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Leaf gas exchange and water relations of grapevines grown in three different conditions

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Abstract

Diurnal and seasonal changes in the leaf water potential (Ψ), stomatal conductance (g_s), net CO_2 assimilation rate (P_N), transpiration rate (E), internal CO_2 concentration (C_i), and intrinsic water use efficiency (P_N/g_s) were studied in grapevines (*Vitis vinifera* L. cv. Touriga Nacional) growing in low, moderate, and severe summer stress at Vila Real (VR), Pinhão (PI), and Almendra (AL) experimental sites, respectively. In VR and PI site the limitation to photosynthesis was caused more by stomatal limitations, while in AL mesophyll limitations were also responsible for the summer decline in P_N.

Additional key words: drought; leaf gas-exchange; mesophyll limitations; net photosynthetic rate; stomatal conductance; transpiration rate; water potential; *Vitis vinifera* L.; water use efficiency.

Introduction

In Mediterranean field conditions, as in Port Wine Region, water deficits usually develop gradually during summer and are normally associated with high temperature and high irradiance stresses (Chaves *et al.* 2002). Large midday decreases in leaf water content can occur even if the soil is moist due to a high evaporative demand. In *Vitis vinifera*, as in many other species, photosynthetic activity decreases during typical summer days due to stomatal (stomatal closure) and non-stomatal (biochemical reactions) limitations (Chaves *et al.* 1987). The relative contribution of these limitations depends of the severity of stress conditions. Stomatal closure, in response to chemical signals synthesized in dehydrating roots (including abscisic acid), is generally accepted to be the main determinant for decreased photosynthesis under mild to moderate summer stress (Chaves 1991, Medrano *et al.* 2002). When the drought period is lengthened, dehydration is more severe, or other environmental stresses are superimposed, a decline in mesophyll photosynthesis may occur because of a down-regulation process (Chaves 1991, Flexas *et al.* 1998, Escalona *et al.* 1999). According to Flexas *et al.* (2002), stomatal conductance (g_s) could be a good indicator of water stress intensity in relation to photosynthesis, serving to unify data from different experiments and allowing the examination of a continuous gradient of water stress. Brodribb (1996) already proposed the use of g_s as an indicator to assess the inflexion point between stomatal and non-stomatal limitations to photosynthesis under drought.

The main objective of the present study was to characterise the short-term regulation of gas exchange in native grapevine cultivar Touriga Nacional growing under increasing summer stress conditions, specifically in three locations in Port Wine Region (Vila Real, VR, Pinhão, PI, and Almendra, AL, respectively).

Materials and methods

Plants: Grapevines (*Vitis vinifera* L.) of a common Portuguese cultivar, Touriga Nacional grafted on 1103P, were used. This cultivar is universally recognized as the finest grape for Porto wine, grows well in all three sub-regions, and flourishes even under very dry conditions. The experiments were undertaken in three commercial vineyards (1st trial – *Campus* of *UTAD* at *Vila Real*, 41°19'N, 7°44'W, *Baixo Corgo* sub-region; 2nd trial –

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Quinta do Seixo at *Pinhão*, 41°10'N, 7°33'W, *Cima Corgo* sub-region; 3rd trial – *Quinta da Leda* at *Almendra*, 41°00'N, 7°04'W, *Douro Superior* sub-region) in summer of 2002. The vines, 15 years old, were trained to a bilateral cordon system and the spurs were pruned to two nodes each with 14–16, 10–12, and 8–10 nodes per vine on 1st, 2nd, and 3rd trial, respectively. The soil is typical schistous. The plants were managed without irrigation. Diurnal field data were measured on sun exposed and fully expanded leaves at the middle of the shoots (usually between 8th and 11th nodes on the shoot axes). Two sampling periods were fixed: the first corresponding to veraison and the last corresponding to the ripeness period.

Environment: The reports of the three local climatic conditions show that total precipitation decreases from Vila Real (1 018 mm) to Pinhão (658 mm) and to Almendra site (437 mm). Rain falls during the winter months and very little between June and September (Fig. 1). The monthly mean air temperature is lower in Vila Real and differs little between Pinhão and Almendra, although the average summer temperature tends to be higher in Almendra.

