Role of xanthophyll cycle-mediated photoprotection in *Arbutus unedo* plants exposed to water stress during the Mediterranean summer

R. BARALDI[*,++], F. CANACCINI[**], S. CORTES[***], F. MAGNANI[**], F. RAPPARINI[*], A. ZAMBONI[**], and S. RADDI[+]

Istituto di Biometeorologia, Consiglio Nazionale delle Ricerche, Via Gobetti 101, 40129 Bologna, Italy[*]
Dipartimento di Colture Arboree, Università degli Studi di Bologna, Viale Fanin 46, 40127 Bologna, Italy[**]
Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano, 85100 Potenza, Italy[***]
Dipartimento di Scienze e Tecnologie Ambientali Forestali, Università degli Studi di Firenze, Via S. Bonaventura 13, 50145 Firenze, Italy[+]

Abstract

We analyzed the response of potted strawberry tree (*Arbutus unedo* L.) seedlings exposed to water stress by withholding water for 10 d (WS). Leaf water potential, net CO_2 assimilation, and stomatal conductance decreased with increasing water deficit. A 30 % reduction of chlorophyll (Chl) content in the antenna complexes was observed in WS-plants. Simultaneously, a decline of photochemical efficiency (F_v/F_m) occurred as a result of an excess of solar radiation energy when carbon assimilation was limited by stomata closure due to soil water deficit. The non-photochemical quenching of Chl fluorescence (Φ_{NPQ}) significantly increased, as well as the leaf contents of zeaxanthin (Z) and antheraxanthin (A) at the expense of violaxanthin during the WS-period. Elevated predawn contents of de-epoxidized xanthophyll cycle components were associated with a sustained lowering of predawn photosystem 2 efficiency; this suggested an engagement of Z+A in a state primed for energy dissipation. Thus, the ability of strawberry trees to maintain the functionality of the xanthophyll cycle during the Mediterranean summer is an efficient mechanism to prevent irreversible damages to the photosynthetic machinery through thermal energy dissipation in the antenna and the reduction in photochemical efficiency.

Additional key words: chlorophyll; fluorescence induction; net photosynthetic rate; photochemical efficiency; photoprotection; photosystem 2; stomatal conductance; strawberry tree; violaxanthin; water potential; zeaxanthin.

Introduction

In Mediterranean-type climates, plants are typically exposed to water stress during the summer (Mooney 1981, Nahal 1981). In this season, the scarcity of precipitation is generally associated with increased day length and solar altitude which translate into high irradiance and high temperature (Comstock and Mahall 1985). The high irradiances usually exceed the capacity for utilizing the energy absorbed by photochemistry (photochemical quenching, Φ_{PS2}) or for dissipating it through photoprotective processes (non-photochemical quenching, Φ_{NPQ}). Thus it may result in potentially harmful reactive oxygen species (Smirnoff 1993) and a long-lasting damage to photosynthetic machinery (Powels 1984, Osmond 1994). The problem is particularly severe under drought, when carbon assimilation is limited by the decrease in stomatal conductance (g_s) and

Received 8 November 2007, *accepted* 2 May 2008.
[++]Corresponding author; fax: +39 051 6399024, e-mail: r.baraldi@ibimet.cnr.it
Abbreviations: A − antheraxanthin; C − control leaves; $\Delta F/F_m'$ − photochemical efficiency of photosystem 2 in the light; F_m − maximal fluorescence in the dark; F_v/F_m − photochemical efficiency of PS2 in the dark; F_0 − minimal fluorescence in the dark; g_s − stomatal conductance; HPLC − high-performance liquid chromatography; PFD − photon flux density; PS2 − photosystem 2; Q_B − secondary plastoquinone acceptors of PS2; RC – reaction centre; V − violaxanthin; VAZ − total amount of xanthophyll cycle components; VPD − vapour pressure deficit; WS − water-stressed leaves; Z − zeaxanthin; $\Phi_{f,D}$ − sum of fluorescence and constitutive thermal dissipation; Φ_{NPQ} − quantum yield of xanthophyll-regulated thermal energy dissipation; Φ_{PS2} − quantum yield of photochemistry.
Acknowledgments: We are grateful to Dr Gianpaolo Bertazza for his help with the use of HPLC. We thank Josep Peñuelas for helpful comments and critical review of the manuscript.

chloroplasts may be subjected to an excess of energy resulting in a down-regulation of photosynthesis or in photoinhibition (Masojídek *et al*. 1991, Long *et al*. 1994, Demmig-Adams *et al*. 1996b). Photoinhibition of photosynthesis is characterized by a sustained decrease in the efficiency of photon utilization by photosystem 2 (PS2) photochemistry. It can be associated with damage to the photosynthetic apparatus and also with an increased thermal dissipation, which is a photo-protective process (Choudhury and Behera 2001). Photoinhibition comprises different events such as photoinactivation of electron transport activity, and damage and repairs of proteins of the PS2 reaction centres (RCs). The extent of photoinhibition depends on the balance between the rate of photodamage to PS2 RCs and the repair capacity (Greer *et al.* 1986, Murata *et al*. 2007). Different environmental stresses affect photoinhibtion by primarily inhibiting the repair capacity of PS2 (Murata *et al*. 2007). The relationship between photoinhibition and D1 protein, the core heterodimeric polypeptide of the PS2 RC complex, is discussed often: protein D1 may play a minor role in photoinhibition (Demmig-Adams and Adams 1992) or its turnover may be the main molecular mechanism solving this phenomenon (Aro *et al*. 1993, Tyystjärvi and Aro 1996).

A strong correlation between the ability to dissipate energy *via* Φ_{NPQ} and the increase of de-epoxidized xanthophyll cycle components, antheraxanthin (A) and zeaxanthin (Z), was found in different plant species (Demmig *et al.* 1988, Björkman and Demmig-Adams 1994, Demmig-Adams and Adams 1996a, Demmig-Adams *et al.* 1996a, Gilmore 1997, Niyogi *et al*. 1998). Thus the xanthophyll cycle, which consists in the conversion (de-epoxidation) of violaxanthin (V) to Z *via* A under excess irradiance and in the reversed reaction (epoxidation) under low irradiance, plays a central role in photoprotection (Demmig-Adams and Adams 1992, 1996b, Pfündel and Bilger 1994).

