BIOLOGIA PLANTARUM 51 (4): 741-745, 2007

BRIEF COMMUNICATION

Chlorophyll fluorescence parameters of three Mediterranean shrubs in a summer-autumn period in central Portugal

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Abstract

The photosynthetic performance of *Cistus crispus*, *Centaurea africana* and *Thymus villosus,* three shrubs of the Mediterranean-type maquis vegetation of Central Portugal, were studied between July and December 2003 by means of *in vivo* chlorophyll *a* fluorescence measurements. In *C. africana* total leaf decay occurred between August and October, in *T. villosus* no clear pattern of variation of the basal fluorescence (F_o) was observed, and in *C. crispus* an increase of this parameter was observed in late autumn. In all species, the limitation to photosynthesis by photoinhibition and the contribution of non-reversible processes to total photoinhibition were more pronounced in the summer than in the autumn. However, a pronounced decrease of the effective quantum yield of photosystem 2 (Φ_{PS2}) was found in late autumn, probably due to a decrease of the carboxylation efficiency of the Calvin-Benson cycle, which was also supported by a parallel decrease of the photochemical quenching coefficient (qP).

Additional key words: *Centaurea africana, Cistus crispus,* Mediterranean climate, photoinhibition, photosynthetic performance, *Thymus villosus.*

Cistus crispus L., *Centaurea africana* Lam. and *Thymus villosus* L. subsp. *villosus* are three shrubs present in the Mediterranean-type maquis vegetation of Central Portugal. *C. crispus* is an endemic chamaeophyte in Portugal. Among the three species studied, it has the most important ecological role providing an important part of the diet of small herbivores (Marques and Mathias 2001). *C. africana* is an endemic hemicryptophyte from the circummediterranean north African areas and has in Portugal its northern limit of distribution, where it is considered to be endangered. *Thymus villosus* ssp. *villosus* is a chamaeophyte protected under the Habitats Directive (Annex IV). Due to the interest in its essential oils, it has been studied with a respect to its phytochemical perspective (Alonso and Neguerela 1984) and submitted to the chemotaxonomic analysis (Salgueiro *et al.* 2000). Although their conservation and ecological importance are very high, the physiology of these species is practically unknown and, as far as we know, their photosynthetic performance has not been studied yet. As photosynthesis highly determine plant productivity and ability to survive and reproduce this adequate knowledge is essential to prepare conservation plans (*e.g.* Iriondo 1996).

The photosynthetic activities of green plants are related, in a complex manner, with the *in vivo* fluorescence emitted by chlorophyll *a*. Since the discovery of the phenomenon by Kautsky and Hirsch (1934) it has been found to be a versatile tool to study photosynthesis (Rosenqvist and Van Kooten 2003). The ecophysiological impact of abiotic stresses such as low temperature (Cavaco *et al.* 2003), nitrogen starvation (Marques da Silva and Arrabaça 1992), water deficiency (Marques da Silva and Arrabaça 2004) and high irradiance (Fuentes *et al.* 2005, Keller and Lüttge 2005) have been studied using this technique. The main instruments currently available

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Received 22 January 2005, *accepted* 30 June 2007.

Abbreviations: F_0 - basal fluorescence; qF_0 - quenching of basal fluorescence; qP - photochemical quenching; qN - non-photochemical quenching; S_m - normalized area above the fluorescence rise curve Φ_{PS2} - effective quantum yield of PS 2 photochemical energy conversion.

Acknowledgements: I acknowledge the financial support by NAER - Novo Aeroporto, S.A.. I am grateful to Prof. Maria José Costa for the opportunity to carry out this investigation, Vanda Alves Monteiro for the grammar corrections and João Serôdio for help in the field work. Credits are paid to Instituto de Meteorologia for making public the climatic data.

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are "modulated" fluorometers designed to resolve the photochemical and non-photochemical utilization of the excitation energy by the well established quenching analysis (Schreiber *et al.* 1986). Moreover, some commercial "modulated" fluoro-meters are now equipped with a kinetic module for the study of the rapid phase of the Kautsky kinetics (Strasser and Govindjee 1992). In this study, we used both techniques of chlorophyll *a* fluorescence measurements in order to understand the photosynthetic performance of three maquis' shrubs in a summer to autumn period in the Portuguese Mediterranean-type climate.

