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Gas exchange in olive fruit

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Abstract

Dry matter (DM) of olive fruit (cv. Leccino) constantly increased from fruit-set (mid-June) to the end of October. The oil content increased rapidly from the beginning of August, about 40-50 d after full bloom (AFB), to the end of October. As the oil content increased, the saccharide content decreased. On a DM basis, fruit dark respiration rate (R_D) and stomatal conductance (G_s) were high soon after fruit-set, then strongly decreased. Gross photosynthetic rate (P_G) in full sunlight was high in the first 3 weeks after fruit-set, when the chlorophyll (Chl) content and the ratio between fruit surface area and volume were high, then it progressively decreased. The fruit intercellular CO_2 concentration (C_i) was always relatively high, particularly from September onwards. The P_G increased following the increase of irradiance (I). The daily P_G trend was similar to the I and temperature trends, showing the maximum values at 14:00 h. For a large part of the fruit growing period, during daylight, the CO_2 intake by a fruit permitted the reassimilation of a large part (40-80 %) of the CO_2 produced by R_D. The stomata in the first stages of fruit growth were oval and surrounded by guard cells, two months later they lost their shape and were covered by wax. The reduction in fruit P_G during fruit growth could be connected to the reduction of the ratio between fruit surface area and fruit volume and the cellular differentiation, whereas the constant high C_i seems to exclude the influence of G_s decrease. Even if olive fruit is highly heterotrophic organ, its photosynthesis can considerably reduce the use of assimilates for respiration and favour fruit maintenance and growth.

Additional key words: chlorophyll; fruit growth; gross photosynthetic rate; irradiance response curve; *Olea europaea* L.; olive oil; respiration rate; saccharides; stomatal conductance; transpiration rate.

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Introduction

Research on green plant parts from various species has shown that in the fruit a certain photosynthetic activity takes place (Bazzaz *et al.* 1979, Blanke and Lenz 1989, Xu *et al.* 1997). Little information is available on gas exchanges of olive fruit, which is green during most of its developmental cycle. This suggests that the fruit has active chloroplasts capable of CO_2 fixation in the light which contribute significantly to fruit development and oil synthesis (Maxie *et al.* 1960, Proietti 1990, Sanchez 1994, Sanchez and Salas 1997, Tombesi *et al.* 1997).

In central Italy, olive trees bloom at the beginning of June. Flowers are produced on inflorescences in the leaf axils, on shoots that grew the previous year. Blooming is scalar and, on a single tree, lasts about 2 weeks. The fruit is a drupe which results from the growth of the ovary in which only one ovule develops. Fruit development, from anthesis to ripening, lasts 25-30 weeks. After a month of intense cellular division, exocarp, mesocarp, and endocarp can be identified. The first is formed by a layer of epidermal cells rich in chloroplasts with the wall covered by cutin and by a thin membrane, the cuticle. The mesocarp cells are rich in protoplasm. The endocarp cells show a thickened parietal membrane. Some time later, the mesocarp cells tend to increase the vacuole size, while those of the endocarp become stony. About 60 d AFB, droplets of fat detach from the protoplasm of mesocarp cells. This reaction continues without interruption for about 4 months (Duran Grande *et al.* 1964). The maturation process is characterised by a change in fruit colour from green to purple or black, with different intensities in different cultivars.

The aim of the present research was to better characterise the magnitude of P_N and R_D in olive fruit during the overall fruit development cycle and during the day in order to define the significance of photosynthesis in fruit and the possible causes of its limitation.

