

# Relationship between photosynthetic activity and chlorophyll content in an isolated *Quercus ilex* L. tree during the year

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## Abstract

The relationship between chlorophyll (Chl) content and net photosynthetic rate ( $P_N$ ) in an isolated *Quercus ilex* tree, growing inside Villa Pamphili Park in Rome, was explored. The highest  $P_N$  was in March, May, and September ( $10.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ , maximum rate).  $P_N$  decreased by 65 % (with respect to the yearly maximum) when leaf temperature reached  $34^\circ\text{C}$ , and by 50 % when leaf temperature was  $9^\circ\text{C}$ . The highest Chl contents were in April, October [ $1.47 \text{ g kg}^{-1}$ (d.m.), maximum value], and December. The lowest Chl content was found in July ( $0.78 \text{ g kg}^{-1}$ ). The decrease of  $P_N$  in July was in close connection with the decrease of Chl content. On the contrary, the high Chl content during winter did not correspond with  $P_N$  of this season. Discordances between Chl content and  $P_N$  over the year influenced the regression analysis, which although positive did not show very high correlation coefficients ( $r = 0.7$ ). The high Chl ( $a+b$ ) content during most of the year indicated that the photosynthetic apparatus remained basically intact also during stress periods.

*Additional key words:* net photosynthetic rate; oak; regression analysis; seasonal course; stomatal conductance.

## Introduction

Plants develop morphological, physiological, and chemical traits in response to their growth conditions. Leaves are able to regulate the level of excitation energy, and such regulation can occur by changes in  $P_N$  and by dissipation of excess energy through various pathways within chloroplasts (Björkman and Demmig-Adams 1994). In nature, a high degree of regulation by radiant energy is achieved by changes in the amount of photosynthetic enzymes and electron carriers relative to Chl; an increase in this ratio results in a higher photosynthetic capacity (Björkman and Demmig-Adams 1994). Chl, being an unstable molecule with a relatively short life, is a good

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short-term indicator of the plant state (Gracia 1984). Increase in the relative proportion of Chl *b* to Chl *a* under low irradiance is an adaptive response maximizing the light-harvesting capability of chloroplasts (Chaves 1991). The Chl *a/b* ratio can be used therefore as a bioassay for the irradiance environment of plants (Dale and Causton 1992).

Margaris (1977) refers to the correlation between  $P_N$  and Chl content in certain seasonal dimorphic species. Šesták and Václavík (1965) demonstrated that its linear expression depends on morphological and physiological leaf traits. A positive correlation was found also for tropical species (Naidu and Swamy 1995), and there were evidences of a strong relationship between  $P_N$  and Chl content per leaf mass of *Hedera helix* L. (Bauer and Bauer 1980). However, the decrease of  $P_N$  by low air temperature in olive tree was only at some extent linked with changes in Chl content (Laouar and Vieira Da Silva 1981).

In Mediterranean climate the much more gradual seasonal changes allow evergreen sclerophyllous species to acclimate to the thermal regime at any time of the year (Björkman and Demmig-Adams 1994), and plants respond to environmental changes with adjustments of photosynthesis and Chl content, in response to water availability and air temperature. Chl content of Mediterranean species decreases under water deficit (Núñez-Olivera and Escudero García 1990, Núñez-Olivera *et al.* 1994), but its slight variation during moderate water stress enables the total functionality after rewetting (Stuhlfauth *et al.* 1990). This suggests that evergreen sclerophyllous species have developed efficient mechanisms for drought resistance (Spyropoulos and Mavrommatis 1978). Evergreen sclerophyllous species suffer a 5-10 % damage when their relative water content decreases from about 50 to 17 % (Larcher 1995).

The aim of this paper was to determine  $P_N$  and Chl content of *Quercus ilex* L. during the year, and to investigate the relationship between them.