A semi-natural area located in the Lisbon District, Portugal, was choosen for performing all measurements. Monthly averaged minimal, maximal and mean temperatures and monthly total precipitation and insolation were obtained from the Portuguese Institute of Meteorology. Fluorescence measurements were made monthly, between July and December 2003, with a *PAM-210* modulated fluorometer (*Walz*, Effeltrich, Germany). The instrument was connected to a personal computer and controlled by the data acquisition software *DA-TEACH* (version *1.01*) (*Walz*). Mature fully-developed leaves of the three species were collected in the field, always between 10:00 and 12:00, and kept in darkness under a humid atmosphere. The samples were transferred to the laboratory, where they were still kept in darkness at room temperature under a water vapour saturating atmosphere for 24 h.

The leaves were then illuminated for 5 min with an actinic light of high (1850 µmol m^{-2} s^{-1}) photosynthetic photon flux density (PPFD) and a saturating pulse of 3500 µmol m^{-2} s^{-1} was applied. This measuring protocol allowed the automatic calculation of the photochemical (qP) and non-photochemical (qN) quenching coefficients and of the effective quantum yield of photosystem 2 (PS 2) photochemical energy conversion (Φ_{PS2}). From the values of basal fluorescence measured after dark adaptation (F_0) and after actinic illumination (F'_0), the quenching of the basal fluorescence (qF_0) was calculated as the ratio $(F_0 - F'_0)/F_0$ (Bilger and Schreiber 1986). Finally, after a new period of 30 min in darkness under a humid atmosphere, a new saturating pulse was applied for 2 s and the rapid Kautsky kinetics was recorded during 7 s. The area above the fluorescence rise curve (A_{max}) was automatically calculated by the *DA-TEACH* software (Strasser *et al.* 2000). The normalized area (S_m) was calculated as the ratio of A_{max} and variable fluorescence (F_v). One-way *ANOVA* (random factors) was used to evaluate significant effects of the month in the year (fixed factor) on the fluorescence parameters. Due to a total leaf decay in *C. Africana,* the months as August, September and October were excluded from the analysis of all parameters. Post-hoc comparison LSD tests were used to detect significant differences between group means within the *ANOVA* setting (Sokal and Rohlf 1995). All the statistical analysis were performed using the software *Statistica 4.0* (*Statsoft*, Tulsa, OK, USA).

The warmest month in 2003 was August (monthly mean temperature 25.4 °C) and the coldest one December, which was the only month with a monthly average minimum temperature bellow 10 °C (8.9 °C) (Table 1). The summer of 2003 was unusually hot in Portugal, with a peak absolute maximum temperature in Lisbon of 40.6 °C, obtained in August 2. The driest month was July (2 mm precipitation) and the rainiest one was October (175 mm). A dry period from July to September, with a total precipitation of 42 mm, is distinguished from a rainy period from October to December with a total precipitation of 401 mm. The highest insolation was found in July (350 h) and the lowest in December (140 h).

The seasonal variation of F_0 was highly significant for *C. crispus* ($P < 0.0001$), significant for *T. villosus* ($P < 0.05$) and not significant for *C. africana* ($P > 0.05$) (Table 2), but its pattern was complex. In *C. crispus* there was an increase from August to September and no significant changes afterwards. In *C. africana* total leaf decay occurred in August, September and October and thereby no measurable basal fluorescence was found. In November and December, the values were again similar to the ones found in July. These months only were used in the *ANOVA* setting and thereby no statistical seasonal variation of F_0 was found. In *T. villosus* F_0 did not change significantly except for the lower value found in August. It has been shown that in most situations F_0 is highly correlated with the chlorophyll content of the photosynthetic structures (Serôdio 2003, Elias *et al.* 2004). However, under certain circumstances, an increase (*e.g.* due to heat stress; Schreiber 1986) of F_0 does occur.

Table 1. Temperature, precipitation and insolation data for Lisbon (airport meteorological station) between July and December 2003.

