

F_v/F_m acclimation to the Mediterranean summer drought in two sympatric *Lasallia* species from the Iberian mountains

M. VIVAS, S. PÉREZ-ORTEGA, A. PINTADO and L. G. SANCHO

Abstract: Photosynthetic performance in lichens can vary throughout the year. We investigate the variation in the PSII quantum efficiency as a proxy for the physiological state of the photosynthetic apparatus in two umbilicate species from the genus *Lasallia*. Temporal variation in F_v/F_m in both species was monitored at a field site in Central Spain where both species coexist. Subsequent measurements were carried out in the laboratory after 48 h preconditioning. Both species showed clear variation during the year in PSII performance, with a marked depression in F_v/F_m during the summer. *Lasallia pustulata* consistently had higher F_v/F_m values than *L. hispanica*. Both species reached higher F_v/F_m values after 48 h of preconditioning in the laboratory and this recovery was particularly notable in the summer months. F_v/F_m was highly related to antecedent weather conditions during the two days prior to measurement.

Key words: chlorophyll fluorescence, lichens, PSII, quantum efficiency, seasonal effects

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Introduction

The Mediterranean region is one of the most biologically diverse in the world (Prendergast *et al.* 1993). It is characterized by a distinctive climate with marked seasonality in rain pattern. Precipitation is almost completely absent in summer, hampering the survival of species in the middle of the growing season. Several studies that have examined the effect of seasonality on lichens, have identified contrasting responses between species from Mediterranean and boreal regions (e.g. MacKenzie *et al.* 2001, 2002; Vrábliková *et al.* 2006; Baruffo &

Tretiach 2007; Tretiach *et al.* 2007; Pirintsos *et al.* 2011). A marked depression of maximum PSII efficiency (F_v/F_m) during the Mediterranean summer has been observed in several epiphytic foliose species (Baruffo & Tretiach 2007; Pirintsos *et al.* 2011), matching the period of water unavailability. Furthermore, the amplitude in F_v/F_m response in Mediterranean species was much wider than that previously found in boreal lichens (Vrábliková *et al.* 2006). So far, the effects of summer drought on lichens have been studied in epiphytic lichens, and it is not known whether similar responses would be found in saxicolous species.

Previous studies on seasonal variation in the physiological performance of lichens have mostly relied on the use of chlorophyll fluorescence. This is a powerful tool, highly sensitive and non-invasive, with which to assess the effectiveness of photosystem II, as a proxy for photosynthesis (Maxwell & Johnson 2000). For this reason, it has been widely used to (1) assess lichen response to environmental stresses (e.g. Bilger *et al.* 1989; Scheidegger *et al.* 1997; Barták *et al.* 2003), (2) detect lichen physiological activity, (3) deduce carbon budgets and potential growth in

M. Vivas: Laboratorio de Fisiología y Biología Molecular Vegetal, Instituto de Agroindustria, Departamento de Ciencias Agronómicas y Recursos Naturales, Facultad de Ciencias Agropecuarias y Forestales, Universidad de la Frontera, Temuco, Chile; Ecobiosis, Dpto. de Botánica, Facultad de Ciencias Naturales y Oceanografía, Universidad de Concepción, Concepción, Chile; and Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, E-28040, Madrid, Spain. Email: meru.vivas@gmail.com

A. Pintado and L. G. Sancho: Departamento de Biología Vegetal II, Facultad de Farmacia, Universidad Complutense de Madrid, E-28040, Madrid, Spain.

S. Pérez-Ortega: Real Jardín Botánico, CSIC, E-28014, Madrid, Spain.

long-term monitoring experiments (Schroeter *et al.* 1991; Lange *et al.* 1999; Raggio *et al.* 2014), (4) assess the effect of pollution (Calatayud *et al.* 1996; Niewiadomska *et al.* 1998; Candotto Carniel *et al.* 2015) and (5) determine the effectiveness of biocide treatments (Speranza *et al.* 2012).

