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Diurnal and seasonal changes in *Prunus amygdalus* gas exchanges

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Abstract

Diurnal courses in net photosynthetic rate (P_N), stomatal conductance (g_s), leaf water potential (ψ), internal CO_2 concentration (c_i), and water use efficiency (WUE) were studied as season progressed, in relation to environmental factors in field grown *Prunus amygdalus*. In sun leaves P_N reached maximum between 09:00 to 11:00 h and subsequently declined when high temperature and low humidity occurred. An increase was observed late in the afternoon. A decrease in g_s and ψ was found as season progressed in both years of measurements. In periods of high evaporative demand, ψ was very low, however, it did not explain the reductions of P_N in all the three periods (spring, early and late summer). Midday depression of P_N and g_s seemed to be related with leaf temperature (T_l) and high irradiance. Increase in c_i and F_0 and decrease in F_v/F_m found between 12:00 and 14:00 h corresponded to the decrease in P_N. Therefore, a transient modification of photosynthetic machinery might be considered. WUE was negatively correlated with vapour pressure difference of leaf to air, that decreased during the day. The September values, higher than in the previous months, were due to the lower seasonal decreases in P_N than in g_s.

Additional key words: diurnal course; drought; internal CO_2 partial pressure; irradiance; net photosynthetic rate; seasonal course; temperature; water relations; water stress; water use efficiency.

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Abbreviations: c_i - internal CO_2 concentration; Chl - chlorophyll; E - transpiration rate; g_s - stomatal conductance for water vapour diffusion; PAR - photosynthetically active radiation; P_N - net photosynthetic rate; T_l - leaf tempearture; VPDLA - vapour pressure difference leaf to air; WUE - water use efficiency; ψ - leaf water potential.

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Introduction

Drought stress has profound effects on *P. amygdalus* productivity and growth. The first symptoms of water stress are expressed at the level of stomata which reduce their aperture to prevent desiccation (Tenhunen *et al.* 1987). As a result, P_N is unavoidably reduced due to the decrease of CO_2 availability at chloroplast level (Wong *et al.* 1985). These are the slighter effects of drought. However, nonstomatal effects may occur as has been reported for olive trees by Angelopoulos *et al.* (1996) and for grapevine by Quick *et al.* (1992). Nonstomatal effects at mild stress (lesser than 30 % leaf water deficit) have been ascribed to photoinhibitory effects due to high air temperature coupled with high irradiance (Björkman and Powles 1984). Decreases in CO_2 concentration at chloroplast level may cause the reduction of photochemical yield of open PS2 centres, due to an increase of thermal deactivation of excitons, an increase of photorespiration relative to CO_2 assimilation, and a decline of two important enzymes, nitrate reductase and sucrose phosphate synthase (Cornic 1994). According to Baker (1993), increases of products of photosynthetic electron transport system (ATP and reductants) if not utilized by other metabolic processes, *e.g.*, photorespiration and Mehler reaction, may induce an excess of excitation energy, which if not dissipated as heat, can damage antenna pigments.

The contribution of nonstomatal components to the decline of P_N can be measured by calculating c_i at chloroplast level (Caemmerer and Farquhar 1981, Jones 1985). This method has been questioned (Cornic 1994) due to the occurrence of patches of stomatal closure in the leaf. However, according to their results in field-grown plants and to those of Gunasekera and Berkowitz (1992), Ort *et al.* (1994) state that patches probably do not occur frequently in the field. The measurement of chlorophyll (Chl) fluorescence yield is an alternative method.

The aim of this work was to find the effect of environmental factors on gas exchanges and water content of almond trees under natural conditions. We tried to explain the decrease in P_N occurring in midday and afternoon, by means of c_i and Chl fluorescence analysis. To facilitate the presentation of results, only means of measurements done on seven cultivars are shown. The cultivars were previously compared in Matos *et al.* (1997).

Materials and methods

The measurements were made in spring and summer, on three-year-old trees of *P. amygdalus* Batsch. Plants have been planted in shallow schistic soil, cambric-eutric leptosol (marginal for annual crops) on the northeast of Portugal around 41°10'N latitude and 7°12'W longitude. The Mediterranean climate is characterized by low rainfall during the year, and summers with high temperature and low humidity. Cultivars Ferragnes, Ferrastar, Marcona, Garrigues, and Non Pareil grafted on two rootstocks (Garrigues, almond, and GF677, peach-almond hybrid), and cultivars Ferraduel and Casa Nova grafted on GF677 were compared. Trees were planted in a randomized complete block design with 6×5 m spacing, in plots of four trees with three replications for each rootstock-scion combination. Guard rows were used to preclude edge effects. The trees were irrigated when planted and once at the beginning of summer; annual fertilization was applied according to soil analysis. Afterwards, the trees grew under natural conditions.