## Materials and methods

Field experiments were made during the year 1995 on an isolated *Quercus ilex* L. tree growing inside Villa Pamphili Park (Rome, Italy). The tree was 13.5 m high, its basal girth was 2.6 m, with following characteristics per leaf: fresh mass  $215 \pm 31$  mg, dry mass  $133 \pm 21$  mg, surface area  $7.9 \pm 1.2$  cm<sup>2</sup>, specific leaf area  $5.94 \pm 1.84$  m<sup>2</sup> kg<sup>-1</sup>, leaf mass per area  $168 \pm 52$  g(d.m.) m<sup>-2</sup>. One hundred branches from the external medium-low portion of the crown, formed from the previous year growth, were permanently labelled. On each sampling occasion, fifty one-year-old leaves (spring leaves) were collected and kept in a cold-box during transportation to the laboratory.

Leaf surface area was measured with the Image Analysis System (*Delta-T Devices*, England). Leaf dry mass was calculated after drying at 90 °C until a constant mass. Specific leaf area (SLA) was calculated as the ratio of unifacial leaf area to its dry mass (Beadle *et al.* 1985). The ratio of dry mass to its unifacial leaf area was calculated according to Reich *et al.* (1992).

Chl was extracted with acetone solution adding a pinch of magnesium carbonate to

prevent pheophytin formation (Linder 1974). Extracts were centrifuged with a refrigerated centrifuge 4237R (ALC, Italy). Measurements of absorbance of the extracts were conducted with *Jasco 7800* spectrophotometer (LCD, Japan). Chl *a* and *b* contents were calculated according to MacLachlan and Zalik (1963). On each sampling occasion the average value of five replicates was utilized.

PAR,  $P_N$ , stomatal conductance ( $g_s$ ), and leaf temperature ( $T_l$ ) were monitored in the field from 09:00 to 11:30 h, using an *ADC-LCA3* open system infrared  $\text{CO}_2$  gas analyzer (Hoddesdon, UK). On each sampling occasion 12 leaves were measured.

On June 20, July 26, October 5, November 7, November 29, December 6, and December 20, the same leaves used to measure  $P_N$  were collected for determining Chl content. All measurements were utilized and tested by regression analysis between Chl *a*, Chl *b*, Chl (*a+b*), and  $P_N$ .

## Results

Fig. 1 shows monthly air temperatures and trends of monthly total rainfall trends during the year 1995 (values collected by the Collegio Romano Meteorological Station). The mean total year rainfall of the period 1983-1995 was 614 mm, the mean minimum air temperature of the coldest month (January) was 4.8 °C, and the mean maximum air temperature of the hottest month (July) was 31.1 °C. The highest annual rainfall values were in autumn.

The dense crown of the *Q. ilex* tree under study was characterized by thick and small leaves. The highest  $P_N$  values were observed in March, May, and September (Fig. 2A), with a maximum ( $10.1 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at the end of September and a minimum ( $3.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) at the end of July.  $P_N$  decreased by 65 % (with respect to the yearly maximum) when  $T_l$  reached 34 °C (Fig. 2B), and by 50 % when leaf temperature was 9 °C.  $g_s$  (Fig. 2B), on the average, showed the same trend, decreasing by 75 and 63 % (according to the yearly maximum) in June and January, respectively.

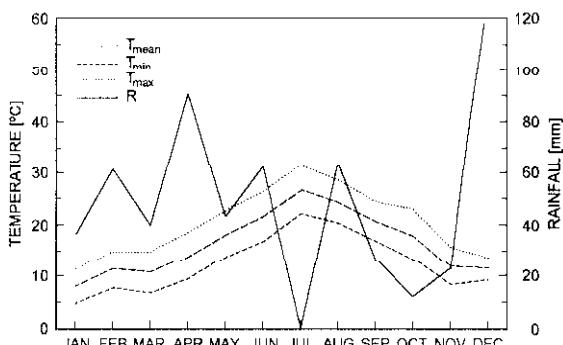


Fig. 1. Monthly trends of air temperature and total rainfall during 1995.  $T_m$  = mean air temperature;  $T_{\min}$  = minimum air temperature;  $T_{\max}$  = maximum air temperature;  $R$  = rainfall. The mean yearly air temperature was 16.2 °C, the absolute minimum and maximum air temperatures were -0.8 °C (January 17) and 35 °C (July 20), respectively. Total yearly rainfall was 586 mm.

