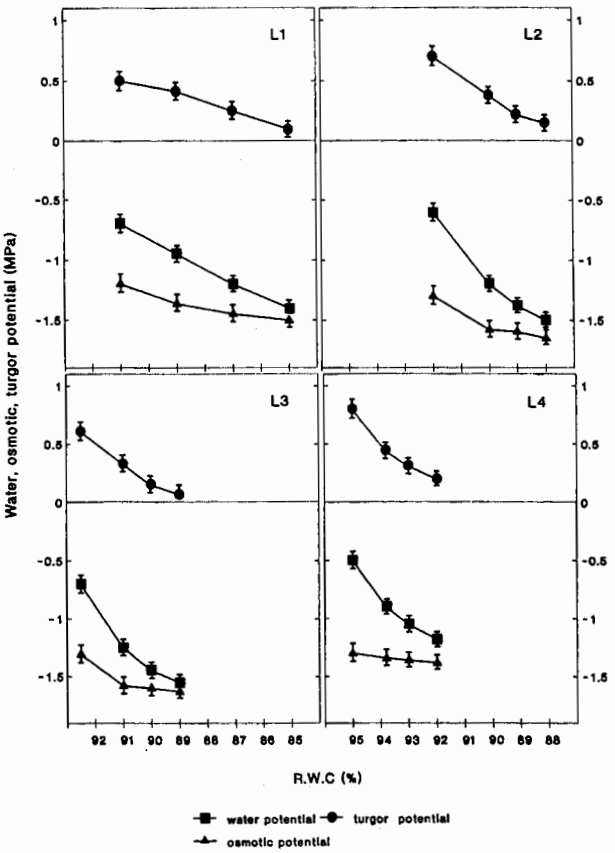

Fig.1: Leaf water potential components in relation to relative water content (RWC) of leaves differing in their age. Bars indicate the standard error of the mean of 5 replicates. L_1, L_2, L_3, L_4: see Table.

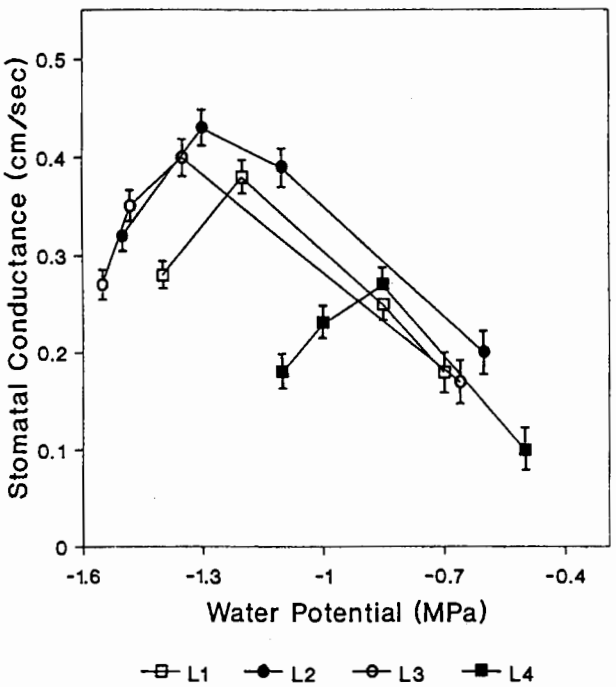

Fig. 2: Diurnal changes in stomatal conductance in relation to the water potential of leaves differing in their age. Bars indicate the standard error of the mean of 7 replicates. L_1, L_2, L_3, L_4: see Table.

Table

Values of symplasmic water fraction (SWF), relative lowering of symplasmic water (RLSW) and bulk modulus of elasticity ($\varepsilon_{max.}$) of leaves differing in their age. Different letters within columns indicate significant differences at the 5% level. L_1: young leaves; L_2, L_3: mature leaves; L_4: old leaves (see Material and methods)

Leaf age	SWF (%)	RLSW (%)	ε_{max} (MPa)
L_1	78 a	17 a	12.7 a
L_2	69 b	14.5 b	22.5 b
L_3	66 b	12 c	25 b
L_4	62 c	11.2 c	28 c
L.S.D (a=0.05)	3.21	1.43	2.72

