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Diurnal and seasonal variations in chlorophyll *a* fluorescence in two Mediterranean-grassland species under field conditions

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Abstract

Seasonal and daily variations in chlorophyll (Chl) fluorescence were studied in two representative species of Mediterranean grasslands, *Tuberaria guttata* (an annual) and *Chamaemelum nobile* (a perennial), in order to assess physiological responses to climatically induced stresses during the growing season. The photochemical efficiency of photosystem (PS) 2 in dark-adapted leaves was measured by the Chl fluorescence ratio F_v/F_p. This ratio decreased progressively from December to July, as the effects of increasing solar radiation and summer drought became more severe. The seasonal decline was observed particularly as a depression of morning and midday values, when photoinhibition was more evident. In both species, the extent of this diurnal depression increased with midday irradiance throughout winter and spring. After sunset, there was complete recovery to optimum values. Towards the end of the life cycle, increased irradiance did not affect the midday decline further but F_v/F_p measurements in the morning and evening never regained their optimum values, indicating the accumulation of photodamage in the reaction centres of PS2. The half-rise time of F_p ($T_{1/2}$), used to estimate the size of the plastoquinone pool, showed little daily variation in *C. nobile* throughout the most important part of its seasonal cycle. However, towards the end of its life cycle (June and July) $T_{1/2}$ values ranged from *ca.* 200 ms before sunrise to near zero at midday on the same day. The annual species, *T. guttata*, showed similar disregulation in energy transmission rate both at the seedling stage and at end of its life cycle. Thus seedlings and reproductive

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Abbreviations: Chl = chlorophyll; F_p, F_0, F_v = peak, initial, and variable Chl fluorescence; $T_{1/2}$ = half-rise time for peak of fluorescence; PS = photosystem; PPFD = photosynthetically active photon flux density; Q_A and Q_B = primary and secondary, stable, quinone acceptors of photosystem 2; PQ = plastoquinone.
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plants in particular are sensitive to environmental conditions (extremes of temperature and drought) and cannot maintain consistent electron flow throughout the day.

Additional key words: Chamaemelum nobile; photochemical efficiency; photoinhibition; photosystem 2; *Tuberaria guttata.*

Introduction

Mediterranean field studies are particularly suitable for investigating combinations of stresses under natural conditions. The limiting availability of water has long been recognized as a key factor in determining the general character of Mediterranean vegetation; it experiences no rainfall when potential evapotranspiration is very high but the winter rains are accompanied by temperatures that are frequently sub-optimal for plant growth (Aschmann 1973).

During the growing season many environmental factors may cause stress and potentially affect population sizes (Lichtenthaler 1996, Figueroa *et al.* 1997). Among these, the high solar irradiation is important. Photon flux density displays strong variations over seasons, days, and even shorter periods. In southern Europe, plants are exposed to saturating actinic irradiance in the middle of the day for much of the year, except on cloudy days. Frequently, there is an imbalance between energy input and its utilization. External protection mechanisms, such as leaf movements, are ineffective when plants are very small. Consequently, when the amount of absorbed radiation exceeds the ability of the photosynthetic apparatus to use it, the surplus energy has to be quenched in order to avoid photooxidative damage to thylakoid membranes (Bolhàr-Nordenkampf *et al.* 1991). This causes a reduction in the efficiency of energy trapping, called photoinhibition (Krause and Weiss 1991, Osmond 1994, Osmond and Grace 1995, Govindjee 1995).

In the nature, several other environmental factors (*e.g.*, drought and temperature) and physiological properties (*e.g.*, C_3 or C_4 pathway, ontogenetic stage, sun or shade acclimation, Chl content) may interact to affect the activity of PS2 (Osmond 1994, Raghavendra *et al.* 1995). Many combinations of stress factors that enhance the sensitivity of the photosynthetic apparatus to photoinhibition have been reported (Epron *et al.* 1992). The activity of PS2 is very sensitive to environmental perturbation (Lichtenthaler 1988, Lichtenthaler and Rinderle 1988, Baker 1991), and it is significant that 90 % or more of the total fluorescence emitted at air temperature comes from PS2 (Govindjee 1995, Gitelson *et al.* 1998).

The use of Chl fluorescence from intact plant leaves is one of the most important non-intrusive methods for monitoring photosynthetic events and judging the physiological state of the plant (Lichtenthaler 1988, Lichtenthaler and Rinderle 1988, Baker 1991, Long *et al.* 1994, Mohammed *et al.* 1995, Srivastava *et al.* 1995, Figueroa *et al.* 1997). Chl fluorescence emitted by dark-adapted leaves may reveal information about the two main classes of photoinhibition on the basis of relaxation time: dynamic photoinhibition or photosynthetic down regulation, a readily reversible process that provides photoprotection to PS2, and chronic photoinhibition, a slowly reversible depression of photosynthesis associated with photodamage (Osmond 1994).