This xanthophyll-mediated protective mechanism responds not only to irradiation (diurnal fluctuation, sunflecks, sun-shade), but also to a combination of temperature (both extreme high and low temperatures), water shortage, and nutrient availability (Demmig-Adams *et al.* 1996b). Several studies investigated the role of xanthophyll cycle during winter stress in various conifers, broad-leaved evergreen species, shrubs, and herbs and reported a nocturnal retention of Z during the coldest night, which remained engaged in a state primed for energy dissipation (Adams and Demmig-Adams 1994, 1995, Adams *et al*. 1995, Verhoeven *et al.* 1996, Barker *et al*. 1998, Garcia-Plazaola *et al.* 1999a,b, Verhoeven *et al.* 1999, Varone and Gratani 2007). This conclusion was supported by the evidence of a sustained decrease of the PS2 photochemical efficiency at predawn (F_v/F_m) associated with the Z retention. The same results have been found for the effects of combined high irradiance and water shortage in *Nerium oleander*, *Zea mays*, and *Rosmarinus officinalis* (Demmig *et al.* 1988, Saccardy *et al*. 1998, Munné-Bosch and Alegre 2000). Barker *et al.* (2002) observed the same correlation between the de-epoxidation of V and the F_v/F_m at predawn only during cold winter days in two *Yucca* species but not during hot summer when, in spite of a nocturnal retention of Z, the correlation with F_v/F_m was not observed. The authors suggested that Z+A retention and engagement are two separate processes that are controlled by separate factors.

Arbutus unedo (strawberry tree) is a drought tolerant evergreen sclerophyll native to the Mediterranean regions that exhibits several mechanisms of drought stress resistance. The effects of drought and excessive radiation on photochemical efficiency, photosynthesis, and carotenoid composition of strawberry trees are known (Demmig-Adams *et al.* 1989, Werner *et al.* 1999). Munné-Bosch and Peñuelas (2004) reported an increase of Z and ascorbate contents and a decrease of chlorophyll (Chl), lutein, and β-carotene contents in strawberry tree plants exposed to a combination of severe water deficit, high irradiance, and high temperature. A predawn retention of the de-epoxidized A and Z was also reported (Levizou *et al*. 2004) as the effect of other micro-environmental conditions (hypoxia, high CO_2 concentration, and increased red to blue photon ratios) within strawberry tree twigs.

The main purpose of this study was to investigate the adaptive strategies of *A. unedo* potted plants to cope with high irradiance and extreme water stress induced by withholding water for 10 d, and to elucidate the mechanisms that might confer protection from photoinhibition. The physiological responses were monitored by measuring gas exchange, Chl fluorescence, and photosynthetic pigments with an emphasis on the role of the xanthophyll cycle in the dissipation of excess photon energy.

Materials and methods

Plants: Twenty strawberry tree seedlings were grown for 2 years in 5 000 cm^3 pots containing a commercial mixture of sand and peat, and covered with organic mulching in order to reduce soil evaporation. At the beginning of the experiment, plants about 60 cm tall were paired for dimensions and randomly allocated to one of two treatments: control plants (C) were watered daily to field capacity, whilst water was withheld from water-stressed plants (WS). The experiment lasted for 10 d, from 22 June to 2 July, 2003. Since plants were grown outside, they also experienced a combination of high irradiance and high temperature, which are typical of summer WS-conditions in the Mediterranean region; maximum daily photon flux density (PFD), air temperature, and vapour pressure deficit (VPD) over the course of the experiment were 1 200±87 μmol m^{-2} s^{-1}, 38.5±0.9 °C, and 4.8±0.3 kPa, respectively, with minimum temperature of 13.1±0.9 °C and VPD of 0.07±0.01.

Eco-physiological measurements: Leaf water potential, gas exchange, modulated Chl fluorescence, and contents of photosynthetic pigments were measured on one leaf per plant ($n = 10$) at predawn and at midday over the course of the experiment; all measurements were repeated on pre-marked, fully expanded sunlit leaves of the same age. The diurnal course of the same variables was also measured on both treatments at the beginning and at the end of the experiment.

Leaf water potentials were determined with a pressure chamber (model *3000*, *Soil Moisture*, Santa Barbara, CA, USA). Net photosynthetic rate (P_N) and g_s were measured with a portable gas-analyzer (*Li-6400*, *Li-Cor*, Lincoln, NE, USA). Chl fluorescence was assessed with a modulated fluorimeter (*PAM-2000*, *Walz*, Effeltrich, Germany), and the quantum yields of photochemistry (Φ_{PS2}), xanthophyll-dependent dissipation as heat (Φ_{NPQ}), and the sum of constitutive thermal dissipation and fluorescence ($\Phi_{f,D}$) computed from steady-state and saturated fluorescence according to Hendrickson *et al.* (2004).

The dynamics of leaf Chl content were also assessed spectroscopically *in vivo* over the course of the experiment; leaf reflectance in the 380-1 000 nm range was measured with a spectral resolution of 2 nm *FWHM* on one marked leaf per plant using a *USB-2000* spectrometer (*Ocean Optics*, Dunedin, FL, USA). Leaf Chl *a*+*b* content was computed from the Red-Edge Index Position (REIP) using the formula proposed by Richardson *et al.* (2002). Measurements were made at midday, so as to minimize the confounding effects of Chl fluorescence (Zarco-Tejada *et al.* 2000).

Pigment analysis: On selected days at the beginning (day 1 at sunrise) and at the end of the experiment (day 11 at sunrise), leaf samples were collected for the determination of xanthophyll contents and de-epoxidation state in response to drought. Triplicate samples were collected from each of three plants per treatment in coincidence with eco-physiological measurements at predawn and at midday; at the end of the experiment, the diurnal cycle of xanthophyll DEPS was also analyzed. Leaf discs (0.64 cm^2, about 20−80 mg fresh mass) were frozen in liquid nitrogen and stored in the dark at −80 °C until extraction. Leaf discs were then ground in a mortar in liquid nitrogen, under dim light, and the collected powder was extracted with 1.5 cm^3 cold HPLC grade 100 % acetone. In order to avoid traces of acid in the acetone used for the extraction, calcium carbonate was added (García-Plazaola and Becerril 1999).

