ELSEVIER

Scientia Horticulturae 85 (2000) 63–73

SCIENTIA HORTICULTURÆ

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Influence of leaf position, fruit and light availability on photosynthesis of two chestnut genotypes

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Accepted 1 November 1999

Abstract

The effects of canopy leaf position, light availability and the presence of fruit on leaf net photosynthesis rate (P_n) were evaluated in a chestnut orchard located in Central Italy. The study was carried out on *Castanea sativa* Mill. cv. 'Marrone di Stroncone', native to the Umbria Region, and on an interspecific hybrid *Castanea crenata* × *Castanea sativa*, cv. 'Marigoule'. 'Marrone di Stroncone' had a higher mean P_n than 'Marigoule'. In both genotypes, P_n changed during the growing season in response to phenological stages and, in particular, was higher during the main fruit growth period. From July to October the part of the shoot with higher P_n moved progressively from the base to the tip. During the day, P_n was high in the morning, and decreased progressively in the afternoon. The nearby presence of fruit increased P_n of leaves on shoots exposed to full sunlight, especially during the morning, but did not significantly influence the leaf chlorophyll (Chl) or carbohydrate contents. Light saturation for P_n was relatively high and there was a drastic reduction in P_n in leaves which, due to their position, intercepted a photosynthetic photon flux density (PPFD) lower than 300–400 μmol photon m^{-2} s^{-1} during the day. Shaded leaves were ca. 26% thinner than sunlit ones. Both the epidermal and mesophyll tissues were thicker in sunlit leaves, and the ''density'' of cells was higher in sunlit leaves. Morpho-anatomical and physiological adaptations allow the chestnut to optimise its use of the limited radiant energy available, but shading greatly reduces productivity. From the results it is affirmed that studies which consider photosynthetic assimilation must take into account the genotype, time of the day, growing season, leaf position and influence of the fruit. In order to increase the efficiency of the tree, it is important to ensure optimal

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PII: S0304-4238(99)00129-6

conditions for whole canopy P_n (light interception, water and nutrient availability, leaf integrity, etc.), particularly during those times when demand for assimilates is high. The chestnut genotypes studied have a very low P_n compared to other temperate fruit and nut trees.

Keywords: *Castanea sativa* Mill.; *Castanea crenata* Sieb and Zucc.; Leaf age; Daily photosynthesis; Shading; Chlorophyll and carbohydrate content; Leaf anatomy

1. Introduction

The formation of photosynthates is the basis of plant growth and production. Net photosynthesis (P_n) is influenced by environmental factors (light, temperature, CO_2 concentration, water, fertility of the soil, etc.) as well as by plant-related factors (morphology, structure and age of the leaf, presence of sinks, etc.). For chestnut there is little information on the relationship between gas exchange and the exogenous and endogenous factors of leaves (Deweirdt and Carlier, 1988; Araujo-Alves et al., 1993; Biricolti et al., 1993a,b; Mousseau, 1993; Lauteri et al., 1993; El-Koen et al., 1994; Frenguelli et al., 1995; Antognozzi et al., 1997; Palliotti et al., 1997). In order to increase our knowledge about such aspects, the influence of leaf age and its position on the shoot and in the crown, the presence of fruit, and light availability on seasonal and daily P_n were studied. It is useful to understand how such factors affect photosynthesis in order fully to comprehend this important physiological process. In turn this is helpful for improving productive models through the correct choice of the environment and the correct use of cultural practices.

The study was carried out on *Castanea sativa* Mill. and on an interspecific hybrid *Castanea crenata* × *Castanea sativa*. The European chestnut, *Castanea sativa*, probably the most economically important species of the genus *Castanea*, produces good quality fruit (the largest is called marron). The trees are very large and cause problems in orchard management because suitable dwarfing rootstocks are not available. The Japanese chestnut, *Castanea crenata* Sieb and Zucc., is a modest-sized tree with abundant fruit production, but the nuts are smaller and less flavoursome than those of the European chestnut. The hybrids *Castanea crenata* × *Castanea sativa* are less vigorous than the European chestnut but have better fruit quality than the Japanese chestnut and so have a high potential for new orchards.