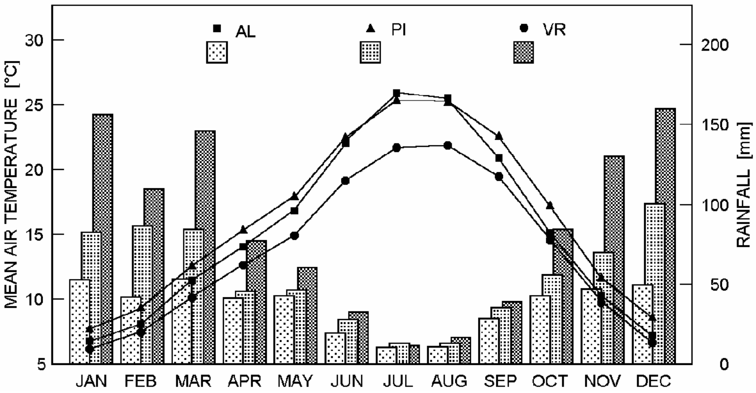

Fig. 1. Mean air temperature (*lines*) and monthly precipitation (*columns*) for the period 1931–1960 in the three experimental sites (*Almendra*, AL, *Pinhão*, PI, and *Vila Real*, VR) (database of *Instituto de Meteorologia* in "*O Clima de Portugal*", vol. XV, 1965).

Leaf water relations: Leaf water potential (Ψ) was determined with a pressure chamber (*ELE International*, Bedfordshire, England), according to the method of Scholander *et al.* (1965). Measurements were performed on fully expand leaves at predawn (Ψ_{PD}) (1 h before sunrise) and at midday (Ψ_{MD}) (between 14:00 and 15:00 h, just after gas-exchange measurements). Care was taken to minimise water loss during transfer of the leaf to the chamber by enclosing it in a plastic bag immediately after excision.

Leaf gas-exchange rates were measured at natural incident photosynthetic photon flux density (PPFD) in the field using a portable gas exchange system (*LCA-3, Analytical Development Co.*, Hoddesdon, England), operating in the open mode, and a leaf chamber clip (*PLC*, surface: 6.25 cm^2, volume: 16 cm^3) with a quantum sensor, and temperature and humidity sensors. Measurements were performed on fully expanded leaves at morning (between 09:00 and 10:30 h), at midday (between 14:00 and 15:30 h), and in the afternoon (between 16:30 and 18:00 h). PPFD incident on the leaves was always greater than 1 000 $\mu mol\ m^{-2}\ s^{-1}$, which is above photosynthesis saturation in these plants (Flexas *et al.* 2002). Net CO_2 assimilation rate (P_N), stomatal conductance (g_s), transpiration rate (E), and internal CO_2 concentration (C_i) were estimated from gas exchange measurements using the equations developed by Caemmerer and Farquhar (1981). Intrinsic water use efficiency was calculated as P_N/g_s. Mesophyll conductance to CO_2 (g_m) was calculated as the ratio of $P_N/(C_i - C_c)$ assuming that CO_2 partial pressure at the site of carboxylation (C_c) is zero (Candolfi-Vanconcelos and Koblet 1991).

Air and leaf temperature: Diurnal courses of air and leaf temperature were followed using a dry bulb thermometer and an infrared thermometer (*Infratrace KM800S*, England) with a 15° field view, respectively. The mean temperature of leaves was obtained by holding the thermometer at about 1 m above the foliar surface and the emission of the canopy was assumed to be 0.97 (Heilman *et al.* 1994).

Statistical analyses: Values were compared by a one-way ANOVA test and mean differences were determined using Duncan's multiple range test.

