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Gas exchange in senescing leaves of *Olea europaea* L.

P. PROIETTI

Istituto di Coltivazioni Arboree, University of Perugia, 06121, Perugia, Italy

Abstract

From the beginning of olive leaf yellowing to leaf fall (1÷3 months), there was a general trend from anabolism to catabolism. Rates of net photosynthesis (P_N) and respiration, areal dry mass, and contents of pigments, particularly of chlorophyll (Chl) *a*, starch, and above all nitrogen (N) decreased. The detachment force decreased dramatically only in completely chlorotic leaves. Chl $a:b$ ratio only declined in the last 10-20 d of senescence, when the total Chl contents diminished by about 70 %, after which the N content, P_N, and efficiency of the photochemical energy conversion of the remaining Chl and N dramatically declined. Consequently, for most of the natural course of senescence P_N remained relatively high. The reduction in P_N was associated with the decreases in transpiration rate (E) and stomatal conductance (g_s), but these probably did not cause the decline of P_N. The recycling of saccharide compounds was low, while 50 % of the total N on a leaf area basis was relocated back before leaf abscission, changing the leaf from a carbon source to a mineral source. Therefore, considering that senescing leaves in olive trees contribute to carbon gain and allow the recycling of resources, it is essential to prevent the premature leaf abscission by avoiding deficits of water and mineral nutrients and by using pruning and training systems that allow good irradiation of all leaves in the crown.

Additional key words: carotenoids; chlorophyll; detachment force; nitrogen content; olive; respiration rate; saccharides; stomatal conductance; transpiration rate.

Introduction

Leaf age must be considered in all studies of physiological and biochemical processes and when modelling canopy assimilation (Šesták 1977, Tichá *et al.* 1985, Proietti *et al.* 1995, Gucci *et al.* 1997). During the life span of a leaf the general trend is a rapid increase in P_N in a young leaf, almost in parallel to the increase in leaf area, to a maximum that is maintained for a long period of time followed by a slower

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Fax: (+39) 75 5856255; e-mail: ASEP@UNIPG.IT
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decline in senescing leaves (Šesták *et al.* 1985). Dark respiration (R_D) is very high in new leaves, but decreases rapidly as the leaves grow and mature, and usually increases slightly in senescent leaves thus resembling the respiratory climateric shown by some fruits (Tetley and Thimann 1974). During senescence numerous other metabolic changes occur that are connected to assimilate production and use, but some are yet to be elucidated and the causes and effects of relationships are not clear (Mae *et al.* 1987).

The olive is an evergreen tree with leaves, most of which persist for 2-3 years, of increasing age from the tip to the base of shoot. The olive periodically abscises a portion of its leaves and only a portion of the total leaf mass is renewed each year. Abscission of old leaves, that occurs mainly in June of their third year, is irregular and therefore all leaf age stages are present at all times. The changes during leaf development and maturation in olive have been studied (Niavis and Kousounis 1981, Bongi *et al.* 1987, Proietti *et al.* 1995, Gucci *et al.* 1997). During the first two weeks after full leaf expansion, P_N markedly increases. Leaves that are 1-year-old or more and particularly mature young leaves (from 1 to 4 months of age) have a higher P_N and g_s than the younger ones. Leaf areal dry mass and Chl content increase with leaf age, but soluble sugar and starch contents are higher in young leaves.

The purpose of this paper is to consider the activity of olive leaves during senescence because this phase has not been studied in this species. The progress of leaf senescence was determined by estimating Chl loss because, before abscission, the aging leaves gradually lose their green colour and turn yellow due to Chl loss and unmasking of carotenoids (Cars). In particular, the relationships between leaf Chl content and gas exchanges, detachment force, saccharides and nitrogen (N) contents were studied.

Materials and methods

The trial was made in central Italy (Foligno, 43°N latitude), in a 12-year-old non-irrigated olive grove, growing in clay loam soil, with trees trained to the vase system and spaced 5×5 m. Measurements were made on 2- to 3-year-old leaves with different Chl contents (*i.e.*, nonsenescing and in various stages of senescence, from green to completely chlorotic). The leaves were taken from five trees of Frantoio cultivar (for comparison of its photosynthetic activity with another olive cultivar see Proietti and Palliotti 1997), with similar vegetative and productive characteristics in mid-June (spring growth flush) and mid-October (shoot lignification) of 1995 and 1996.