2. Materials and methods

The trial was carried out in 1995 in a chestnut orchard in Central Italy (Terni, Umbria Region) on adult trees of *Castanea sativa*, cv. ‘Marrone di Stroncone’

found in the Umbria Region, and on *Castanea crenata* × *Castanea sativa*, cv. 'Marigoule'. The rate of net photosynthesis was measured between 8:30 and 10:00 in mid-July (end of blooming–fruit-set), mid-August (fruit growth) and mid-October (reserve accumulation and shoot lignification) on apical, middle and basal leaves of shoots with and without fruit (about two clusters/shoot), located in external, sunlit parts of the crown (sunlit leaves). For middle leaves only, P_n was also measured on shoots in internal, shaded crown portions (shaded leaves). In mid-August, on sunlit and shaded leaves inserted in the middle portion of shoots, the photosynthetic light response curve and, on sunlit leaves only, the photosynthetic daily trend were determined.

Measurements were made on cloudless days on leaves from five trees (three replications per tree) of each genotype with similar vegetative and productive characteristics, using an LCA-2 portable gas exchange analyzer (Analytical Development Company, Hoddesdon, UK) and a Parkinson leaf chamber type PLC(*n*). The leaf, immediately after detachment, was placed in the chamber and exposed perpendicular to the sun rays, with an incoming photosynthetic photon flux density (PPFD) of 1300–1500 μmol photon m^{-2} s^{-1}. Leaves were detached to allow easier measurement of P_n, since preliminary experiments showed that the detachment of leaves did not cause significant changes of P_n during the time the measurements were performed (30–50 s). The flow rate of air passing through the chamber was kept at 5 ml s^{-1} (about 0.5 ml s^{-1} cm^{-2} of leaf area). During gas exchange measurements, the external concentration of CO_2 was about 370 mg l^{-1} and the air temperature inside the leaf chamber was 2–3°C higher than the atmospheric temperature, varying from 26 to 32°C in July and August and from 19 to 22°C in October. The light response curve was determined by covering the leaf chamber with neutral shading nets.

After making the gas exchange measurements the leaves were taken to the laboratory in a portable refrigerator for the determination of specific leaf weight (SLW), carbohydrate and chlorophyll (Chl) content, and cyto-histo-anatomical investigation. Leaf area was measured using a leaf area meter (Hayashi Denkoh Co., Model AAM-7). Total, a and b Chl content were determined on one disc (1.13 cm^2), removed from each leaf according to the Bruinsma (1963) method. Half of the leaves were then used to determine soluble sugar and starch content (Morris, 1948) and to make transverse and tangential sections of the lamina. The remaining leaves were weighed and dried to constant weight in a forced air oven at 90°C to determine SLW (leaf dry weight/leaf area). For the sections, small portions (1–2 mm^2) of leaf lamina were collected and fixed for 5 h at room temperature in glutaraldehyde in 0.075 M cacodylate buffer at pH 7.0. The material was washed overnight in the same buffer, post-fixed for 2 h in buffered 1% OsO_4 and then dehydrated in a graded ethanol series. After treatment with propylene oxide, samples were embedded in an Epon mixture. Sections, about 2 μm thick, were stained in Toluidine blue for general purposes or periodic acid-

Schiff (PAS) reaction for localization of insoluble polysaccharides. Cyto-histo-anatomical investigations were carried out using a light microscope (×1000), equipped with an ocular micrometer to determine cellular dimensions of the different tissues in the leaf cross-section. The values reported are the average of about 15 observations.

The data collected were subjected to analysis of variance; for P_n a factorial scheme was used. For mean separation the Student–Newman–Keuls Test was used ($P \leq 0.05$). An exponential rise to maximum function [$y = a\,(1-e^{-bx}) + c$] provided the best fit for the relationship between PPFD and P_n (Goudriaan, 1979), where x is irradiance in μmol photons $m^{-2}\ s^{-1}$, e is the base of natural logarithms with values of 2.718 and a, b and c are coefficients.