Chl fluorescence parameters may change during the course of the day, with a marked reduction in efficiency at midday. This has been observed among higher plant species (Xu and Wu 1996), and the decline is not only detectable in Chl fluorescence ratio but also in xanthophyll pigments and CO_2 uptake (Schindler and Lichtenthaler 1996). Such transient diurnal changes have been referred to as "diurnal photoinhibition" (Ögren and Evans 1992, Long *et al.* 1994) or "dynamic photoinhibition" (Osmond 1994), but according to Demmig-Adams *et al.* (1996) it is better to use terms such as "diurnal PS2 regulation" or diurnal changes in the "efficiency with which excitation energy is delivered to open PS2 centres", because the decrease in PS2 efficiency simply reflects an adjustment of PS2 to the different fractions of absorbed radiation that can be utilized in electron transport over the course of a day (Adams *et al.* 1989, 1995, Björkman and Demmig-Adams 1994). Longer term, seasonal changes in Chl fluorescence have been reported in trees (Lichtenthaler *et al.* 1989).

The aim of this work was to examine changes in the photochemical efficiency of PS2 over the phenological cycle of representative annual and perennial Mediterranean grassland species in the field, by examining the diurnal changes in individual fluorescence parameters.

Materials and methods

Plants: The study site at Alía (Cáceres, in south-west Spain) was selected as one of the oldest undisturbed areas of semi-arid grassland ("dehesa") in Extremadura. A detailed description of the site and the species present is given by Figueroa and Davy (1991). The grassland studied has a strong seasonality, typical of the Mediterranean climate. Irradiance and temperature are high during summer, with practically no precipitation between June and October, whereas winters are wet and cool. Accordingly, the dry, hot summer, with a high evaporative demand, is the most stressful period for the vegetation. In addition, the Mediterranean summer is practically cloudless, imposing a potential light stress (Figueroa *et al.* 1997).

We selected two abundant, representative species: *Chamaemelum nobile* (L.) All. (*Asteraceae*) is perennial and *Tuberaria guttata* (L.) Fourr. (*Cistaceae*) is a typical winter annual. Measurements were made five times during the growing season of the plants. The records started in late autumn, after the establishment of the seedlings and new shoots in the field (December). Subsequent samples were taken in the middle of winter (February), the spring (March and May), and early summer (June). The last measurement was taken in the first days of July when only few species had live vegetative shoots. All measurements were made on young leaves with the same orientation relative to light, on plants selected from the same area, to avoid effects of microclimatic variations.

Chl fluorescence measurements in the field were made throughout each sampling day. The first measurement was made before sunrise. Then we made measurements every two hours until the last one at sunset. This yielded seven values in December and nine in July. With each Chl fluorescence measurement, the PPFD was also

measured using a quantum sensor and meter (*LiCor Li-189*). Additional measurements were made to establish the maximum irradiance at noon when the Chl fluorescence measurement did not coincide with this. Values for daily precipitation and daily maximum and minimum temperature were obtained from the Guadalupe (Cáceres) number 2 meteorological station, about 15 km from the research site.

Chl fluorescence kinetics of intact leaves were measured at the prevailing air temperature using a portable non-modulated fluorimeter (Plant Stress Meter, *PSM Mark II, Biomonitor S.C.I. AB*, Umeå, Sweden) and a white lightweight clamp cuvette (*Biomonitor 1020*) for dark adaptation (Öquist and Wass 1988, Bolhàr-Nordenkampf *et al.* 1989, Mohammed *et al.* 1995). Leaves were dark pretreated for 20 min before measurement. The Chl fluorescence transient over 2 s was determined after an actinic pulse of 400 μmol m^{-2} s^{-1}. Ten measurements were made at each sampling time on young leaves of randomly chosen plants.

Initial fluorescence (F_0) was determined when all PS2 reaction centres were an "open" state and the rate of photochemistry was not limited. F_0 depends on the size of PS2 Chl antenna and on the functional integrity of PS2 reaction centres (Krause and Weis 1991). Superimposing a flash of actinic radiation yielded a peak of fluorescence (F_p) dependent on the level of actinic stimulation. Variable fluorescence (F_v) was determined as the change in fluorescence emission between the two defined states, F_0 and F_p. The half-time for the transition from F_0 to F_p ($T_{1/2}$) was determined, and this is related to the reduction rate of the first electron acceptors, Q_A, Q_B, and PQ; it has been used to determine the size of the PQ pool (Krause and Weis 1991, Bolhàr-Nordenkampf and Öquist 1993).