Results and discussion

The seasonal trends of predawn (Ψ_{PD}) and midday (Ψ_{MD}) leaf water potential are illustrated in Fig. 2. Ψ_{PD} is presumed to correspond to the equilibrium between soil and plant water potentials (Van Zyl 1987) and is often reported to control the daily maximum g_s (Correia *et al.* 1995). According to that, at veraison the VR and PI plants were grown under better soil water availability (Ψ_{PD} = –0.2 and –0.3 MPa, respectively, $p>0.05$) than AL plants (–0.6 MPa). However, at ripeness stage, due to a higher depletion of soil water, Ψ_{PD} of PI plants was lower and similar to Ψ_{PD} of AL plants (–0.7 and –0.8 MPa, respectively, $p>0.05$) relatively to Ψ_{PD} (–0.3 MPa) measured in VR plants. Differences in Ψ_{MD} between VR, PI, and AL plants were always significant ($p<0.05$), decreasing mean values from VR to PI and AL plants and mean differences between the three sites from veraison to ripeness stage, mainly due to a higher declining in VR plants (–70.6 % *versus* –17.8 and –5.0 % in PI and AL plants, respectively). This higher decrease of Ψ_{PD} may be explained by higher E and probably by a higher leaf area per vine. This hypothesis is supported by pruning differences in the vines present in the three experimental sites (see Materials and methods). Particularly at the ripeness stage, also the diurnal pattern of Ψ of VR plants showed a higher decrease (346 %) from predawn to midday, whereas in PI and AL plants this daytime decrease was more gradual (127 and 111 %, respectively). The capacity for lowering the osmotic potential might be a strategy for better restricting the leaf water losses in grapevines growing under water stress (Patakas and Noitsakis 1999).

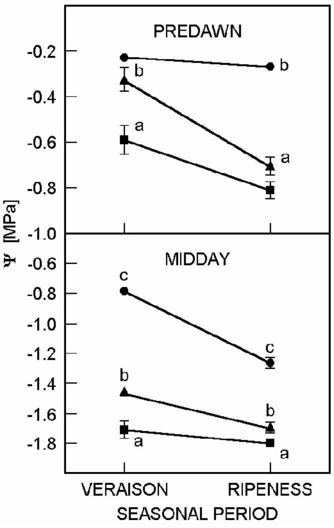

Fig. 2. Seasonal pattern of predawn and midday leaf water potential (Ψ) of grapevines in the three experimental sites (AL, *squares*; PI, *triangles*; VR, *circles*). *Vertical bars* (not shown if smaller than symbols) indicate the S.E. of the mean of 12 leaves. Within each seasonal and diurnal period, symbols followed by the same letter are not significantly different ($p<0.05$) according to Duncan's test.

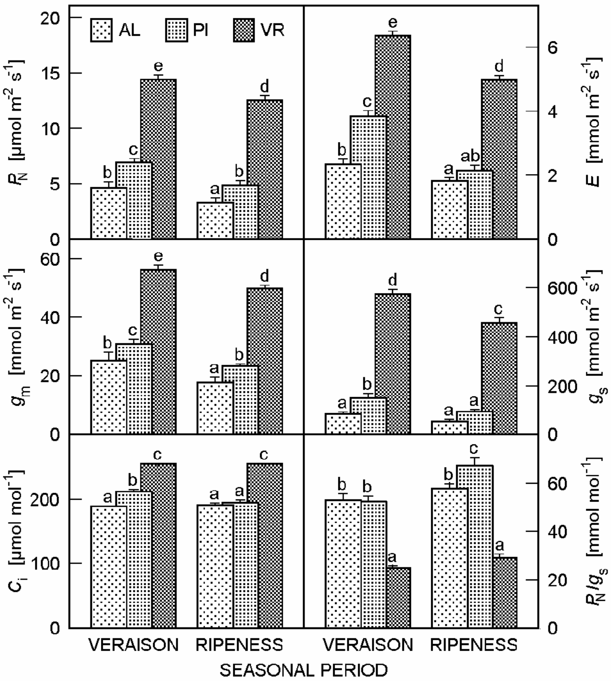

Fig. 3. Seasonal changes of mean daily net CO_2 assimilation rate (P_N), mesophyll conductance to CO_2 (g_m), internal CO_2 concentration (C_i), transpiration rate (E), stomatal conductance (g_s), and intrinsic water use efficiency (P_N/g_s) of grapevines in the three experimental sites (*Almendra*, AL, *Pinhão*, PI, and *Vila Real*, VR). *Columns* are means and *vertical bars* represent the S.E. measurements of 48 different leaves. At each graph, columns followed by the same letter do not differ significantly ($p<0.05$) according to Duncan's test.