Values were always collected on cloudless days, in the morning from 09:00 to 10:30 h. Detachment force (applied perpendicularly to the shoot) was measured on about 40 leaves using the *Carpo* dynamometer (*Carpano et Pons*, Thiez, France) modified by *CCS Caltor* (Torino, Italy). In the same leaves, P_N, E, g_s, and substomatal CO_2 concentration (C_i) were measured using a *LCA-2* portable gas exchange analyser (*Analytical Development Co.*, Hoddesdon, U.K.) and a Parkinson leaf chamber type *PLC(n)*. The detached leaf was enclosed in the chamber and

exposed perpendicularly to sun rays (incoming photosynthetic photon flux density 1300-1500 μmol m^{-2} s^{-1}). The flow rate of air passing through the chamber was kept at 5 cm^3 s^{-1} [about 10 000 cm^3 s^{-1} m^{-2}(leaf area)]. During gas exchange measurements, the external concentration of CO_2 was about 370 cm^3 m^{-3}, and the air temperature inside the leaf chamber was 2-3 °C higher than the atmospheric temperature, varying from 26 to 28 °C in June and from 20 to 23 °C in October. The R_D was measured by covering the chamber with a black cloth screen. Recordings were taken under steady-state conditions.

After gas exchange measurements, the leaves were immediately transferred to the laboratory in a portable refrigerator for other determinations. Leaf area was measured using a leaf area meter (*Hayashi Denkoh Co.*, model *AAM-7*). Chl *a* and *b* and Cars contents were determined on one disc (1.13 cm^2) removed from each leaf, with a representative colour of the whole blade. According to Bruinsma (1963), pigment contents of each leaf disc, previously weighed and frozen in liquid nitrogen, were determined by grinding and mixing with 10 cm^3 of 80 % acetone in a small mortar. The samples were sealed in beakers with *Parafilm* to prevent evaporation, and were extracted overnight at 4 °C. The absorbance of extracts was measured at 480, 645, and 663 nm by a spectrophotometer. Pigment contents were calculated according to Holm (1954).

Half of the leaves were then used to determine soluble sugar and starch contents (Morris 1948). Dry mass and water content of the remaining leaves were determined using drying to constant mass in a forced air oven at 90 °C. The dry leaves were used to determine total organic N level by the micro-Kjeldahl procedure of Nelson and Sommers (1973).

Values obtained during the two years of sampling were considered together and an exponential rise to maximum function [$y = a\ (1 - e^{-bx}) + c$] or linear regression provided the best fit for the relationship between leaf age (Chl content) and leaf characteristics.

Results

In green nonsenescing leaves, the total Chl content was 0.8-1.0 g m^{-2}, in completely yellow leaves it was less than 0.1 g m^{-2}. Twenty days to 3 months passed from the onset of yellowing to leaf fall, with the shortest times in June. For about 70 % of this time the leaf Chl content was higher than 0.3 g m^{-2}. The Chl *a* : *b* ratio declined with advancing senescence only when the total Chl content dropped below 0.2-0.3 g m^{-2} (Fig. 1*A,B*). In October, Chl decline was associated with the decrease in Cars content, while in June the Cars content, lower than in October, was constant during leaf senescence (Fig. 1*C,D*). Leaf detachment force varied greatly and decreased dramatically in the completely chlorotic leaves (Chl content lower than 0.1 g m^{-2}) (Fig. 1*E,F*).

In both periods considered, P_N decreased with the Chl content loss during leaf senescence until abscission occurred (Fig. 2*A,B*). However, P_N declined more rapidly when total Chl content descended under 0.3-0.4 g m^{-2}. In fact the photosynthetic

efficiency of a unit Chl amount ("assimilation number") did not decline with advancing senescence except in leaves having a total Chl content under 0.3 g m^{-2} (Fig. 2*C,D*), which corresponded to the onset of Chl *a* : *b* ratio decrease (Fig. 1*A,B*).

