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Stomatal and mesophyll limitations to photosynthesis in one evergreen and one deciduous Mediterranean oak species

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Abstract

In the evergreen *Quercus rotundifolia* and the co-existing deciduous *Q. faginea* we studied the diurnal variations in photosynthetic capacity (P_{max}), measured as the rate of O_2 evolution at photon and CO_2 saturation, and in the rate of net CO_2 assimilation (P_N) in the field during the period of maximum photosynthetic activity. Our aim was to check the contribution of stomatal and non-stomatal limitations to the diurnal variation in photosynthesis, and to study the differences between both species. *Q. faginea* leaves displayed lower mass per unit area and higher nitrogen content than *Q. rotundifolia* leaves. The maximum stomatal conductance and P_N in the field were higher in *Q. faginea* than in *Q rotundifolia.* Also P_{max} of *Q. faginea* was higher than that of *Q. rotundifolia.* Both species attained in the field a high percentage of the P_{max} (around 82 % for *Q. faginea* and 73 % for *Q. rotundifolia*). This indicates reduced stomatal limitation of photosynthesis under favourable conditions, especially in *Q. faginea.* P_N underwent a sharp decrease towards mid-day in association with increase in the atmospheric vapour pressure deficit and decrease in the leaf water potential. P_{max} was also reduced during mid-day. This demonstrated the contribution of mesophyll limitations to the P_N in the two species under stress. The mesophyll limitation of photosynthesis seemed to be similar for both species, independently from the differences in leaf traits between them.

Additional key words: field gas exchange; leaf mass per unit area; leaf nitrogen content; leaf water potential; photosynthetic capacity; *Quercus faginea*; *Quercus rotundifolia*; vapour pressure deficit.

Introduction

In areas with a cold Mediterranean climate both low winter temperatures and summer drought limit the duration of the growth season to a short period lasting from the end of spring to the beginning of summer (Mitrakos 1980). To compensate for the short duration of the photosynthetically active period, it is crucial for tree species to achieve high maximum photosynthetic rates during the favourable period. Nevertheless, a decrease in photosynthetic activity around mid-day during the spring has been reported for Mediterranean species, even under well-watered conditions (Lange *et al.* 1982, Tenhunen *et al.* 1984, Faria *et al.* 1996). Since the CO_2 assimilation rate (P_N) and stomatal conductance (g_s) usually decrease in parallel, the decrease in the intracellular concentration of CO_2 in the leaves as a consequence of the reduction in g_s is usually considered the main factor responsible for the inhibition of photosynthesis (Jones 1992, Tourneaux and Peltier 1995, Sánchez-Rodríguez *et al.* 1999). However, there may be important non-stomatal limitations to photosynthesis and mesophyll limitations may be of greater importance than stomatal limitations with regard to the reduction in P_N in certain conditions (Ni and Pallardy 1992, Kubiske and Abrams 1993).

Measurements of O_2 evolution rate in saturating CO_2 conditions with the oxygen electrode (P_{max}) and its comparison with P_N obtained in field conditions are adequate to investigate limitations to photosynthesis. In principle, under saturating CO_2 conditions the limitations to photosynthesis by stomatal restriction of the availability of CO_2 are removed (Torneaux and Peltier 1995). Accordingly,

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Abbreviations: g_s = stomatal conductance; LMA = leaf mass per unit leaf area; P_{max} = photosynthetic capacity measured at photon and CO_2 saturation; P_N = maximum net CO_2 assimilation rate in the field; P_N/g_s = intrinsic water-use efficiency; PPFD = photosynthetically active photon flux density; T_{air} = ambient air temperature; VPD = atmospheric vapour pressure deficit; Ψ_{leaf} = leaf water potential; Ψ_{pd} = predawn leaf water potential.

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any decrease in $P_{\max}$ during the day could be interpreted as a result of the mesophyll inhibition of photosynthesis.