P_N, transpiration rate (E), g_s, leaf (T_l) and air (T_a) temperatures, relative humidity (RH), photosynthetically active radiation (PAR), and external CO_2 concentrations were determined under natural conditions using a portable CO_2/H_2O gas exchange system (*LI 6200*; *LI-COR*, Lincoln, USA). P_N, g_s, and c_i were calculated according to the equations of Caemmerer and Farquhar (1981). Plant water status was followed by monitoring ψ with a pressure chamber, on two detached leaves per tree, according to Scholander *et al.* (1965). Measurements from predawn to sunset were always made on adjacent leaves before the gas exchange determinations. The measurements were made on individual attached sun exposed and fully expanded leaves from the middle of the canopy, in a 1000 cm^3 well stirred cuvette to minimize boundary layer resistance.

Chl fluorescence parameters, basal fluorescence (F_0), and variable to maximum fluorescence ratio (F_v/F_m) were measured by a portable Plant Stress Meter *Mark II (BioMonitor S.C.I. AB,* Sweden). Before measurements, leaves (near to those for gas exchange measurements) were dark adapted for 30 min.

Diurnal courses of all parameters were followed in the middle of May, end of June (1994), beginning of July (1995), and first days of September. Each month was represented by measurements on two successive days.

Results were statistically analyzed by means of the *Statistix* 4.1, 1985, 94 *Analytical Software*, and *Systat for Windows* version 5.

Results and discussion

In May 1994 the mornings were partially clouded, whereas in July and September the days were clear dry and hot (Fig. 1*E,F,G*). In 1995 the days of measurements were milder and maximum air temperatures did not attain so high values as in the previous year (Fig. 2*E,F,G*).

Predawn ψ in 1994 (Fig. 1*C*) reduced three fold from May to September, while in 1995 (Fig. 2*C*) due to unusual precipitation, ψ decreased only by 16 % from May to July and maintained this value until September. Diurnal changes were higher as the season progressed and less evident in 1995 (Figs. 1 and 2).

P_N changed along the growing season and had different daily patterns (Figs. 1*A*, 2*A*). In spring, early in the morning, low temperatures sometimes limited P_N; later, under mild air temperature and humidity, P_N increased. However, in May 1994 when the days of measurement were clouded in the morning, P_N was lower than in the afternoon when high irradiances appeared. The highest values of P_N were found in the morning (09:00-11:00 h) in all days of measurement, with the exception of clouded days of May 1994. Midday decreases in P_N, evident in June 1994, were accompanied with low g_s. Similar behaviour has been reported for several plants growing in Mediterranean (Schulze *et al.* 1974, Pereira *et al.* 1986, Tenhunen *et al.*

1987) and Indian (Singh *et al.* 1996) climates. In *Olea europaea* L. (Angelopoulos *et al.* 1996) P_N reached maximum early in the morning and subsequently gradually declined, both in watered or drought-stressed plants. The low P_N that occurred at midday and afternoon in June and September 1994 could be ascribed to low g_s under the decreasing CO_2 concentration at chloroplast level that modulates chloroplast activity to maintain stable c_i (Schulze 1986, Kaiser 1987). However, we observed an increase in c_i that was maintained during the afternoon. This behaviour can be ascribed to effects on photosynthetic machinery. Similar results were found in *Arbutus unedo* by Beyschlag *et al.* (1987) in June. According to Pearcy *et al.* (1977), the inactivation of photosynthesis may be due to damages at chloroplast level induced by high temperature. The effect of high temperature on the reduction of mesophyll photosynthetic capacity and increase of respiration have also been suggested by Beyschlag *et al.* (1987). Also Lauer and Boyer (1992) related increases