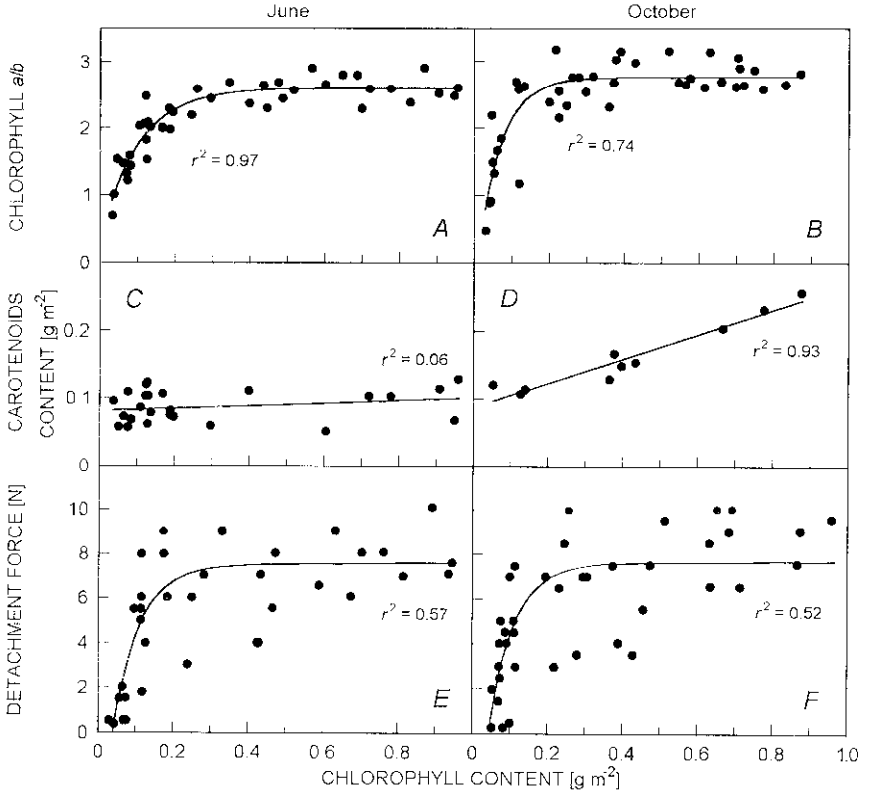

Fig. 1. Relationships between the total chlorophyll content and *a* : *b* ratio (*A, B*), carotenoid contents (*C, D*), and the detachment force (*E, F*) in olive leaf in mid-June (*A, C, E*) and mid-October (*B, D, F*).

Therefore, according to Šesták *et al.* (1985), it seems that the difference between the "assimilation number" of mature and old leaves is due to the changes in relative contents of Chl *a* and *b*. These values are consistent with a previous study in *Platanus occidentalis* and indicate that the efficiency for photochemical energy conversion of the remaining Chl was maintained high for most of the natural course of senescence (Adams *et al.* 1990). R_D, slightly higher in June than in October, in both periods decreased slightly and gradually with senescence (Fig. 2*A,B*).

During leaf aging, in both periods C_i increased, but g_s and E decreased (Figs. 2*E,F* and 3). Leaf water content tended to decrease only in the last part of leaf senescence (Fig. 4*A,B*). The areal dry mass of leaves, higher in June than in October, remained almost constant for the most part of senescence, then fell by about 20 % (Fig. 4*C,D*). Total Kjeldahl N content decreased with aging from about 2.5 g m^{-2} to 1.0-1.5 g m^{-2} (Fig. 4*E,F*). The P_N per unit N decreased with advancing senescence when total leaf

Chl content fell below 0.3-0.4 g m^{-2} (Fig. 5*A,B*). The contents of saccharides did not vary substantially during senescence: starch slightly decreased and reducing sugars slightly increased (Fig. 5*C,D*).