Very little work has been carried out in the Mediterranean environment addressing photosynthetic rates measured more or less simultaneously in the same photosynthetic material under field conditions and in a CO_2-saturated atmosphere at the laboratory (Di Marco *et al.* 1990, Quick *et al.* 1992, Faria *et al.* 1996). Furthermore, although there are numerous studies comparing gas-exchange rates in different Mediterranean *Quercus* species (Lange *et al.* 1982, Tretiach 1993, Damesin *et al.* 1998), we have not found other comparative studies of the two species studied here: the evergreen *Quercus rotundifolia* Lam. and the deciduous *Q. faginea* Lam. In a previous study (Mediavilla *et al.* 2001), we observed that leaf N content, specific leaf area, and maximum CO_2 assimilation rates were higher in *Q. faginea* than in *Q. rotundifolia.* Our purpose in the present study was to analyse the effects of stomatal and mesophyll limitations on the differences in photosynthetic rates of both species and on the mid-day inhibition of photosynthesis. Although both species coexist in large areas in the interior Iberian Peninsula, *Q. rotundifolia* tends to occupy more xeric sites than *Q. faginea* (Tutin *et al.* 1964). We anticipated that the evergreen species should maintain a higher stomatal control of transpiration during periods of high atmospheric evaporative demand (Nardini *et al.* 1999, Kolb and Stone 2000), and that, as a consequence, *Q. rotundifolia* should experience a greater stomatal limitation and a smaller mesophyll limitation than *Q. faginea* during periods of stress.

Materials and methods

Study species and area: Gas-exchange measurements were made on leaves of the evergreen *Quercus rotundifolia* Lam. [= *Quercus ilex* L. ssp. *ballota* (Desf.) Samp.] and the deciduous species *Q. faginea* Lam. Both species were studied in a site close to the City of Salamanca in central-western Spain. *Q. rotundifolia* is an evergreen species with mean leaf longevity of around 735 d. *Q. faginea* is a deciduous species, with a mean leaf life span of around 208 d.

The site consisted of sparse populations (about 50×10^{-4} trees per m^2) of isolated mature trees over 100 years old with open pasture areas among them. These savannah-like formations ("dehesas") are very frequent in the western part of the Iberian Peninsula. Trunk diameter at 1.3 m height ranged from 20 to 60 cm and mean heights were about 6-10 m. Height above sea level in the study area is 790 m. The climate is cold Mediterranean, with a mean annual temperature of between 11-12 °C. The mean maximum monthly temperature ranges between 13 °C in the coldest month (December-January) and 35 °C in the hottest month (July-August). Mean minimum monthly temperature ranges between –5 °C (December-January) and 9 °C (July-August). Annual precipitation ranges between 500-600 mm, although there is always a summer drought period (data supplied by the National Institute of Meteorology, Valladolid Centre).

Photosynthetic capacity measurements: A leaf disc oxygen electrode (*Hansatech*, Kings Lynn, UK) was used for measurements of gas exchange rates (O_2 evolution) in controlled, elevated CO_2 concentration (of *ca.* 15 % CO_2; cf. Quick *et al.* 1992). Air entering the chamber was previously humidified to saturation to avoid leaf desiccation during measurements. The temperature of the water-jacketed leaf chamber was maintained at 25 °C by circulating water from a water bath. Leaf discs were irradiated at gradually increasing PPFD until a maximum rate of O_2 evolution was achieved (at about 800 $\mu mol\ m^{-2}\ s^{-1}$).

The measurements were made on a total of 20 dates through the months of May and June of 1996, before the onset of summer drought. Four trees of each species were randomly selected at each measurement date. Totally expanded, current-year leaves were taken from sunlit branches at a mid-height in the canopy from shortly after sunrise to about 09:00 (solar time) thus avoiding the time of the day with heat stress. Once collected, the leaves were immediately taken to the laboratory to make measurements of O_2 evolution. Owing to calibration difficulties with the O_2 electrode, the values corresponding to leaves that could be damaged by an excessive delay in the start of the measurements were discarded. From all the measurements made, only approximately 40 measurements of O_2 evolution rate for each species were used for estimating mean photosynthetic capacity. Additional measurements were also taken through the day on some sampling dates in order to observe diurnal changes in the photosynthetic capacity, resulting from water, heat, or radiation stress. A total of 12 diurnal curves were elaborated for each species. A single tree of each species was used for elaborating the 12 diurnal curves.