3. Results and discussion

The ecotype of *Castanea sativa* 'Marrone di Stroncone' showed a much higher P_n than the interspecific hybrid *Castanea crenata* × *Castanea sativa*, cv. 'Marigoule' (Table 1). The higher P_n observed in 'Marrone di Stroncone' was not associated with a higher leaf Chl content. The leaf carbohydrate content and the SLW were not statistically different in the two genotypes.

During the growing season the highest P_n was observed in August, lower values were measured in mid-July, while the lowest values were found in October (Table 2). The P_n rates observed in this study were slightly lower than those observed in other chestnut cultivars (Biricolti et al., 1993b; Palliotti et al., 1997) and are low in comparison with many other fruit and nut species in the temperate zone. The low P_n rates observed in chestnut compared to other species, according to Andersen (1994), could be primarily attributed to non-stomatal limitations to CO_2 diffusion and fixation.

The leaf soluble sugar and starch content was higher in October than in July and August, although the total Chl content (Table 2) and Chl a/b ratio (data not

Table 1
Net photosynthesis rate, specific weight and total chlorophyll and carbohydrate content of sunlit leaves in 'Marrone di Stroncone' and 'Marigoule' genotypes[a]

Genotype	Net photosynthesis rate (μmol CO_2 $m^{-2}\ s^{-1}$)	Total chlorophyll (mg dm^{-2})	Soluble sugars (mg cm^{-2})	Starch (mg cm^{-2})	Specific weight (mg cm^{-2})
'M. Stroncone'	5.81 b	3.92 a	1.56 a	0.41 a	10.01 a
'Marigoule'	3.42 a	4.27 a	1.43 a	0.41 a	9.93 a

[a] Average values of apical, middle and basal leaves with and without fruit during all seasons are given. In each column, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 2
Net photosynthesis rate, specific weight and total chlorophyll and carbohydrate content of sunlit leaves during the growing season[a]

Time	Net photosynthesis rate (μmol CO_2 m^{-2} s^{-1})	Total chlorophyll (mg dm^{-2})	Soluble sugars (mg cm^{-2})	Starch (mg cm^{-2})	Specific weight (mg cm^{-2})
Mid-July	4.99 b	4.25 b	1.16 a	0.28 a	9.79 a
Mid-August	6.01 c	4.20 b	1.56 ab	0.39 ab	9.76 a
Mid-October	2.83 a	3.83 a	1.77 b	0.55 b	10.44 a

[a] Average values of apical, middle and basal leaves with and without fruit of both genotypes are given. In each column, means followed by the same letter are not significantly different at $P \leq 0.05$.

shown) were lower; 'Marrone di Stroncone' had a lower ratio than 'Marigoule' in October which may have been due to different degrees of leaf senescence. The SLW did not change significantly during the season. The effect of inflorescences or fruit on the shoot on leaf P_n changed during the growing season (interaction effect). In July, the nearness of inflorescences or recently set fruit did not significantly influence leaf P_n (Table 3); in August, leaves on fruiting shoots showed higher P_n than leaves on shoots without fruit; while in October, even at lower P_n, the presence of fruit on the shoot increased P_n. Also the effect of leaf position depended on the time of measurement (interaction effect). In July, the middle and especially the basal leaves had higher P_n than the apical ones (Table 4); in August and October the middle and the apical leaves, respectively, had the highest P_n. The presence/absence of fruit and leaf position did not substantially influence the leaf Chl and carbohydrate contents or SLW (data not shown).