Extracts were centrifuged at 5 000×*g* at 0 °C for 4 min and the supernatants were stored in ice. The pellets were re-suspended with small amounts of acetone (*e.g.* 0.5 cm^3) until the supernatants remained colourless. Water was added to the combined supernatants to give a final concentration of acetone 80 % (v/v). The pigment solutions were finally filtered through a 0.45 µm syringe-filter (*Chemtek Analitica*, Bologna, Italy) and stored in the dark at −20 °C until injection into HPLC. To avoid any possible sample degradation or concentration by solvent evaporation, injection was made within two days from extraction. According to García-Plazaola and Becerril (1999), storage of extracts at –20 °C for 4 weeks results in an average loss of compounds of 3.1 %.

Pigment composition was analysed with reversed-phase HPLC according to Niinemets *et al.* (1998) using a *Hypersil ODS* column (particle size 5 µm, 250×4.6 mm; *Alltech Italia*, Sedriano, Milan, Italy), which was thermostated at 15 °C, in combination with a guard column (5 µm, 7.5×4.6 mm; *Alltech*). The HPLC system was equipped with two pumps *LC-10AD*, a mixer *FCV-10AL*, an oven *CTO-10AS*, a degasser *Gastorr 154* (*Shimadzu Italia*, Milan, Italy), and an *UV6000 LP* photodiode array detector (*Finnigan SpectraSYSTEM*, Milan, Italy).

The pigments were eluted at a flow-rate of 1.5 cm^3 min^{-1}. Two solvents with different polarities were used for separation: Solvent A was 10 mM tris(hydroxymethyl)aminomethane buffer, pH 8.0, and solvent B was 100 % acetone, HPLC grade. The mixture of 75 % solvent B and 25 % solvent A was run isocratically for the first 7.5 min, followed by a linear gradient to 100 % B for the next 9.5 min. Then, solvent B ran isocratically for 3 min. This was followed by a change in the eluent composition to 25 % A and 75 % B with a linear gradient for 2 min and then the column was equilibrated for 8 min before the next sample injection. The injection volume was 20 μm^3. For overnight storage the column was flushed with methanol : water (50/50 v/v).

Peaks were detected and integrated at 445 nm for carotenoid and Chl *b* contents, and at 410 nm for Chl *a* and pheophytin *a*. Their concentrations were calculated from the corresponding peak area (Munné-Bosch and Alegre 2000). We calculated the conversion state of the xanthophyll cycle as the ratio (Z+0.5A)/(VAZ), following Müller *et al.* (2006).

The calibration was performed using commercially available pigment standards (neoxanthin, V, Z, and lutein; *DHI Water & Environment*, Denmark). The calibration factor for V was also used for A. Standards of lutein were injected periodically in order to correct the conversion factors, assuming that the extent of changes would be the same for all coefficients, as described by de las Rivas *et al.* (1989).

Statistics: Data of physiological variables were analysed by ANOVA using *PC SAS* version *8.2* (*SAS Institute*, Cary, NC, USA). Treatments were compared using the General Linear Model Procedure with the LSD test. Differences referred to as significant had a $p<0.05$.

Results

Water status and pigments during WS: After 4 d of water shortage, the predawn leaf water potential of droughted plants begun to decrease, reaching a minimum value of –5.82 MPa by the end of the experiment (Fig. 1*A*). At that time, droughted leaves were severely wilted and yellow. Chl bleaching was confirmed by both spectroscopic measurements of leaf reflectance (data not shown) and HPLC (Fig. 1*B*): since day 4, Chl *a*+*b* content (on a leaf area basis) also declined in WS-leaves and by the end of the experiment a significant Chl loss of about 30 % was observed. In contrast, the total amount of xanthophyll cycle components (VAZ) per unit leaf area remained constant during the experiment in both treatments (Table 1).

