Laboratory of Plant Physiology, Department of Biology, University of Patras, Patras 26500, Greece

Abstract

Photochemical efficiency of photosystem 2 (PS2), assessed from *in situ* chlorophyll (Chl) fluorescence measurements, was seasonally monitored in five evergreen sclerophyll and five malacophyllous drought semi-deciduous species, co-occurring in the same Mediterranean field site. In evergreen sclerophylls, a considerable drop in the variable (F_v) to maximum (F_m) Chl fluorescence ratio coincided with the lowest winter temperatures, indicating low PS2 efficiency during this period. Summer drought caused a comparatively slight decrease in F_v/F_m and only in three of the five evergreen sclerophyll species tested. In drought semi-deciduous shrubs, the winter drop in F_v/F_m was much less conspicuous. During the summer, and in spite of the severe and prolonged desiccation of their malacophyllous leaves, F_v/F_m was maintained high and only in one species the PS2 efficiency was transiently suppressed, when the leaf relative water content became lower than 30 %. Thus evergreen sclerophylls are more prone to photoinhibition by low winter temperatures, while the sensitivity of drought semi-deciduals depends on the extent and duration of summer drought.

Additional key words: *Arbutus*; chlorophyll fluorescence kinetics; *Cistus*; *Genista*; *Nerium*; *Phillyrea*; *Phlomis*; *Pistacia*; relative water content; *Sarcopoterium*.

Introduction

Mediterranean climate is characterized by mildly cold, humid winters and long, dry, hot, and sunny summers. Therefore, Mediterranean ecosystems are dominated by soft-leaved drought avoiders (winter annuals or perennials whose above-ground

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*Author for correspondence; e-mail: Y.Manetas@upatras.gr
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transpiring surfaces are shed during the summer) and evergreen sclerophylls, the leaves of which possess several xeromorphic attributes appropriate for life under water stress. Between these two extremes, a third group of malacophyllous shrubs applies a semi-decidual habit, shedding more than 60 % of their transpiring surfaces by early summer (Orshan *et al.* 1989). Plants possessing leaves and growing only during the summer, are scarce. The traditional conclusion arising from this brief overview of the dominant life forms is that Mediterranean summer is the main unfavourable season limiting photosynthesis and growth (Mooney 1987). Accordingly, the investigations have mainly focused on behaviour of stomata, showing a strict stomatal control of transpiration for both evergreen sclerophylls and semi-deciduous plants during the dry period (Beyschlag *et al.* 1986, Harley *et al.* 1987, Lange 1988, Grammatikopoulos *et al.* 1995).

Some studies, however, have shown that low winter temperatures may also limit the growth of Mediterranean plants. Physiological criteria like CO_2 assimilation rates (Larcher 1961, Eckardt *et al.* 1975, Beyschlag *et al.* 1990, Grammatikopoulos *et al.* 1995, Gratani 1997) or frost resistance of plant organs (Larcher 1981) and biogeographical criteria (Mitrakos 1980) have been used by these investigators. However, the number of species tested was small to permit any firm conclusion.

In the present study we used Chl fluorescence transients in order to assess *in situ* the seasonal changes in PS2 photochemical efficiency in ten Mediterranean plant species. We took advantage of the fact that modern, portable, Chl fluorescence meters allow for quick screening of a large number of samples, improving the statistical potential of the measurements. The measured ratio of variable to maximum Chl fluorescence (F_v/F_m) is linearly correlated to PS2 photochemical efficiency (Butler and Kitajima 1975), and there is a general consensus that PS2 is the target for many environmental stresses, including low temperatures stress (Mohammed *et al.* 1995).

Materials and methods

Plants and sampling site: The study site was located 2 km east of the Patras University campus (38.3°N, 29.1°E) where the vegetation is characterized by the presence of both evergreen sclerophylls and drought semi-deciduous shrubs. The species selected for the study are those which dominate in the area. Five mature individuals from each species were tagged and used throughout the sampling period. These were the sclerophylls *Arbutus unedo* L., *Arbutus andrachne* L., *Phillyrea latifolia* L., *Pistacia lentiscus* L., and *Nerium oleander* L., and the drought semi-deciduous shrubs *Cistus creticus* L., *Cistus salviifolius* L., *Genista acanthoclada* L., *Sarcopoterium spinosum Spach.*, and *Phlomis fruticosa* L. Meteorological data were provided by the University weather station (Figs. 1 and 2).