Chlorophyll fluorescence is not without problems when used for the study of cryptogams. Unlike vascular plants (Demmig & Björkman 1987; Genty *et al.* 1989), there is no direct and clear relationship in bryophytes and lichens between ETR (electron transport rate, as a parameter obtained from fluorescence measurements) and CO₂ fixation (Leisner *et al.* 1997; Green *et al.* 1998; Proctor & Smirnov 2011). Owing to different photo-protective mechanisms operating in lichens, an F_v/F_m (maximum quantum efficiency of PSII) as high as that for vascular plants (*c.* 0.83) is not to be expected, with normal values for lichens lying between 0.63 and 0.76 (Demmig-Adams *et al.* 1990) and only exceptionally exceeding 0.80 (Baruffo & Tretiach 2007). Moreover, in spite of the extensive literature on chlorophyll *a* (Chl*a*) fluorescence, knowledge of the factors that influence it is still poor. For example, it is not known how weather conditions prior to measurement could influence the results, masking the state of PSII and thus making comparisons among species or seasons unreliable (Gauslaa *et al.* 2001). For instance, Baruffo & Tretiach (2007) observed that the light regime in the days preceding field measurements influenced the functionality of PSII of *Parmelia subrudecta*.

In the present work, we studied the temporal variation in F_v/F_m in two sibling species of *Lasallia* which show different ecological preferences and distribution ranges. We aimed to (1) discover whether co-occurring populations of these species show Chl*a* fluorescence acclimation to seasonal changes, (2) analyse the influence of antecedent climatic conditions on F_v/F_m , (3) assess the effect on F_v/F_m of a short period of acclimation under controlled laboratory conditions and (4) identify any physiological differences between the species. To achieve these aims we measured F_v/F_m values throughout a year, both in the field and in the laboratory.

Material and Methods

Lichen material

The study site is located in Central Spain (Silla de Felipe II, El Escorial, 40°34'5"N, 9°8'45"W, 1070 m a.s.l.). A *Quercus pyrenaica* forest, rich in epiphytic bryophyte and lichen species, dominates the landscape. Pyrenean oak has marcescent foliage, especially in young branches. In this locality, new leaves appear at the end of April and flowering takes place shortly after. There are numerous granite boulders in the forest, harbouring a rich saxicolous lichen community, among which *Lasallia pustulata* (L.) Mèrat and *L. hispanica* (Frey) Sancho & Crespo are two of the most abundant macrolichen species. The coexistence in this area of these sibling species (Davydov *et al.* 2010) may be explained by their niche segregation since they rarely share the same microhabitat (Scott & Larson 1986; Sonesson *et al.* 2011). *Lasallia hispanica* is a Mediterranean endemic, occurring from 1000 m a.s.l. to the highest summits of Iberian Sistema Central (Almanzor Peak, 2592 m), on vertical, very exposed and windy surfaces. *Lasallia pustulata* is a widely distributed species that is usually found in more protected habitats (Sancho & Crespo 1989; Codogno & Sancho 1991). Samples of each species were selected from a single population of each species growing on two nearby boulders to minimise physiological variation that might result from differences in microhabitat conditions. *Lasallia pustulata* samples were taken from a population of more than 100 individuals growing on a boulder protected by the canopy. *Lasallia hispanica* samples were collected from a boulder in an open position being more exposed to wind and irradiation, but with the same orientation (facing north) as that of *L. pustulata*. Owing to their different population sizes, thalli of *L. pustulata* could be collected monthly for laboratory analyses, whereas thalli of *L. hispanica* were collected every two months.

Fluorescence measurements

Field and laboratory fluorescence measurements were carried out using a MINI-PAM fluorimeter (Walz, Effeltrich, Germany). Eight healthy thalli of *Lasallia hispanica* and *L. pustulata* were selected and their physiological status monitored by making sequential measurements throughout the year. The maximum photochemical quantum yield of PS II, F_v/F_m ($= (F_m - F_0)/F_m$), was measured at 10 points on each thallus in order to minimize error estimates owing to intrathalline variability (F_0 : minimum Chl*a* fluorescence of a dark-adapted sample, F_m : maximum Chl*a* fluorescence of a dark-adapted sample). F_m was determined on thalli that had been fully hydrated by spraying and after dark adaptation (20 min covered with a black velvet cloth) using a saturating pulse (8000 μmol photosynthetically active photons m⁻² s⁻¹, lasting 0.8 s). Field measurements were carried out on the first day of each month, always around 11:00 a.m. In order to compare field measurements with acclimated thalli in the laboratory, 8 thalli were collected each month on the same day as the field measurements and kept for two days in a chamber at 10°C, 65% relative humidity (RH), with a 12 h photoperiod at a photosynthetic photon flux density

