ARTICLES

Mediterranean Botany
ISSNe 2603-9109

EDICIONES
COMPLUTENSE

https://dx.doi.org/10.5209/mbot.62325

Physiological, morphological and anatomical leaf traits variation across leaf development in *Corylus avellana*

Rosangela Catoni[1], Francesco Bracco[1], Loretta Gratani[2] & Mirko Umberto Granata[1]

Received: 8 November 2018 / Accepted: 14 March 2019 / Published online: 7 November 2019

Abstract. The study analyzed the variations of physiological, morphological and anatomical leaf traits during its development in *Corylus avellana* L. saplings. Three different phases were identify during leaf development: the first phase (hereafter IP) considered in the developing leaves, the second phase (IIP) in the mature green leaves and the third phase (IIIP) in the senescent leaves. In particular, variations in parameters estimated from the photosynthetic light response curves, in chlorophyll fluorescence variables and in morphological leaf traits were analyzed during the three phases. The principal component analysis (PCA) carried out using all the considered morphological characters (leaf mass per area – LMA, and leaf tissue density – LTD) and physiological traits (the maximum net photosynthetic rates – A_{Nmax}, dark respiration rates – R_{D}, light compensation point – LCP, light saturation point – LSP, maximum quantum yield – $\phi_{\max}$, fluorescence-based maximum quantum yield of PSII photochemistry – F_v/F_M and effective quantum yield of PSII photochemistry – ϕ_{PSII}) showed a complete separation among the considered phases. The results showed that the major differences occur between the phases IP and IIP. In particular, a greater variation was found for LMA, A_{Nmax}, R_{D}. On the contrary a lower variation was observed for $\phi_{\max}$ which remain quite constant from IP to IIP indicating that chloroplasts present in juvenile leaves are fully functional.

Keywords: Hazelnut; Leaf mass per area; Light compensation point; Maximum photosynthetic rates.

Variaciones fisiológicas, morfológicas y anatómicas en el desarrollo de las hojas de Corylus avellana

Resumen: Este trabajo analiza la variación de las características fisiológicas, morfológicas y anatómicas de las hojas durante su desarrollo en los plantones de *Corylus avellana* L. Se identificaron tres fases diferentes durante el desarrollo de la misma: la primera (IP) para las hojas en desarrollo, la segunda (IIP) para las hojas verdes maduras y la tercera (IIIP) para las hojas senescentes. En particular, se analizó la variabilidad de los parámetros estimados a partir de las curvas de respuesta a la luz fotosintética, de las variables de fluorescencia de la clorofila y de los rasgos morfológicos de las hojas durante las tres fases. El análisis de componentes principales (PCA) que se llevó a cabo utilizando todos los caracteres morfológicos estudiados (masa de la hoja por área – LMA y densidad del tejido de la hoja – LTD) y características fisiológicas (tasas netas de fotosíntesis máxima – A_{Nmax}, tasas de respiración oscura – R_D, el punto de compensación de luz – LCP, el punto de saturación de luz – LSP, el rendimiento cuántico máximo – $\phi_{\max}$, el rendimiento cuántico máximo basado en fluorescencia de la fotoquímica PSII – F_v/F_M y el rendimiento cuántico efectivo de la fotoquímica PSII – ϕ_{PSII}), mostraron una completa separación entre las fases estudiadas. Los resultados mostraron que las principales diferencias se producen entre las fases IP y IIP. En particular, se encontró una mayor variación para LMA, A_{Nmax}, R_{D}. Por el contrario, se observó una variación más baja para $\phi_{\max}$ que permanece bastante constante desde la IP hasta la IIP, lo que indica que los cloroplastos presentes en las hojas juveniles son completamente funcionales.

Palabras clave: Avellano; masa foliar por área; punto de compensación de luz; tasas de máxima fotosíntesis.

Introduction

Leaf development comprises an important period of leaf lifetime (Miyazawa *et al.*, 1998; 2003). It is a genetically controlled process (Tardieu *et al.*, 1999; Sugiyama, 2005; Varone & Gratani, 2009) which depends on several environmental conditions, such as photon flux density, air temperature and soil water status (Cookson *et al.*, 2005; Sui *et al.*, 2012).

Morphological, anatomical and physiological traits significantly vary during leaf development (Marchi *et al.*, 2008), which can be generally divided into three phases (Čatský & Šesták, 1997). The first phase concerns leaf blade formation. In this phase leaf is a net carbon-importing structure with low net assimilation rates (A_N) and high dark respiration rates (R_D) (Cai *et al.*, 2005) to sustain the construction cost of leaf structural compounds process (Armstrong *et al.*, 2006). In the following phase, leaf reaches the maximum expansion and becomes a photosynthetic exporting organ while its assimilation rates reach their peak (Sui *et al.*, 2012). The third phase is leaf senescence, in which leaf progresses into a period of massive mobilization and export of carbon and minerals to others plant compartments (Sui *et al.*, 2012). The A_N

---

[1] University of Pavia, Department of Earth and Environmental Sciences. Via S. Epifanio 14. 27100 Pavia, Italy. Corresponding author, Email: rosangela.catoni@unipv.it

[2] La Sapienza University of Rome, Department of Environmental Biology. P. le A. Moro, 5. 00185 Rome, Italy.

decline during leaf senescence was one of the earlier observations of the process in plants and has now been documented for a large number of species (Čatský & Šesták, 1997; Yoo *et al.*, 2003; Sui *et al.*, 2012).