Sampling: At the indicated dates, four exposed, south facing leaves from each individual were cut. Two of them were put in air-tight plastic bags and immediately transferred to the laboratory for measurement of leaf relative water content (RWC).

The other two were inserted in the appropriate leaf clips of the fluorimeter and put in a light-tight box. Chl fluorescence signals were taken 25-30 min after darkening the

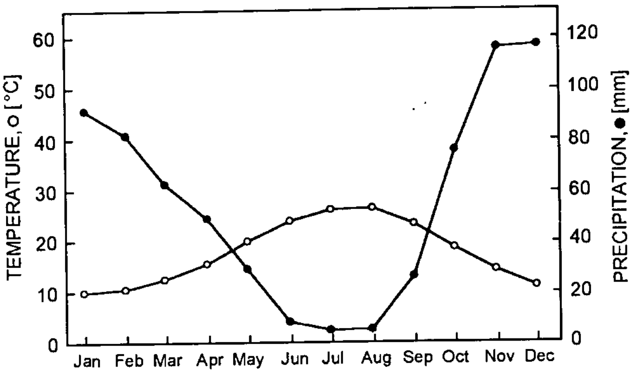

Fig. 1. Ombrothermic diagram for the experimental area (mean of thirty-seven years, 1955-1992).

leaves. In all cases, sampling was performed around mid-day, on clear days, and the fluorescence signals were obtained from the upper, exposed surface of the leaves.

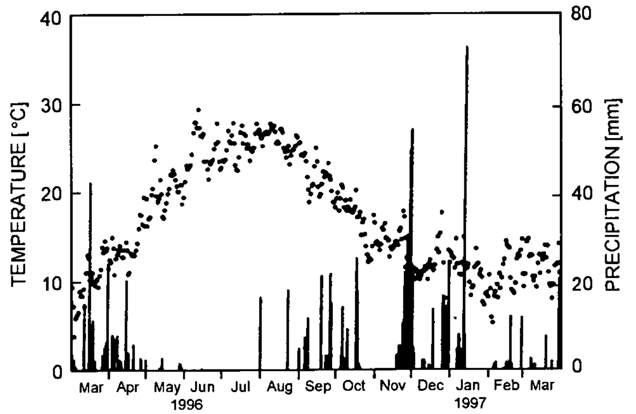

Fig. 2. Mean daily temperatures (*dots*) and total daily precipitation (*bars*) for the sampling period.

Measurements: RWC was measured with the leaf disc floating method of Turner (1981) but the equilibration time over distilled water was extended to 18 h (Kyparissis and Manetas 1993). For Chl fluorescence, a time resolving, non-modulated fluorimeter (*Hansatech*, Plant Efficiency Analyser) was used. Fluorescence signals (Kautsky curves) were obtained by irradiating the pre-darkened leaves with red radiation at 1500 μmol m^{-2} s^{-1} for 4 s. In preliminary experiments this photon fluence rate was saturating for all species.

Statistics: The results are means ± SD from 10 leaves (2 for each tagged shrub) from each species and sampling date. When needed, ANOVA between successive sampling dates was performed and the levels of significance are shown in the figures.

Results

Evergreen sclerophylls: Mid-day RWC in most evergreen sclerophylls was progressively reduced during the summer dry period, attaining minima in late August, just before the first rains (Fig. 3). RWC recovered during the autumn and high values were maintained throughout the winter and spring. Unexpectedly, the leaf RWC of *P. lentiscus* was kept comparatively constant during the whole year. In spite of the considerable water loss during the summer, the corresponding reductions in F_v/F_m were not so pronounced and occurred only in three of the tested species, *i.e.*, *P. latifolia, A. unedo,* and *A. andrachne*. If, for example, we compared the F_v/F_m in June, *i.e.*, at the start of the dry period, to that at the late August, the coresponding reductions were 9, 7, and 4 %, respectively (Fig. 3). However, a decreasing trend was evident in all cases during the winter, with the minimum values for all plants occurring at late January/early February 1997 (Fig. 3). The winter minimum was much deeper, the mean reduction was 26±2 % compared to the autumn maximum (Fig. 3), and coincided with the lowest attained temperatures of winter (Fig. 2).