The ratio of variable to peak Chl fluorescence [$F_v/F_p = (F_p - F_0)/F_p$] was used as a measure of photochemical efficiency of PS2 (Björkman and Demmig 1987, van Kooten and Snel 1990). This ratio correlates with the number of functional PS2 reaction centres (Öquist *et al.* 1992) and has been used to quantify photoinhibition (Osmond 1994, Krivosheeva *et al.* 1996).

Results were analyzed by analysis of variance (ANOVA). Means were compared using the Tukey test. Differences were considered significant at $p<0.01$. Paired *t*-tests were used to test differences between the solar times during the life cycle.

Results

Climate: Daily minimum and maximum temperatures and total daily precipitation between October 1993 and July 1994 (corresponding with the Mediterranean growing season) at the Guadalupe 2 meteorological station are shown in Fig. 1.

Daily and seasonal changes in photochemical efficiency (Fig. 2): *C. nobile* and *T. guttata* did not show a significant noon depression of F_v/F_p in December. The sampling day in December was typically foggy and so PPFD was consistently low. In March and May, PPFD progressively increased (sampling was in full sunshine), and both species showed marked reductions in photochemical efficiency at noon of about 12-15 %, as compared to the morning values. These were partly reversed by the late

afternoon. By June, PPFD was close to its maximum of about 2 000 μmol m^{-2} s^{-1}. At this time, the midday depression of F_v/F_p was very pronounced, and values for photochemical efficiency in both species were 40 % less than those recorded before sunrise. In July, only *C. nobile* remained; its night value for photochemical efficiency was reduced in comparison with those previously, although midday values were similar to those in June.

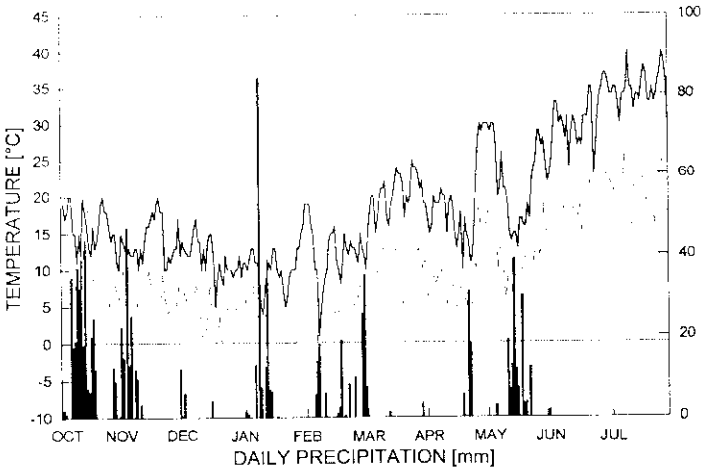

Fig. 1. Climatic values for Guadalupe No. 2 meteorological station (Cáceres) from October 1993 until July 1994: weekly means of daily minimum (···) and maximum (—) air temperature and daily precipitation (*vertical bars*).

Whatever the midday depression, in all diurnal courses recovery of F_v/F_p at night was complete. Recovery started as soon as PPFD began to fall, and F_v/F_p returned to the previous morning values by the end of the night.

Daily and seasonal trends in fluorescence kinetics (Figs. 3 and 4): From autumn to mid spring, F_0 maintained very constant values throughout the day, with a slight decrease at noon. F_v, on the other hand, tended to decrease at midday, and this was the reason for the depression in F_v/F_p (Fig. 3). Both F_0 and F_v reached annual maxima in May. By June, F_v values were significantly lower throughout the day, although with some recovery at night. The lowest F_v recorded was for *C. nobile* in July, and this showed no recovery at sunset or dawn. In June and July F_0 tended to increase at midday, achieving values similar to F_v, and this is an important component of the midday depression in F_v/F_p.

C. nobile showed little variation in $T_{1/2}$ during the day and little variation with season, from December until May (Fig. 4). In June the diurnal variations in $T_{1/2}$ developed a wide amplitude, with high values in the morning and lower ones at noon. Between December and March the highest value for $T_{1/2}$ was recorded near to noon, while in June and July the value at noon was the lowest of the day. $T_{1/2}$ for *T. guttata* in March and May showed little difference between the morning records and the ones at noon, as in *C. nobile* throughout much of its growing season. The measurements

taken in December and June had significant variations between noon and morning or evening. However, these sampling dates differed in the time of day in which the plant achieved the maximum value of $T_{1/2}$; in December the maximum was recorded at noon and in June it was in the morning.