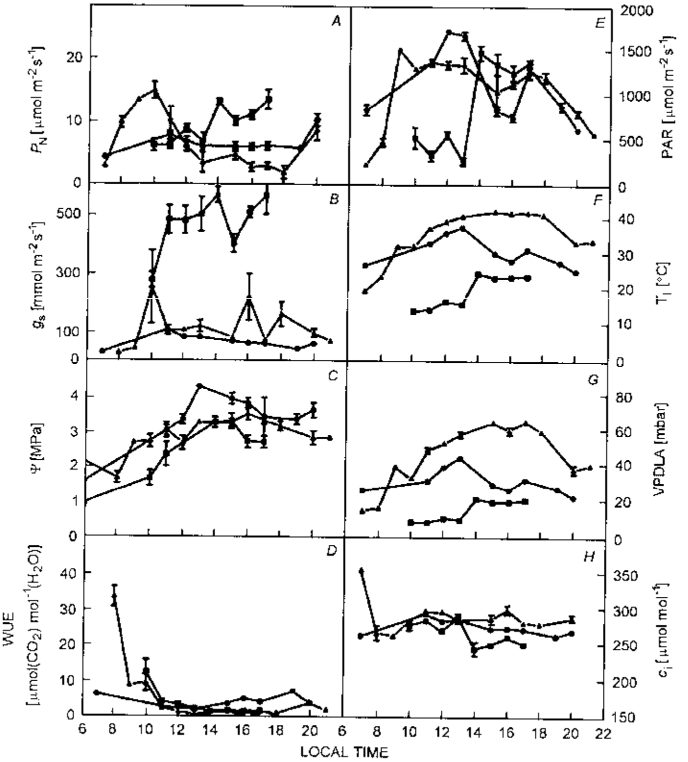

Fig. 1. Diurnal changes in net photosynthetic rate, P_N (*A*), stomatal conductance, g_s (*B*), leaf water potential, ψ (*C*), water use efficiency, WUE (*D*), photosynthetically active radiation, PAR (*E*), leaf temperature, T_l (*F*), vapour pressure difference between leaf and the air, VPDLA (*G*), and leaf internal CO_2 partial pressure, c_i (*H*) of field grown *Prunus amygdalus* leaves (means of 7 cultivars) measured in spring (■), early summer (▲), and late summer (●) in 1994. Vertical bars indicate standard error of means.

in c_i with the water deficit-induced decreases in P_N of sunflower, bush bean, and soybean.

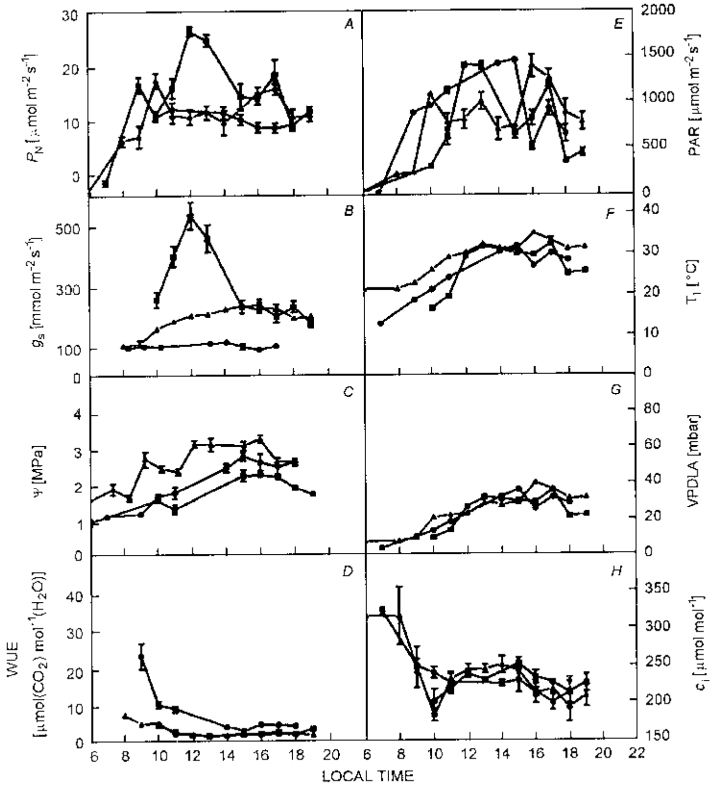

Fig. 2. Diurnal changes in the same parameters as in Fig. 1, but measured in 1995. Vertical bars indicate standard errors of means.

