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Changes in photosynthetic performance of *Ceratonia siliqua* in summer

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Abstract

In carob tree (*Ceratonia siliqua*) radiant energy saturated net photosynthetic rate (P_N) during summer was about 10 % of the spring values. This was accompanied by a reduction in stomatal conductance (g_s), which only partially explains the strong reduction in P_N. Photosynthetic capacity (P_{max}) and quantum yield (Φ), both measured under saturating CO_2, had the maximum in spring (about 34 μmol m^{-2} s^{-1} and 0.08 mol mol^{-1}, respectively) and both decreased in late summer to about 55 % of their spring values. Despite strong decreases in Φ, photoinhibition of photosystem 2 (PS2) was negligible or easily reversible in carob leaves subjected to summer drought, since F_v/F_m, measured in the morning, did not show appreciable changes. The recovery of affected parameters was very rapid after the first rains in late October. The chlorophyll (Chl) *a*/*b* ratio in the end of the summer was 2.6, a value significantly lower than 3.6 obtained in the spring, suggesting that Chl *a* was preferentially reduced.

Additional key words: carob tree; chlorophyll; drought; gas exchange; mesophyll impairments; net photosynthetic rate; photosystems; quantum yield; soil water; stomatal conductance.

Introduction

Carob tree (*Ceratonia siliqua* L.) is well adapted to the Mediterranean climates, which are characterised by hot and dry summers. Nunes and Linskens (1980) showed that at high temperatures stomata stay open provided that leaf water content is also high. Results obtained with plants in pots (Nunes and Correia 1980, Nunes and Matos 1987) indicate that the species is particularly tolerant to drought. The minimum P_N (less than 10 % of the maximum) of mature trees in the field occurs at the end of summer when nearly four months of drought cause negligible g_s. In the present work, photosynthetic performance during the hot and dry periods was further studied.

Materials and methods

Plants: The measurements were performed in trees growing in the neighbourhood of Lisbon in a calcareous soil and climate similar to the conditions in the south of the country where large areas are cultivated with carob. The dry period coincides with summer beginning in May and often lasts until October, with a mean maximum temperature of 26.4 °C. Mature leaves from the last growing period, placed in the periphery of the canopy, were measured between 10:00 and 13:00, at least 3 d per month.

Gas exchange and fluorescence parameters: P_N, g_s, and apparent quantum yield (Φ_a) were measured in the field, under full sunlight, using a portable photosynthetic system *Li-6200* (*Li-Cor*, Lincoln, USA).

Measurements of oxygen evolution expressing photosynthetic capacity (P_{max}) and quantum yield (Φ) were performed in leaf discs (1.5 cm^2) under saturating CO_2 (*ca.* 7 %), saturating PPFD of 1800 μmol m^{-2} s^{-1} provided by a halogen lamp, and temperature of 25 °C,

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Abbreviations: Chl - chlorophyll; F_v and F_m - variable and maximal fluorescence; P_{max} - photosynthetic capacity; P_N - net photosynthetic rate; PPFD - photosynthetic photon flux density; PS - photosystem; SW - soil available water content; Φ - quantum yield of O_2 evolution, obtained at saturating CO_2 conditions; Φ_a - apparent quantum yield, obtained at ambient CO_2; Ψ_{md} - midday leaf water potential.

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using an oxygen electrode *LD2/2* (*Hansatech*, King's Lynn, UK).

Chl fluorescence parameters were measured in the early morning, in dark-adapted mature leaves, using a *PAM 2000* system (*H. Walz*, Effeltrich, Germany). To obtain F_m, a saturating flash of 7000 μmol m^{-2} s^{-1} of actinic radiation was applied to the leaf.

Chl content was estimated spectrophotometrically from leaf extracts in 80 % acetone using the formulae of Arnon (1949).

Leaf water potential (Ψ_{md}) was determined with the Schölander's pressure chamber (*Manofrigido*, Lisboa, Portugal).