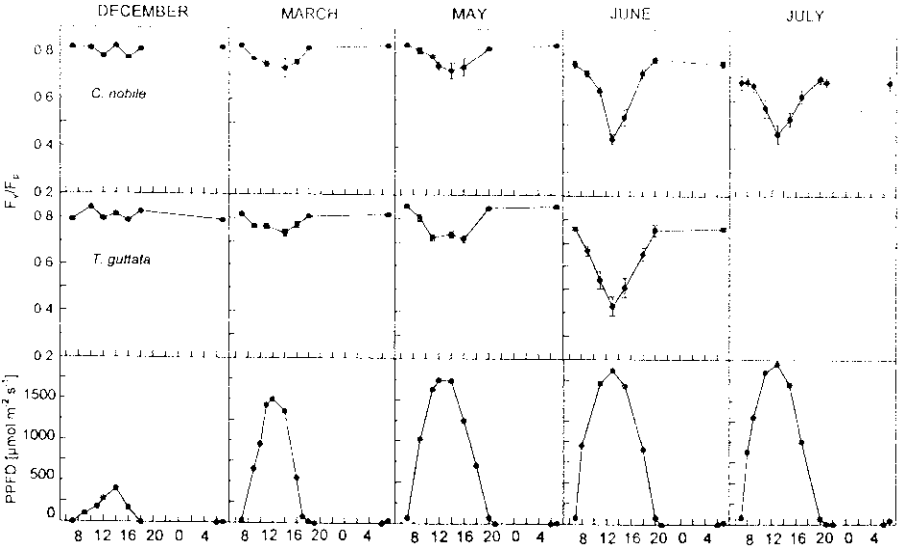

Fig. 2. Diurnal time course of changes in F_v/F_p in *Chamaemelum nobile* (*top*), *Tuberaria guttata* (*middle*), and photosynthetic photon flux density, PPFD (*bottom*), during the growing season. Each value for F_v/F_p is the mean of 10 measurements. Vertical bars indicate SE.

Discussion

We found a strong and reversible depression in the photochemical efficiency of PS2 of two Mediterranean grassland species in the field, and this occurred concomitantly with the diurnal increase and decrease in radiation. Photochemical efficiency of PS2 usually decreases at the same rate as the photon yield of O_2, reflecting a diurnal limitation in CO_2 assimilation (Adams *et al.* 1990, Bilger *et al.* 1995).

F_v/F_p recorded before sunrise had the same nearly optimum level over the greater part of the growing season. Only under the high temperatures, high insolation, and drought of mid-summer did these values start to decline, reflecting recovery from the midday decline (but lower to physiological optimum) and indicating an accumulation of photodamage from one day to another. The measurements taken at midday showed a more or less pronounced reduction of F_v/F_p relative to sunrise as a response to the radiation.

The diurnal decrease in photochemical efficiency of PS2 recorded in December and March was due to a quenching in F_0 and mainly in F_v. This process has the characteristics of an increase in the rate of radiationless energy dissipation in the antenna pigments, and should be accompanied by an increase in zeaxanthin content or other quenching mechanism (Demmig-Adams *et al.* 1989, 1996, Schindler and

Lichtenthaler 1994, 1996). This result has been reported previously for different plants subjected to a combination of different stress factors (Adams and Demmig-Adams 1992). This quenching tends to divert excitation energy away from the PS2 reaction centres so gives protection to photosynthetic membranes against the adverse effects of the accumulation of excess excitation energy (Krause 1988, Long *et al.* 1994, Demmig-Adams and Adams 1996). At this time of year, there is no reduction in electron transport in thylakoid membranes at midday, since $T_{1/2}$ only shows slight variations during the day (Bolhàr-Nordenkampf *et al.* 1991).