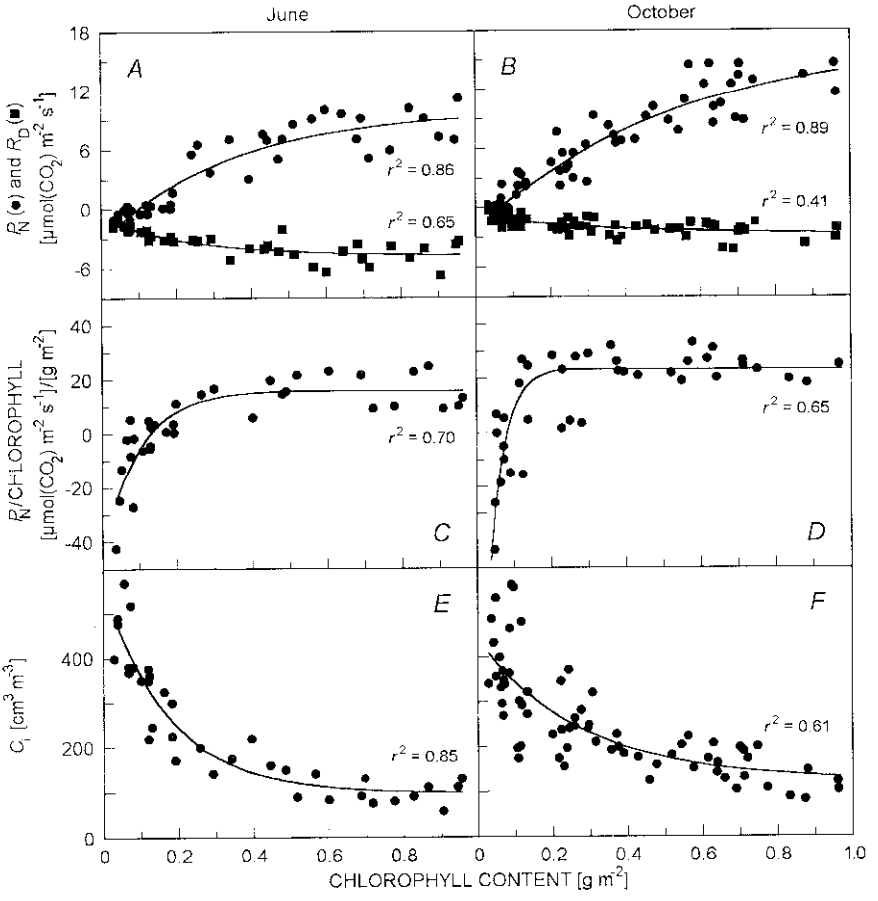

Fig. 2. Relationship between total chlorophyll content and the net photosynthetic rate (P_N) under full irradiance related either to leaf area (*A, B*) or to chlorophyll content (*C, D*), dark respiration rate (R_D) (*A, B*), and the substomatal CO_2 concentration (C_i) (*E, F*) in olive leaf in mid-June (*A, C, E*) and mid-October (*B, D, F*).

Discussion

Aging olive leaves gradually lose their Chl, whereas the detachment force dramatically decreases only in completely chlorotic leaves, so the detachment force is not a good index to evaluate the leaf aging progress in olive. As reported by Šesták (1978) for other species, the leaf Cars content in olive also changed during vegetation season, with higher amounts in October than in June. Furthermore, no common

pattern of changes in Cars during leaf senescence was observed at the different times:

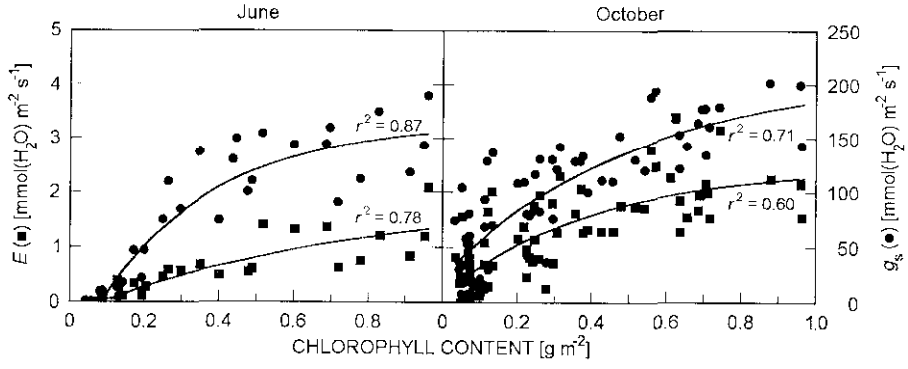

Fig. 3. Relationship between total chlorophyll content and the transpiration rate (E) or stomatal conductance (g_s) in olive leaf in mid-June and mid-October.

