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BRIEF COMMUNICATION

The photosynthetic performance of sterile and fertile sporophytes in a natural population of the fern *Dryopteris affinis*

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Abstract

We studied the photosynthetic performance of sterile and fertile sporophytes in a natural population of the fern *Dryopteris affinis* growing within a riparian forest (Central Italy) using chlorophyll (Chl) *a* fluorescence transients, the OJIP phase, where O is for the minimum fluorescence, P is for the peak (the maximum), and J and I are inflections. The "vitality" of the samples was assessed by the maximum quantum yield of primary photochemistry obtained indirectly from the fluorescence data (F_v/F_m); in the same way, the so-called performance index (PI_{ABS}) was obtained from fluorescence data. The photosynthetic performance (inferred from PI_{ABS}) of *D. affinis* changed significantly with the seasonal development of the fronds. The highest photosynthetic performance was recorded in the summer, corresponding to the period of spore release. The photosynthetic performance decreased in the winter, down to the minimal values of senescent fronds reached at the end of the seasonal cycle (May–June). On the whole, during the seasonal development, sterile and fertile fronds had a similar photosynthetic behaviour, as inferred from fluorescence data. At the end of spore maturation and dispersal (September–October), the fertile fronds showed somewhat lower photosynthetic performance than the sterile fronds, as revealed by PI_{ABS}. Being a long-lived fern, confined to humid and undisturbed sites in the Mediterranean, *D. affinis* deserves to be further investigated as a potential indicator of ecological continuity in Mediterranean riparian forests.

Additional keywords: chlorophyll fluorescence induction; JIP-test; Mediterranean area; performance index; photosynthesis; pteridophytes.

Ferns are sensitive components of ecosystems. Similar to other plants, they display a seasonal acclimation of photosynthetic activity to changing environmental conditions (Volkova *et al.* 2011), with a transition from the sterile to the fertile phase (Bauer *et al.* 1991). Stress conditions, such as during dry or extremely cold periods, may lead to different physiological responses during different growth phases (Pereira and Chaves 1995, Gratani *et al.* 1998).

In this study, we investigated the photosynthetic performance, through Chl *a* fluorescence measurements, of the sterile and fertile sporophytes in a natural population of the fern *D. affinis* (R. Lowe) Fras.-Jenk. ssp. *affinis* (hereafter referred as *D. affinis*) in a humid Mediterranean environment (Central Italy). Little is known about the photosynthetic performance of *D. affinis* in its habitat. The objective of the study was to determine whether its photosynthetic performance changed with the seasonal development of the fronds between sterile and fertile sporophytes.

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Abbreviations: ABS - absorbed energy flux; ABS/RC − absorbed energy flux per active reaction centre of photosystem II; Chl − chlorophyll; EM – emergent fronds; ET − energy flux of electron transport; F_0 and F_m − minimum and maximum Chl *a* fluorescence; $F_v = (F_m - F_0)$ − variable fluorescence; LED – light emitting diode; PI_{ABS} − performance index (potential) for energy conservation from exciton trapping to the reduction of the intersystem electron; Q_A − primary quinone electron acceptor of PSII; PSII – photosystem II; RC − reaction centre; SE – senescent fronds; SU – summer fronds; TR − energy flux for trapping; $\gamma_{RC} = Chl_{RC}/Chl_{total}$ − probability that PSII Chl molecule functions as RC; $\varphi_{P0} = TR_0/ABS = F_v/F_m$ − maximum quantum yield of primary photochemistry of PSII; WI – winter fronds; $\psi_{Eo} = ET_0/TR_0$ − probability that an electron is transferred further than to the electron acceptor Q_A.

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D. affinis is a long-lived herbaceous fern, with monomorphic fronds subdivided into pinnae and small pinnulae (no morphological differences between the fertile and sterile frond), that commonly grows on moist soils in woodlands throughout Europe. In Mediterranean ecosystems, it is mainly confined to very humid sites.

Sporophytes of *D. affinis* were investigated within a population extending over an area of 10 × 30 m along a narrow stream inside the Biogenetic Reserve "Tocchi" (43°13'68"N, 11°25'35"E, Tuscany, Central Italy). This population is located in a riparian forest dominated by *Alnus glutinosa*. The climate is Mediterranean, characterized by dry summers and rain during the spring and the autumn seasons. The hottest months are July and August, and the coldest being January and February. Referring to the study period, the annual precipitation was approximately 1,100 mm and the mean annual temperature was 12.1°C. However, the climate is mitigated by microclimatic characteristics of the riparian forest: all the ferns are confined to very humid and shaded sites, sheltered from direct solar radiation, living under tree canopies during the summer. The sun irradiance was recorded during Chl fluorescence measurements; values ranged between 100–700 μmol(photon) m^{-2} s^{-1} PAR in the winter and 500–1,000 μmol(photon) m^{-2} s^{-1} PAR in the summer. Furthermore, a tree coverage (95–98%), soil characteristics within a depth of 20 cm (pH = 4), and topography (altitude = 350–365 m, slope 18–20°, east orientation) were uniform throughout the site.