P_N, g_s, g_m, and E followed seasonal patterns similar to Ψ (Fig. 3). There was a marked reduction in these parameters from the lower to the higher dry site (VR > PI > AL). The relationship between g_s and P_N for VR, PI, and AL plants assessing the inflexion point between stomatal and non-stomatal limitations of photosynthesis under drought (Brodribb 1996, Flexas *et al.* 2002) is shown in Fig. 4. All the data best fitted a hyperbolic function (r^2 = 0.94) and show that the linear relationship between the two parameters was different on the three experimental sites. Of these functions, the VR values predominate on the horizontal part of the hyperbola, while AL values were restricted to the vertical zone and PI data were scattered on this zone and on the inflexion zone [at g_s around 200 mmol(H_2O) m^{-2} s^{-1}]. From VR to PI and from PI to AL the slope of first-order linear regression between g_s and P_N increased (0.011 > 0.026 > 0.045), which means that, for a given variation of g_s, the AL plants exhibited greater variations of P_N than plants of the two other sites. In relation to P_N/g_s this behaviour implied an opposite trend, *i.e.* the lowest values were observed in VR plants. From veraison to ripeness stage, those values increased at all sites, but more expressively in PI plants (27 % against 7 and 14 % in AL and VR plants, respectively). The lower increase of P_N/g_s in AL plants, associated to the slight increase of C_i (+2.4 % against –2.0 and –8.3 % in VR and PI, respectively) evidences that, with the progress of the summer season, the non-stomatal limitations also contributed to the highest reduction of P_N (Schultz *et al.* 1996, Medrano *et al.* 2002). In contrast, in PI plants under moderate drought, non-stomatal limitations had a lower impact on the reduction of photosynthesis because the greatest increase of P_N/g_s was associated with a decrease of C_i (Chaves 1991, Flexas and Medrano 2002). The C_i values should be treated with some caution, due to the non-uniform aperture of stomata over the surface of a vine leaf (patchiness) when environmental stresses (water stress, salinity, low humidity) are present (Düring 1992). In these conditions,

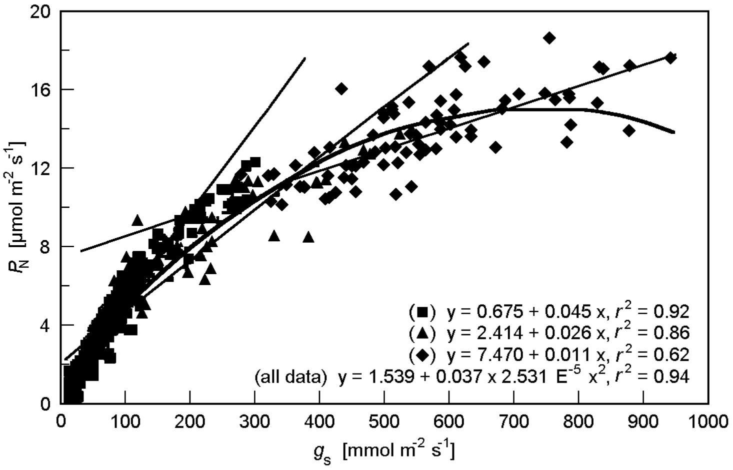

Fig. 4. Relationship between net CO_2 assimilation rate (P_N) and stomatal conductance (g_s) in AL (*squares*), PI (*triangles*), and VR grapevines (*lozenges*) over the two days of the present study.