The highest Chl (*a+b*) contents were found in April, October, and December, with a maximum ( $1.47 \text{ g kg}^{-1}$ ) in October; the minimum ( $0.78 \text{ g kg}^{-1}$ ) was in July (Table 1). Chl *a* and Chl *b* contents showed a similar trend during the year, although Chl *a*

Table 1. Average contents of chlorophylls (Chl) *a*, *b*, and (*a+b*) [ $\text{g kg}^{-1}$ (f.m.)] and Chl *a/b* ratio of one-year-old spring leaves of *Quercus ilex*. Each value is the mean of five replications with standard error.

	Chl <i>a</i>	Chl <i>b</i>	Chl ( <i>a+b</i> )	Chl <i>a/b</i>
January 23	0.830±0.009	0.230±0.016	1.060±0.025	3.6
March 9	0.730±0.013	0.220±0.008	0.950±0.021	3.3
March 31	0.760±0.016	0.240±0.021	1.000±0.037	3.2
April 29	1.000±0.083	0.320±0.042	1.320±0.125	3.1
May 25	0.940±0.071	0.290±0.007	1.230±0.078	3.2
June 2	0.690±0.001	0.240±0.004	0.930±0.005	2.9
June 20	0.690±0.011	0.210±0.009	0.900±0.020	3.3
July 19	0.570±0.005	0.210±0.004	0.780±0.009	2.7
July 26	0.650±0.049	0.250±0.002	0.900±0.051	2.6
September 30	0.840±0.008	0.300±0.004	1.140±0.012	2.8
October 23	1.150±0.073	0.320±0.013	1.470±0.086	3.6
November 7	0.840±0.007	0.280±0.005	1.120±0.012	3.0
November 29	0.890±0.009	0.240±0.007	1.130±0.016	3.7
December 6	0.910±0.074	0.290±0.019	1.200±0.093	3.1
December 20	1.070±0.062	0.290±0.011	1.360±0.073	3.7

had more pronounced peaks than Chl *b*. Chl *a* and Chl *b*, respectively, decreased to 50.4 and 34.4 % in July (with respect to the yearly maximum). Chl *a/b* ratio ranged from 2.6 to 3.7 during the year: these values are typical of sun leaves according to Eliáš and Masarovičová (1980) and Gratani (1995, 1997). The decrease of Chl contents in July was in close connection with the decrease of  $P_N$  and it attested

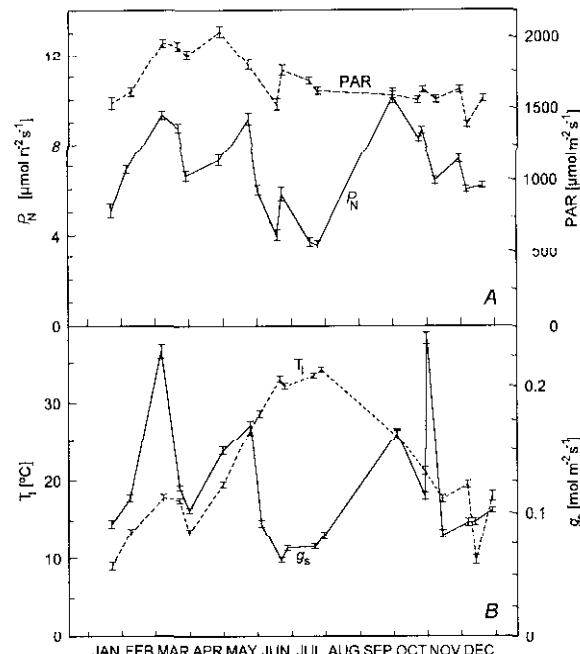


Fig. 2. Trends of net photosynthetic rate ( $P_N$ ) and PAR (A), and stomatal conductance ( $g_s$ ) and leaf temperature ( $T_l$ ) (B) measured from 09:00 to 11:30 h during the year 1995. Standard errors are shown.

the pronounced decline of the mesophyll activity (Anderson *et al.* 1988). The highest correlation coefficients were observed between Chl (*a+b*) content and  $P_N$  (Fig. 3).

