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

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**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*\(_{\text{max}}\)*, dark respiration rates – *R*\(_{\text{D}}\), light compensation point – LCP, light saturation point – LSP, maximum quantum yield – *ϕ*\(_{\text{psII}}\), fluorescence-based maximum quantum yield of PSII photochemistry – *F*\(_{\text{v}}*\), and effective quantum yield of PSII photochemistry – *F*\(_{\text{m}}*\) 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*\(_{\text{max}}\), *R*\(_{\text{D}}\). On the contrary a lower variation was observed for *ϕ*\(_{\text{psII}}\) 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*\(_{\text{max}}\), tasas de respiración oscura – *R*\(_{\text{D}}\), el punto de compensación de luz – LCP, el punto de saturación de luz – LSP, el rendimiento cuántico máximo – *ϕ*\(_{\text{psII}}\), el rendimiento cuántico máximo basado en fluorescencia de la fotoquímica PSII – *F*\(_{\text{v}}*\), y el rendimiento cuántico efectivo de la fotoquímica PSII – *F*\(_{\text{m}}*\)) 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*\(_{\text{max}}\), *R*\(_{\text{D}}\). Por el contrario, se observó una variación más baja para *ϕ*\(_{\text{psII}}\) 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*\(_{n}\)
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 (Catský & 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 (45°11'8.93"N / 9°09"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 (IIIP, i.e. mature-green leaves) and at the third phase (IIIP, i.e. senescent leaves) according to Catský & Šesták (1997).

Microclimate measurements were carried out over the study period. Air temperature (T, °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(photon)s m⁻² s⁻¹) was recorded at 5 min intervals by Sunshine Sensor BF3 (Delta-T Devices, 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, cm²) 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 (LCPro+, 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 (Aᵣ) [µmol(CO₂) m⁻² s⁻¹] and photosynthetic photon flux density (PPFD) [µmol(photon)s 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(photon)s 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(photon)s m⁻² s⁻¹.

The mathematical models used to fit Aᵣ/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(photon)s m⁻² s⁻¹]; LSP = light saturation point [µmol(photon)s m⁻² s⁻¹]; A_max = maximum net photosynthetic rate [µmol(CO₂) m⁻² s⁻¹]; R_d = dark respiration rate [µmol(CO₂) m⁻² s⁻¹]; ϕ_P = maximum quantum yield [µmol(CO₂) µmol(photon)s⁻¹], namely a measure of photosynthetic efficiency expressed in moles of photons absorbed per mole of CO₂ fixed (Skillman, 2008). Moreover, A_max and R_d were also expressed on mass-based by use of LMA (Wright et al., 2004). The ratio between R_d and A_max 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 (Fv/Fm, i.e. the optimal quantum efficiency of PSII in dark-adapted leaves), effective quantum yield of PSII photochemistry (Fv'/Fm') 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:

$$F_{V}/F_{M} = (F_M - F_0)/F_M$$

$\Phi_{PSII}$ 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 ($\sim 8000 \mu\text{mol m}^{-2}\text{s}^{-1}$) and $F_s$ was the steady-state fluorescence of illuminated leaves ($1600 \mu\text{mol m}^{-2}\text{s}^{-1}$).

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 ($A_{\text{max}}$, $R_d/A_{\text{max}}$, $R_D$, $R_{D}$ and $A_{\text{max}}$ expressed on a mass-base, Chl content, $F_{V}/F_{M}$, LCP, LSP, $\Phi_{PSII}$, $\Phi_{\text{max}}$) 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 $12.9 \pm 1.9^\circ C$, leaf expansion ended at the third decade of May, when mean air temperature was $23.0 \pm 2.0^\circ C$, and leaves approaching the senescence phase at the end of September when air temperature was $16.6 \pm 1.3^\circ C$ (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 ($T_{\text{mean}}$), minimum air temperature ($T_{\text{min}}$), maximum air temperature ($T_{\text{max}}$) 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.](image)

Values of morphological parameters are show in Table 1. In particular, LMA showed the lowest value in IP $(3.3 \pm 0.1 \text{ mg cm}^{-2})$ increasing by more than 100% in IIP and IIIP. LTD was $405 \pm 13 \text{ mg cm}^{-2}$ 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 ≤ 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 \(A_\text{/PPFD}\) curves (i.e. \(A_{\text{max}}, R_\text{d}, \text{LCP}, \text{LSP} \text{ and } \phi_{\text{max}}\)) are shown in Table 2. Among the three phases, IP showed the highest \(R_\text{d}\) associated with the lowest \(A_{\text{max}}\) resulting in the highest \(R_\text{d}/A_{\text{max}} (1.163 ± 0.058)\) (Figure 2). The highest \(A_{\text{max}}\) was monitored in IIP associated to a low \(R_\text{d}\) as attested by the lowest \(R_\text{d}/A_{\text{max}} (0.053 ± 0.004)\). When expressed on a mass-base, \(R_\text{d}\) 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. \(A_{\text{max}}\) 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. \(\phi_{\text{max}}\) 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 \([A_{\text{max}}, \mu\text{mol}(\text{CO}_2) \text{ m}^2 \text{s}^{-1}]\); dark respiration rate \([R_\text{d}, \mu\text{mol}(\text{CO}_2) \text{ m}^2 \text{s}^{-1}]\); light compensation point \([\text{LCP}, \mu\text{mol}(\text{photons}) \text{ m}^2 \text{s}^{-1}]\); light saturation point \([\text{LSP}, \mu\text{mol}(\text{photons}) \text{ m}^2 \text{s}^{-1}]\); maximum quantum yield \([\phi_{\text{max}}, \mu\text{mol}(\text{CO}_2) \mu\text{mol}(\text{photons})^{-1}]\) 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 ≤ 0.05) among the three phases. Mean values (± SE) are shown (n = 10).