With regard to the leaf morphological traits variation during leaf development, leaf mass per area (LMA) which is a pivotal trait for the carbon-fixation 'strategy' (Wright *et al.*, 2004) increases during leaf development due to the accumulation of carbon-rich compounds and the increase of leaf tissue density (LTD) (Niinemets, 2001).

Corylus avellana L. (hazelnut) is a shade-tolerant species (Kull & Niinemets, 1993; Catoni *et al.*, 2015a,b) which is able to colonize large gaps showing capacity to perform well in full sunlight (Kull & Niinemets, 1993; Catoni *et al.*, 2015a). It grows in the understory of European deciduous forests (Tutin *et al.*, 2001). This species is one of the major world's nut crops cultivated in orchard in full sunlight in several countries (Bignami *et al.*, 2009).

The aim of this study was to analyze leaf physiological, morphological and anatomical traits variation in *C. avellana* during leaf development, in order to understand which of the investigated traits undergoes the major variations.

Materials and methods

Study site and plant material

The experiment was carried out from March to September 2017 on *Corylus avellana* (hereafter *Corylus*) cv ´Tonda Gentile Trilobata´ saplings (10 three-year-old plants with a mean height of 70 ± 5 cm) growing in the open, at the Botanical Garden of the University of Pavia (45º11'8.93"N / 9º09"48.47"E). Five branches with vegetative buds were labelled for each of the considered saplings at the beginning of the growing season (i.e. beginning of March). Morphological and physiological measurements were carried out during leaf development at the first phase (IP, i.e. developing leaves), at the second phase (IIP, i.e. mature-green leaves) and at the third phase (IIIP, i.e. senescent leaves) according to Čatský & Šesták (1997).

Microclimate measurements were carried out over the study period. Air temperature (T_a,°C) and relative humidity (RH, %) were recorded at 5 min intervals by HOBO data loggers (H08–003–02, Onset HOBO Data Loggers, Cape Cod, MA); the photosynthetic photon flux density [PPFD, μmol(photons) m^{-2} s^{-1}] was recorded at 5 min intervals by Sunshine Sensor BF3 (Delta-T Device, UK). Moreover, during the course of the experiment, when necessary, hazelnut saplings were irrigated to maintain the soil at 100% of field capacity in order to avoid the contrasting effects due to low water availability, being hazelnut a sensitive species to water stress by its low capacity of stomatal control (Cristofori *et al.*, 2014; Catoni *et al.*, 2017).

Leaf morphology

Five leaf samples (on each sampling occasion), per each of the considered saplings were collected. The following parameters were measured: projected fresh leaf surface area excluding petioles (LA, cm^2) using Image Analysis System (Delta-T Devices, UK) and leaf dry mass (DM, mg) drying leaves at 80 °C to constant mass. Leaf mass per unit of leaf area (LMA, mg cm^{-2}) was calculated. Leaf tissue density (LTD, mg cm^{-3}) was calculated by the ratio of LMA and leaf thickness, this last measured by leaf sections from fresh of the selected saplings and measured using a light microscope.

Gas exchange measurements and chlorophyll content

Gas exchange measurements were carried out on five labeled leaves in each sampling–occasion by an infrared gas analyzer (LC*Pro*+, ADC, UK) equipped with a leaf chamber (PLC, Parkinson Leaf Chamber, UK). Measurements were performed in the morning (9.00h-12.00h) during two consecutive days for each developmental phase.

The leaf temperature at the time of all measurements varied between 18–25°C. The relationship between net assimilation rates (A_N) [μmol(CO_2) m^{-2} s^{-1}] and photosynthetic photon flux density (PPFD) [μmol(photons) m^{-2} s^{-1}] was performed using the auto-program function. In particular, nine steps in ascending order were selected: 0, 25, 50, 100, 200, 500, 750, 1000, and 1500 μmol(photons) m^{-2} s^{-1}, with a minimum wait time of 10 min and a maximum wait time of 20 min for the step at 0 μmol(photons) m^{-2} s^{-1}. The mathematical models used to fit A_N/PPFD curves were selected according to Lobo *et al.* (2013). From the selected model the following estimated parameters were considered: LCP = light compensation point [μmol(photons) m^{-2} s^{-1}]; LSP = light saturation point [μmol(photons) m^{-2} s^{-1}]; A_{Nmax} = maximum net photosynthetic rate [μmol(CO_2) m^{-2} s^{-1}]; R_D = dark respiration rate [μmol(CO_2) m^{-2} s^{-1}]; ϕ_{max} = maximum quantum yield [μmol(CO_2) μmol(photons)$^{-1}$], namely a measure of photosynthetic efficiency expressed in moles of photons absorbed per mole of CO_2 fixed (Skillman, 2008). Moreover, A_{Nmax} and R_D were also expressed on mass-based by use of LMA (Wright *et al.*, 2004). The ratio between R_D and A_{Nmax} indicative of leaf carbon balance was calculated (Loveys *et al.*, 2002).

Total chlorophyll (Chl) content was determined using a chlorophyll meter (SPAD-502, Minolta, Japan). Measurements were carried out on the same leaves used for gas-exchange (five leaves per each of the considered saplings, on each sampling occasion).

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was used to measure the following parameters: fluorescence-based maximum quantum yield of PSII photochemistry (F_V/F_M, i.e. the optimal quantum efficiency of PSII in dark-adapted leaves), effective quantum yield of PSII photochemistry (ϕ_{PSII}) and electron transport rate [ETR, μmol(e^-) m^{-2} s^{-1}] by using a portable modulated fluorometer (OS5p, Opti-Sciences, USA) on the same leaves used for gas exchange measurements.