Materials and methods

The experiments were done in central Italy (Foligno, 43°N latitude, 300 m altitude), in a 30-year-old non-irrigated olive orchard, growing in clay loam soil, with trees trained to the vase system and spaced 5×5 m. From full bloom (around mid-June) to harvest (end of November), every 15 d six samples of 100 fruits each were taken from six trees of cv. Leccino, with similar vegetative and productive characteristics (medium fruit load), in order to determine the transversal and longitudinal diameter, fresh mass, dry mass after drying at 85 °C for at least 72 h, and the oil, soluble sugars, and starch contents. On the same days, 3 samples of 4-10 fruits each (beginning with 10 fruits and progressively reducing the fruit number as fruit mass increased) were used to determine the CO_2 exchange rates in full sunlight and in the dark, photosynthetic I-response curves, transpiration rate (E), G_s, C_i, and Chl content. Because the CO_2 given off by the fruit was always higher than that assimilated, the photosynthetic activity, calculated on the basis of reduction of the released CO_2 induced by irradiation, was expressed as P_G calculated by summing net photosynthesis and R_D, assuming that dark and light respiration do not vary. Values were always collected on cloudless days, in the morning from 09:00 to 10:30 h.

Twenty-three days AFB, when fruit photosynthetic activity was fairly high, the gas exchanges were determined every 2 h during the day, from 08:00 to 18:00. Twenty-three, 45, and 132 d AFB, fruit samples were examined by scanning electron microscope (SEM) to check the nature of natural openings at the epicarp level.

Total Chl content was determined according to Bruinsma (1963). Fruits, previously weighed and frozen in liquid nitrogen, were ground and mixed with 20 cm^3 of 80 % acetone in a 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 645 and 663 nm by spectrophotometric analysis and Chl was calculated using the constants of Holm (1954).

Soluble sugar and starch contents were determined according to Morris (1948). Oil content was measured using a *Foss-let 1531* apparatus (*Foss Electric,* Hilleröd, Denmark).

Gas exchanges were determined using a *LCA-3* portable gas exchange analyser (*Analytical Development Co.*, Hoddesdon, England) using a Parkinson leaf chamber type *PLC-3FM*. The detached fruits were enclosed in the chamber, and the rate of CO_2 exchange was determined first in full sunlight (incoming photosynthetic photon flux density 1300-1500 $\mu mol\ m^{-2}\ s^{-1}$) and then in the dark. The flow rate of dried air passing through the chamber was kept at 6.7 $cm^3\ s^{-1}$. 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 2-4 °C higher than the atmospheric temperature, varying from 26 to 29 °C in June, from 28 to 36 °C in July-September, from 19 to 25 °C in October, and from 13 to 17 °C in November. The photosynthetic I-response curves were determined by covering the chamber with neutral shading nets to progressively decrease I until darkness (temperature decreased about 4 °C as I passed from 1300 to 0 $\mu mol\ m^{-2}\ s^{-1}$). Recordings were taken under steady-state conditions. In the linear portion of the photosynthetic I-response curve, from 0 to 160 $\mu mol\ m^{-2}\ s^{-1}$, apparent quantum yield (Y_Q) was calculated as the ratio between the increase of photosynthetic rate and of I ($Y_Q = \Delta P_N/\Delta I$).

For the SEM observations, four fresh fruit samples per time were fixed in 2.5 % glutaraldehyde for 2 h, rinsed in 0.1 M phosphate buffer (pH 7.2), and post-fixed in 1 % osmium tetroxide for 1 h. Samples were dehydrated in a series of increasing ethanol concentrations, at critical point dried in an oven (*Balzers Union*) placed on an aluminium mount, and gold coated in a SEM coating unit *E5100* (*Polaron Equipment*). Since the fruit surface of the 132-d-AFB-sample was covered by wax, a part of the fruit sample was washed with soap before fixing. The samples were then observed with a *501-B Philips* SEM.

The fruit parameters were referred to fruit DM and, only for the gas exchanges, also to one fruit and to fruit surface area. The fruit surface area was estimated assuming that the fruit was cylindrical in the middle part with spherical caps at the extremities.

Values are expressed as the means ± standard error. A cubic function was used to fit the relationship between I and CO_2 exchange rate.