Month	Temperature [°C] mean	mean minimum	absolute minimum/(d)	mean maximum	absolute maximum/(d)	Precipitation [mm] total precipitation	absolute precipitation/(d)	Insolation [h $month^{-1}$]
July	22.3	17.9	15.4/(16)	26.7	37.4/(31)	2	1.5/(15)	350
August	25.4	20.8	17.6/(20)	30.0	40.6/(2)	33	24.6/(31)	345
September	23.2	18.9	16.3/(24)	27.6	35.6/(13)	7	4.7/(30)	260
October	18.0	14.6	9.7/(24)	21.3	27.0/(10)	175	37.2/(2)	210
November	15.2	12.4	10.3/(24)	18.0	21.9/(7)	132	48.8/(22)	155
December	11.7	8.9	6.9/(23)	14.6	17.6/(16)	94	17.1/(9)	140

Table 2. Seasonal variation of individual fluorescence parameters for *Centaurea africana*, *Cistus crispus* and *Thymus villosus*. Results are means ± SD of 6 replicates. Different letters indicate statistically significant differences ($P < 0.05$). Abbreviations: F_0 - basal fluorescence; qF_0 - quenching of basal fluorescence; Φ_{PS2} - effective quantum yield of PS 2 photochemical energy conversion (measured at PPFD of 1850 μmol m^{-2} s^{-1}); qP - photochemical quenching; qN - non-photochemical quenching; S_m - normalized area above the fluorescence rise curve.

Parameter	Species	July	August	September	October	November	December
F_0	*C. africana*	0.324±0.099a	-	-	-	0.345±0.008a	0.366±0.027a
	C. crispus	0.163±0.056a	0.200±0.039a	0.266±0.017b	0.283±0.024b	0.259±0.025b	0.295±0.026b
	T. villosus	0.322±0.121a	0.177±0.071b	0.379±0.139a	0.309±0.114a	0.313±0.027a	0.375±0.062a
qF_0	*C. africana*	0.428±0.104a	-	-	-	0.194±0.033b	0.177±0.027b
	C. crispus	0.224±0.153a	0.102±0.048a	0.345±0.369a	0.091±0.026a	0.142±0.038a	0.222±0.017a
	T. villosus	0.486±0.176a	0.350±0.168b	0.562±0.060a	0.151±0.140b	0.149±0.065b	0.258±0.044b
Φ_{PS2}	*C. africana*	0.152±0.026a	-	-	-	0.059±0.011b	0.058±0.011b
	C. crispus	0.186±0.038a	0.084±0.038b	0.079±0.013b	0.096±0.025b	0.059±0.017b	0.068±0.006b
	T. villosus	0.171±0.038a	0.045±0.028b,c	0.034±0.007b,c	0.054±0.025b	0.043±0.01b,c	0.017±0.02c
qP	*C. africana*	0.472±0.054a	-	-	-	0.156±0.033b	0.125±0.0298b
	C. crispus	0.393±0.073a	0.289±0.136b	0.239±0.028b,c	0.221±0.070b,d	0.124±0.035d	0.194±0.023c,d
	T. villosus	0.554±0.177a	0.226±0.062b	0.170±0.029b,c	0.171±0.073b,c	0.108±0.024c,d	0.048±0.064d
qN	*C. africana*	0.886±0.039a	-	-	-	0.878±0.017a	0.845±0.013a
	C. crispus	0.837±0.019a,c	0.871±0.050b,c	0.890±0.022b	0.822±0.036a	0.819±0.020a	0.904±0.004b
	T. villosus	0.906±0.045a	0.904±0.034a	0.960±0.010c	0.890±0.023a,b	0.859±0.017b,d	0.889±0.016a,d
S_m	*C. africana*	16000±2700a	-	-	-	37200±15500b	89100±13200b
	C. crispus	15900±5300a	23700±13100a	41800±4000b	36900±4500b	36600±8300b	74100±5000c
	T. villosus	9400±5100a	14600±10200a	28300±12500b	28500±11900b	18700±4800a,b	43900±5400c

The increase of F_0 found in *C. crispus* may be due to both increased chlorophyll content and heat damage. On the contrary, the large variation found in *C. africana* is due to the total decay of the leaves which have started in the middle of the summer and the recovery of F_0 was due to the growth of new leaves in the middle of the autumn.

No significant variation of qF_0 was found in the studied period in *C. crispus* ($P > 0.05$) (Table 2). On the contrary, the variation of qF_0 was highly significant in *C. africana* ($P < 0.0001$, Table 2), although, due to the leaf decay between August and October, only July, November and December were used in the *ANOVA* setting. Also in *T. villosus* a highly significant variation of the qF_0 was found ($P < 0.0001$; Table 2), with significantly higher values found in July and September. The extent of F_0 quenching after actinic illumination is an indicator of the non photochemical quenching at the antenna level, which may be related with reversible or non-reversible photoinhibition. The pattern of variation was very different among the three species.