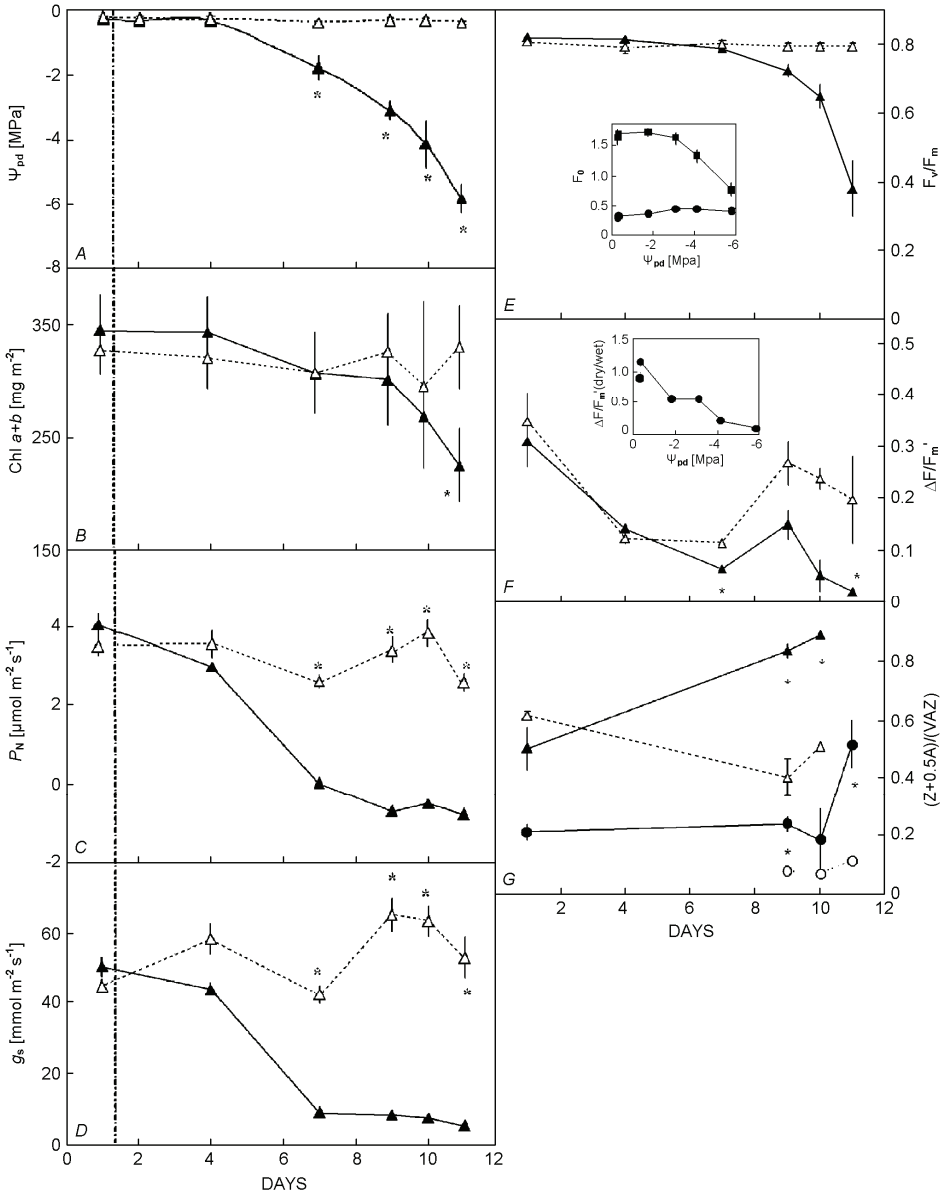

Fig. 1. Dynamics of (*A*) predawn leaf water potential (Ψ_{pd}), (*B*) chlorophyll (Chl) *a*+*b* content estimated from midday leaf reflectance, (*C*) midday net photosynthetic rate (P_N), (*D*) stomatal conductance (g_s), photochemical quantum yield of PS2 in the dark (*E*, F_v/F_m, predawn measurements) and in the light (*F*, $\Delta F/F_m'$, midday measurements), and (*G*) conversion state of the xanthophyll cycle [(Z+0.5)/(VAZ)] in well-watered, C (*open symbols*) and water-stressed, WS (*solid symbols*) plants, over the course of the experiment. *The dashed vertical line* marks the beginning of the drought stress period. In *G*, data were not available for control plants on the first day of the experiment, due to technical problems. *The inset* in *E* shows the evolution of F_0 (minimal fluorescence in the dark, ■) and F_m (maximum fluorescence in the dark, ●) in WS-plants as a function of Ψ_{pd}. *The inset* in *F* displays the trend of $\Delta F/F_m'$ in WS-plants, normalized to the concurrent value in control plants in order to remove environmental effects. Means±SE (n = 10), $^*p<0.05$.

Table 1. Xanthophyll pigment (A – antheraxanthin, V – violaxanthin, Z – zeaxanthin) contents in well-watered (C) and water stressed (WS) leaves determined in samples collected at midday of the 1st and 9th d from the beginning of the WS-period. Means±SE (n = 3). *Significant differences at the 5 % level between C- and WS-plants within the same day.

Pigment [μmol m^{-2}]	Time of WS [d]			
	1		9	
	C	WS	C	WS
Z	12.75±2.02	11.98±4.19	8.80±1.47*	23.90±2.29
A	4.18±0.82	6.11±0.06	4.07±1.42	3.15±0.38
V	7.21±1.55*	10.91±0.58	9.05±0.74*	1.85±0.26
[VAZ]	24.14±4.39	29.00±3.72	21.92±3.62	28.90±2.43

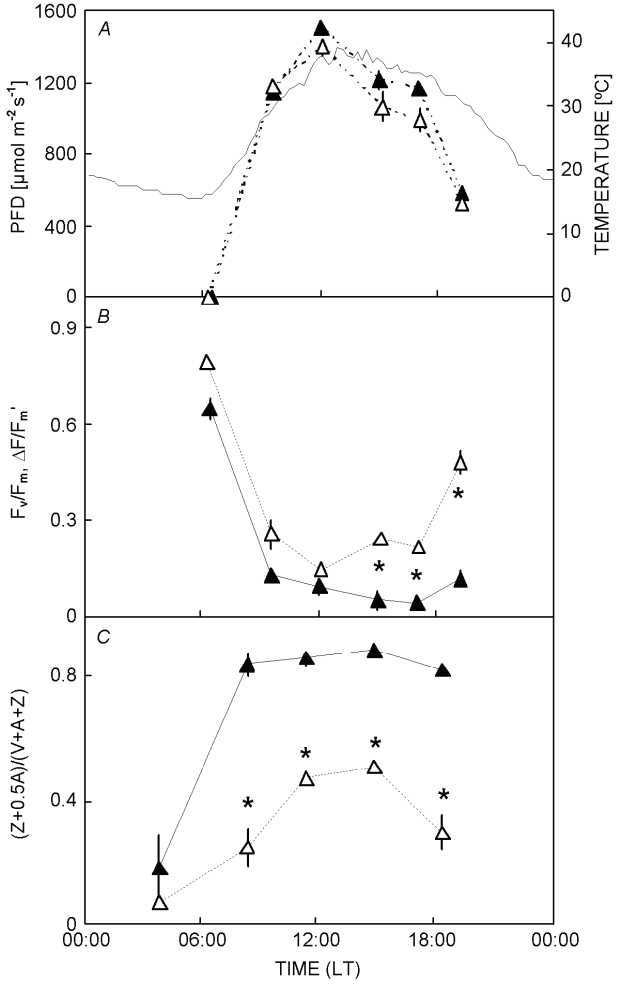

Fig. 2. Diurnal characterization of (*A*) photon flux density, PFD (*triangles*) and air temperature (—) continuously measured, (*B*) PS2 efficiency in the dark (F_v/F_m, predawn measurements) and in the light ($\Delta F/F_m'$, the rest of measurements), and (*C*) the conversion state of the xanthophyll cycle in well-watered, C (*open symbols*) and water-stressed, WS (*solid symbols*) leaves at day 10 from the beginning of the drought stress period (1 July). *Error bars* represent the SE of the experiment. *p<0.05

Drought-induced changes in P_N, g_s, PS2 efficiency, and xanthophyll cycle: WS resulted in a marked reduction in leaf gas exchange. Midday P_N strongly decreased over the experiment in WS-leaves and became negative 7 d after the beginning of the WS-period (Fig. 1*C*). On the contrary, P_N remained almost stable in C-leaves. Such a strong decrease in P_N was mostly due to the substantial stomatal closure induced by water shortage (Fig. 1*D*). The g_s began to drop in WS-leaves since day 4, together with P_N, whereas no limitation was observed in control plants.