Field gas exchange and water potential measurements: Gas-exchange measurements were made in the field on sun-exposed, current-year leaves on the same dates and from the same trees and the same positions in the crowns as those collected for the determination of photosynthetic capacity at the laboratory. Measurements were also taken from shortly after sunrise to 09:00 (solar time), to obtain an estimate of the maximum photosynthetic rate under field conditions. In order to compare the diurnal patterns of field gas exchange and photosynthetic capacity, measurements were also made during the day on the same dates used to elaborate diurnal curves of O_2 evolution in the laboratory. Accordingly, also a total of approximately 40 leaves were included in the final estimates of maximum field assimilation rate and 12 diurnal curves were elaborated for each species.

The measurements were made with a portable photosynthesis system (*LI-6200, Li-Cor*, Lincoln, NE, USA) at ambient CO_2 concentration (around 360 µmol mol^{-1}) and saturating photosynthetic photon flux density (PPFD *ca.* 1 100 µmol m^{-2} s^{-1}). Besides the necessary information for calculating stomatal conductance for water vapour (g_s), the measuring instrument provided air and leaf temperature and atmospheric vapour pressure deficit (VPD).

After the gas exchange measurements both in the field and at the laboratory, leaf area (using a *Delta-T Image Analysis System*, *Delta-T Devices*, Cambridge, UK), dry mass (after drying at 70 °C until constant mass), and leaf N content (with a *CE-Instruments NA-2100* autoanalyser, *ThermoQuest*, Milan, Italy) were determined. Leaf mass per unit leaf area (LMA), leaf N content per unit area, CO_2 assimilation rate per unit area (P_N), and O_2 evolution rate per unit area (P_{max}) were derived from these data. The P_N/g_s ratio was taken as an estimate of water use efficiency for a given vapour pressure deficit (intrinsic water-use efficiency).

The leaf water potential (Ψ_{leaf}) was determined using a *Scholander* pressure chamber (*Mod 1001*, *PMS Instruments*, Corvallis, OR, USA) on twigs taken from the same trees and from the same positions in the crowns as those we used for the gas exchange measurements.

Statistical analyses: We used the *SPSS Statistical Package* (*SPSS*, Chicago, IL, USA). One-way analysis of variance and Fisher's protected LSD test were used to establish significant differences at $p \leq 0.05$ between means after applying the Levene test to check for homogeneity of variances. The relationships among the different parameters estimated were described using linear regression analysis.

Results

Environment and water status: On all the sampling dates, the PPFD surpassed 1 100 µmol m^{-2} s^{-1} at around 07:00 (Fig. 1). Air temperature increased by about 10 °C along the day, from minimum values in the 21-24 °C range in the morning to about 30-33 °C, normally reached between 12:00 and 14:00 (Fig. 1). Like temperature, the atmospheric vapour pressure deficit (VPD) increased as the season and day progressed, reaching maximum values around mid-day (Fig. 1).

Although the pre-dawn water potential (Ψ_{pd}) decreased throughout the sampling period, it never fell below –0.7 MPa in *Q. faginea* and –1.1 MPa in *Q. rotundifolia*. The Ψ_{pd} values were always lower in the evergreen species. Concomitant with increasing leaf and air temperatures and atmospheric VPD, the leaf water potential (Ψ_{leaf}) underwent a marked decrease from pre-dawn values until shortly after midday when minimum values close to –3 MPa were reached (Fig. 1).

Leaf characteristics: The evergreen species displayed a higher mass per unit leaf area (LMA) than *Q. faginea* (Table 1). However, the leaf N content per unit area was significantly higher in *Q. faginea* than in *Q. rotundifolia* (Table 1) owing to the higher content of N per unit leaf mass in the leaves of *Q. faginea* (values not shown). There were no significant differences in the leaf traits studied between the set of leaves used in field gas-exchange and the set of leaves used for oxygen electrode measurements (*Anova* not shown).

Table 1. Mean ± SE ($n = 40$) leaf mass per unit area (LMA), leaf N content per unit area, maximum stomatal conductance (g_s), and net photosynthetic rate (P_N) at ambient CO_2 concentration, and net photosynthetic capacity (O_2 evolution rate) in saturating CO_2 concentration (P_{max}) measured with oxygen electrode. For each variable, means not followed by the same letter are significantly different ($p < 0.05$).