The fraction of available soil water (SW) present in the soil where the trees were grown was calculated for the top layer of 50 cm depth, using the curve determined in the laboratory, which relates the water content in a soil sample with the suction pressure applied to the sample. The total soil available water range corresponds to the difference in moisture retained after the application of 30 and 1585 kPa as extraction pressures to a saturated soil sample.

Potential evapotranspiration was obtained by equation of Penman (1948) using temperature, wind, air relative humidity, and radiation from Lisbon records (20 km from the site). The soil-water balance was calculated by the method of Thornthwaite and Mather (1957). Precipitation values were obtained from local measurements.

Results

In the middle of May the SW was still above 50 % (Fig. 1*A*) and declined sharply, being totally depleted from the beginning of July onwards. The apparent efficiency of carbon assimilation (Φ_a), which was measured at ambient CO_2 and low irradiance, declined more slowly and reached about 55 % when SW disappeared. At this time of the year, P_N was reduced by *ca.* 40 % (Fig. 1*B*). After that, P_N and Φ_a decreased gradually, reaching minimum values in October, which represent in both cases about 8 % of their spring values (Fig. 1*A*,*B*). The decreases of P_N and Φ_a paralleled the decrease of g_s to about 10 % of the maximum annual value, indicating the interference of stomatal closure in those parameters (Fig. 1*B*).

The results of Φ and P_{max} that were obtained under saturating CO_2 concentrations in air (Fig. 1*C*) showed that the performance of the mesophyll processes was also affected by drought. In October, before the first rains, Φ and P_{max} were reduced to 58 and 50 %, respectively, of their highest values observed in spring.

When the Ψ_{md} decreased to -2.5 MPa, the values for P_N decreased to about half (Fig. 2) and at the peak of dehydration in the end of September (Ψ_{md} *ca.* -2.9 MPa), P_N represented only about 8 % of the highest values, observed for well-watered plants. At that time, P_N values represented about 10 % of P_{max}, whereas when plenty of water was available in the soil (Ψ_{md} *ca.* -1.2 MPa) P_N represented 65 % of P_{max}.

Photochemical efficiency of photosystem 2 (PS2), given by F_v/F_m, did not decrease during summer (Fig. 1*D*), which indicates that PS2 machinery was not inhibited at the time of measurement, *i.e.*, in the early morning.

The relative concentrations of Chl *a* and *b* (Chl *a*/*b* ratio) changed along the year showing maximal values of *ca.* 3.6 around April and May and minimum of *ca.* 2.6 from July to October (Fig. 1*D*).

Discussion

Soil-plant water relationships during summer: Ψ_{md} decreased progressively during the summer reaching values around -2.9 MPa in the end of September, which is in agreement with the observations in nature (Correia and Martins-Loução 1995). These authors observed that Ψ_{md} values obtained in the end of July were better correlated with atmospheric parameters than with the irrigation treatments, and suggested that the trees explored soil water resources below the irrigation layer. The present work also suggests that the trees got advantage from exploring water resources below the 50 cm profile, since the estimated water available in this layer was almost zero since the end of July, *i.e.*, at least 10 weeks before the minimum Ψ_{md} was reached. This may also explain, why, at the limited capacity of a pot, Ψ_{md} in 3-years-old carob plants was as low as -4.0 and -3.6 MPa (Nunes and Correia 1980, Nunes *et al.* 1989).

Values of Ψ_{md} around -3 MPa are low for most of the plants, either herbaceous or trees. However, they must be considered moderate if other plants of the Mediterranean *maquis* are exclusively compared. Shrubs such as *Cistus monspeliensis* and *Cistus albidus* can reach Ψ_{md} less than -6 MPa (Werner *et al.* 1999) and the tree *Pistacia lentiscus* can show in nature Ψ_{md} values lower than -4 MPa (Correia *et al.* 1994).

Nunes and Correia (1980) measured relative water contents together with water potentials. They demonstrate that the minimum Ψ_{md} (-4 MPa) is achieved when the tissue still presents 60 % of relative water content, a situation also found in the drought-resistant tree *Acacia aneura* of the arid zones (Slatyer 1960). The present work shows that reduction in g_s can be detected early in May (Fig. 1*B*) and this is expected to reduce water consumption through transpiration and contribute to the retention in the leaf. This may be a key fact to avoid drastic effects of drought on growth and photosynthesis.