In WS-plants, maximum photochemical efficiency of PS2 in dark-adapted leaves (F_v/F_m) exhibited a drop starting from day 7 of the experiment; values around 0.38 were reached by the end of the experiment, representing about 52 % of the control values (~0.8) that were typical of healthy non-photoinhibited leaves (Fig. 1*E*). As drought progressed, the maximum fluorescence in the dark (F_m) fell considerably while the minimal fluorescence in the dark (F_0) remained constant or rose slightly (*inset* in Fig. 1*E*).

In both treatments, the photochemical efficiency of PS2 at midday ($\Delta F/F_m'$) showed marked day-to-day differences, as a result of variation in environmental conditions (*i.e.* variation of irradiance and temperature during partially cloudy days; Fig. 1*F*). Starting from the 7th d of the experiment, however, a significant decrease of $\Delta F/F_m'$ in WS compared to C-leaves was observed (Fig. 1*F*); the impact of drought was apparent when the effects of other environmental factors were removed by normalizing values from WS-plants over C-ones (*inset* in Fig. 1*F*).

The de-epoxidation state of xanthophyll cycle pigments [DEPS = (Z+0.5A)/(VAZ)] generally exhibited higher values in WS-leaves compared to C-leaves, and rose as the WS became more severe (Fig. 1*G*). Values of 0.89 were reached at midday of day 10 in WS-leaves indicating that almost all the V was de-epoxidized to Z (Fig. 1*G* and Table 1). Predawn values in WS-leaves also increased, reaching a value of 0.18 by the end of the experiment, as the result of Z retention which was not converted into V overnight.

Diurnal changes in environment, PS2 efficiency, and xanthophyll cycle: On the last day of the experiment, the diurnal course of eco-physiological parameters and xanthophyll DEPS was also measured in WS- and C- plants

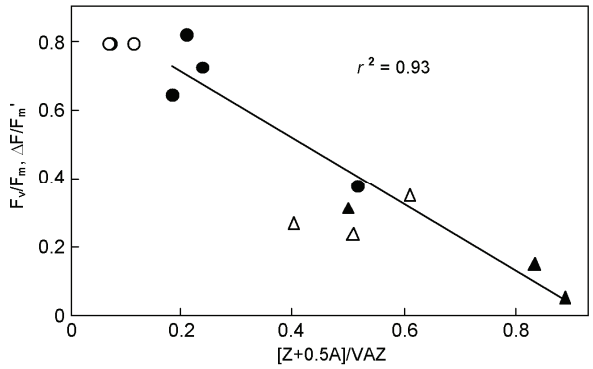

Fig. 3. Relationship between the efficiency of photosystem 2 (PS2) and the conversion state of xanthophyll cycle in well-watered (*open symbols*) and water-stressed (*solid symbols*) leaves. Samples collected and measured prior to sunrise denoted by circles indicate the relationship between the fraction of the xanthophyll cycle present as Z+0.5A and the PS2 efficiency in the dark (F_v/F_m); while samples collected and measured at midday are denoted by triangles and indicate the relationship between Z+0.5A and the PS2 efficiency in the light ($\Delta F/F_m'$). Data from Fig. 1.

(Fig. 2). Maximum PFD and air temperatures over the day were 1 454 μmol m^{-2} s^{-1} and 38.9 °C, respectively (Fig. 2*A*). The diurnal pattern of Φ_{PS2} closely tracked the course of incident radiation in C-plants (Fig. 2*B*); in WS-plants, however, Φ_{PS2} not only started from lower predawn values, but also failed to recover after midday, so that by the end of the following night a low value of 0.38 was still observed (Fig. 1*G*). Xanthophyll DEPS also tracked the diurnal course of irradiance in C-plants, with a maximum of 0.51 at midday and a minimum of 0.07 before dawn (Fig. 2*C*). Xanthophyll de-epoxidation state in stressed leaves increased faster during the morning in response to irradiance, reaching values as high as 0.89 already at 08:30, whereas in C-leaves xanthophyll cycle activation occurred more gradually. In the afternoon, the DEPS of WS-leaves failed to recover, and by the end of the night and thus at predawn of the next day a DEPS of 0.18 was still recorded.

Relationship between the PS2 efficiency and the conversion state of the xanthophyll cycle: Changes in Φ_{PS2} over the course of the experiment closely tracked the parallel pattern of xanthophyll DEPS. The photochemical efficiency of PS2 and the conversion state of the xanthophyll cycle, measured prior to sunrise and at midday during the experimental period, are shown in Fig. 3. The decrease in PS2 efficiency was linearly related to the increase in the de-epoxidized fraction of xanthophylls for both treatments ($r^2 = 0.87$), even though the correlation was more evident in WS-leaves ($r^2 = 0.93$); the relationship between xanthophyll DEPS and Φ_{PS2} was similar, irrespective of the source of variation (drought alone in predawn measurements, irradiance, or a combination of irradiance and drought in midday values). WS-plants, which showed a remarkable decrease in predawn F_v/F_m at the end of the experiment, concomitantly displayed higher xanthophyll de-epoxidation state.

Discussion

Water deficit, generally associated with high solar irradiance and high temperatures during the summer, is the main limiting factor for plant growth in Mediterranean-type ecosystem (di Castri 1981). In our investigation, the photosynthetic capacity of *A. unedo* plants subjected to severe WS, as indicated by extreme value of leaf water potential (−5.82 MPa), strongly decreased in response to water shortage. Simultaneously, degradation of Chls in the antenna complexes was observed in WS-plants (30 % decrease). This significant loss of Chls is not only a sign of damage induced by stress, but also of a photoprotective adaptive mechanism that stressed plants apply to reduce the amount of photon energy intercepted by the leaves (Munné-Bosch and Alegre 2000). However, the leaf content of xanthophyll pigments (VAZ) remained almost stable during the experiment.

In WS-plants, the observed photosynthetic decline was probably a consequence of low carbon availability due to drought-induced stomatal closure and of the non-stomatal limitation presumably related to the down-regulation of photochemistry. Strong limitations of P_N may lead to an imbalance between the photochemical activity of PS2 and the electrons required for photosynthesis which provokes an over-excitation and thus photodamage to PS2 (Powles 1984, Kaiser 1987). In order to prevent this, an increase of the proportion of radiation that is thermally dissipated is required. Our results showed an enhancement of xanthophyll de-epoxidation in water stressed plants which probably contributed to the dissipation of excess energy in thylakoids as heat.