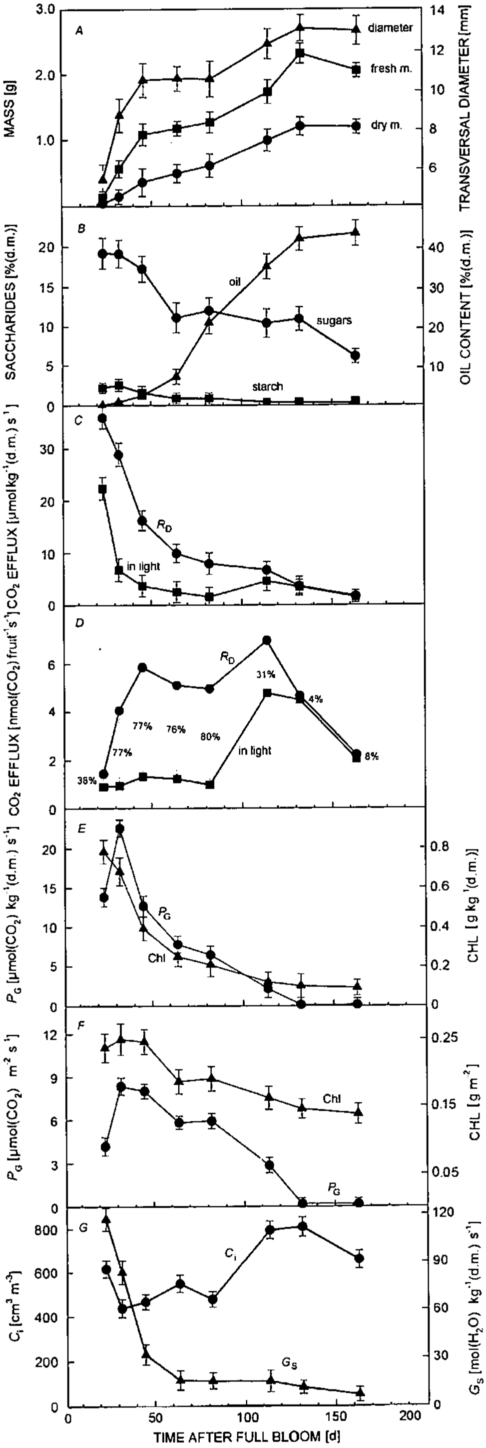

Fig. 1. Fruit mass and transversal diameter (*A*), saccharide and oil contents on a dry matter (DM) basis (*B*), CO_2 efflux in full sunlight and in the dark (dark respiration, R_D) on DM basis (*C*) or per fruit (*D*) or its % reduction in full sunlight with respect to R_D (*D*), gross photosynthetic rate (P_G) and chlorophyll (Chl) content on a DM (*E*) or surface area (*F*) bases, and intercellular CO_2 concentration (C_i) and stomatal conductance (G_s) on a DM basis (*G*) during fruit development. Each point is the mean of 6 (*A*, *B*) or 3 (*C*, *D*, *E*, *F*, *G*) replicates ± standard error.

Results

The pattern of fruit fresh mass and transversal diameter exhibited a double sigmoid curve (Fig. 1*A*). After a rapid increase up to 45 d AFB (3rd decade of July), there was a stasis of about 40 d followed by another increase until the end of October; fruit fresh mass decreased afterwards. The fruit dry mass showed a more linear increase from fruit-set to the end of October with a stasis afterwards.

The oil content increased rapidly from the beginning of August to the end of October (Fig. 1*B*). The soluble sugar content rapidly decreased from fruit-set to mid-August. After a stasis of about 2 months, it continued to decrease until the end of November. The starch content decreased from mid-July to the beginning of October, then it remained almost constant (about 0.5 % of DM).

The fruit R_D, on a DM basis, was high after fruit-set, then it rapidly decreased by more than 70 % in about 45 d, and finally slowly decreased to very low values (Fig. 1*C*). Fruit photosynthesis substantially reduced fruit CO_2 efflux. Considering one fruit, the photosynthetic activity in full sunlight reduced CO_2 efflux with respect to R_D by about 40 % during the initial phase of fruit growth, then, for about 2 months, the reduction was 75-80 %, and successively, during the last part of the fruit development cycle, the difference between CO_2 efflux in the dark and in the light decreased and became very small (Fig. 1*D*).