(PPFD) of $100 \mu\text{mol photons m}^{-2} \text{s}^{-1}$, provided by a metal halide lamp, and sprayed once a day with mineral water (Schroeter *et al.* 1995; Pintado *et al.* 2005). Spraying took place 1 h after the lights turned on with sufficient water for the thalli to reach maximum water content and to remain wet for the remainder of the light period. F_v/F_m was measured after similar dark adaptation and full hydration as in the field.

Meteorological data

Climatic data were provided by the State Meteorological Agency (AEMET) for the sampling period from September 2008 to September 2009 with daily values for precipitation, temperature and irradiance (Fig. 1). The meteorological station is located 1 km from the study site. Due to the high continentality of the study site and the usually low ambient RH, dewfall seldom occurs, being detected on only 18 days during the study period, scattered between January and March and between November and December. Data given for any particular month are data for the previous month (i.e. data labelled as January in Fig. 1 are those gathered in December). The reason for this labelling is to keep the same month labels for all the data, since samples from January were those collected at the beginning of January, and hence exposed to the antecedent climatic conditions in December.

Statistical analyses

Seasonal variation was assessed by repeated measures ANOVA after checking normality and sphericity.

Differences between months were tested by pairwise comparisons with the Bonferroni correction for the P -value using the car package (Fox & Weisberg 2011) in R 3.1.1 (R Core Team 2014). A paired t -test was performed to check the differences between field and laboratory acclimated values. Correlation tests were performed between F_v/F_m values and climatic variables measured for different durations, from 1 to 30 days, in order to assess the influence on F_v/F_m of the antecedent climatic conditions, as well as the period over which they were measured (Hmisc R package, Harrell *et al.* (2014)).

Results

Lasallia hispanica and *L. pustulata* showed similar patterns regarding seasonality, with a marked depression in F_v/F_m during the summer months followed by a recovery at the beginning of autumn (Fig. 2). The absence of field data for *L. hispanica* in December is owing to frozen thalli preventing fluorescence measurements. Field data from October are missing due to a software failure. The highest F_v/F_m field values were recorded in November in both species (0.647 for *L. hispanica* and 0.733 for *L. pustulata*, both within the values that indicate a healthy state of PS II, according to Jensen (2002)), while the lowest values were