In addition, progressively ascending values of F_v/F_m were observed in all plants (except *A. andrachne*) during early spring of 1996 (Fig. 3), following the low winter temperatures (Fig. 2). Apparently, minimum values of F_v/F_m during the winter of 1996 were lower than the first reported values in Fig. 3 (March 1996), further strengthening the view that low winter temperatures may suppress PS2 photochemical efficiency in evergreen sclerophylls.

Drought semi-deciduous: Compared with the corresponding values for evergreen sclerophylls, the summer decrease of RWC in the drought semi-deciduous shrubs was more dramatic and persistent (Fig. 4). In *P. fructicosa*, RWC dropped to less than 30 % during August 1996. However, the fluctuations in F_v/F_m in most of the cases were far less conspicuous (Fig. 4). Although a trend for reduction during the cold days of winter of 1997 was observed, it was not so intense as in evergreen sclerophylls. For example, the mean minimum value of F_v/F_m of the semi-deciduous reached 91.4±0.9 % of their maxima, while the corresponding value for evergreen sclerophylls was 73.8±4.8 % (Fig. 3). In *C. creticus, C. salviifolius, G. acanthoclada,* and *S. spinosum,* although the leaves were severely desiccated during the summer (RWC about 40-50 %), the photochemical efficiency of PS2 remained unaffected. However, in *P. fruticosa* a steep drop in F_v/F_m was observed in August, coinciding with the minimum RWC values. In this plant, the F_v/F_m measured before the occurrence of the minimum RWC (*i.e.*, during June and July) and immediately after the rains of August (see Fig. 2) was fairly high, in spite of the fact that the corresponding values of RWC were very low. In *P. fruticosa*, for example, the RWC during June and July was around 35 % and after the August rain was less than 50 %. However, the F_v/F_m values were maintained higher than 0.72, indicating that a severe suppression of PS2 photochemical efficiency occurs only when the RWC becomes lower than a critical value.