Species	Field gas exchange				Oxygen electrode		
	LMA [g m^{-2}]	N content [g m^{-2}]	P_N [µmol m^{-2} s^{-1}]	g_s [mol m^{-2} s^{-1}]	LMA [g m^{-2}]	N content [g m^{-2}]	P_{max} [µmol m^{-2} s^{-1}]
Q. rotundifolia	189.31±2.00 a	2.35±0.05 b	9.52±0.57 b	0.140±0.012 b	193.45±2.59 a	2.42±0.06 b	13.10±0.66 b
Q. faginea	146.25±3.30 b	2.70±0.06 a	15.10±0.63 a	0.251±0.016 a	144.30±3.78 b	2.62±0.06 a	18.50±0.72 a

Field gas exchange: *Q. rotundifolia* and *Q. faginea* showed very similar diurnal gas exchange patterns, although g_s and P_N of *Q. faginea* were always higher than those of *Q. rotundifolia* (Fig. 1). In both species, maximum P_N was reached during the first hours of the morning. A later decrease in P_N accompanied the increase in VPD and the decrease in Ψ_{leaf} (see Fig. 1). This decrease in P_N was prolonged until approximately mid-day (Fig. 1), when VPD and the water potential reached almost maximum and minimum values, respectively (Fig. 1). These maximum VPD and minimum Ψ_{leaf} values persisted into the late afternoon, when measurements ended as the PPFD had begun to decrease significantly (Fig. 1). Up to that time, we failed to detect any recovery in P_N from their lowest values. Thus, the last values of P_N obtained were reduced to 41 % in the case of *Q. faginea* and to 45 % in the case of *Q. rotundifolia* with respect to the maximum P_N recorded during the first hours of the day (Fig. 1). However, re-hydration overnight obviously permitted the recovery of P_N, and maximum assimilation rates at the morning were similar in two consecutive days. The same diurnal pattern was observed for g_s (Fig. 1), which underwent decreases similar to those observed for P_N.

Maximum g_s and P_N, calculated as an average of the values obtained between sunrise and 09:00, were significantly higher in *Q. faginea* than in *Q. rotundifolia* (Table 1). For both species a multiple regression with Ψ_{leaf} and VPD as independent variables explained most of the variation in P_N of each species (*Q. rotundifolia*, $r^2 = 0.71$, $p = 0.0001$; *Q. faginea*, $r^2 = 0.62$, $p = 0.0001$). Similar relationships were observed between g_s and Ψ_{leaf} and VPD (values not shown).

Photosynthetic capacity: *Q. faginea* reached maximum O_2 evolution rates significantly higher than *Q. rotundifolia* (Table 1). In both species, P_{max} was higher during the first hours of the morning and decreased considerably during the hottest part of the day (Fig. 1). The magnitude of this decrease (around 50 % with respect to the maximum values) was such that, at the times of strongest inhibition, the photosynthetic rates obtained at the laboratory were not very different from those measured in the field. However, the reduction in P_{max} was clearly reversible, increasing once again, as the evening approached, to values representing more than 80 % of the morning maxima in both species (Fig. 1).