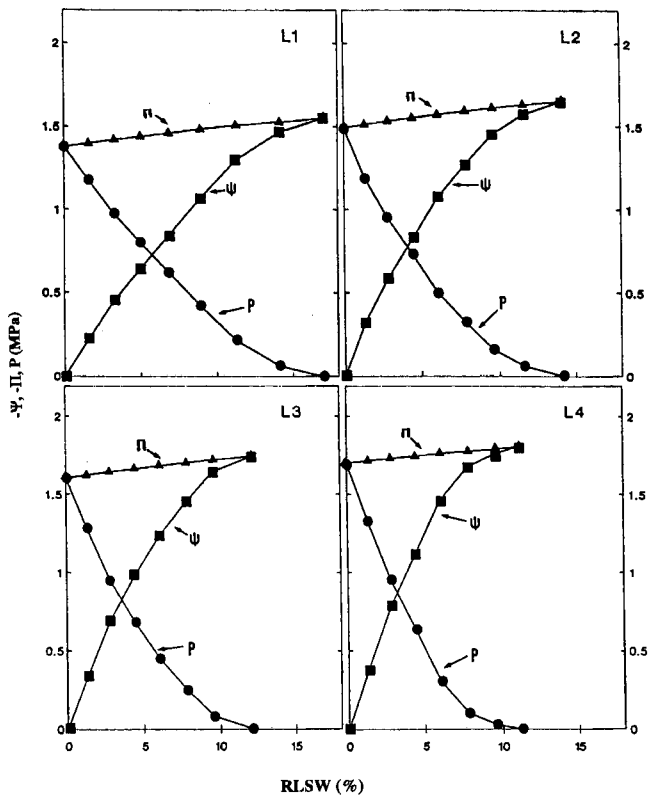

Fig.3: Changes in water potential (ψ), osmotic potential (Π), and turgor potential (P) in relation to relative lowering of symplasmic water (RLSW) of leaves differing in their age. L_1, L_2, L_3, L_4: see Table.

The diurnal decrease of Π as affected by water loss tended to be greater in young and mature leaves compared to old leaves (Fig. 1). A more detailed description of osmotic changes requires a distinction between changes due to the removal of water from the tissue symplasm („concentration effect") and those due to an accumulation of solutes. Comparing the net increase of solutes in leaves of different age, the ability of the leaves to osmoregulate can be determined (Hsiao *et al.* 1985; Noitsakis and Tsiouvaras 1990). The calculated net increase of solutes in cells was significantly higher in mature (16 %) and young leaves (14 %) than in old leaves (1.8 %).

Discussion

About 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 from 5 to 45 % (Vos and Oyartzun 1988; Andersen *et al.* 1991). Values of the apoplasmic and symplasmic fractions seem to be strongly related to leaf age. Fully expanded and old leaves showed significant lower values compared to young leaves both in the symplasmic water fraction and in the fraction of water lost to reach turgor zero (Table). This suggests that water moves out from the symplasm into apoplasm as leaves get older. This change in the allocation of water will increase the concentration of solutes in the symplasm and therefore will result in a decrease of Π even without changes of the amount of net solutes. This suggestion would explain the lower values in ψ and Π at full turgor and along the whole range of tissue dehydration to turgor zero of mature and old leaves (Fig. 3).

Of greater interest are the diurnal changes in Π which are quite large in mature and young leaves when measured by thermocouple psychrometry (Fig. 1). The diurnal reduction in osmotic potential was not only due to a passive "concentration effect" as cell water decreased but it was also due to an accumulation of solutes. The accumulation of solutes in old leaves was very low. These results indicate that the ability to osmoregulate is reduced with increasing leaf age (Smart and Coombe 1983, Düring 1984).

Compared to mature and young leaves older leaves (L_4) showed higher RWC values. This can be attributed to their significantly lower transpiration rates (Fig. 2). Similar results were reported for various plant species (Dufrene and Saugier 1993). In addition, stomatal closure started at higher values of ψ in old leaves thereby restricting water loss effectively, a fact that contributes to turgor maintenance under water stress conditions.

We expected that the higher RWC values in mature leaves (L_2, L_3) compared to young leaves (L_1) were due to reduced stomatal conductance (Fig. 1). However, mature leaves exhibited higher stomatal conductance than young leaves (Fig. 2). This response might be explained by the greater changes in ψ probably due to greater changes in P and/or Π. Since the ability to osmoregulato was almost the same in mature and young leaves, the greater changes in ψ in mature leaves could be attributed to the higher changes in P 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, higher values of ε in mature leaves are responsible for the higher changes in P in relation to relative symplasmic water loss.

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 could be interpreted as a physiological mechanism which enables the mature vine leaves to change rapidly their water potential (high dψ) in response to small leaf water losses (low dRWC). Furthermore, the drastic decrease in ψ in mature leaves could help to maintain water uptake from drying

soil without undergoing a large tissue water deficit (ABRAMS and MENGES 1992).

Conversely, young vine leaves are characterized by more elastic cell walls. Lower ε in young 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). The higher cell wall elasticity of young leaves 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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Erratum

In the paper

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the term "Relative lowering of symplasmic water (RLSW)" (see text, Table and Fig. 3) was misnamed and should be replaced by "Relative symplasmic water loss (RSWL)".