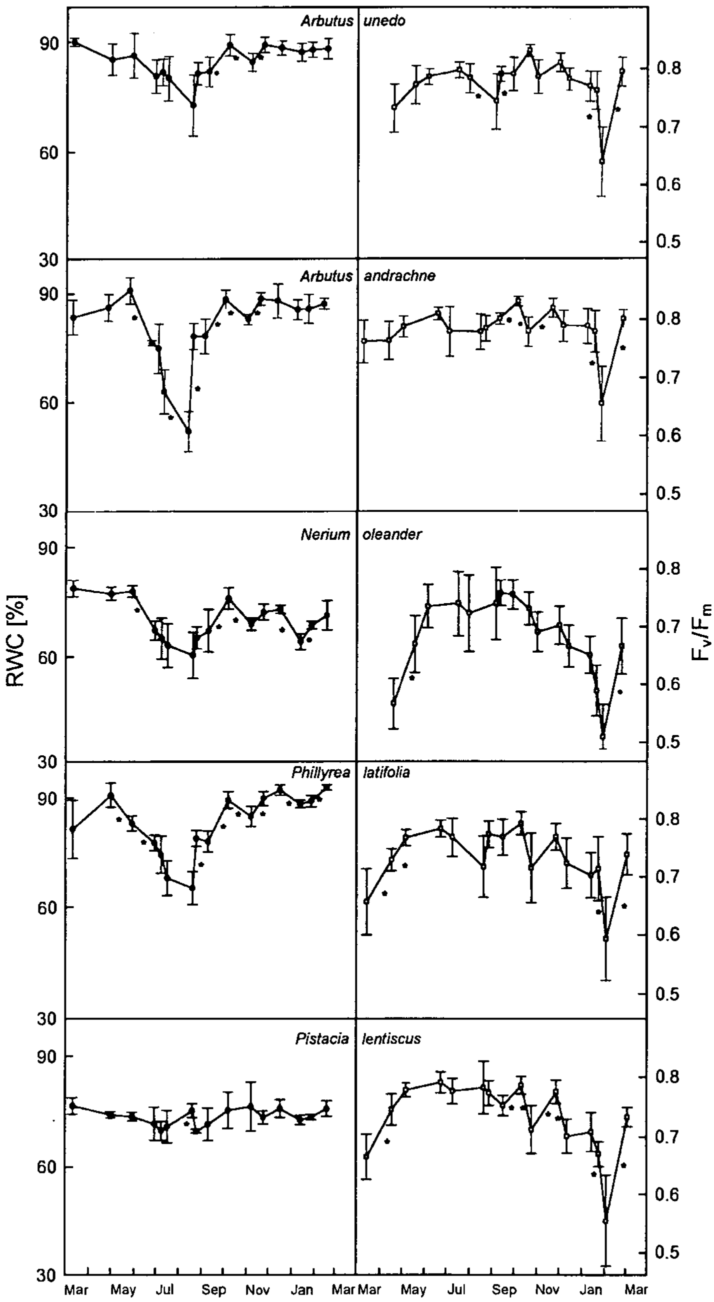

Fig. 3. Seasonal variation in leaf relative water content and in mid-day values of F_v/F_m in evergreen sclerophylls. The asterisks between two successive sampling dates denote corresponding statistically significant differences at $p < 0.05$.

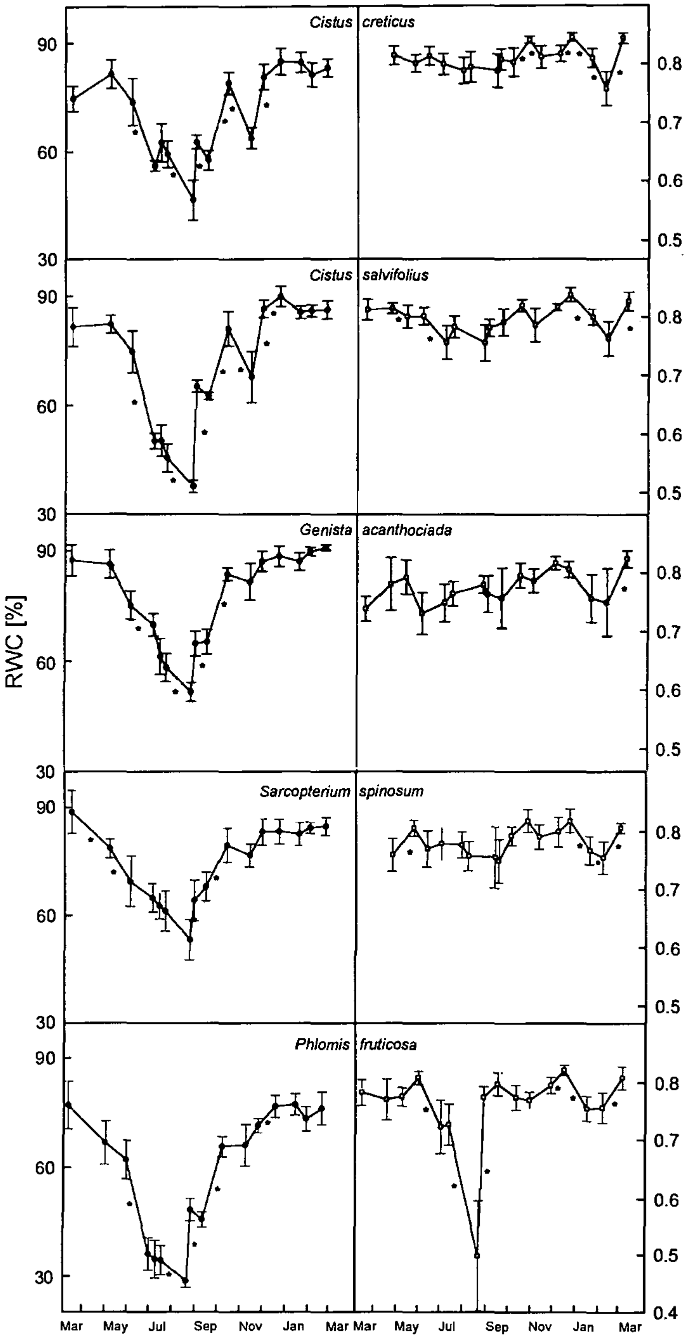

Fig. 4. Seasonal variation in leaf relative water content and in mid-day values of F_v/F_m in drought semi-deciduous shrubs. Symbols as in Fig. 3.