Within the population, five sterile (immature, 2–7 years old) and five fertile (mature or generative, more than 10 years old) sporophytes were randomly selected. Each sporophyte is a rosette of fronds (or ramets) arising by a vegetative reproduction or from the spores. Fully expanded fronds have a dark green lamina that lasts for 13–14 months, until the late spring, when a set of new fronds begin unfolding (emergent fronds, with a light green lamina). During the months of May and June, both old senescent fronds (when at least half of the lamina is dark brown) and the new emergent fronds are present on the same plant. The sterile sporophytes were characterized by the following average values (given by the first number, followed by min–max values, *i.e.*, the number of fronds: 4, 2–5; the length of fronds: 34, 20–48 cm; the width of fronds: 11, 9–15 cm. Further, the fertile sporophytes had the following average min–max values, *i.e.*, the number of fronds: 8, 6–9; the length of fronds: 102 (83–124) cm; the width of fronds: 28 (22–34) cm. Three fronds for each plant were randomly selected and tagged for measurements. The experiment lasted from February 2010 to January 2011. Measurements of Chl *a* fluorescence were carried out once a month (last week, during sunny days, between 10 in the morning and the midday with a *Plant Efficiency Analyser Handy PEA (Hansatech Ltd.*, Norfolk, UK). New fronds emerged between April and May and measurements started as soon as the lamina was fully expanded.

After a dark adaptation for 10 min using a clip placed at the base of the pinnule (a midpoint of the longest pinna on the right), samples were illuminated for 1 s with a saturating excitation pulse [3,000 μmol(photon) s^{-1} m^{-2}] of red light (650 nm) from a LED into the fluorometer sensor. All fluorescence induction curves were recorded up to 1 s. Up to ten fluorescence curves were recorded per each sporophyte and analysed by means of the so-called JIP-test (Strasser *et al.* 2000; Stirbet and Govindjee 2011), which is used to translate original fluorescence data to biophysical parameters that quantify energy fluxes and their ratios, physiological states, conformation, and overall photosynthetic performance of the samples.

The "vitality" of the samples was expressed by the maximum of quantum yield of primary photochemistry as inferred from fluorescence data: $\varphi_{Po} = (F_m - F_0)/F_m = F_v/F_m$. In addition, the performance index (PI_{ABS}) was calculated from another set of equations: $\gamma_{RC}/(1 - \gamma_{RC})\cdot\varphi_{Po}/(1 - \varphi_{Po})\cdot\psi_{Eo}/(1 - \psi_{Eo})$, where, $\gamma_{RC} = Chl_{RC}/Chl_{total}$ is the probability that a PSII Chl molecule functions as RC; $\gamma_{RC}/(1 - \gamma_{RC})$ is approximated in the JIP-test by RC/ABS, with ABS/RC the inferred absorbed energy flux per active RC of PSII; $\varphi_{Po} = TR_0/ABS$ (= F_v/F_m) is the inferred maximum of quantum yield of primary photochemistry; $\psi_{Eo} = ET_0/TR_0$ is the probability that an electron moves further than the electron acceptor Q_A (Strasser *et al.* 2000, 2004; Stirbet and Govindjee 2011).

Differences in the inferred photosynthetic parameters based on fluorescence measurements (F_v/F_m and PI_{ABS}) between the sterile and fertile fronds (within each month) were analysed using nonparametric *Mann-Whitney's U*-test, since normality and homogeneity of variance assumptions were not satisfied.

The maximal quantum yield of primary photochemistry ($\varphi_{Po}= F_v/F_m$) and the performance index (PI_{ABS}) were higher in the summer than in the winter, both in the sterile and fertile sporophytes (Fig. 1*A,B*). Sori appeared in June and spores were released between July and October; this period corresponded to the highest photosynthetic performances, as inferred from fluorescence measurements. The decay of the performance index in the old fronds was sudden, and occurred during the development of the new emerging fronds (May−June).

The OJIP transient curves are clearly observed, when the measured fluorescence values are plotted against time on a log scale (Strasser *et al.* 1995). Taking into consideration the seasonal development of the fronds, and accounting for differences in PI_{ABS} values, fluorescence transients were grouped as shown in Fig. 2. We accepted that the fluorescence induction curves reflected different photosynthetic behaviour of the samples, reflecting a seasonal acclimation of the ferns. Further direct measurements of photosynthesis are needed to confirm these results.