At the end of the dark period, when a recovery of rapidly reversible effects of photon yield is normally allowed, the measured F_v/F_m was significantly depressed in WS-leaves, indicating a sustained reduction in photochemical efficiency throughout the experiment (Demmig *et al.* 1988). Differences between Mediterranean species in the allocation of absorbed photon energy for photochemistry and for dissipation in association with differences in the conversion state of the xanthophyll cycle have been reported by Faria *et al.* (1998). In particular, the values of photons utilized for photosynthesis processes ranged from 20 % in *Olea europea*, similarly to what we observed in droughted *Arbutus* plants, to maximum values of 50 % in *Eucalyptus globulus*.

In our experiment, the proportions of Z and V were largely altered by the WS-treatment which slowed down the reconversion of Z to its precursor, resulting in a considerable retention of Z and A during the night as the

drought stress became more intense. This nocturnal retention was previously reported both during the summer in *N. oleander*, *Z. mays*, *R. officinalis*, and *Yucca* species (Demmig *et al*. 1988, Saccardy *et al*. 1998, Munné-Bosch and Alegre 2000, Barker *et al*. 2002) and during the winter in several conifers, broad-leaved evergreen species, shrubs, and herbs (Adams and Demmig-Adams 1994, 1995, Verhoeven *et al.* 1996, 1998, 1999, Barker *et al.* 1998, Logan *et al*. 1998, Garcia-Plazaola *et al.* 1999a,b, Barker *et al.* 2002) as a response to different environmental types of stress.

Since we found a strong negative correlation ($r^2 = 0.93$) between F_v/F_m and the amount of de-epoxidized xanthophyll cycle components at predawn, we conclude that there was a nocturnal retention of Z+A in a state primed for energy dissipation, as suggested by several authors (Adams and Demmig-Adams 1994, 1995, Verhoeven *et al.* 1996, 1999, Garcia-Plazaola *et al.* 1999, Levizou *et al*. 2004). A similar relationship between predawn Z+A and predawn F_v/F_m has been reported for two *Yucca* species only in winter (Barker *et al*. 2002), while during hot summer night in spite of a nocturnal retention of Z, the correlation with F_v/F_m was not observed. The authors suggested that whereas an increased demand for thermal energy dissipation in either summer or winter led to overnight retention of Z+A, these carotenoids remained engaged in the thermal dissipation only on cold winter but not during hot summer nights, when, instead, the possible involvement of Z in stabilization of the thylakoid membrane at elevated temperature is hypothesized. This different plant response might be the physiological adaptation of plants grown at high environmental stresses typical of a desert environment with a combination of high air temperature (up to 45 °C) and extremely low humidity in summer.

In our study we did not attribute photoinhibition of PS2 to photodamage but to an increase in the rate of thermal energy dissipation (Björkman 1987, Demmig and Björkman 1987) mediated by Z (Demmig *et al.* 1988). Although a specific recovery study has not been carried out, the tested plants survived the imposed stress. The strong midday decrease in $\Delta F/F_m'$ presumably reflects increased thermal dissipation of excess excitation energy before it reaches the RCs. This mechanism of photoprotection of the photosynthetic apparatus has been reported in *N. oleander* and *Z. mays* (Demmig *et al.* 1988, Saccardy *et al.* 1998) when water deficit occurred at relatively moderate temperatures. In our experiment F_0 remained almost constant or rose slightly during the WS-treatment whereas, according to Adams and Demmig-Adams (1994) and Verhoeven *et al.* (1996), we would expect a net decrease following the reduction in F_m, as an evidence of photoprotection. A similar trend in F_0 was evident also in WS-leaves of *N. oleander* and the authors suggested a contribution of a second not specified process to the decrease in F_v/F_m (Demmig *et al*. 1988). Another interpretation has been suggested by Zhu *et al*. (2005), who instead explained the slight increase of F_0 by the reduction in the size of peripheral antenna relative to core antenna and by an increase in the concentration of Q_B-non-reducing PS2 RCs. The heterogeneity of PS2 RCs was reported by Krause and Weis (1991) and Lavergne and Briantais (1996). The model of Zhu *et al*. (2005) assumed that the excitation energy incident on Q_B-non-reducing PS2 RCs is not transferred to Q_B-reducing PS2 RCs for charge separation but is dissipated only as heat or fluorescence. As a consequence, there might be an increase in F_0. Also Bertamini and Nedunchezhian (2004) found that photoinhibition could result from the formation of PS2 inactive RCs besides from a possible slower rate of D1 protein degradation, which in turn means a slower total repair cycle of photodamaged PS2 RCs (Kyle *et al.* 1984, Aro *et al*. 1993, Barber 1995, Carpentier 1995). Similarly, our results indicate the primary involvement of the xantophyll cycle in the photoprotection mechanism of the photosynthetic apparatus, although the role of D1 protein turnover and in particular of its synthesis can not be ruled out. PS2 reorganization with enhanced D1 turnover has also been proposed by Giardi *et al.* (1996) as a possible mechanism to counteract the inactivation of part of the PS2 core centres in WS-plants.

In conclusion, the ability of strawberry trees to maintain the functionality of the xanthophyll cycle during the Mediterranean summer appears as an efficient mechanism to prevent irreversible damages to the photosynthetic machinery through the process of thermal energy dissipation in the antenna and the reduction in photochemical efficiency. In addition, the nocturnal retention of Z and A confers a greater capacity of photoprotection in the early morning and thus contributes to the survival of severely stressed plants.

References

Adams, W.W., III, Demmig-Adams, B.: Carotenoid composition and down regulation of photosystem II in three conifer species during the winter. − Physiol. Plant. **92**: 451-458, 1994.

Adams, W.W., III, Demmig-Adams, B.: The xanthophyll cycle and sustained thermal dissipation activity in *Vinca minor* and *Euonymus kiautschovicus* in winter. − Plant Cell Environ. **18**: 117-127, 1995.

Adams, W.W., III, Demmig-Adams, B., Verhoeven, A.S., Barker, D.H.: Photoinhibition during winter stress: involvement of sustained xanthophyll cycle-dependent energy dissipation. − Aust. J. Plant Physiol. **22**: 261-276, 1995.