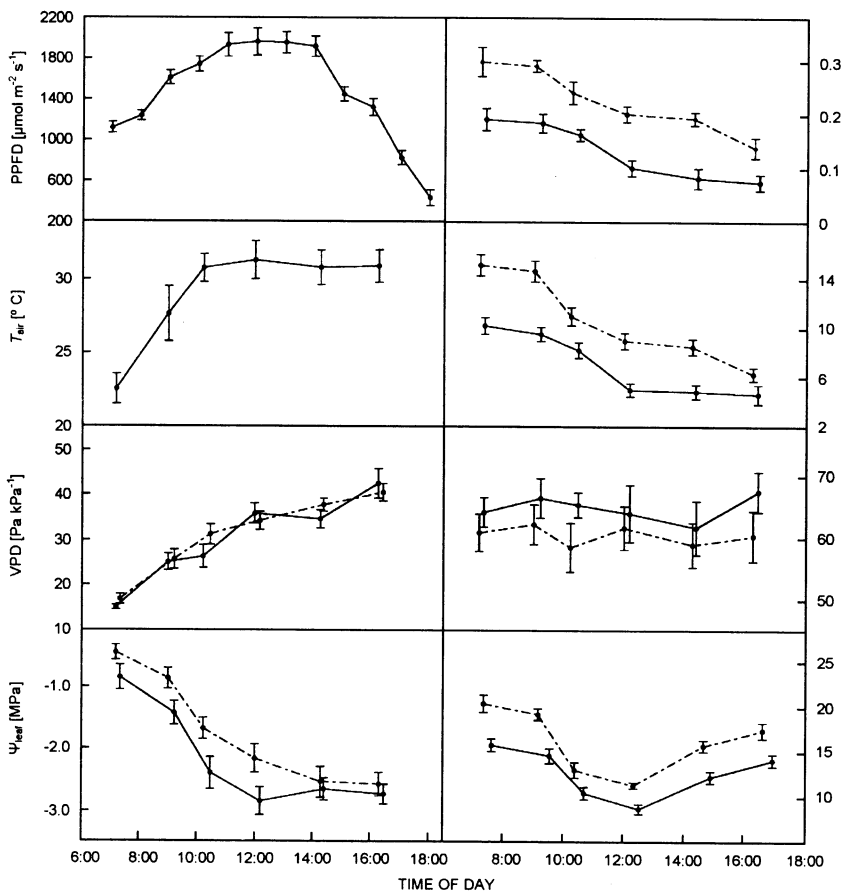

Fig. 1. Daily time courses (mean ± S.E., $n = 12$) of photosynthetic photon flux density on leaf surface (PPFD), air temperature (T_{air}), atmospheric vapour pressure difference (VPD), leaf water potential (Ψ_{leaf}), stomatal conductance (g_s), net photosynthetic rate (P_N), intrinsic water use efficiency (P_N/g_s), and net O_2 evolution rate in saturating CO_2 concentration (P_{max}), of *Q. faginea* (*dashed line*) and current-year leaves of *Q. rotundifolia* (*continuous line*).

Discussion

Photosynthetic capacity and maximum field gas exchange: interspecific comparisons: *Q. faginea* showed a P_{max} and a maximum field P_N higher than *Q. rotundifolia* (Table 1). Our results then revealed a negative relationship between leaf longevity and photosynthesis, as many other authors have reported (*e.g.* Gower *et al.* 1993, Reich *et al.* 1997). For *Q. faginea* the mean P_N and P_{max} values were very similar to the values observed in other deciduous Mediterranean oak species by Tretiach (1993), Epron *et al.* (1994), Valentini *et al.* (1995), and Damesin *et al.* (1998). However, for *Q. rotundifolia* our P_N and P_{max} values were lower than the maxima reported by other authors in other evergreen oak species (Tenhunen *et al.* 1984, Caldwell *et al.* 1986), including the similar *Q. ilex* ssp. *ilex* (Di Marco *et al.* 1990, Tretiach 1993, Damesin *et al.* 1998). These differences between *Q. rotundifolia* and *Q. ilex* ssp. *ilex* may be the result of the more extreme environmental conditions characteristic of the interior regions in which *Q. rotundifolia* is distributed, by comparison with the littoral or sub-littoral zones populated by *Q. ilex* ssp. *ilex*.

The maximum P_N reached in the field during the well-watered period represents on average about 82 % of the P_{max} obtained with the oxygen electrode for *Q. faginea* and 73 % for *Q. rotundifolia* (Table 1). These high percentages reveal that the stomatal limitation to CO_2 assimilation under favourable conditions is fairly low in the two species. Furthermore, when comparing both kinds of measurements, one has to take into account the enhancement of carboxylation rate by the high CO_2 concentrations in the oxygen electrode, as well as the inhibition of photorespiration (Faria *et al.* 1996, Muraoka *et al.* 2000) and the effects of processes not accompanied by carbon dioxide fixation, such as nitrate reduction in the chloroplasts (Björkman and Demmig 1987), that are not detected by the methods used normally in the field measurements. All these processes may help explain part of the difference between the maximum photosynthetic rates obtained in the field and those obtained with the oxygen electrode, further reducing the significance of the effects of stomatal limitations in our two species.