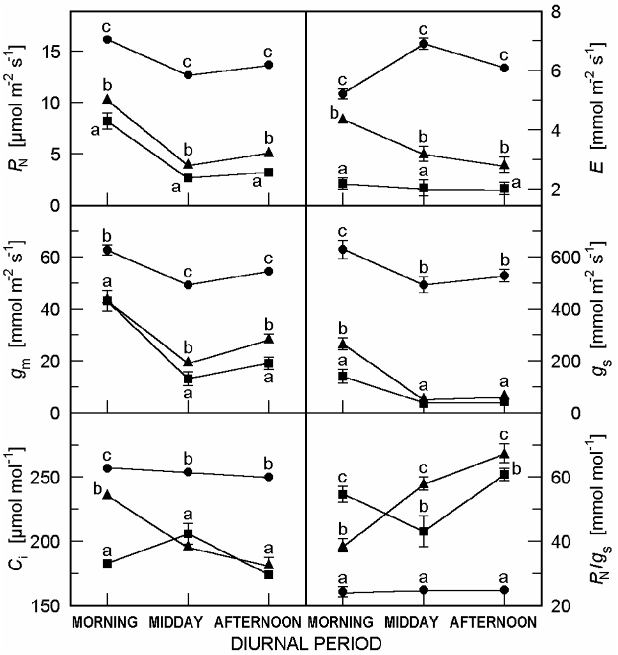

Fig. 5. Diurnal changes in net CO_2 assimilation rate (P_N), mesophyll conductance to CO_2 (g_m), internal CO_2 concentration (C_i), transpiration rate (E), stomatal conductance (g_s), and intrinsic water use efficiency (P_N/g_s), measured at grape veraison period, for main leaves of AL (*squares*), PI (*triangles*), and VR (*circles*) grapevines. Within each diurnal period mean ± S.E. (n = 16) values followed by the same letter are not significantly different (p<0.05) according to Duncan's test.

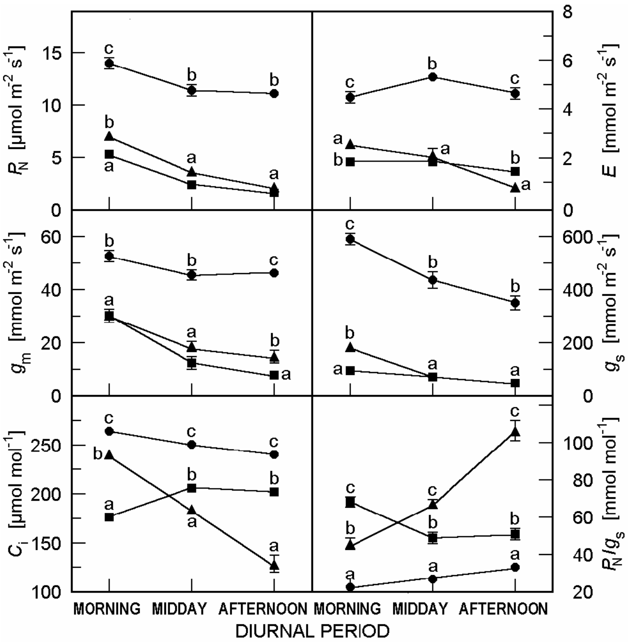

Fig. 6. Diurnal changes in net CO_2 assimilation rate (P_N), mesophyll conductance to CO_2 (g_m), internal CO_2 concentration (C_i), transpiration rate (E), stomatal conductance (g_s), and intrinsic water use efficiency (P_N/g_s), measured on fruit ripeness period, for main leaves of AL (*squares*), PI (*triangles*), and VR (*circles*) grapevines. Within each diurnal period mean ± S.E. (n = 16) values followed by the same letter are not significantly different (p<0.05) according to Duncan's test.

according to Downton *et al.* (1988), C_i calculated from gas-exchange measurements can be overestimated and lead to erroneous conclusions of non-stomatal limitation of photosynthesis. However, Escalona *et al.* (1999) reported that in grapevines undergoing drought in field the effect of patchy stomatal closure on gas-exchange is experiments less expressive than in the potted experiments. The diurnal course of P_N (Figs. 5 and 6) in the veraison and ripening phases showed the similar trend in all sites. However, at veraison, there were significant differences between VR, PI, and AL plants, but at ripeness these differences were restricted to the morning period. At midday and afternoon, P_N of PI and AL plants was not significantly different and, such as in VR plants, exhibited a continuous gradual decline. Correia *et al.* (1990) suggested a direct effect of high PPFD to explain the midday and afternoon depression of leaf P_N, even under non-restricting water availability in the soil.