For F_V/F_M determination, leaves were dark-adapted for 30 min using dark leaf clips. Afterwards, a saturating pulse was applied to measure initial (F_0) and maximum (F_M) fluorescence.

F_V/F_M was estimated as:

$$F_V/F_M = (F_M - F_0)/F_M.$$

ϕ_{PSII} was determined on light-adapted leaves, according to Genty *et al.* (1989) as:

$$\phi_{PSII} = (F_{M'} - F_s)/F_{M'}$$

where $F_{M'}$ was the maximum fluorescence obtained with a light-saturating pulse (~8000 μmol m^{-2} s^{-1}) and F_s was the steady-state fluorescence of illuminated leaves (1600 μmol m^{-2} s^{-1}).

Data analysis

The obtained data (expressed as mean ± standard error (±SE)) were analyzed by one-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test to compare differences (at $p \leq 0.05$) between means of the considered parameters among the three phases. All the statistic tests were performed by a statistical software package (PAST, version 3.1). A principal component analysis (PCA) was carried out in order to summarize the considered morphological (LMA and LTD) and physiological (A_{Nmax}, R_D/A_{Nmax}, R_D, R_D and A_{Nmax} expressed on a mass-base, Chl content, F_V/F_M, LCP, LSP, Φ_{PSII}, Φ_{max}) leaf traits into major components which explained their variation in the three phases.

Results

Environmental conditions and leaf morphology

Corylus bud break occurred at the third decade of March, when the mean air temperature was 12.9 ± 1.9°C, leaf expansion ended at the third decade of May, when mean air temperature was 23.0 ± 2.0°C, and leaves approaching the senescence phase at the end of September when air temperature was 16.6 ± 1.3 °C (Figure 1). Specifically, in the course of the leaf development, IP was considered 7 days after bud break (i.e. 27–28 March 2017), IIP after 65 days (i.e. 25–26 May 2017) and IIIP after 172 days (28–29 September 2017). Over the entire study period, microclimate measurements at individuals level showed a T_a range from 10.8°C to 28.4°C, a RH from 48% to 97%, and a PPFD from 1200 to 1650 μmol(photon) m^{-2} s^{-1}.

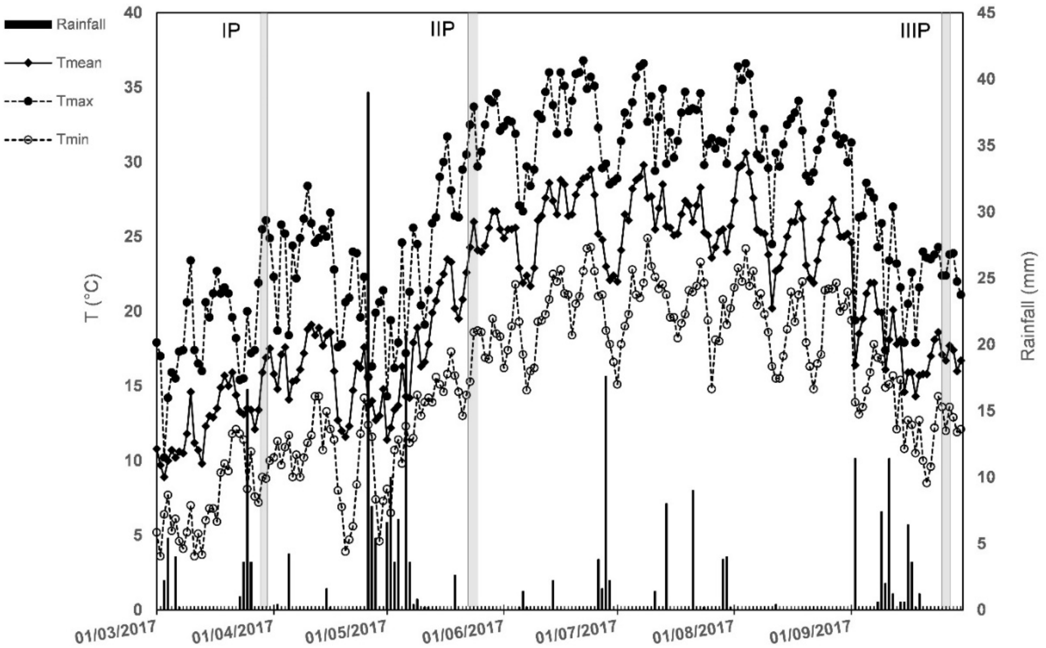

Figure 1. Trend of mean air temperature (T_{mean}), minimum air temperature (T_{min}), maximum air temperature (T_{max}) and rainfall during the study period (from the beginning of March to the end of September 2017). Grey bars indicated the sampling days for each of three phases considered during leaf development in *Corylus*. IP = first phase at developing leaf; IIP = second phase at fully developed leaf; IIIP = third phase at leaf senescence. Data from Lombardia Regional Agency for Environmental Protection, Meteorological Station of Pavia (v. Folperti), daily data for the period 1/03/2017 –30/09/2017.

Values of morphological parameters are show in Table 1. In particular, LMA showed the lowest value in IP (3.3 ± 0.1 mg cm^{-2}) increasing by more than 100% in IIP and IIIP. LTD was 405 ± 13 mg cm^{-3} in IP increasing by 70% and 63% in IIP and IIIP, respectively.