Discussion

Apart from the stomatal limitations, photosynthetic CO_2 assimilation can be suppressed by internal limitations, *i.e.*, by carboxylation efficiency (related to the activity of carboxylating enzymes) and/or the impairment of electron flow due to the low efficiency of PS2 photochemical conversion. If the carboxylation phase is limited (for example by low temperatures, high resistance of CO_2 diffusion, *etc.*), the excess excitation energy may cause chronic photoinhibitory damage, unless it can be lost as heat through the activation of dissipating mechanisms (down-regulation; Long *et al.* 1994). The result is the reduction in CO_2 assimilation and growth (Ögren and Sjöström 1990).

Our results show that winter temperatures, although mildly cold, are considerably more stressful for the PS2 photochemical efficiency of evergreen sclerophylls, than the long summer drought. Adams *et al.* (1990) showed that F_v/F_m is highly correlated to the quantum yield of net photosynthesis (Φ_i). In *A. unedo* (Beyschlag *et al.* 1990), Φ_i was considerably depressed both during the low winter temperatures and the summer dry period. With our plants, considerable summer decreases in PS2 photochemical efficiency were found only in the semi-deciduous *P. fruticosa*, while in sclerophylls, trends for lower F_v/F_m during the summer were observed only in *A. unedo, A. andrachne,* and *P. latifolia*. At first sight, this was unexpected since, apart from the already mentioned extensive seasonal study of Beyschlag *et al.* (1990), other investigators have shown considerable reduction in F_v/F_m when Mediterranean evergreen sclerophylls were drought stressed under high irradiance. Björkman and Powles (1984) for example, working with potted *Nerium oleander,* found considerable decreases in F_v with increasing water deficit. In addition, Demmig-Adams *et al.* (1989) working with field-grown *A. unedo,* observed a 50 % mid-day reduction of F_v/F_m on exposed leaves, coinciding with the mid-day depression of stomatal apperture. However, in both cases fluorescence signals were obtained after 2-5 min of leaf predarkening. In *A. unedo*, a recovery of F_v/F_m was observed upon further time in the dark indicating that the low initial values could be due to a temporary down-regulation of photosynthesis related to zeaxanthin formation (Long *et al.* 1994). Our results, however, are in agreement to those of Garcia-Plazaola *et al.* (1997) who observed a slight (*ca.* 12 %) mid-day reduction in F_v/F_m during the summer but a considerable (*ca.* 31 %) reduction during the winter in the evergreen sclerophyll *Quercus suber* growing in the field in Portugal. These authors used a 30 min predarkening period as we did and, accordingly, the sustained values of F_v/F_m were measured.

Our results do not permit to distinguish whether the low winter PS2 efficiency is due to photoinhibition or down-regulation of photosynthesis. Sustained low values of F_v/F_m in predarkened leaves are an indication of photoinhibitory damage (Long *et al.* 1994). Alternatively, they may indicate increased zeaxanthin concentration in the chloroplast (Demmig-Adams and Adams 1992). Zeaxanthin may mediate the harmless heat dissipation of extra excitation energy in the pigment bed, thus protecting chloroplast membranes from photodamage (Young 1991). Darkness or low irradiance quickly restore low contents of zeaxanthin through its epoxidation to violaxanthin, while F_v/F_m increases to its maximum (Demmig-Adams and Adams 1992). Under low freezing temperatures, however, zeaxanthin epoxidation may be inhibited (Adams *et al.* 1995). Indeed, Adams and Demmig-Adams (1994) found that conifer needles during the cold winters maintain high zeaxanthin levels throughout the 24-h-cycle, affording the opportunity of high photodissipative capacity during the high irradiance and freezing temperatures winter's day. In addition, Leverenz and Öquist (1987) have found a considerable drop in F_v/F_m during the freezing winter temperatures in the cold-resistant *Pinus silvestris*. Freezing temperatures are scarce in the Mediterranean. However, the extent of sensitivity to low temperatures is apparently species specific and correlates well with the environmental history of plants (Larcher 1995).

Further field and laboratory work is needed to elucidate these points. However, we may reasonably conclude that, at least for the Mediterranean evergreen sclerophylls, chilling winter temperatures are more stressful for PS2 function than the lack of water during the summer.

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