On a DM basis, the P_G was high in the first 3 weeks after fruit-set, then it progressively decreased, and became negligible in the last part of the fruit development cycle (Fig. 1*E*). The fruit Chl content, which at the beginning of development was relatively high, decreased rapidly during fruit ontogeny, with a trend similar to that of P_G. On a fruit surface area basis, P_G rapidly increased after fruit-set, remained at relatively high values for about 2 months, and then decreased (Fig. 1*F*). The Chl had a similar trend, but from September onwards its reduction rate was less than that of P_G.

In full sunlight, the fruit G_s per DM was very high at the beginning of fruit development, decreased markedly until mid-August, and then remained constant or decreased slightly (Fig. 1*G*). The fruit C_i was always relatively high, particularly from September onwards.

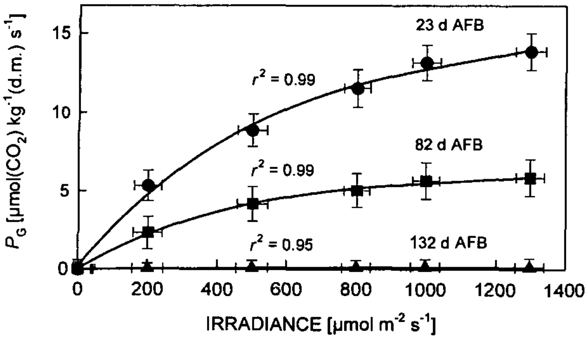

Fig. 2. Response of fruit gross photosynthetic rate (P_G) on a dry matter (DM) basis to irradiance by photosynthetic photon flux 23, 82, and 132 d after full bloom (AFB). Each point is the mean of 3 replicates ± standard error.

The fruit P_G was strongly influenced by I. At the beginning of fruit development it did not reach saturation in full sunlight (Fig. 2). The fruit P_G response to I decreased with fruit development, and at 132 d AFB (end of October) the P_G showed almost no response to increasing I. During fruit growth, Y_Q per fruit DM strongly decreased and was 23.0, 11.0, and 0.3 pmol(CO_2) s^{-1} per µmol(photon) m^{-2} s^{-1}, at 23, 82, and 132 d AFB, respectively.

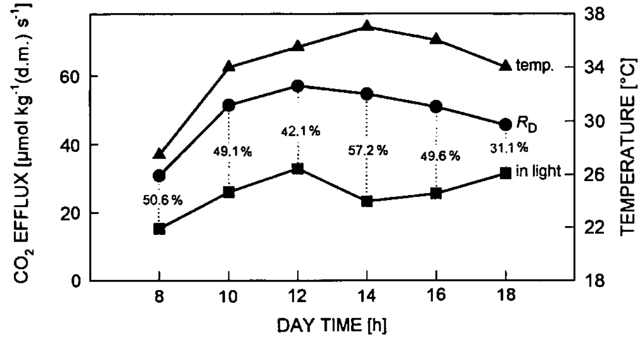

Fig. 3. Daily fruit CO_2 efflux in the full sunlight and in the dark (dark respiration, R_D) on a dry matter (DM) basis and temperature 23 d after full bloom. The % value is the reduction of CO_2 efflux in full sunlight with respect to R_D. For CO_2 efflux each point is the mean of 3 replicates.