Table 1. Values of leaf mass per unit of leaf area (LMA, mg cm^{-2}) and leaf tissue density (LTD, mg cm^{-3}) in the three phases considered during leaf development in *Corylus*. IP = first phase at developing leaf; IIP = second phase at fully developed leaf; IIIP = third phase at leaf senescence. Different letters indicate significant differences (ANOVA, $p \leq 0.05$) across the three phases. Mean values (± SE) are shown (n = 50).

	LMA mg cm^{-2}	LTD mg cm^{-3}
IP	3.3 ± 0.1 a	405 ± 13 a
IIP	7.9 ± 0.2 b	688 ± 14 b
IIIP	7.1 ± 0.2 c	662 ± 16 b

Gas exchange measurements and chlorophyll content

Parameters estimated from the A_N/PPFD curves (i.e. A_{Nmax}, R_D, LCP, LSP and ϕ_{max}) are shown in Table 2. Among the three phases, IP showed the highest R_D associated with the lowest A_{Nmax} resulting in the highest R_D/A_{Nmax} (1.163 ± 0.058) (Figure 2). The highest A_{Nmax} was monitored in IIP associated to a low R_D as attested by the lowest R_D/A_{Nmax} (0.053 ± 0.004). When expressed on a mass-base, R_D showed the highest rate in IP [138 ± 2 μmol(CO_2) kg^{-1} s^{-1}] decreasing by 91% and 93% in IIP and IIIP, respectively. A_{Nmax} expressed on a mass-base showed the highest rate in IIP [212 ± 4 μmol(CO_2) kg^{-1} s^{-1}] decreasing by 41% and 27% in IP and IIIP, respectively. The highest LCP was found in IP [89.6 ± 4.3 μmol(photons) m^{-2} s^{-1}] decreasing by 82% and 92% in IIP and IIIP, respectively. The highest LSP was found in IIP [1278.5 ± 11.5 μmol(photons) m^{-2} s^{-1}], decreasing 63% and 29% in IP and IIIP, respectively. ϕ_{max} was the highest in IIP [0.061 ± 0.001 μmol(CO_2) μmol(photons)$^{-1}$] decreasing by 8% and 44% in IP and IIIP, respectively. Chl content was the highest in IIP (31.6 ± 0.5 SPAD unit) followed by IIIP (17.3 ± 0.7 SPAD unit) and IP (14.0 ± 0.6 SPAD unit) (Figure 3).

Table 2. Values of maximum net photosynthetic rate [$A_{Nmax,}$ μmol(CO_2) m^{-2} s^{-1}]; dark respiration rate [R_D, μmol(CO_2) m^{-2} s^{-1}]; light compensation point [LCP, μmol(photons) m^{-2} s^{-1}]; light saturation point [LSP, μmol(photons) m^{-2} s^{-1}]; maximum quantum yield [$\phi_{max,}$ μmol(CO_2) μmol(photons)$^{-1}$] in the three phases considered during leaf development in *Corylus*. IP = first phase at developing leaf; IIP = second phase at fully developed leaf; IIIP = third phase at leaf senescence. Different letters indicate significant differences (ANOVA, $p \leq 0.05$) among the three phases. Mean values (± SE) are shown (n = 10).

	IP	IIP	IIIP
A_{Nmax} [μmol(CO_2) m^{-2} s^{-1}]	4.28 ± 0.15a	16.72 ± 0.37b	10.89 ± 0.78c
R_D [μmol(CO_2) m^{-2} s^{-1}]	4.85 ± 0.04a	0.91 ± 0.07b	0.61 ± 0.02c
LCP [μmol(photons) m^{-2} s^{-1}]	89.6 ± 4.3a	15.7 ± 0.7b	7.1 ± 0.4c
LSP [μmol(photons) m^{-2} s^{-1}]	468.5 ± 9.6a	1278.5 ± 11.5b	909.6 ± 14.3c
ϕ_{max} [μmol(CO_2) μmol(photons)$^{-1}$]	0.056 ± 0.001a	0.061 ± 0.001b	0.034 ± 0.001c

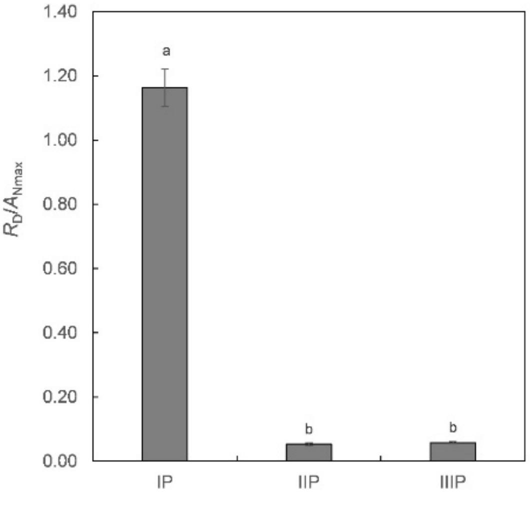

Figure 2. Values of the ratio of dark respiration rate to maximum net assimilation rate (R_D/-A_{Nmax}) in the three phases considered during leaf development in *Corylus*. IP = first phase at developing leaf; IIP = second phase at fully developed leaf; IIIP = third phase at leaf senescence. Different letters indicate significant differences among the three phases (ANOVA, p<0.05). Mean values (± SE) are shown (n = 10).