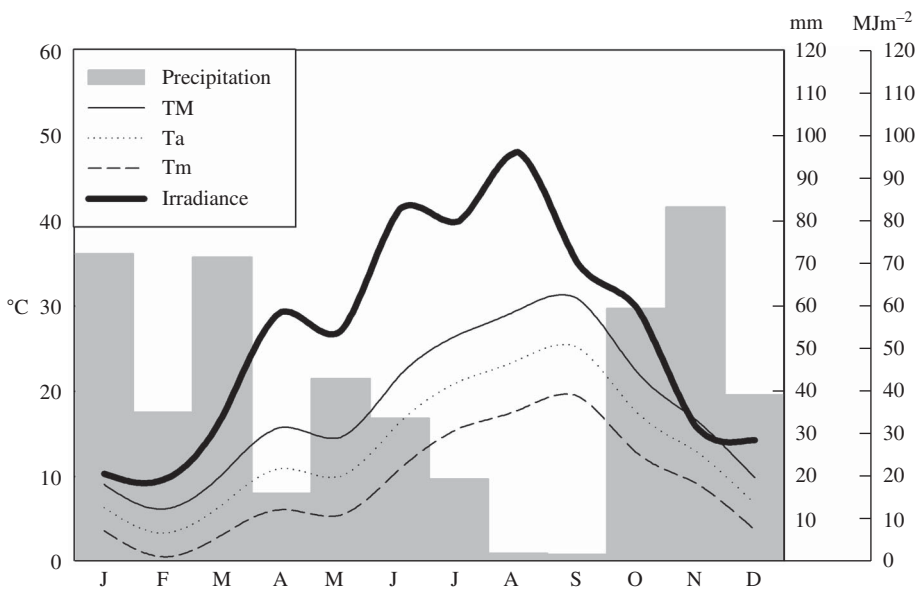


FIG. 1. Climatic conditions recorded in the study area during the investigation. Monthly means of daily minimum air temperature (Tm); daily maximum air temperature (TM); mean daily air temperature (Ta); monthly summation of irradiance and precipitation. (Data from State Meteorological Agency, AEMET.)

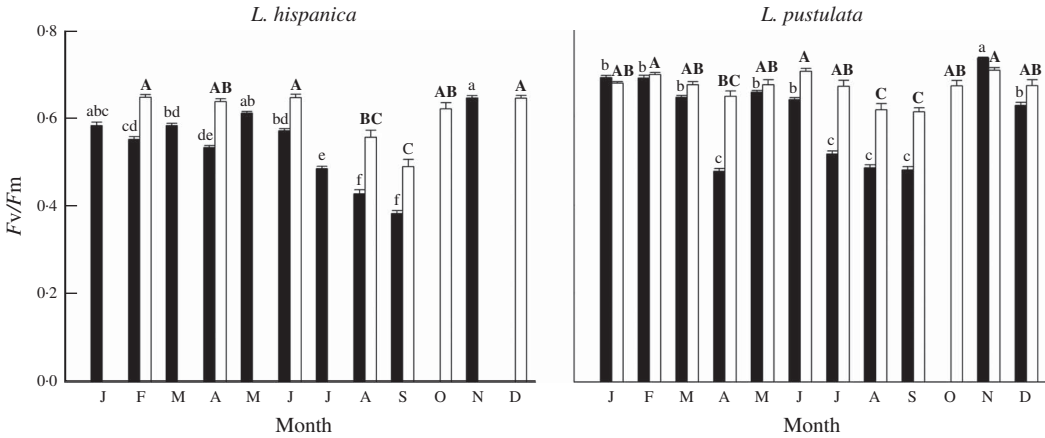


FIG. 2. F_v/F_m values obtained in the laboratory (open columns) and in the field (closed columns) for *Lasallia hispanica* (left) and *L. pustulata* (right). Repeated measures ANOVAs were performed in each case and differences between months were tested by a pairwise comparison with a Bonferroni correction for the P -value. Values with the same lower case or uppercase letters are not significantly different at the $P \leq 0.05$ level. Mean values are plotted (\pm 1 SEM)

TABLE 1. Analyses of temporal changes in F_v/F_m and of differences between field and laboratory measurements. Field and Laboratory rows: analyses of differences in F_v/F_m over a year for each species by repeated measures ANOVA tests (statistic = F). Field vs. laboratory row: analysis of differences between field and laboratory measurements by paired t test (statistic = t).

	<i>Lasallia hispanica</i>		<i>Lasallia pustulata</i>	
	df	F or t statistic	df	F or t statistic
Field	9	47.32***	10	38.97***
Laboratory	6	15.95***	11	2.74*
Field vs. laboratory	4	-12.17***	10	-2.94**

* $P = <0.02$; ** $P = <0.01$; *** $P = <0.001$

reached in September (0.384 and 0.479, respectively). *Lasallia pustulata* field values for April were also low (0.481). In the laboratory both *L. hispanica* and *L. pustulata* similarly had the lowest F_v/F_m values in September (0.494 and 0.611, respectively) while the highest values were reached in February (0.646) and November (0.705), respectively. Repeated measures ANOVA tests revealed a significant change with time in both field and laboratory measurements for both species (Table 1).

Higher F_v/F_m values were always obtained after preconditioning in the laboratory, with the exception of January and November in *L. pustulata*. The differences were statistically significant for both species using a paired t -test

(Table 1). The disparity tended to be larger in summer in the case of *L. pustulata* (e.g. 26% in July vs. 0.73% in February), while it was fairly similar across the year for *L. hispanica* (ranging from 15% to 22%). Differences between species were also observed: F_v/F_m values in *L. pustulata* were on average 12% higher than those of *L. hispanica* for both field and laboratory data (Fig. 1).