The daily R_D had a trend similar to the temperature one: it was relatively low in the morning, then rapidly rose to values around 55 µmol(CO_2) kg^{-1}(DM) s^{-1} at 12:00 h followed by a slow decrease (Fig. 3). Fruit photosynthesis in full sunlight reduced diurnal CO_2 efflux with respect to R_D by about 50 % from 08:00 to 16:00 h, and by about 30 % at 18:00. The maximum P_G was observed at 14:00 h, and the lower ones, probably due to the low I, were observed early in the morning and late in the afternoon (Fig. 4*A*). In the light, the fruit G_s followed the magnitude of CO_2 intake (Fig. 4*B*). The fruit E increased greatly from 08:00 to 10:00 h, remained at high

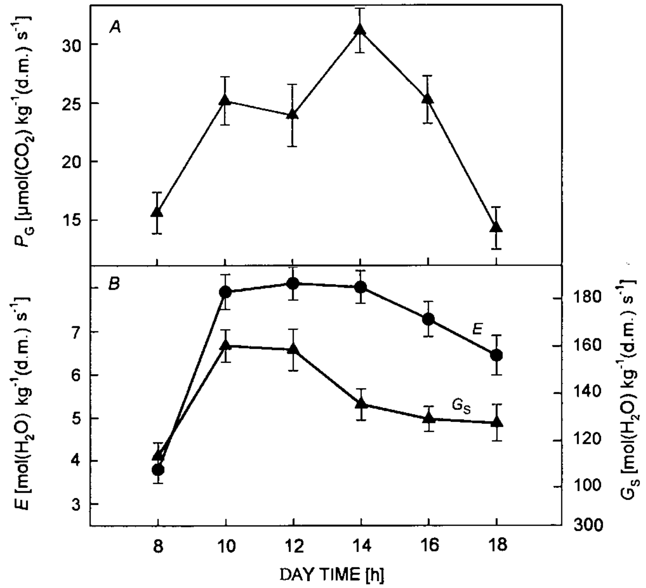

Fig. 4. Daily fruit gross photosynthetic rate (P_G) (*A*), and transpiration rate (*E*) and stomatal conductance (G_s) (*B*) on a dry matter (DM) basis 23 d after full blom. Each point is the mean of 3 replicates ± standard error.

values until 14:00, and then decreased slightly in the afternoon, following the temperature trend.