The higher P_n observed in August, corresponding to strong fruit growth, and in mid-July, corresponding to the end of blooming–fruit-set, indicates that the

Table 3
Effect of growing season time and of the presence of fruit on net photosynthesis rate of sunlit leaves[a]

Time	Shoot	Net photosynthesis rate (μmol CO_2 m^{-2} s^{-1})
Mid-July	With fruit	5.10 c
	Without fruit	4.88 c
Mid-August	With fruit	6.52 e
	Without fruit	5.50 d
Mid-October	With fruit	3.13 b
	Without fruit	2.62 a

[a] Average values of apical, middle and basal leaves of both genotypes are given. In each column, means followed by the same letter are not significantly different at $P \leq 0.05$.

Table 4
Effect of time of year and of leaf position on net photosynthesis rate[a]

Time	Leaf	Net photosynthesis rate (μmol CO_2 m^{-2} s^{-1})
Mid-July	Apical	4.28 c
	Middle	5.25 d
	Basal	5.45 d
Mid-August	Apical	5.75 d
	Middle	6.85 e
	Basal	5.43 d
Mid-October	Apical	3.45 bc
	Middle	2.65 ab
	Basal	2.43 a

[a] Average values of leaves with and without fruit of both genotypes are given. In each column, means followed by the same letter are not significantly different at $P \leq 0.05$.

metabolite demand, associated with blooming, fruit-set and fruit growth can stimulate P_n. The lower P_n values observed in October, considering that the temperatures are still favorable for photosynthesis, are probably due to the lower assimilate demand, consequent to the end of fruit growth, and above all to the beginning of leaf senescence. In October, the higher leaf carbohydrate content could also be a result of the lower assimilate demand. In general, the presence of fruit stimulates P_n, however since no substantial differences in leaf starch content were observed, the lower P_n of leaves on non-fruiting shoots does not seem to be connected to the excessive accumulation of leaf starch as observed in other species (Flore and Gucci, 1988). This suggests that even in the absence of fruit, assimilates are very rapidly transferred from leaves to other tree organs. During the growing season the part of the shoot with higher P_n progressively shifts from the base to the tip of the shoot. Evidently, mature leaves have a higher P_n than younger ones until senescence begins.

In both genotypes, leaves in the inner portions of the crown, where the light availability was 40–100 μmol photon m^{-2} s^{-1} (equivalent to 5–8% of the full sunlight), showed a P_n 50% lower than the outside leaves at light saturation (Table 5). The pattern of photosynthetic response to light intensity of leaves developed in conditions of high and low light availability was similar in the two genotypes (Fig. 1). In the sunlit leaves the saturation PPFD was 1000–1200 μmol photon m^{-2} s^{-1}; at 300 and 400 μmol photons m^{-2} s^{-1}, respectively, for 'Marigoule' and 'Marrone di Stroncone', the P_n was half that at light saturation. Leaves grown at low light availability had lower values for photosynthetic light saturation point, dark respiration (about -1.1 vs. -2.2 μmol CO_2 m^{-2} s^{-1}) and light compensation point (about 30 vs. 70 μmol photon m^{-2} s^{-1}) than sunlit

Table 5
Net photosynthesis rate measured at light saturation, area, specific weight and total chlorophyll and carbohydrate content of middle leaves grown in inner, shaded, and outer, sunlit, portions of the canopy[a]

Leaf	Net photosynthesis rate (μmol CO_2 m^{-2} s^{-1})	Area (cm^{-2})	Total chlorophyll (mg dm^{-2})	Soluble sugars (mg cm^{-2})	Starch (mg cm^{-2})	Specific weight (mg cm^{-2})
Sunlit	4.92 b	52.5 a	4.21 a	1.53 b	0.37 b	10.3 b
Shaded	2.23 a	74.5 b	3.83 a	0.43 a	0.14 a	4.18 a

[a] Average values of both genotypes are given. In each column, means followed by the same letter are not significantly different at $P \leq 0.05$.

leaves; on the other hand, leaves grown at low light availability had a higher apparent quantum yield (about 0.049 vs. 0.040 mol CO_2 mol^{-1} photon). Compared to sunlit leaves the shaded ones were larger and had a higher Chl content on dry weight basis (the difference disappeared on leaf area basis) and a lower Chl a/b ratio (−20–40%), soluble sugar and starch content and SLW.