All correlations between antecedent meteorological data and F_v/F_m values in each species, both in the field and after laboratory preconditioning, were highly significant with positive coefficients for precipitation and negative coefficients for temperature and irradiance (Table 2). Correlations between

TABLE 2. Pearson's product moment correlation coefficient (r) between F_v/F_m measurements in the field and the laboratory for *Lasallia hispanica* and *L. pustulata* and values of precipitation, average temperature and total irradiance during 2 and 5 days prior to the measurements.

Period of preconditioning (days)		<i>Lasallia hispanica</i>		<i>Lasallia pustulata</i>	
		Field	Laboratory	Field	Laboratory
2	Precipitation (mm)	0.69	0.42	0.69	0.60
	Average temp. (°C)	-0.61	-0.36	-0.64	-0.66
	Irradiance (MJm ⁻²)	-0.59	-0.31	-0.52	-0.40
5	Precipitation (mm)	0.31	0.26	0.28	0.26*
	Average temp. (°C)	-0.59	-0.31	-0.60	-0.56
	Irradiance (MJm ⁻²)	-0.59	-0.28	-0.44	-0.40

*correlation is significant at the $P \leq 0.01$ level. All other correlations are significant at the $P \leq 0.001$ level.

precipitation, mean temperature and irradiance were all stronger for a 2-day than for a 5-day antecedent period. In *L. hispanica* the correlation coefficients between F_v/F_m values and climate variables were on average 73% higher for laboratory measurements while, by contrast, r values for *L. pustulata* were broadly similar for field and laboratory data, with those calculated for field data being only 7% higher.

Discussion

The study of F_v/F_m by Chla fluorescence in *Lasallia hispanica* and *L. pustulata* over one year suggests that photosynthetic capacity in saxicolous lichens in a Mediterranean area might have a seasonal pattern. They displayed a marked depression of F_v/F_m in summer (Fig. 2) and recovered their optimum fluorescence values in autumn, as has already been reported for epiphytic lichens (Baruffo & Tretiach 2007; Pirintsos *et al.* 2011). This pattern therefore seems to be characteristic of lichens in the Mediterranean area and does not occur in boreal regions (MacKenzie *et al.* 2002; Vrábliková *et al.* 2006). Mediterranean summers are characterized by high temperatures, severe drought and high irradiance. These factors have a negative influence on the fluorescence performance of lichens (Gauslaa *et al.* 2001; MacKenzie *et al.* 2002; Barták *et al.* 2008; Larsson *et al.* 2009) and vascular plants (e.g. Gulías *et al.* 2002; Tausz *et al.* 2004) and this study further supports those observations (Table 2). Tretiach *et al.* (2012) reported a

similar seasonal trend in fluorescence values for *Flavoparmelia soredians*, attributing most of the decrease in F_v/F_m to the activation of a non-photochemical quenching mechanism during desiccation; this is progressively, but not immediately, deactivated when water becomes available again. This mechanism probably influences the observed decrease in F_v/F_m in the present study. The effect of irradiance on F_v/F_m was investigated by MacKenzie *et al.* (2002) who reported seasonal changes in fluorescence variables in a population of *Lobaria pulmonaria* growing in a deciduous forest but who did not observe such changes in the same species living in an evergreen forest with a permanently closed canopy. The considerable decrease in *Lasallia pustulata* F_v/F_m field values in summer, when the tree canopy is fully developed, points to prolonged water stress in the light being a more important factor than the direct effect of high irradiance, in accordance with the results shown by Gauslaa *et al.* (2012). This hypothesis is further supported by the stronger positive correlation between F_v/F_m and precipitation than that between F_v/F_m and irradiance (Table 2). In addition, the results for November, the only month where field values were higher than laboratory preconditioned values, indicate that the water regime in the field might have been more suitable than in other months. Water deprivation might also underlie the low F_v/F_m values measured for *L. pustulata* in the field in April, owing to relatively low precipitation

and higher irradiance compared to values for March (Fig. 1) under a still undeveloped canopy. Tretiach *et al.* (2013) also showed a lower physiological performance in *Parmelia sulcata* in the absence of a canopy.