Aro, E.-M., McCaffery, S., Anderson, J.M.: Photoinhibition and D1 protein degradation in peas acclimated to different growth irradiances. − Plant Physiol. **103**: 835-843, 1993

Barber, J.: Molecular basis of photoinhibition. − In: Mathis, P. (ed.): Photosynthesis, From Light to Biosphere. Vol. IV. Pp.

159-164. Kluwer Academic Publ., Dordrecht – Boston – London 1995.

Barker, D.H., Adams, W.W., III, Demmig-Adams, B., Logan, B.A., Verhoeven, A.S., Smith, S.D.: Nocturnally retained zeaxanthin does not remain engaged in a state primed for energy dissipation during the summer in two *Yucca* species growing in the Mojave Desert. − Plant Cell Environ. **25**: 95-103, 2002.

Barker, D.H., Adams, W.W., III, Logan, B.A., Demmig-Adams, B.: Photochemistry and xanthophyll cycle-dependent energy dissipation in differently oriented cladodes of *Opuntia stricta* during the winter. – Aust. J. Plant Physiol. **25**: 95-104, 1998.

Bertamini, M., Nedunchezhian, N.: Photoinhibition and recovery of photosynthesis in leaves of *Vitis berlandieri* and *Vitis rupestris*. − J. Plant Physiol. **161**: 203-210, 2004.

Björkman, O.: High-irradiance stress in higher plants and interaction with other stress factors. − In: Biggins, J. (ed.): Progress in Photosynthesis Research. Vol. 4. Pp. 11-18. Martin Nijhoff, Dordrecht – Boston – Lancaster 1987.

Björkman, O., Demmig-Adams, B.: Regulation of photosynthetic light energy capture, conversion and dissipation in leaves of higher plants. − In: Schulze, E.D., Caldwell, M.M. (ed.): Ecophysiology of Photosynthesis. Pp. 17-47. Springer-Verlag, Berlin 1994.

Carpentier, R.: Influence of high light intensity on photosynthesis: photoihibition and energy dissipation. − In: Pessarakli, M. (ed.): Handbook of Photosynthesis. Pp. 443-450. Marcel Dekker, New York – Basel – Hong Kong 1995.

Choudhury, N.K., Behera, R.K.: Photoinhibition of photosynthesis: Role of carotenoids in photoprotection of chloroplast constituents. – Photosynthetica **39**: 481-488, 2001.

Comstock, J.P., Mahall, B.E.: Drought and changes in leaf orientation for two California chaparral shrubs: *Ceanothus megacarpus and Ceanothus crassifolius.* – Oecologia **65**: 531-535, 1985.

de las Rivas, J., Abadía, A., Abadía, J.: A new reversed phase-HPLC method resolving all major higher plant photosynthetic pigments. − Plant Physiol. **91**: 190-192, 1989.

Demmig, B., Björkman, O.: Comparison of the effect of excessive light on chlorophyll fluorescence (77K) and photon yield of O_2 evolution in leaves of higher plants. − Planta **171**: 171-184, 1987.

Demmig, B., Winter, K., Krüger, A., Czygan, F.-C.: Zeaxanthin and the heat dissipation of excess light energy in *Nerium oleander* exposed to a combination of high light and water stress. − Plant Physiol. **84**: 17-24, 1988.

Demmig-Adams, B., Adams, W.W., III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Physiol. Plant mol. Biol. **43**: 599-626, 1992.

Demmig-Adams, B., Adams, W.W., III: Xanthophyll cycle and light stress in nature: uniform response to excess direct sunlight among higher plant species. − Planta **198**: 460-470, 1996a.

Demmig-Adams, B., Adams, W.W., III: The role of xanthophyll cycle carotenoids in the protection of photosynthesis. − Trends Plant Sci. **1**: 21-26, 1996b.

Demmig-Adams, B., Adams, W.W., III, Barker, D.H., Logan, B.A., Bowling, D.R., Verhoeven, A.S.: Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. – Physiol. Plant. **98**: 253-264, 1996a.

Demmig-Adams, B., Adams, W.W., III, Winter, K., Meyer, A., Schreiber, U., Pereira, J.S., Krüger, A., Czygan, F.-C., Lange, O.L.: Photochemical efficiency of photosystem II, photon yield of O_2 evolution, photosynthetic capacity, and carotenoid composition during the midday depression and net CO_2 uptake in *Arbutus unedo* growing in Portugal. − Planta **177**: 377-387, 1989.

Demmig-Adams, B., Gilmore, A.M., Adams, W.W., III: *In vivo* functions of carotenoids of higher plants. − FASEB J. **10**: 403-412, 1996b.

di Castri, F.: Mediterranean-type shrublands of the word. − In: di Castri, F., Goodall, D.W., Specht, R.L. (ed.): Mediterranean-Type Shrublands. Pp. 1-52. Elsevier, Amsterdam 1981.

Faria, T., Silvério, D., Breia, E., Cabral, R., Abadia, A., Pereira, J.S., Chaves, M.M.: Differences in the response of carbon assimilation to summer stress (water deficits, high light and temperature) in four Mediterranean tree species. − Physiol. Plant. **102**: 419-428, 1998.

García-Plazaola, J.I., Artetxe, U., Becerril, J.M.: Diurnal changes in antioxidant and carotenoid composition in the Mediterranean sclerophyll tree *Quercus ilex* (L) during winter. − Plant Sci. **143**: 125-133, 1999.

García-Plazaola, J.I., Becerril, J.M.: A rapid high-performance liquid chromatography method to measure lipophilic antioxidants in stressed plants: Simultaneous determination of carotenoids and tocopherols.− Phytochem. Anal. **10**: 307-313, 1999.

Giardi, M.T., Cona, A., Geiken, B., Kučera, T., Masojídek, J., Mattoo, A.K.: Long-term drought stress induces structural and functional reorganization of photosystem II. − Planta **199**: 118-125, 1996.

Gilmore, A.M.: Mechanistic aspects of xanthophyll cycle-dependent photoprotection in higher plant chloroplasts and leaves. – Physiol. Plant. **99**: 197-209, 1997.

Greer, D.H., Berry, J.A., Björkman, O.: Photoinhibition of photosynthesis in intact bean leaves: role of light and temperature, and requirement for chloroplast-protein synthesis during recovery. − Planta **168**: 253-260, 1986.

Hendrickson, L., Furbank, R.T., Chow, W.S.: A simple alternative approach to assessing the fate of absorbed light energy using chlorophyll fluorescence. − Photosynth. Res. **82**: 73-81, 2004.