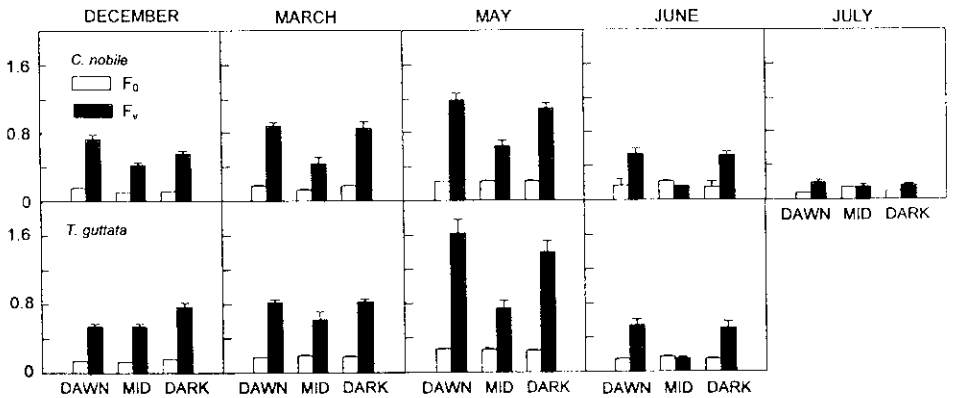

Fig. 3. Changes in F_0 and F_v in *Chamaemelum nobile* and *Tuberaria guttata* during the growing season at different times throughout the day (± SE, n = 10).

The increase in F_0 at midday after May indicates damage to PS2 centres (Krause 1988). Also after this date, a considerable variation in $T_{1/2}$ was recorded, which suggests a reduction in the number of functional centres of PS2 and the PQ pool. Hence, we can distinguish two parts in the life cycle of these species (Figueroa *et al.* 1997). In the first part, repair mechanisms are able to maintain high photochemical efficiency and a constant electron transport during the day, whereas in the second part, repair mechanisms are not able to avoid damage at midday. In this latter part, the recovery from photoinhibition never reached the optimum values; even in the morning photochemical efficiency of PS2 was suboptimal. At this level, there were no differences in photochemical efficiency between the species studied.

The most important difference between the two species is related to the seasonal variations in $T_{1/2}$ at particular times of day. *C. nobile* is the more tolerant species to such environmental stress. It displays little diurnal variation in $T_{1/2}$, suggesting an almost constant D1-turnover and PQ pool. This could be due to a high rate of substitution of damaged proteins. As a result, most of the trapped energy is used in photosynthesis, and this is reflected in the rapid growth observed in the field. After June, however, at midday the half-time to maximum fluorescence fell to values near zero. This suggests limited energy transmission between photosystems and of course to the photosynthetic process. At this time, the only useful ways to divert energy are related to thermal deactivation in the inactive PS2 reaction centres and the diversion of energy to additional pigments, as in the xanthophyll cycle (Anderson and Aro 1994, Long *et al.* 1994). However, photochemical and nonphotochemical

deactivation mechanisms are not sufficient to prevent the damage to the antenna complex that is reflected in an increase in initial fluorescence. In July, this situation is more pronounced, with maximum values for $T_{1/2}$ in the morning. Although the highest diurnal variation in $T_{1/2}$ in *C. nobile* was recorded in summer, PPFD had reached 1500 μmol m^{-2} s^{-1} by March, and so the effects were not exclusively due to irradiance, but to its interactions with temperature, drought, and senescence (for the effects of leaf age on fluorescence see Šesták and Šiffel 1997).

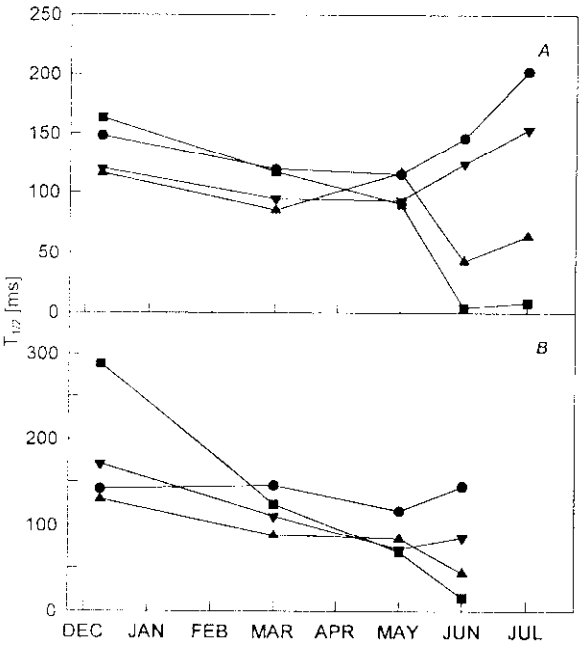

Fig. 4. Seasonal variations in $T_{1/2}$ as measured at different times of the day: morning (●), noon (■), evening (▲) and night (▼) in *Chamaemelum nobile* (*A*) and *Tuberaria guttata* (*B*). $n = 10$.

The annual species, *T. guttata*, showed greater diurnal oscillation in $T_{1/2}$. In June, the reason for this variation was the same as for *Chamaemelum*. However, in December the higher diurnal oscillation could be related to the delicate ontogenetic state of the plants as seedlings. Further studies are necessary to generalize these ideas to plants of Mediterranean habitats.

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