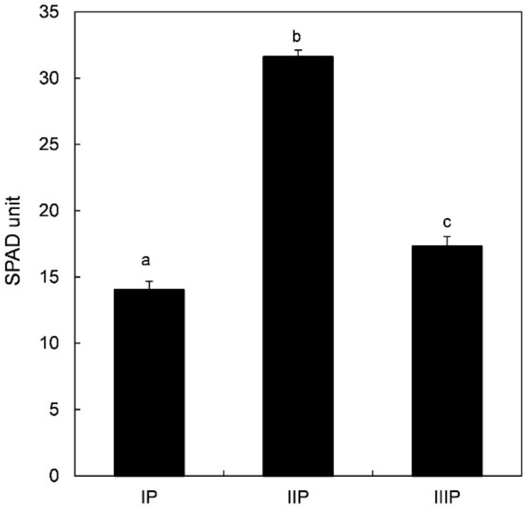

Figure 3. Values of chlorophyll content in three phases considered during leaf development in *Corylus*. IP = first phase at developing leaf; IIP = second phase at fully developed leaf; IIIP = third phase at leaf senescence. Different letters indicate significant differences among the three phases (ANOVA, p<0.05). Mean values (± SE) are shown (n = 50).

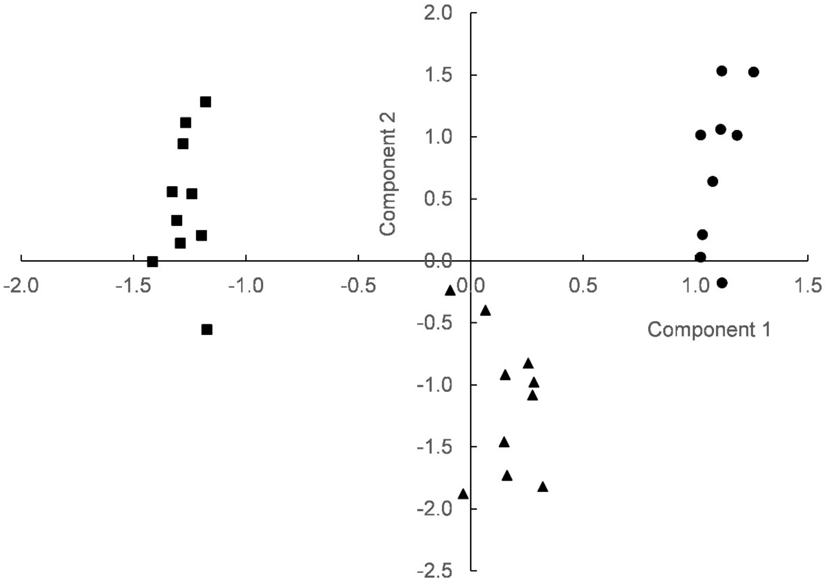

Figure 4. Principal component analysis (PCA) carried out using morphological (LMA and LTD) and physiological (A_{Nmax}, R_D/A_{Nmax}, R_D, R_D and A_{Nmax} expressed on a mass-base, Chl content, F_V/F_M, LCP, LSP, Φ_{PSII}, Φ_{max}) variables for the three phases of leaf development in *Corylus*. The 1st component, accounting for 96% of the total variance, was positively related to LSP, A_{Nmax}, F_V/F_M, Chl content, A_{Nmax} on a mass-base, LMA and LTD and negatively to LCP, R_D, R_D/A_{Nmax} and R_D on a mass-base. The 2nd component explained 3% of the total variance and it was correlated to Φ_{max}. ■ = first phase at developing leaf (IP), ● = second phase at fully developed leaf (IIP); ▲ = third phase at leaf senescence (IIIP).

Chlorophyll fluorescence measurements

Parameters obtained from chlorophyll fluorescence measurements are shown in Table 3. F_V/F_M was the highest in IIP (0.776 ± 0.007) decreasing by 11% and 9% in IP and IIIP, respectively. ϕ_{PSII} were the highest in IIP (0.343 ± 0.028) decreasing by 4% and 7% in IP and IIIP, respectively. ETR ranged from 157 ± 17 (IP) to 202 ± 16 (IIP).

Table 3. Values of fluorescence-based maximum quantum yield of PSII photochemistry (F_V/F_M), effective quantum yield of PSII photochemistry (ϕ_{PSII}) and electron transport rate [ETR, µmol(e$^-$) m^{-2} s^{-1}] in the three phases considered during leaf development in *Corylus*. IP = first phase at developing leaf; IIP = second phase at fully developed leaf; IIIP = third phase at leaf senescence. Different letters indicate significant differences (ANOVA, p ≤ 0.05) among the three phases. Mean values (± SE) are shown (n = 10).

	IP	IIP	IIIP
F_V/F_M	0.692 ± 0.011a	0.776 ± 0.007b	0.711 ± 0.009a
Φ_{PSII}	0.328 ± 0.027a	0.343 ± 0.028a	0.317 ± 0.027a
ETR [µmol(e$^-$) m^{-2}s^{-1}]	157 ± 17a	202 ± 16a	193 ± 16a

Principal Component Analysis

The PCA highlighted that the first two principal components accounted for 99% of the total variance. The first component explained 96% of the total variance and it was positively related to LSP (r = 0.997), A_{Nmax} (r = 0.953), F_V/F_M (r = 0.707), Chl content (r = 0.861), A_{Nmax} on a mass-base (r = 0.825), LMA (r = 0.920), LTD (r = 0.906) and negatively related to LCP (r = −0.846), R_D (r = −0.881) and R_D/A_{Nmax} (r = −0.896) and R_D on a mass-base (r = −0.901). The second component explained 3% of the total variance and it was correlated only to Φ_{max} (r = 0.825). A distinct split of the three phases was shown (Figure 4) with IIP displaying the greatest values related to the first component and IP the lowest ones, while IIIP was in the middle. Along the second component IIIP showed the lowest value and IP and IIP were found in the same position.