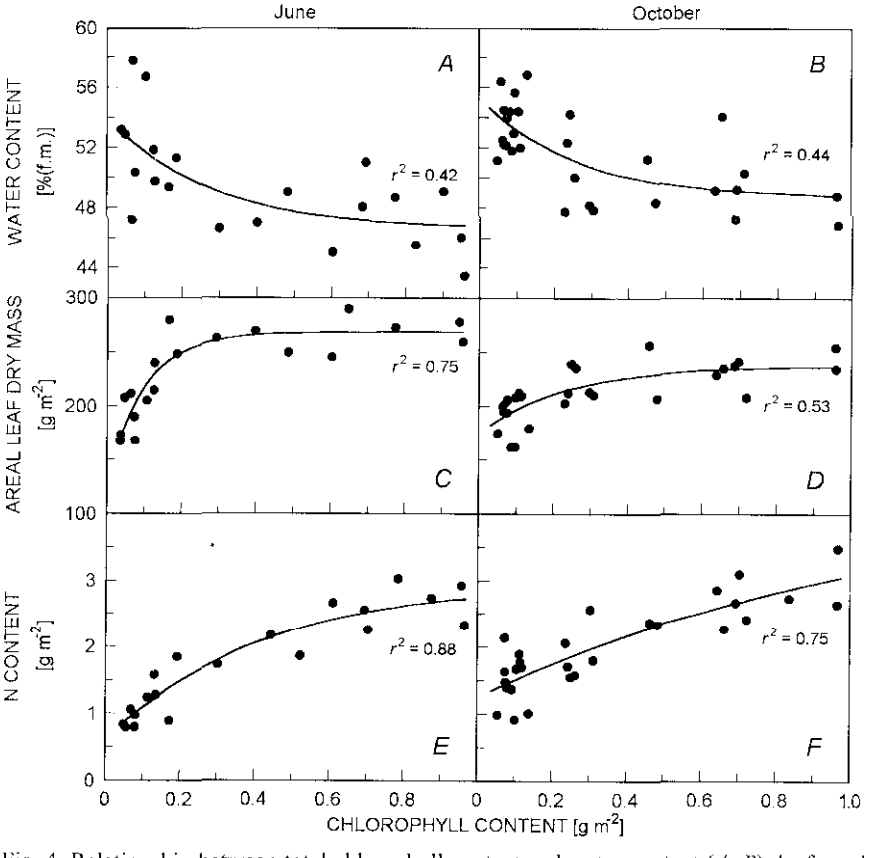

Fig. 4. Relationship between total chlorophyll content and water content (A, B), leaf areal dry mass (C, D), and nitrogen content (E, F) in olive leaf in mid-June (A, C, E) and mid-October (B, D, F).

the Cars content declined during leaf senescence in October, whereas in June it remained constant. Chl $a : b$ ratio declined only in the last part (10-20 d) of the senescence period (1-3 months), beginning when the total Chl contents dropped by