To confirm the assumed reduction of mesophyll photosynthetic capacity, Chl fluorescence was measured in the second year of experiments. Diurnal reductions of F_v/F_m accompanied by increases in F_0 that occurred in the afternoon, very evident in September 1995 (Fig. 3), may be due to interactive effects of high irradiance and temperature. Decreases in photochemical efficiency F_v/F_m and increases in F_0 may reflect photoinhibitory or thermal injuries to PS2 (cf. Xu and Wu 1996). However, when these conditions became mild, a gradual recovery was observed. According to Burke (1990), the inactivation of CO_2 fixation at elevated temperature is caused by the inhibition of ribulose-1,5-bisphosphate carboxylase activity. The different effect of temperature on CO_2 assimilation and photorespiration may be caused by the different solubility of CO_2 and O_2, besides the changes on kinetic parameters of the reactions catalyzed by ribulose-1,5-bisphosphate carboxylase (Burke 1990, Hall and Keys 1983).

In September 1994 P_N was gradually reducing from 11:00 to 19:00 h, and g_s followed a similar pattern, with values at 11:00 h significantly higher than those measured afterwards. Significant reductions in P_N between June and September 1994 were ascribed to the lower g_s at this period of the year, dryness of the soil, and leaf ageing.

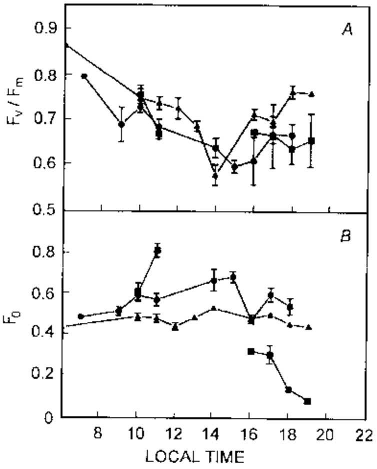

Fig. 3. Diurnal changes in photochemical efficiency of photosystem 2, F_v/F_m (*A*) and basal fluorescence, F_0 (*B*) of field grown *Prunus amygdalus* leaves (means of 7 cultivars) measured in spring (■), early summer (▲), and late summer (●) in 1995. Vertical bars indicate standard errors of means.

Another hypothesis is that photosynthetic machinery, namely PS2, can be reversibly affected by the lower c_i at chloroplast level when under mild water stress, high irradiance, and high temperature. According to Baker (1993), at irradiances higher than correspondent to optimum quantum efficiency of photosynthesis, leaves use some mechanism to down-regulate photosynthesis, and hence avoid overexcitation of PS2 reaction centres. When under water stress, as it was the case in midday and afternoon in summer, at very low values of ψ and low g_s, an increase of electron flux to O_2 can occur to dissipate the effect of excitation energy. However, when water stress is high, PS2 reaction centres may be damaged. This was probably not the case in the present study as shown by the tendency to recover F_0 and F_v/F_m late in the afternoon.

Lower values of g_s than those of May and July were also evident in September 1995. These may not be ascribed to ψ that was significantly higher than in July, but may be due to climatic factors and leaf ageing.

In July 1995, the low values of P_N between 11:00 and 14:00 h were due to a cloud. The dependence of P_N on irradiance followed the model $P_N = 1.879 + 0.011$ PAR, $r^2 = 0.802$. At this period, leaf temperature did not overcome 34.57 ± 0.50 °C, what was not the case in June 1994 where maximum leaf temperature attained 41.01 ± 0.42 °C. In this case the main factor responsible for changes in P_N was not irradiance, but leaf temperature, according to $P_N = 52.144 - 1.190\ T_f$, $r^2 = 0.927$, adjusted to the points between 09:00 and 18:00 h when irradiance was $\geq 1000\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$.

WUE was negatively correlated with VPDLA, decreasing during the day in May and June of 1994 and 1995. In September 1995, WUE was significantly higher than in the other months due to a significant decrease in g_s ($p = 0.01$). The relationship between P_N and VPDLA was linear according to the equation $P_N = 18.17 - 0.23$

VPDLA, $r^2 = 0.33$, $p = 0.00$, $n = 191$. In global analysis of the results, the dependence of P_N on T_a could be established according to the equation $P_N = -4.550 + 1.144\ T_a - 0.024\ T_a^2$, $r^2 = 0.472$, $n = 463$.

Leaf water potential did not explain the reductions on P_N in all the three experimental periods.

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