Discussion

The results show significant leaf traits variations during *Corylus* leaf development. In particular, LCP, LSP, A_{Nmax} and R_D, obtained from the light-response curves, highlight physiological variations during the three phases. A low A_{Nmax} in the first phase (i.e. developing leaves) is associated with the highest R_D resulting in a high R_D/

A_{Nmax} (1.163 ± 0.058), according with the results of Cai *et al.* (2005). The highest R_D rates are thought to facilitate leaf/shoot growth (Amthor, 2000) as indicate by low LMA (3.3 ± 0.1 mg cm^{-2}) which will increase more than 100% in the subsequent phases of leaves development (i.e. mature green leaves, IIP and senescent leaves, IIIP). The variations in LMA are linked to an increase in tissue density as confirmed by the lowest LTD, increasing by 70% and 63% in mature and senescent leaves (i.e, IIP and IIIP, respectively) compared to the developing leaves (i.e. IP). The highest R_D expressed on a mass-base [138 ± 2 μmol(CO_2) kg^{-1} s^{-1}], in developing leaves, stresses the metabolic expenditure of photosynthates in leaf since the mass-based measures may be more directly linked to chemistry and metabolism compared to area-based expressions (Reich & Walters, 1994).

Furthermore, the higher respiratory CO_2 evolution in the IP by reducing $A_{\text{Nmax,}}$ increases the light compensation point of photosynthesis (Cai *et al.*, 2005) which shows, in this phase, the highest value [LCP = 89.6 ± 4.3 μmol(photons) m^{-2} s^{-1}]. Despite at IP A_{Nmax} represents only the 25% of the its maximum value, reached in the subsequent phase (i.e. IIP), F_V/F_M, which represents the original activity of PSII (Hulsebosch *et al.* 1996), is 11% lower compared to the value measured in IIP (0.776 ± 0.007). This small change in F_V/F_M between IP-IIP indicates that young leaves have almost the same activity of primary charge separation as in mature leaves (Sui *et al.*, 2012). Thus, according to Weng *et al.* (2005) we can suppose that the activity of PSII might not be the major limiting factor of A_N in young leaves. Moreover, ϕ_{max} is quite constant from the first phase to the second phase, suggesting that photosynthetically active chloroplasts with fully coupled light and dark reactions of photosynthesis are present in juvenile leaves (Cai *et al.*, 2005). This result is also corroborate by the stable value of ϕ_{PSII}, which not show any significant differences among the considered phases. In fact, considering that ϕ_{PSII} measures the proportion of the light absorbed by chlorophyll associated to PSII that is used in photochemistry (Maxwell & Johnson, 2000), its stability across the phases suggests that the absorbed irradiance is used *via* photochemical reaction already at the first stage of leaf development until the senescence phase.

At the following phase (i.e. IIP) when leaves are mature and then completely structured, the shift from carbohydrate import (sink) to export (source) is reflected by the decrease of light compensation point [LCP = 15.7 ± 0.7 μmol(photons) m^{-2} s^{-1}] according to Cai *et al.* (2005).

This LCP value is lower compared to the value reported by Hampson *et al.* (1996) for hazelnut leaves grow in full sunlight, probably due to the lower R_D rates found in our study. Nevertheless, the observed R_D value [0.91 ± 0.07 μmol(CO_2) m^{-2} s^{-1}] falls within the range of sun plants [from 0.67 to 1.33 μmol(CO_2) m^{-2} s^{-1}] rather than in that for shade plants [0.06 – 0.16 μmol(CO_2) m^{-2} s^{-1}] as reported by Boardman (1977). This is in line with the results reported by Hampson *et al.* (1996), and seems to be related the facultative behavior of *C. avellana*, that, although it prefers to grown in shade conditions as just said, it performs well also under sunlight according to the results of Hampson *et al.* (1996), Valentini *et al.* (2009) and Catoni *et al.* (2015a). The 81% R_D decrease from IP to IIP reflects a lower ATP request (Armstrong *et al.*, 2006), and as the photosynthetic system matures, the requirement for respiratory energy decreases rapidly, resulting in a lower R_D/A_{Nmax} (0.053 ±0.004). In this phase, both Chl content and A_{Nmax} reached their highest values [31.6 ± 0.5 SPAD units and 16.72 ± 0.37 μmol(CO_2) m^{-2} s^{-1}, respectively] indicating that the total photochemical capacity, as well as the light-harvesting capacity, are increased. The lower proportion of absorbed photons with a slightly higher Fv/F_M in mature green leaves has been also observed by Cai *et al.* (2005) in tropical tree species.

At the last phase of leaf development (i.e. IIIP), a decrease of Chl content (by 45% compared to IIP) is the clear indicator of leaf senescence (Thomas & Stoddart, 1980). Moreover, the decline of chlorophyll content is accompanied by a decrease of both A_{Nmax} and R_D (by 35% and 33%, respectively, compared to IIP). It can be supposed that the decline in the photosynthesis at this phase may be mostly due to the decrease in carbon assimilation capacity, rather than the limitation of photochemical efficiency of PSII (Cai *et al.*, 2005), as highlighted by a slightly lower decrease (by 8%) of F_V/F_M.