Both genotypes responded to different light availability during leaf growth with modified lamina thickness, which greatly influenced the SLW (Tables 5 and 6). The epidermal cells were thicker in sunlit leaves than in shaded ones; this difference was greater in the upper than in the lower epidermis. The mesophyll tissue (two layers of palisade cells and spongy parenchyma) was also thicker in sunlit leaves. Moreover, in sunlit leaves the "density" of palisade and spongy tissues, as measured by intercellular space, was higher than in the shaded ones (Table 7); this contributed to the reduction in SLW of shaded leaves. On the whole, the morpho-anatomical and physiological modifications observed in

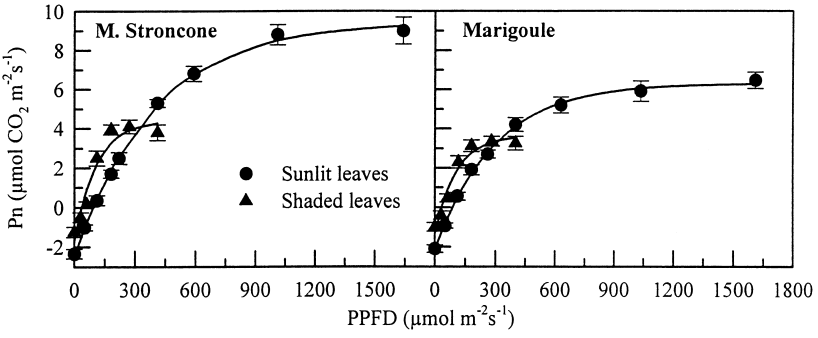

Fig. 1. Photosynthetic light response curve of sunlit and shaded middle leaves in 'Marrone di Stroncone' and 'Marigoule'. Bars represent standard error. Measurements were taken in mid-August.

Table 6
Cellular thickness in the different tissues identified in the transverse section of middle leaves grown in inner, shaded, and outer, sunlit, portions of the canopy[a]

Leaf	Lamina thickness (μm)	Upper epidermis (μm)	Palisade first layer (μm)	Palisade second layer (μm)	Spongy parenchyma (μm)	Lower epidermis (μm)
Sunlit	182 b	28.3 b	52.7 b	30.9 b	55.5 b	13.7 a
Shaded	135 a	24.3 a	35.0 a	22.8 a	40.5 a	12.9 a

[a] Average values of both genotypes are given. In each column, means followed by the same letters are not significantly different at $P \leq 0.05$.

Table 7
Percentage of intercellular space in the middle leaf tangential section in 'Marrone di Stroncone' and 'Marigoule' genotypes[a]

Leaf	Spongy parenchyma	Palisade first layer	Palisade second layer
Sunlit	53.4 a	15.6 a	33.4 a
Shaded	57.3 b	20.3 b	38.3 b

[a]The tangential section was taken as 100%. Average values of both genotypes are given. In each column, means followed by the same letter are not significantly different at $P \leq 0.05$.

shaded chestnut leaves, in agreement with Palliotti et al. (1997) and Frenguelli et al. (1995), show a good capacity to adapt to low light availability, facilitating interception of the low light available and reducing maintenance costs. No differences in lamina thickness and thickness of epidermis, palisade and spongy parenchyma between the two genotypes were observed (Table 8). This suggests that P_n differences between the two genotypes are not due to different thicknesses of leaf photosynthetic tissues. The presence of fruits on shoots located in the internal zone of the crown did not substantially affect P_n, suggesting that in such circumstances light is the main limiting factor (data not shown).