Mediterranean lichens deal with the harsh summer conditions by entering a prolonged desiccated, metabolically inactive state. Desiccated lichens are resistant to high irradiance because desiccation reduces light transmission through the cortex (Gauslaa & Solhaug 2001) and stabilizes the photosynthetic apparatus by causing a functional disconnection of its components (Bilger *et al.* 1989; Demmig-Adams *et al.* 1990). However, during long dry periods when the weather remains too dry for the repair mechanisms to become activated, damage gradually accumulates (Gauslaa & Solhaug 1999). The absence of dewfall in this area adds to this problem. However, acclimation to the Mediterranean climate is evidenced by the ability of these *Lasallia* species to recover normal F_v/F_m values after the summer period, a recovery not shown by boreal species (Gauslaa *et al.* 2001).

This ability to recover is also evidenced by the difference between field and preconditioned laboratory thalli (Fig. 2). The laboratory thalli reach maximum F_v/F_m in a short period, with the exception of August and September when damage might have been greater owing to harsher conditions. After protracted periods of drought, it might take longer to recover than the 48 h preconditioning period, but they finally do so and this ability is probably the reason for their survival in this climate. A boreal population of *Lobaria pulmonaria* affected by high irradiance and drought was found to have a much slower rate of recovery of F_v/F_m , reaching 50% of control thalli values after 40 days under natural conditions followed by 48 h of laboratory acclimation under low light and frequent moistening (Gauslaa & Solhaug 2000). On the other hand, Pellegrini *et al.* (2014) reported damage by low light on thalli of *Flavoparmelia caperata* from an ash forest in Italy and then a full recovery of these thalli after two days of full hydration. The capacity for a fast response shown in this study is supported by the stronger positive correlation between F_v/F_m and precipitation during the preceding

2 days than that during the preceding 5 days. (Table 2). A quick physiological response in a climate such as the Mediterranean, with frequent unpredictable wet periods, could be advantageous for growth and development.

Although both species showed a similar seasonal pattern, notable differences between them were observed, probably resulting from their microhabitat preferences and their different stress tolerance. *Lasallia hispanica* seems to be in a suboptimal state under natural conditions as it only reached the apparent maximum F_v/F_m in the field in November, whereas *L. pustulata* maintained apparent maximum values in the field during almost the whole year (Fig. 2). The results are consistent with the increase in F_v/F_m in response to the 48 h preconditioning period (Fig. 2). However, the values obtained from laboratory acclimated *L. hispanica* were consistently higher than the respective field values, while in *L. pustulata* the differences between field and laboratory measurements were often small. This might indicate an efficient and fast activation of the repairing mechanisms in *L. hispanica*, resulting in a substantial increase in F_v/F_m under laboratory conditions during much of the year. This is in accordance with the preference of this species for more exposed habitats (Sancho & Crespo 1989). Moreover, *L. hispanica* has an aero-hygrophytic strategy, taking moisture only from the air, whilst *L. pustulata* has a substratum-hygrophytic one, relying on the moisture in the substratum (Sancho & Kappen 1989). This means that the former dries more rapidly and thus experiences desiccation more frequently, both of which enhance the production of reactive oxygen species, with the subsequent damage to DNA, proteins and lipids, amongst other deleterious effects (Minibayeva & Beckett 2001; Kranner *et al.* 2008). This suggests that *L. hispanica* may be the more tolerant and resistant of the two species to environmental stress.

This study shows clear and similar temporal changes in the F_v/F_m in two co-occurring populations of *Lasallia hispanica* and *L. pustulata* growing in a typical Mediterranean ecosystem and demonstrates the

close relationship between the state of PSII and climatic conditions. The response to summer drought seems to be characteristic of lichenized photobionts when exposed to typical Mediterranean summer conditions. Since both species can also be found in the north of Spain, where Mediterranean climatic features are not so pronounced, a further study with northern populations would be worth conducting in order to understand their adaptation to seasonality.

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