Emergent fronds (May, EM) had a similar behaviour to that of the fully expanded fronds from June until October (referred to as summer, SU). Expanded fronds in

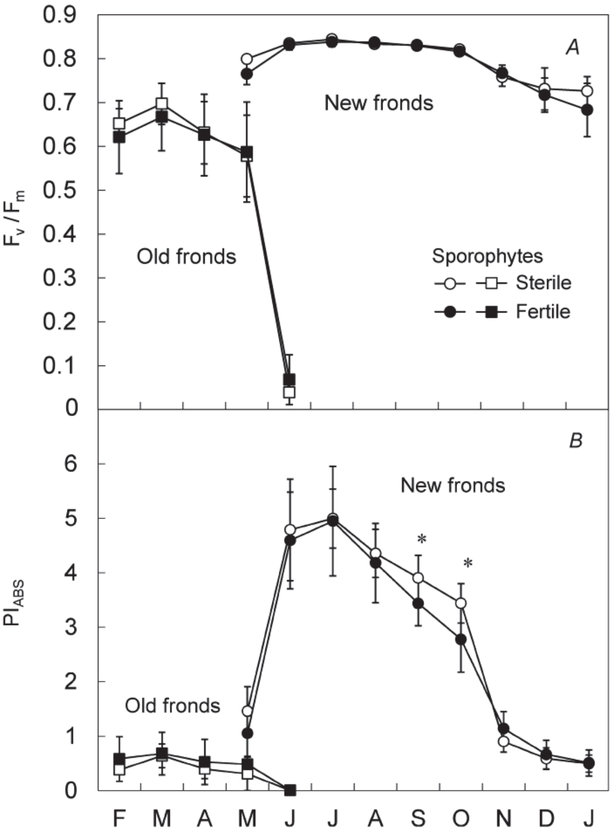

Fig. 1. (*A*) Seasonal variations of the maximum quantum yield of primary photochemistry of PSII (F_v/F_m) as inferred from the ratio of the maximum variable fluorescence (F_v) to maximum fluorescence F_m, and (*B*) seasonal variations of the index of the photosynthetic performance (PI_{ABS}) in sterile and fertile sporophytes in the fern *Dryopteris affinis*. Old fronds (February – June); new fronds (May – January). Mean values (middle point); ± SD (bars); $n = 50$. * – significant differences according to *Mann-Whitney*'s *U*-test, $p<0.05$.

the cold period – from November until April (referred to as winter, WI), showed a lowering fluorescence emission down to the minimal values of the senescent fronds, reached at the end of the seasonal cycle (May–June, SE).

Gratani *et al.* (1998) observed seasonal acclimation of the photosynthetic parameters in rhizomatose ferns from arid environments (*Cheilanthes persica*). They found that the content of photosynthetic pigments and the net photosynthetic rate increased during a frond expansion (the spring), decreased during the dehydration phase (the summer), increased again during the rehydration phase (the autumn), and finally dropped during the declining phase (the winter). Seasonal changes are controlled either by internal factors, such as plant hormones and adaptive traits, or by environmental factors, such as soil characteristics (*e.g.* humidity, pH, and nutrient availability) and microclimatic conditions (Odland 1995, Williams-Linera 1997). Perennial ferns may accumulate substances in rhizomes and overwintering fronds, which may serve as a carbohydrate storage organ for a new frond growth (Tani and Kudo 2003). However, phenological events, such as unfolding and expansion of fronds, as well as a maturation and dispersal of spores, need to be supported by favourable environmental conditions (Barrington 1993). Our values of F_v/F_m and PI_{ABS} in *D. affinis* were taken to reflect a higher photosynthetic performance of the fern during the summer. The likely reason was that *D. affinis* is confined to very humid and undisturbed sites in its natural habitat. Hence, although the summer days were hot, undergrowth in riparian forests was well shaded and the soil was moist, so that water availability was not a limiting factor to the fern life cycle.