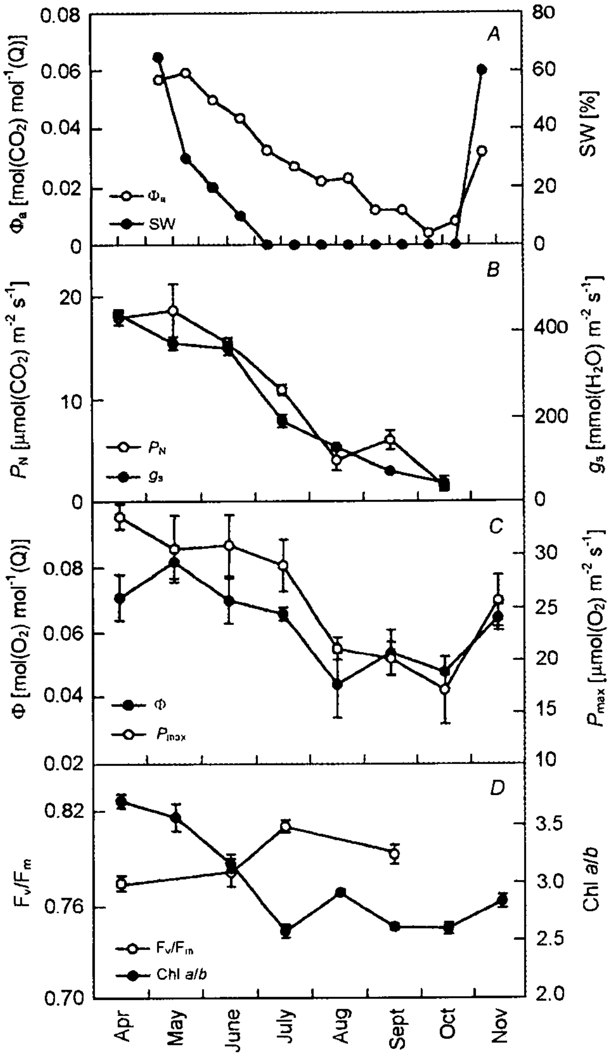

Fig. 1. *A*: Fraction of soil available water (SW) retained in the 50 cm layer of soil and apparent quantum efficiency (Φ_a) in the first pair of mature leaves, measured along the experimental period. Each Φ_a value represents the slope of a linear regression (*n* = 6-15 points) from an irradiance-response curve, obtained under low irradiance (between 40 and 140 µmol m^{-2} s^{-1}). *B*: Net photosynthetic rate (P_N) and stomatal conductance (g_s) measured along the experimental period in natural conditions. Means ± SE (*n* = 8-10). *C*: Photosynthetic capacity (P_{max}) and quantum yield for oxygen evolution (Φ) measured along the experimental period. Means ± SE (*n* = 4-6). *D*: Photochemical efficiency of photosystem 2 (PS2) measured in dark-adapted leaf discs (F_v/F_m) and chlorophyll (Chl) *a*/*b* ratio along the experimental period. Means ± SE (*n* = 4-6).

Lo Gullo *et al.* (1986), working with mature leaves of trees in Sicily during June, observed only slight decreases of g_s at midday; they argue that stomata are not important in the regulation of water. However, water shortage was surely very moderate in these experiments because the minimum leaf water potential was above -1.5 MPa, values that are very high when compared with our results (Fig. 2).

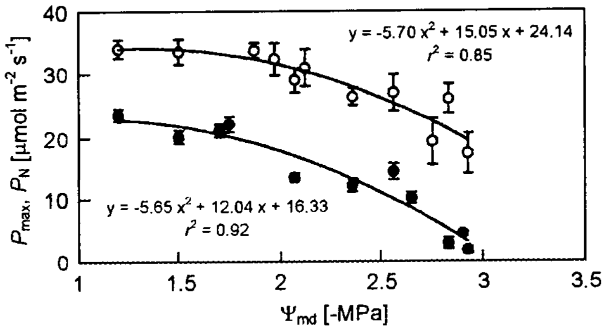

Fig. 2. Relations of photosynthetic capacity (P_{max}, *open circles*) and net photosynthetic rates (P_N, *closed circles*) with leaf water potential at midday (Ψ_{md}), calculated from values obtained in the same leaves. Means ± SE (*n* = 4-8).