The results of the PCA show a clear separation among the three phases, with IP and IIP more spaced indicating that almost all the considered leaf traits show their major variations in the period between the developing leaves and the mature green leaves. In particular, the major differences concern LSP, A_{Nmax}, R_D on a mass-base, LMA and LTD. The greater differences found for R_D on a mass-base, which reflects the metabolic expenditure of photosynthates in leaf, suggest that the major changes in this period are linked to leaf blade maturation, rather than the maturation of the photosynthetic machinery as also underlined by the greater variations in LMA and LTD. In fact, both ϕ_{PSII} and ϕ_{max} are the only parameters not related to the first component, confirming the presence of photosynthetically active chloroplasts and PSII reaction centers, which remain intact and functional across IP and IIP.

Overall, the analysis of leaf development in *C. avellana* underlines the presence of a photosynthetic machinery already functional from the first phase of leaf development. The period from the IP-IIP and the environmental conditions in which occurred represents a critical stage since can affects the achievement of an efficient photosynthesis and the subsequent formation of reproductive organs. Therefore, considering that hazelnut is an important horticultural tree crop, the variations occurring between IP and IIP can be consider crucial for the optimization of nut yield. This could mean that the non-achievement of the optimal maximum photosynthetic rates, particularly in the season that represents the most important period for nut production (i.e. April–May) could results in a high self-abortion rate and thus a lower nut yield. However, based on our results, to prevent this, it is important that the environmental conditions, in terms of photosynthetic photon flux density, air temperature and soil water status fit the species-specific requests since early stages of leaf development. For instance, leaves should receive from the first stages a PPFD around 1000 μmol(photons) m^{-2} s^{-1} in order to obtain the best hazelnut performance, indeed an insufficient light infiltration reduces bud formation, fruit set and quality in orchard crops as highlighted by Snelgar *et al.* (1992). Thus, in this context canopy management to manipulate light penetration results in a very important aspect as suggested by Hampson *et al.* (1996). Additionally, obtaining a high light level immediately in the first stage of leaf development, will allow leaves to acclimate to the light conditions typical of the hazelnut orchards. In this way, it is possible to avoid the transient photo-oxidative damages after transfer in full light conditions (Coopman *et al.*, 2008). Moreover, as a sensitive specie to water stress, water availability is an another important environmental parameter that needs to be checked in hazelnut orchards through the entire period of leaf development aiming to get the maximum efficiency of the photosynthetic machinery in order to achieve the best possible productivity.

Acknowledgments

This work was supported by the ´Natural Reserve Bosco Siro Negri´ funded by The Ministry of the Environment and Protection of Land and Sea of Italy.

References

Amthor, J.S. 2000. The McCree–de Wit–Penning de Vries–Thornley Respiration Paradigms: 30 Years Later. Ann. Bot. 86(1): 1–20.

Armstrong, A.F., Logan, D.C. & Atkin, O.K. 2006. On the developmental dependence of leaf respiration: responses to short– and long-term changes in growth temperature. Am. J. Bot. 93: 1633–1639.

Bignami, C., Cristofori, V., Ghini, P. & Rugini, E. 2009. Effects of irrigation on growthand yield components of hazelnut (Corylus avellana L.) in Central Italy. Acta Hort. 845: 309–314.

Boardman, N.K. 1977. Comparative photosynthesis of sun and shade plants. Ann. Rev. Plant Physiol. 28: 355–377.

Cai, Z.-Q., Slot, M. & Fan, Z.-X. 2005. Leaf development and photosynthetic properties of three tropical tree species with delayed greening. Photosynthetica 43: 91–98.

Catoni, R., Granata, M.U., Sartori, F., Varone, L. & Gratani, L. 2015a. Corylus avellana responsiveness to light variations: morphological, anatomical, and physiological leaf trait plasticity. Photosynthetica 53(1): 35–46.

Catoni, R., Gratani, L., Bracco, F. & Granata, M.U. 2017. How water supply during leaf development drives water stress response in Corylus avellana saplings. Sci. Hort. 214: 122–132.

Catoni, R., Gratani, L., Sartori, F., Varone, L. & Granata, M.U. 2015b. Carbon gain optimization in five broadleaf deciduous trees in response to light variation within the crown: correlations among morphological, anatomical and physiological leaf traits. Acta Bot. Croat. 74 (1): 71–94.

Čatský, J. & Šesták, Z. 1997. Photosynthesis during leaf development. In: Pessarakli, M. (Ed.) Handbook of Photosynthesis. Pp. 633–660. Marcel Dekker, New York, NY, USA.

Cookson, S.J., Van Lijsebettens, M. & Granier, C. 2005. Correlation between leaf growth variables suggest intrinsic and early controls of leaf size in Arabidopsis thaliana. Plant Cell Environ. 28: 1355–1366.

Coopman, R.E., Reyes-Díaz, M., Briceño, V.F., Corcuera, L.J., Cabrera, H.M. & Bravo L.A. 2008. Changes during early development in photosynthetic light acclimation capacity explain the shade to sun transition in Nothofagus nitida. Tree Physiol. 28: 1561–1571.