In a parallel work, all microclimatic parameters recorded in the study area were modelled to see the best predictors of the phenological events in the fern during yearly life cycle (Landi *et al.* 2013). Phenological events

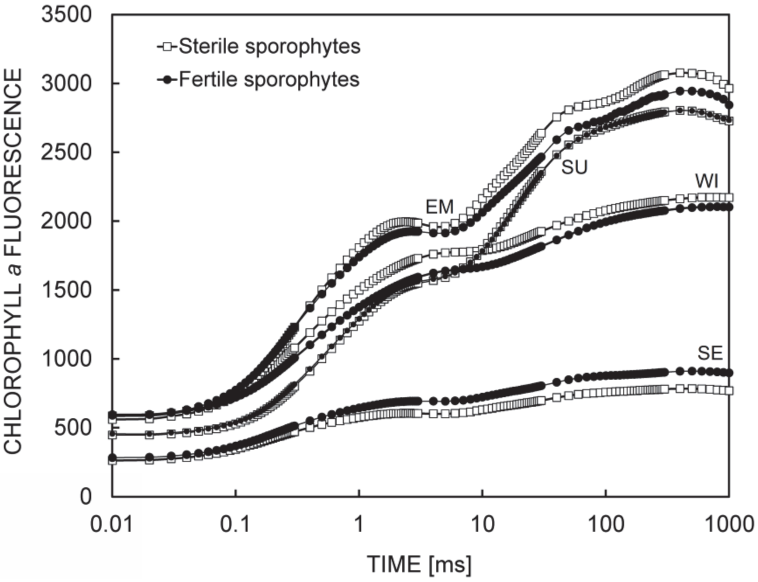

Fig. 2. Chlorophyll *a* fluorescence transients in sterile and fertile sporophytes in the fern *Dryopteris affinis*. Emergent fronds (EM), expanded fronds in summer (SU), expanded fronds in winter (WI), senescent fronds (SE).

in humid habitat were related more to temperature than to water availability. The development of the new fronds (in May) was positively correlated with the increase in the number of days with the mean temperature over 10°C, and with the increase in the sun irradiance [500–1,000 μmol(photon) s^{-1} m^{-2} of PAR]. These parameters contributed also to the senescent phase of the old fronds. The increase in the number of days with the mean temperature over 10°C was the best predictor for the development of sori in fertile fronds (in June). The overall increase of the mean temperature was the best predictor for the start of the release of spores (in July). EM and SU fronds showed an analogous photosynthetic behavior, as inferred from similar fluorescence induction curves (Fig. 2). The end of the release of spores (during September–October) was associated with the increase of precipitation events. From November, with the decrease of temperatures, a general decrease of the "photosynthetic performance" was evident both in the sterile and the fertile fronds.

Earlier measurements of the net photosynthetic rates in the fern *D. filix-mas* suggested that fertile and sterile sporophytes generally behave analogously (Bauer *et al.* 1991). However, differences in a seasonal development should be reflected by the photosynthetic behaviour of the samples in their natural habitat. In our study, we could find significant differences between the sterile and the fertile sporophytes by means of the performance index, PI_{ABS}, at the end of the warm season (September–October). These differences were related to significant changes in the $\gamma_{RC} = Chl_{RC}/Chl_{total}$, a component of PI_{ABS}, approximated in the JIP-test by RC/ABS (*Mann-Whitney*'s *U*-test, $p<0.05$, data not shown) and reflecting a decrease of the density of active RCs in our fertile fronds. The maximal quantum yield of primary photochemistry of PSII ($\varphi_{Po} = F_v/F_m$) and the electron transport probability further than the electron acceptor Q_A (ψ_{Eo}) did not change between the sterile and the fertile fronds (*Mann-Whitney's U* -test, $p<0.05$). Such data suggest that when the fertile sporophytes were at the end of the spore maturation and dispersal period, they might have, on the whole, a lower "vitality" than the sterile samples. The capacity to produce spores leads to the alteration of a seasonal development of the fronds: *e.g.* in the fertile individuals of *D. filix-mas* fronds might begin to unfold with a delay, of about a week, than in the sterile individuals, and in the autumn they decayed up to two months later (Sato 1982, Bauer *et al.* 1991).

Being the long-lived fern, confined to humid and undisturbed sites in the Mediterranean, *D. affinis* is strictly dependent on the availability of suitable microhabitats not threatened by anthropogenic activities (*e.g.* forest management practices).

Spore dispersal and new plant establishment are limited by both the abilities of the fern and the availability of suitable habitats (Tájek *et al.* 2011). Understanding physiological and ecological factors responsible for the distribution of the species is therefore of key importance for its conservation and for planning ecosystem conservation strategies. In this context, *D. affinis* deserves to be further investigated as a potential indicator of ecological continuity in Mediterranean riparian forests.

In summary, the photosynthetic performance of *D. affinis*, as inferred from Chl fluorescence measurements, changed greatly with the seasonal development of the fronds. On the whole, the sterile and the fertile fronds appeared to have a similar photosynthetic behaviour and differences between the sterile and the fertile fronds were related to the ending phase of a spore maturation and dispersal in fertile fronds. Further direct measurements of photosynthesis (CO_2 fixation and oxygen evolution) are needed to support the conclusions reached here.

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