Table 8
Cellular height in the different tissues identified in the transverse section of middle leaves of the 'Marrone di Stroncone' and 'Marigoule' genotypes[a]

Leaf	Lamina thickness (μm)	Upper epidermis (μm)	Palisade first layer (μm)	Palisade second layer (μm)	Spongy parenchyma (μm)	Lower epidermis (μm)
'M. Stroncone'	151.5 a	25.1 a	40.7 a	25.9 a	45.4 a	13.7 a
'Marigoule'	165.5 a	27.4 a	47.0 a	27.9 a	50.6 a	12.8 a

[a] Average values of shaded and sunlit leaves are given. In each column, means followed by the same letter are not significantly different at $P \leq 0.05$.

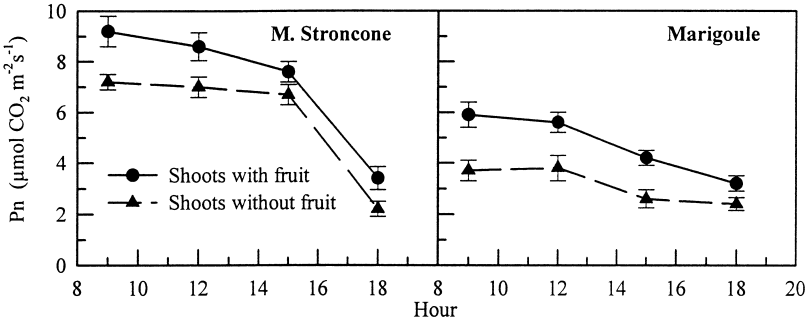

Fig. 2. Photosynthetic daily trend of the sunlit middle leaves on shoots with and without fruit in 'Marrone di Stroncone' and 'Marigoule'. Bars represent standard error. Measurements were taken in mid-August.

During the day, leaves in the outer portions of the crown showed the highest P_n in the morning until 12:00 a.m., then P_n decreased progressively reaching values 70% lower at 6:00 p.m. (Fig. 2). Leaves on fruiting shoots had higher P_n through the day than on shoots without fruit, although differences were greater in the morning than in the afternoon.

In conclusion, the results indicate that there are interesting physiological and biochemical differences between the European and the interspecific hybrid chestnut, with a higher P_n in the first genotype. In both genotypes, during the growing season the P_n changes in the different phenological stages and, in particular, it is higher during the fruit growth period. Measurements of daily P_n, taken in mid-August, show that P_n is high in the morning, then progressively decreases in the afternoon. The presence of fruit increases the P_n of nearby leaves, especially during the morning. Therefore, from these results, it can be affirmed that studies which consider photosynthetic assimilation must take into proper account the genotype, time of the day, growing season, leaf position and influence of the fruit.

Furthermore, it is important to consider that in the chestnut, the P_n light saturation is relatively high and there is a drastic reduction in P_n in leaves which, due to their position, intercept a low PPFD during the day. Therefore, even if morpho-anatomical and physiological adaptations allow the chestnut tree to optimize the use of the limited radiant energy availability, shading strongly reduces productivity. It is also noteworthy that the P_n rates observed in this study are very low in relation to many other fruit and nut species in the temperate zone. Andersen (1994), discussing the results of Deweirdt and Carlier (1988), concluded that chestnut, like citrus, has primarily non-stomatal limitations to CO_2 diffusion and fixation.

In order to increase tree efficiency, it is important to ensure optimal conditions (light interception, water and nutrient availability, protection from diseases and

insects, etc.) especially when the demand for assimilates is high. In particular it is important to manipulate factors such as orchard exposure, row orientation, plant spacing, training system, pruning, etc., which are able to reduce shading in the crown, ensuring that most of the leaves receive more than 300–400 μmol photon m^{-2} s^{-1} for the greater part of the day. This assumes greater importance in the European chestnut than in the interspecific hybrid chestnut because the former is more sensitive to low light availability and, having a high vigor, it has more shading within the crown. Low photosynthetic rate in the crops is not necessarily correlated with low yield, as is shown by citrus (Goldschmidt and Koch, 1996), and good yield of chestnut depends more on optimizing whole canopy P_n, rather than that of individual leaves.

Acknowledgements

Research was supported by MURST of Italy, ex-60% and C.N.R. (MARCFRU) funds.

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