|       | IP        | IIP       | IIIP      |
|-------|-----------|-----------|-----------|
| \(A_{\text{max}}\) [\mu\text{mol}(\text{CO}_2) \text{ m}^2 \text{s}^{-1}] | 4.28 ± 0.15a | 16.72 ± 0.37b | 10.89 ± 0.78c |
| \(R_\text{d}\) [\mu\text{mol}(\text{CO}_2) \text{ m}^2 \text{s}^{-1}] | 4.85 ± 0.04a | 0.91 ± 0.07b | 0.61 ± 0.02c |
| LCP [\mu\text{mol}(\text{photons}) \text{ m}^2 \text{s}^{-1}] | 89.6 ± 4.3a | 15.7 ± 0.7b | 7.1 ± 0.4c |
| LSP [\mu\text{mol}(\text{photons}) \text{ m}^2 \text{s}^{-1}] | 468.5 ± 9.6a | 1278.5 ± 11.5b | 909.6 ± 14.3c |
| \(\phi_{\text{max}}\) [\mu\text{mol}(\text{CO}_2) \mu\text{mol}(\text{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 \(R_\text{d}/A_{\text{max}}\) 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).
Chlorophyll fluorescence measurements

Parameters obtained from chlorophyll fluorescence measurements are shown in Table 3. Fv/FM was the highest in IIP (0.776 ± 0.007) decreasing by 11% and 9% in IP and IIIP, respectively. ΦPSII 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 (Fv/FM), effective quantum yield of PSII photochemistry (ΦPSII) 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                  |
|------------|---------------------|----------------------|-----------------------|
| Fv/FM      | 0.692 ± 0.011a      | 0.776 ± 0.007b       | 0.711 ± 0.009a        |
| ΦPSII      | 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), Amax (r = 0.953), Fv/FM (r = 0.707), Chl content, Amax on a mass-base (r = 0.825), LMA (r = 0.920), LTD (r = 0.906) and negatively related to LCP (r = −0.846), Rd (r = −0.881) and Rd/Amax (r = −0.896) and Rd on a mass-base (r = −0.901). The second component explained 3% of the total variance and it was correlated only to Φmax (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, Amax and Rd obtained from the light-response curves, highlight physiological variations during the three phases. A low Amax in the first phase (i.e. developing leaves) is associated with the highest Rd resulting in a high Rd/Amax.
to be related the facultative behavior of the results reported by Hampson et al. (1996) 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 $R_{ip}$ expressed on a mass-base [138 ± 2 µmol(CO₂) 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 CO₂ evolution in the IP by reducing $A_{max}$ 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(photos) m⁻² s⁻¹]. Despite at IP $A_{max}$ represents only the 25% of the its maximum value, reached in the subsequent phase (i.e. IIP), $V_{m}/F_{M}$ 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 $A_{c}$ in young leaves. Moreover, $\phi_{psii}$ 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 $\phi_{psii}$ which show any significant differences among the considered phases. In fact, considering that $\phi_{psii}$ 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(photos) 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 $R_{ip}$ rates found in our study. Nevertheless, the observed $R_{ip}$ value [0.91 ± 0.07 µmol(CO₂) m⁻² s⁻¹] falls within the range of sun plants [from 0.67 to 1.33 µmol(CO₂) m⁻² s⁻¹] rather than in that for shade plants [0.06 – 0.16 µmol(CO₂) 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 grow 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% $R_{ip}$ 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 $R_{D}/A_{max}$ (0.053 ± 0.004). In this phase, both Chl content and $A_{max}$ reached their highest values [31.6 ± 0.5 SPAD units and 16.72 ± 0.37 µmol(CO₂) 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 $F_{v}/F_{M}$ 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 $A_{max}$ and $R_{ip}$ (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, $A_{max}$, $R_{ip}$ on a mass-base, LMA and LTD. The greater differences found for $R_{ip}$ 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 $\phi_{psii}$ and $\phi_{max}$ 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-abortment 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.

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