In relation to g_s, either at veraison or at ripeness, the three experimental fields were distinct only in the morning period, VR and AL plants exhibiting higher and lower values, respectively (Figs. 5 and 6). In the two other diurnal periods, PI and AL plants had similar and lower g_s values [< 82 mmol(H_2O) m^{-2} s^{-1}], relatively to VR plants [g_s > 345 mmol(H_2O) m^{-2} s^{-1}]. However, while at veraison the midday and afternoon C_i values did also not differ significantly between these two sites, at ripeness the C_i of AL plants was clearly higher than that of the PI plants. This increase, associated with a slight decline of g_m and P_N, can be explained by lower activity of photosynthetic machinery, *i.e.* by the predominance of non-stomatal limitations to photosynthesis (Downton *et al.* 1987, Iacono and Sommer 1996, Medrano *et al.* 2002). These results in field-grown grapevines reveal a pattern of gradual response of photosynthesis to water stress. According to Medrano *et al.* (2002), after an early effect of drought resulting in partial stomatal closure, mainly expressed by PI results, a metabolic adjustment takes place at AL plants through metabolic pathways.

Reduced g_s of the PI plants and mainly of AL plants was linked to smaller E (Figs. 3, 4, and 5), relatively to the VR plants. It evidenced still that at the midday period, E of VR plants increased significantly, relatively to the morning period, while in PI and AL grapevines, mainly at veraison, the diurnal course was clearly decreasing or constant, respectively. The larger water supply of VR plants and the increase in the atmospheric vapour pressure deficit from morning to midday justify this luxurious consumption of water. Particularly at midday period, differences between leaf and air temperature ($T_l - T_a$) showed an inverse relationship with E, that is, the leaves of AL plants were hotter than the leaves of other plant sites (Fig. 7). At this period, the leaves of PI plants had an intermediate behaviour and were more similar to the AL plants. In contrast, in the morning measurements, the $T_l - T_a$ values of PI plants were similar or more reduced than those of the VR plants, as observed at the veraison or ripening periods, respectively. In this second seasonal period these results were related with the nebulosity in the summer morning of the PI region.

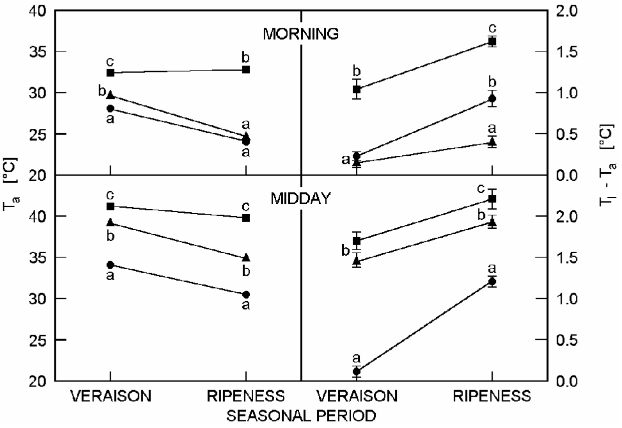

Fig. 7. Seasonal pattern of morning and midday air temperatures (T_a) and differences between leaf and air temperature ($T_l - T_a$) in the three experimental sites (AL, *squares*; PI, *triangles*; VR, *circles*). Within each seasonal and diurnal period mean ± S.E. ($n = 16$) values followed by the same letter are not significantly different ($p<0.05$) according to Duncan's test.

Concerning the diurnal variation of P_N/g_s, there was no variation in VR plants while PI grapevines exhibited a significant increase throughout the day (about 130 %), suggesting a higher drought avoiding strategy (for review see Chaves *et al.* 2003), relatively to AL plants. In these plants, either at veraison or at ripeness, P_N/g_s decreased from morning to midday, recovering or not during afternoon, respectively. This behaviour, mainly at ripening phase when environmental stress was more severe, could suggest that the lower P_N was related with stomatal closure and also with changes in photosynthetic apparatus.

In conclusion, the results indicate a significant influence of the local site on the stomatal and non-stomatal limitations of photosynthesis. The stomatal effects were most important in the drought response of grapevines from VR to PI to AL site, whereas the non-stomatal effects became more expressive in the AL site, where the environmental stress was more severe. Future work should evaluate the photoinhibition of photosynthesis (see also Bertamini and Nedunchezhian 2002) and photorespiration for assessing the impact of environmental conditions of the three experimental sites on vine performance.

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