The higher maximum g_s of *Q. faginea* in the field by comparison with *Q. rotundifolia* (Table 1) would suggest that stomatal limitations might be of lesser significance in *Q. faginea*. However, also P_{max} of *Q. faginea* was significantly higher than P_{max} of *Q. rotundifolia*, indicating that certain characteristics of the photosynthetic system of *Q. rotundifolia* must also contribute to limiting P_N.

Leaves of both species differed in their average nitrogen content, which was higher in *Q. faginea* (Table 1). A direct relationship between N content and P_N has been reported by several authors (Field *et al.* 1983, Strand 1997, Grassi *et al.* 2001, Osaki and Shinano 2001, Weng and Hsu 2001), and results from the major investment of this nutrient in the photosynthetic enzymes (Evans 1989).

The highest LMA of *Q. rotundifolia* (Table 1) may be another factor contributing to the lower P_{max} and P_N of this species. The negative effects of a high LMA on P_N in inter-specific comparisons are a common finding in many studies, and indeed are seen in all types of vegetation and climate (Reich *et al.* 1997). Nevertheless, at the intra-specific level the leaves with a high LMA often show higher assimilation rates than low LMA leaves (Meir *et al.* 2002). A higher internal competition for photons and CO_2 (Poorter *et al.* 1990), higher internal resistance to the transfer of CO_2 (Lloyd *et al.* 1992), a low proportion of leaf nitrogen in photosynthetic enzymes in leaves with high LMA (Evans 1989, Niinemets 1999), and an unequal distribution of nitrogen in the different enzymes involved in CO_2 assimilation (Poorter and Evans 1998) have been proposed as factors responsible for the low P_N in high-LMA leaves.

Photosynthetic capacity and field gas exchange: diurnal trends: Total assimilation in the two species studied was significantly reduced by the mid-day decreases of photosynthesis in response to the increase in VPD and to the decrease in Ψ_{leaf} (Fig. 1). If the decrease in the CO_2 supply as a result of stomatal closure were the main cause of the inhibition of P_N, it could be expected that the P_N/g_s ratio would increase due to the increment in the CO_2 concentration gradient between the air and the sites of carboxylation. However, P_N/g_s remained in both species almost constant along the day (Fig. 1). The constancy in the P_N/g_s values therefore seems to point to the existence of mesophyll inhibition of photosynthesis towards mid-day, which is also demonstrated with the oxygen electrode. Indeed, P_{max} also underwent a considerable decrease during the hottest part of the day, as was also observed by other authors (Beyschlag *et al.* 1987, Demmig-Adams *et al.* 1989, Di Marco *et al.* 1990). This appreciable decrease in photosynthetic capacity in our species suggests a direct injury to the photosynthetic apparatus caused by an enhancement of stress (be this a water deficit, heat stress, irradiance stress, or probably, a combination of all) during mid-day. Contrary to our expectations, the mesophyll limitation of photosynthesis seemed to be similar for both species, independently from the differences in leaf traits between them. The mid-day decrease in g_s would constitute an adjustment that permits the matching of the supply of CO_2 to the capacity of the mesophyll to fix carbon, in agreement with Wong *et al.* (1979) and Farquhar and Sharkey (1982).

The photosynthetic apparatus recovered its carbon-fixing capacity along the afternoons. However, the recovery of mesophyll activity was not accompanied by a recovery of the field P_N (Fig. 1), which Faria *et al.* (1996) also failed to observe in leaves of *Q. suber*. Accordingly, the recovery of P_{max} was more rapid than the recovery of field P_N, which requires re-hydration of the plant overnight. The fact that g_s was not recovered along the afternoons, whereas P_{max} was, suggests that the reduced P_N observed in our two species along the afternoon is mainly governed by stomatal inhibition. Thus, g_s would mainly limit photosynthesis during the first hours of the day and during the afternoon, whereas mesophyll activity is maintained. Nevertheless, stomatal closure does not seem to be a limitation to photosynthesis during mid-day, in which inhibition of mesophyll activity seems to play a more important role.

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