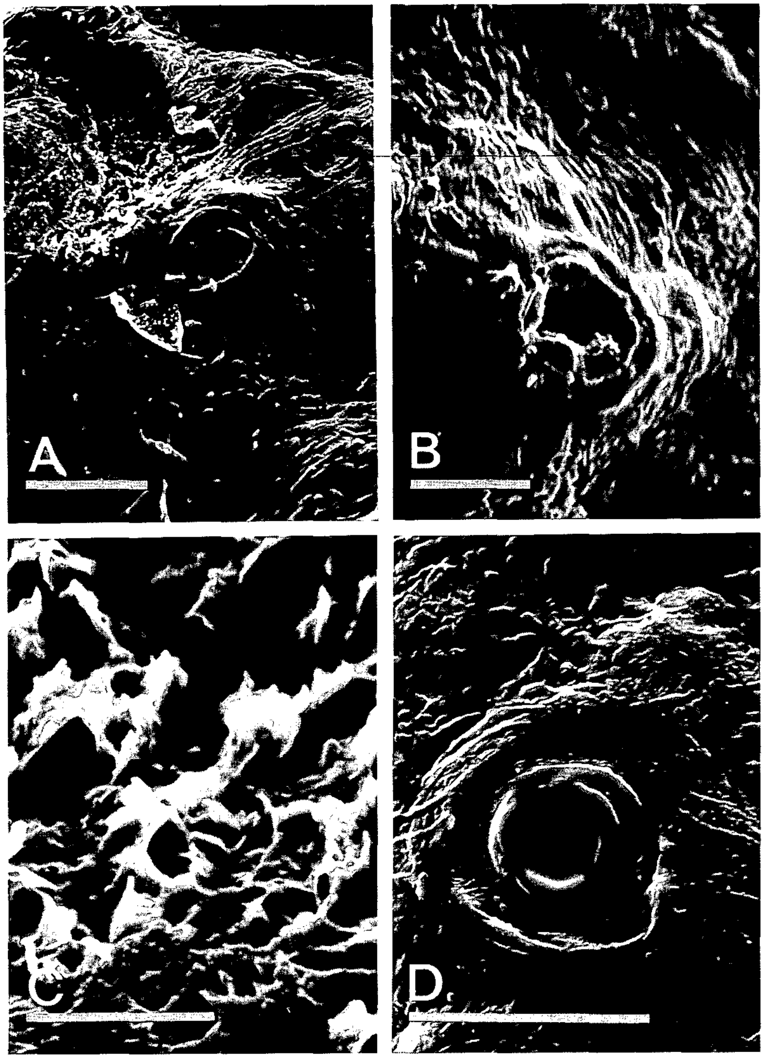

Fig. 5. Scanning electron micrographs of fruit surface: 23 (*A*) or 45 (*B*) d (bar = 50 μm) after full bloom (AFB), and 132 d AFB before (*C*, bar = 5 μm) or after (*D*, bar = 50 μm) fruit washing with soap.

At the beginning of fruit development, oval pores were observed on the fruit surface which were surrounded by cells similar in shape to guard cells of leaf stomata (Fig. 5). Two months later the stomata lost their shape and were successively covered by wax with a complex architecture.

Discussion

In olive fruit, from fruit-set to the end of October, there is a constant acquisition of DM. The first phase is characterised by a rapid increase in fruit size, then, in August, during the lignification of the endocarp cells, the volume increase stops, and then it recommences at the beginning of September until it definitively ceases at the end of October. Oil storage starts about one month AFB, but becomes consistent only 40-50 d AFB.

R_D is very high in the initial stage of development, due to cellular division. Consequently, C_i is also relatively high and, considering that during this period the fruit is rich in Chl, which on a DM basis is 25-30 % of the Chl leaf content, there are no limitations to fruit photosynthesis, with the exception of the capacity of radiant energy to reach the internal, Chl containing fruit layers. P_G increases greatly with the increase of I and, in full sunlight, has the highest values of the whole fruit development cycle.

R_D progressively decreases from few days after fruit-set onwards, due to the reduction of cellular division in internal fruit tissues, while C_i remains fairly high, due to the reduction of fruit photosynthetic activity. The decrease in fruit photosynthesis could be associated with the increase of fruit volume and to cellular differentiation. The increase of fruit volume makes penetration of PAR into the internal tissue layers very difficult. Furthermore, in these tissue layers CO_2 diffusion could also be strongly hindered because in fruit tissues, unlike mesophyll tissue, there is not an adequate intercellular space system, that is indispensable to enable the rapid diffusion of gaseous CO_2 for long distances (Virzo de Santo 1995). However, the difficulty of CO_2 diffusion does not seem to cause a photosynthesis decrease because, considering that the CO_2 produced by the R_D is always higher than that assimilated and due to the impermeability of the cuticle, the fruit accumulates high amounts of CO_2 in its free space (Sanchez 1994). The lignification of endocarp cells and the disposition of internal mesocarp cells for oil storage reduce the contents of Chl and other components of the photosynthetic apparatus in these cells. Consequently, Chl content tends to remain high only in the outer mesocarp layers. According to Sanchez *et al.* (1992), who found much higher photosynthetic activity in the epicarp than in mesocarp of maturing olive fruit, the decrease of P_G and Chl is less evident on a fruit surface area than DM basis until the beginning of September. These observations can explain the progressive reduction of Y_Q on a DM basis and the flattening of photosynthetic I-response curve. A constant high C_i (between 400 and 800 $cm^3\ m^{-3}$) seems to exclude that a decrease in fruit photosynthesis is caused by the reduction of stomatal functionality and the decrease of stomata number per unit fruit surface area. A confirmation of this is the observation that during the intermediate and final periods of fruit growth, P_G progressively decreases, while the G_s remains constant and C_i increases. So the wax covering of the cuticle and stomata does not seem to reduce photosynthesis, but it could be a suitable mechanism for reducing water loss and regulating the influx of nourishing substances through the phloem vessels in the fruit.