Cristofori, V., Muleo, R., Bignami, C. & Rugini, E. 2014. Long term evaluation of hazelnut response to drip irrigation. Acta Hort. 1052: 179–185.

Genty, B., Briantais, J.M. & Baker, N.R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. Biochim. Biophys. Acta 990: 87–92.

Hampson, C.R., Azarenko A.N. & Potter, J.R. 1996. Photosynthetic rate, flowering, and yield component alteration in hazelnut in response to different light environments. J. Am. Soc. Hort. Sci. 121(6): 1103–1111.

Hulsebosch, R.J., Hoff, A.J. & Shuvalov, V.A. 1996. Influence of KF, DCMU and removal of Ca^{+} on the high-spin EPR signal of the cytochrome b-559 heme Fe (III) ligated by OH^{-} in chloroplasts. Biochim. Biophys. Acta Bioenerg. 1277: 103–106.

Kull, O. & Niinemets, Ü. 1993. Variations in leaf morphometry and nitrogen concentration in Betula pendula Roth, Corylus avellana L. and Lonicera xylosteum L. Tree Physiol. 12: 311–318.

Lobo, F.A., de Barros, M.P., Dalmagro, H.J., Dalmolin, Â.C., Pereira, W.E., de Souza, É.C., Vourlitis, G.L. & Rodríguez Ortíz, C.E. 2013. Fitting net photosynthetic light-response curves with Microsoft Excel – a critical look at the models. Photosynthetica 51 (3): 445–456.

Loveys B.R., Scheurwater, I., Pons, T.L., Fitter, A.H. & Atkin. O.K. 2002. Growth temperature influences the underlying components of relative growth rate: an investigation using inherently fast- and slow-growing plant species. Plant Cell Environ. 25(8): 975–988.

Marchi, S., Tognetti, R., Minnocci, A., Borghi, M. & Sebastiani, L. 2008. Variation in mesophyll anatomy and photosynthetic capacity during leaf development in a deciduous mesophyte fruit tree (Prunus persica) and an evergreen sclerophyllous Mediterranean shrub (Olea europaea). Trees 22: 559–571.

Maxwell, K. & Johnson, G.N. 2000. Chlorophyll fluorescence–a practical guide. J. Exp. Bot. 51: 659–668.

Miyazawa, S.I., Makino, A. & Terashima, I. 2003. Changes in mesophyll anatomy and sink–source relationships during leaf development in Quercus glauca, an evergreen tree showing delayed leaf greening. Plant Cell Environ. 26: 745–755.

Miyazawa, S.I., Satomi, S. & Terashima, I. 1998. Slow leaf development of evergreen broad-leaved tree species in Japanese warm temperate forests. Ann. Bot. 82: 859–869.

Niinemets, Ü. 2001. Global-Scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. Ecology 82(2): 453–469.

Reich, P.B. & Walters, M.B. 1994. Photosynthesis–nitrogen relations in Amazonian tree species. II. Variation in nitrogen vis-a-vis specific leaf area influences mass– and area-based expressions. Oecologia 97: 73–81.

Skillman, J.B. 2008. Quantum yield variation across the three pathways of photosynthesis: not yet out of the dark. J. Exp. Bot. 59: 1647–1661.

Snelgar, W.P., Manson, P.J. & Martin, P.J. 1992. Influence of time of shading on flowering and yield of kiwifruit vines. J. Hort. Sci. 67: 481–487.

Sugiyama, S. 2005. Developmental basis of interspecific differences in leaf size and specific leaf area among C3 grass species. Funct. Ecol. 19: 916–924.

Sui, X.-L., Mao, S.-L., Wang, L.-H., Zhang, B.-X. & Zhang, Z.-X. 2012. Effect of low light on the characteristics of photosynthesis and chlorophyll a fluorescence during leaf development of sweet pepper. J. Integr. Agric. 11(10): 1633–1643.

Tardieu, F., Granier, C. & Muller, B. 1999. Modelling leaf expansion in a fluctuating environment: are changes in specific leaf area a consequence of changes in expansion rate? New Phytol. 143: 33–43.

Thomas, H. & Stoddart, J.L. 1980. Leaf senescence. Ann. Rev. Plant Physiol. 31: 83–111.

Tutin, T.G., Heywood, V.H., Burges, N.A., Valentine, D.H., Walters, S.M. & Webb, D.A. 2001. Flora Europaea, Volumes 1–5. Cambridge University Press, Cambridge.

Valentini, N., Caviglione, M., Ponso, C., Lovisolo, C. & Me, G. 2009. Physiological aspects of hazelnut trees grown in different training systems. Acta Hort. 845: 233–237.

Varone, L. & Gratani, L. 2009. Leaf expansion in Rhamnus alaternus L. by leaf morphological, anatomical and physiological analysis. Trees 23: 1255–1262.

Weng, X.Y., Xu, H.X. & Jiang, D.A. 2005. Characteristics of gas exchange, chlorophyll fluorescence and expression of key enzymes in photosynthesis during leaf senescence in rice plant. J. Integr. Plant Biol. 47: 560–566.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J., Diemer, M., Flexas, J., Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B., Lee, T., Lee, W., Lusk, C. & Villar, R. 2004. The worldwide leaf economics spectrum. Nature 428: 821–827.

Yoo, S.D., Greer, D.H., Laing W.A. & McManus, M.T. 2003. Changes in photosynthetic efficiency and carotenoid composition in leaves of white clover at different developmental stages. Plant Physiol. Biochem. 41: 887–893.