In carob trees undergoing slow soil dehydration along the summer, the leaf zero turgor point seems not to be a sharp critical point. Instead, broad changes in the relative osmotic water content correspond to a sustained turgor around 0.2 MPa (Nunes *et al.* 1989). This can be interpreted as an acclimation strategy and was probably obtained without requirement for synthesis of new osmotic solutes, since the authors explained the increase in osmotic potential by the redistribution of water within the cell.

Water deficit and mesophyll processes: Both P_{max} and Φ, which are measured in air with saturating CO$_2$ to overcome the stomatal resistance, did not show significant changes until the complete depletion of SW in July. Therefore, g_s seems to be the main reason for decrease in carbon assimilation until the disappearance of water from the horizon with 50 cm deep (Fig. 1*A*,*B*). After that, significant decreases were observed in P_{max} and Φ, which have to be attributed to mesophyll impairments induced by drought. In August P_{max} and Φ were reduced to about 62 and 54 % of the spring values, while in October they represented 50 and 58 %, respectively. We observed that, at the same time of the year, P_N was reduced much more drastically than P_{max}: to 21 % in August and to 8 % in October. This indicates that stomata, despite the high mesophyll limitation imposed on photosynthesis after July, represent the major component in the reduction of net carbon assimilation. In fact, in well hydrated leaves P_N represents about 65 % of P_{max}, whereas in leaves dehydrated to a water potential of -2.9 MPa it represents only *ca.* 10 % (Fig. 2), *i.e.*, the leaf is working very far from its potential, due to stomatal limitation.

Photosynthetic membranes may recover during the night pause, what could be partially responsible for the stable and high values of F_v/F_m. However, the maintenance of these values suggests that PS2 was not an important target under drought. That invokes processes related with electron transport and/or carbon metabolism to justify the impact observed on P_{max}. Tezara *et al.* (1999) concluded that the inhibition of photosynthesis of sunflower induced by drought was due to the decrease in coupling factor and ATP. According to Gimenez *et al.* (1992), the rapid decrease in ribulose-1,5-bisphosphate content during a mild stress may explain the decrease in photosynthetic activity, also in sunflower. Here we show that, concomitantly with the absence of important damage in PS2, a decrease of Chl *a*/*b* ratio occurred, what can be interpreted as a preferential loss of Chl *a* associated with PS1. This is consistent with results reported for plants under high temperature (Williams *et al.* 1986) and high irradiance (Nunes *et al.* 1993), and might reflect decreases in PS1 complex and in cytochrome b_6f complex (Schmidt and Schäfer 1994, Ramalho *et al.* 1999).

The critical Ψ_{md} related to the beginning of a clear impact of P_{max} in *C. siliqua* may be around -2.5 MPa, which may correspond to turgor pressures of 0.2 MPa, according to previous results (Nunes *et al.* 1989). Therefore, the mesophyll processes of photosynthesis (P_{max} and Φ) were impaired at levels of drought stress close to tissue wilting point.

Integrating the present results with previous data about carob tree we conclude that, in nature, carob leaves do not reach very pronounced dehydration stages, displaying an avoidance strategy in face of water shortage. This includes: (*1*) the ability to explore water in deep soil, (*2*) the existence of a strong stomatal control, and (*3*) the possibility of osmotic adjustment and maintaining turgor above zero in a broad range of low relative osmotic water content. In addition, direct observation of trees in nature reveals the presence of nastic movements of leaflets, which contribute to reduce radiant energy absorption by the leaf. Also, shedding of leaves or even branches may occur at extreme summer drought associated to high temperature, which favours the maintenance of water in the remaining parts of the plant.

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