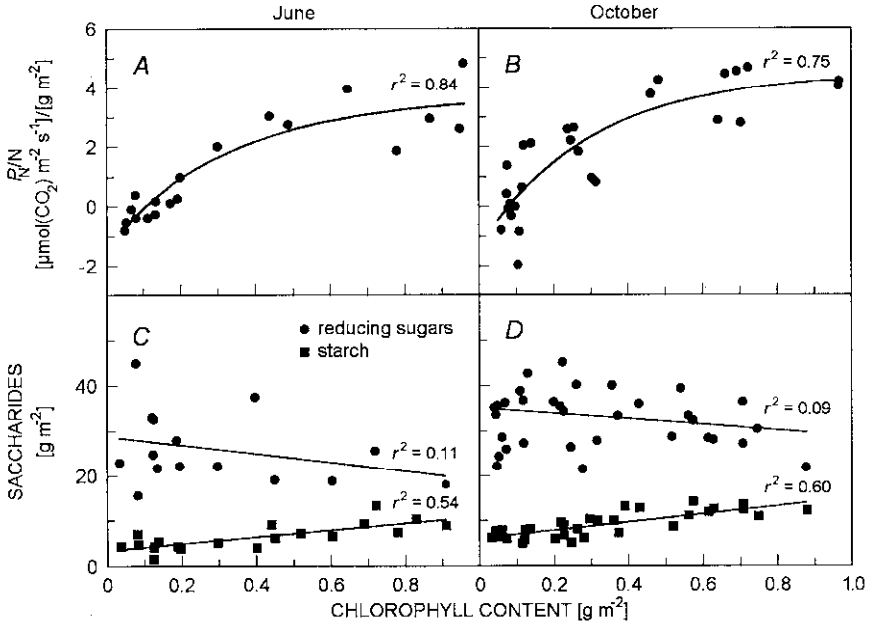

Fig. 5. Relationship between total chlorophyll content and net photosynthetic rate (P_N) under full irradiance related to nitrogen (N) content (*A, B*), and contents of saccharides (*C, D*) in olive leaf in mid-June (*A, C*) and mid-October (*B, D*).

about 70 %. This was a turning point because prior to this the efficiency for photochemical energy conversion of the remaining Chl and the functionality of photosynthetic components in the leaf remained high; after this point they declined, indicating a decreased resource-use efficiency (Adams *et al.* 1990). In addition, starch content slightly decreased and N content dramatically declined. Since a large proportion of the total leaf protein is constituted by RuBP carboxylase and other P_N-related enzymes, after the N content fell below certain limit, some necessary photosynthetic enzymes were probably lost (Titus and Kang 1982). The decline of Chl and protein contents as senescence develops could be structurally associated with the deterioration of chloroplast (Varner 1961). Consequently, for most of the natural course of senescence the P_N decreased very slowly, remaining fairly high, and only when total Chl content dropped below 0.3-0.4 g m^{-2} did it decline rapidly.

The decline in P_N in senescing leaves was associated with the C_i increase. This created conditions under which stomata close with a consequent g_s and E decrease. These reductions therefore do not seem to cause the decline in P_N. In particular, the reduced g_s of senescing leaves does not seem to be caused by the loss of guard cell functionality; in fact the leaf water content did not decrease with leaf senescence, whereas a considerable water stress should occur with loss of stomatal control.

Therefore the presence of functional stomata may optimize the P_N against water loss and probably prevents senescing leaves from premature desiccation (Raschke 1975, Woolhouse and Batt 1976, Heaton *et al.* 1987).

The ability to continue photosynthetic activity is important for carbon gain in olive tree because it, like other evergreen species, has a long leaf senescence phase. The leaf activity during senescence is also important for enhancing the redistribution of resources through a controlled process of degradation and export of nutrients to newly developing leaves or into branches, trunk, and roots during periods of inactive growth (Lloyd 1980, Côté and Dawson 1986, Adams *et al.* 1990). However, in senescing olive leaves the solubilization and export of saccharides are low. This means that unlike other species, in olive the carbon recycling during senescence is not very consistent. In fact, an increased R_D in senescent leaves, such as reported in other species where it resembled the respiratory climateric of some fruits and was ascribed to a functional change in leaf saccharide economy, was not observed (Tetley and Thimann 1974, Lloyd 1980). Rather, the 50 % of total N (based on a unit leaf area) was translocated back before leaf abscission. The N recycling through mobilization from senescing leaves may be very important because it is less costly in the energy balance than fixing of new N (Heaton *et al.* 1987).

In conclusion, during leaf senescence in olive, similarly as in other species, there is a general trend from anabolism to catabolism. Senescing leaves, changing slowly from a carbon source to a mineral source, contribute to the carbon gain and allow the recycling of resources. For this reason, considering that senescence is induced and influenced by plant ontogeny as well as by microclimate (water stress, shading of leaves, *etc.*), it is essential to prevent premature leaf abscission by avoiding deficits of water and mineral nutrients, and shading. Because olive leaves pass from full-irradiance exposition on the shoot tip in the first year to shading position on the branch in the third year of span life, it is important to improve the irradiance of all leaves in the crown by using the right pruning and training system.

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