With the advance of fruit ripening, even if oil storage and protoplasm degradation processes are active, cellular metabolism decreases and both R_D and P_G become very small. During this period, Chl is replaced by other pigments.

Therefore, olive fruit exhibits the maximum CO_2 assimilation after fruit-set, when it has a high R_D, due to the intense metabolism associated with cellular division, an elevated Chl content, and a high ratio between fruit surface area and fruit volume. In this phase, fruit photosynthesis could be very important because, due to the consumption of most of the tree nutritional reserves for flowering and shoot growth, the fruit-set may be limited by competition for assimilates, which are substrates for respiration and energy sources for fruit maintenance and growth. So the fruit, when it is involved in building up a strong sink to compete with other organs or competitors, may obtain consistent help from autotrophic CO_2 assimilation. In this period, considering that the P_G, DM, and surface area of a leaf are about 13 μmol(CO_2) m^{-2} s^{-1}, 0.1 g, and 5 cm^2, respectively, fruit photosynthesis is about 30 and 60 % of that of leaf on a dry matter and surface area bases, respectively. In absolute terms, the CO_2 intake of one fruit is a little higher than that of a half leaf and, considering that in fruit shoots there are generally only 2-4 leaves per fruit, the CO_2 assimilation of lighted fruit could increase the nutritional availability for fruit-set and initial fruit development. Successively, for most of the fruit growing season, even if P_G on a DM basis progressively decreases, during the daylight fruit photosynthesis can enable the reassimilation of a large part (70-80 %) of the CO_2 lost by respiration thereby strongly reducing the fruit maintenance costs. Furthermore, because photosynthesis is performed inside the sink part, it also eliminates the CO_2 produced by the metabolic process (Xu *et al.* 1997).

The exposure of the fruit to sunlight increases not only fruit photosynthetic activity but also fruit *E* and so favours the income of nutritional and hormonal elements transported *via* xylem.

During the final period of fruit growth, due to cell specialisation, fruit photosynthesis has little importance in energetic terms, but the fruit lighting strongly influences oil synthesis (Sanchez *et al.* 1990, Sanchez 1995, Tombesi *et al.* 1997).

The saccharide content in the fruit is relatively high during the initial period of fruit growth, then decreases with the beginning of oil synthesis. This indicates the alternative function between the two substances. However, the greatest part of sugars for oil synthesis flows from nearby leaves (Proietti and Tombesi 1996).

In conclusion, our results suggest that olive fruit, even if it is an highly heterotrophic organ, should not be considered simply as a cost to the carbon budget of the plant. During the 40-50 d AFB, due to its CO_2-fixing capacity in the light, the olive fruit can recover 40-70 % of DM consumed by respiration during the daylight and supply itself with a substantial energetic contribution during a crucial stage of the fruit development cycle, favouring fruit maintenance and cellular division. These effects are very important for the amount of yield that largely depends on fruit number and size. During the subsequent growth period, CO_2 assimilation by the fruit, which strongly reduces the respiratory loss of CO_2 and increases the *E*, can improve fruit growth and oil synthesis. Therefore, the better fruit size and richness in oil observed in the well irradiated drupes (Ortega Nieto 1969, Sanchez 1994, Tombesi *et* *al.* 1997) may depend not only on good irradiance of the leaves near the fruit but also on a contribution by the fruit itself to CO_2 assimilation and on a high *E*. As suggested by Bazzaz *et al.* (1979) for other plant species, in order to improve yield quantity and quality, the extent to which the fruit photosynthetic activity might be improved should perhaps be considered. For this reason, considering that the fruit photosynthetic activity depends on fruit ontogenic stage, fruit temperature, fruit lighting, and probably on the number of competing fruits, the correct choice of the orchard exposure, planting space, training system, pruning, and the other cultural practices should promote not only leaf assimilate formation, but also fruit irradiation.

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