## Discussion

Chloroplasts acclimate to their environment by modulating the stoichiometry of components of thylakoid membranes and stroma, in accordance with the requirements of the plant for the highest photosynthetic efficiency (Anderson 1985, Dale and Causton 1992). Our results show a close connection among Chl contents, air temperature, and rainfall. Chl (*a+b*) content peaked in spring, when leaf temperatures ranged from 17.8 to 26.2 °C, and in October, due to the optimal leaf temperature (20.9 °C) and to the sufficient rainfall of this season.

Like other Mediterranean oak species, *Q. ilex* contains more Chl than necessary for absorbing 99 % of incident radiation (Gracia 1984). In the Mediterranean climate, leaves of evergreen plants are often exposed to potentially photoinhibitory conditions during winter, when low air temperatures are often associated with high irradiances, and much more during summer, when high irradiances and high air temperatures are associated with a water shortage. Probably the high Chl content can be partially involved in the dissipation of harmful excess excitation energy and in the mechanisms of self-photooxidation of Chl (Harbour and Bolton 1978, Frosch *et al.* 1979).

The highest Chl *a/b* in spring indicates the highest photosystem efficiency while the high winter Chl content might be regarded as an adaptation which ensures the stability of the photosynthetic mechanism during this period. This could explain why the winter Chl contents are only to some extent linked with  $P_N$  of this season. In winter there is, according to Powles (1984), a decline in  $P_N$  but low changes in Chl content. Spyropoulos and Mavrommatis (1978) show small changes in Chl (*a+b*) content during water stress in the more xerophytic species. In contrast, our results show a 50.4 % lowering of Chl  $\alpha$  content during summer. Because Chl  $\alpha$  is in the light-harvesting complex and primary antenna of both photosystems (Baker and Markwell 1985), this explains the 65 % decrease of  $P_N$ . The decrease of  $P_N$  in July indicates a reduction of carboxylation efficiency during drought. In close connection with the decrease in Chl content, the decline in carboxylation efficiency may reduce the possibility of photoinhibitory damage to photosynthetic apparatus.

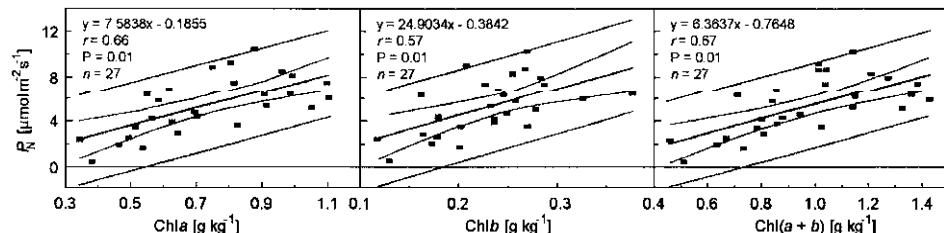


Fig. 3. Correlations between net photosynthetic rate ( $P_N$ ) and chlorophyll (Chl) *a*, *b*, and (*a+b*) contents.

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A small discordance between Chl contents and  $P_N$  during the year influences the regression analysis, which although positive does not show very high  $r$  values. The lowest  $r$  of the correlation between Chl *b* content and  $P_N$  is probably due to the highest stability of Chl *b* compared to the great variability of  $P_N$  during the year.

The high total Chl contents monitored in *Q. ilex* during most of the year indicate that its photosynthetic apparatus remains basically intact also during stress periods, according to Demmig-Adams and Adams (1992) for plants adapted to high irradiance, when protection mechanisms predominate and little injury occurs.

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