The effective quantum yield of PS 2 photochemical energy conversion under high irradiance (Φ_{PS2}) showed the most clear trends among all the fluorescence parameters, in *C. crispus* ($P < 0.0001$), *C. africana* ($P < 0.0001$), and *T. villosus* ($P < 0.0001$) (Table 2). Both in *C. crispus* (0.186) and *T. villosus* (0.171) significantly higher values were found in July than in other months. The lowest values were found in November for *C. crispus* (0.059) and December for *T. villosus* (0.017). Also in *C. africana,* the values measured in July (0.152) were much higher than those values measured in November (0.059) and December (0.058). The physiological performance of both the primary reactions of photosynthesis and of the bioche-mical cycles is integrated by Φ_{PS2}. As the decrease of this parameter was much more pronounced than the variation observed in the previous ones (F_0 and qF_0), it indicates that the Calvin cycle enzymes were much more affected by the seasonal weather conditions.

The results found for qP confirm this possibility, as it showed a very significant and even more pronounced decrease, during the measured period ($P < 0.001$ for *C. crispus*, $P < 0.0001$ for *C. africana* and $P < 0.0001$ for *T. villosus*). In *C. crispus* the highest value was found in July (0.393) and the lowest in November (0.124). In *C. africana* the highest value was found in July (0.472), measurements were not possible between August and October and the lowest value was found in December (0.125). In *T. villosus* the highest value was found in July (0.554) and the lowest one (0.048) in December (Table 2). The photochemical quenching is an indicator of the fraction of the open (reoxidised) reaction centres of PS 2, and its decrease indicates a more reduced state of a plastoquinone pool in the winter, as a potential effect of senescence. The increased reduction state is probably due to a decrease of the electron flow through PS 2, probably due to a lower turnover of electron acceptors as a consequence of a lower activity of the Calvin-Benson cycle and the activation of many reparative and dissipative mechanisms (Table 2). Similar decreases of qP have been previously reported under water stress conditions (Marques da Silva and Arrabaça 2004).

The seasonal variation of qN (Table 2) was also significant for *C. crispus* ($P < 0.001$) and for *T. villosus* ($P < 0.001$) but not for *C. africana* ($P > 0.05$). The values were high in all species due to the high actinic photosynthetic photon flux density used and thus the activation of many non-photochemical processes. In *C. crispus* the lowest value was found in November (0.819) and the highest in December (0.904). In *C. africana* the lowest value was found in December (0.845) and the highest in July (0.886), although in the three months where measurements were possible (July, November and December) no significant variation was found. In *T. villosus* the lowest value was found in November (0.859) and the highest in September (0.960). qN is related to the energy state of the thylakoid membrane, *i.e.*, predominantly to the trans-thylakoid pH gradient. An increased qN indicates the build up of protons in the thylakoids lumen as a result of a decreased turnover of ATP, probably due to a lower Calvin-Benson cycle activity. Photoinhibition may also contribute to higher qN.

The variation of S_m (Table 2) was significant ($P < 0.0001$ for *C. crispus*, $P < 0.0001$ for *C. africana* and $P < 0.001$ for *T. villosus*). In all species a notorious increase of S_m from July to December was found. In *C. crispus*, the lowest value (15900) was recorded in July and the highest (74100) in December. For *C. africana* the July value was 16000 and the highest value (89100) was recorded in December. In *T. villosus*, the lowest value was recorded in July (9400) and the highest in December (43900). S_m is the complementary area above the Kautsky curve normalized by the variable fluorescence, and it is a measure of the energy needed to close all reaction centres, being proportional to the total number of electrons transported through PS 2 during the time of chlorophyll fluorescence induction. It therefore represents a measure of the acceptor pool size of PS 2 (Murata *et al.* 1966). An increase in the acceptor pool size may have been observed in all species in the winter.

In summary, although the strategy of resource allocation to the photosynthetic apparatus is quite different among the three species, in all of them a higher photoinhibitory limitation was found in the summer months, possibly due to the influence of high temperatures and water shortage. Nevertheless, it was found that in the winter all species showed a dramatic decrease of the photosynthetic capacity (estimated from the effective quantum yield of PS 2), probably due to limitations posed by the lower efficiency of biochemical reactions in the Calvin-Benson cycle.

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