Kaiser, W.M.: Effects of water deficit on photosynthetic capacity. – Physiol. Plant. **71**: 142-149, 1987.

Krause, G.H., Weis, E.: Chlorophyll fluorescence and photosynthesis – the basics. – Annu. Rev. Plant Physiol. Plant mol. Biol. **42**: 313-349, 1991.

Kyle, D.J., Kuang, T.-Y., Watson, J.L., Arntzen, C.J.: Movement of a sub-population of the light-harvesting complex (LHC_{II}) from grana to stroma lamellae as a consequence of its phosphorylation. – Biochim. biophys. Acta **765**: 89-96, 1984.

Lavergne, J., Briantais, J.-M.: Photosystem II heterogeneity. − In: Ort, D.R., Yocum, C.F. (ed.): Oxygen Photosynthesis: the Light Reactions. Pp. 265-287. Kluwer Academic Publ., Dordrecht – Boston – London 1996.

Levizou, E., Petropoulou, E., Manetas, Y.: Carotenoid composition of peridermal twigs does not fully conform to a shade acclimation hypothesis. – Photosynthetica **42**: 591-596, 2004.

Logan, B.A., Grace, S.C., Adams, W.W., III, Demmig-Adams, B.: Seasonal differences in xanthophyll cycle characteristics and antioxidants in *Mahonia repens* growing in different light environments. – Oecologia **116**: 9-17, 1998.

Long, S.P., Humphries, S., Falkowski, P.G.: Photoinhibition of photosynthesis in nature. − Annu. Rev. Plant Physiol. Plant mol. Biol. **45**: 633-662, 1994.

Masojídek, J., Trivedi, S., Halshaw, L., Alexiou, A., Hall, D.O.:

Synergistic effect of drought and light stress in sorghum and pearl millet. − Plant Physiol. **96**: 198-207, 1991.

Mooney, H.A.: Primary production in Mediterranean-climate regions. − In: Di Castri, F., Goodall, D.W., Specht, R.L. (ed.): Mediterranean-Type Shrublands. Pp. 249-256. Elsevier, Amsterdam 1981.

Müller, M., Hernández, I., Alegre, L., Munné-Bosch, S.: Enhanced α-tocopherol quinone levels and xanthophyll cycle deepoxidation in rosemary plants exposed to water deficit during a Mediterranean winter. – J. Plant Physiol. **163**: 601-606, 2006.

Munné-Bosch, S., Alegre, L.: Changes in carotenoids, tocopherols and diterpenes during drought and recovery, and the biological significance of chlorophyll loss in *Rosmarinus officinalis* plants. − Planta **210**: 925-931, 2000.

Munné-Bosch, S., Peñuelas, J.: Drought-induced oxidative stress in strawberry tree (*Arbutus unedo* L.) growing in Mediterranean field conditions. − Plant Sci. **166**: 1105-1110, 2004.

Murata, N., Takahashi, S., Nishiyama, Y., Allakhverdiev, S.I.: Photoinhibition of photosystem II under environmental stress. − Biochim. biophys. Acta **1767**: 414-421, 2007.

Nahal, I.: The Mediterranean climate from a biological viewpoint. − In: di Castri, F., Goodall, D.W., Specht, R.L. (ed.): Mediterranean-Type Shrublands. Pp. 63-86. Elsevier, Amsterdam 1981.

Niinemets, Ü., Bilger, W., Kull, O., Tenhunen, J.D.: Acclimation to high irradiance in temperate deciduous trees in the field: changes in xanthophyll cycle pool size and in photosynthetic capacity along a canopy light gradient. − Plant Cell Environ. **21**: 1205-1218, 1998.

Niyogi, K.K., Grossman, A.R., Björkman, O.: *Arabidopsis* mutants define a central role for the xanthophyll cycle in the regulation of photosynthetic energy conversion. − Plant Cell **10**: 1121-1134, 1998.

Osmond, C.B.: What is photoinhibition? Some insights from comparisons of shade and sun plants. − In: Baker, N.R., Bowyer, J.R. (ed.): Photoinhibition of Photosynthesis, from Molecular Mechanisms to the Field. Pp. 1-24. Bios Scientific Publishers, Oxford 1994.

Pfündel, E., Bilger, W.: Regulation and possible function of the violaxanthin cycle. – Photosynth. Res. **42**: 89-109, 1994.

Powles, S.B.: Photoinhibition of photosynthesis induced by visible light. – Annu. Rev. Plant Physiol. **35**: 15-44, 1984.

Richardson, A.D., Duigan, S.P., Berlyn, G.P.: An evaluation of noninvasive methods to estimate foliar chlorophyll content. − New Phytol. **153**: 185-194, 2002.

Saccardy, K., Pineau, B., Roche, O., Cornic, G.: Photochemical efficiency of Photosystem II and xanthophyll cycle components in *Zea mays* leaves exposed to water stress and high light. – Photosynth. Res. **56**: 57-66, 1998.

Smirnoff, N.: The role of active oxygen in the response of plants to water deficit and desiccation. − New Phytol. **125**: 27-58, 1993.

Tyystjärvi, E., Aro, E.M.: The rate constant of photoinhibition, measured in lincomycin-treated, is directly proportional to light intensity. − Proc. nat. Acad. Sci. USA **93**: 2213-2218, 1996.

Varone, L., Gratani, L.: Physiological response of eight Mediterranean maquis species to low air temperature during winter. – Photosynthetica **45**: 385-391, 2007.

Verhoeven, A.S., Adams, W.W., III, Demmig-Adams, B.: Close relationship between the state of the xanthophyll cycle pigments and photosystem II efficiency during recovery from winter stress. – Physiol. Plant. **96**: 567-576, 1996.

Verhoeven, A.S., Adams, W.W., III, Demmig-Adams, B.: Two forms of sustained xanthophyll-cycle dependent energy dissipation in overwintering *Euonymus kiautschovicus*. − Plant Cell Environ. **21**: 893-903, 1998.

Verhoeven, A.S., Adams, W.W., III, Demmig-Adams, B.: The xanthophyll cycle and acclimation of *Pinus ponderosa* and *Malva neglecta* to winter stress. – Oecologia **118**: 277-287, 1999.

Werner, C., Correia, O., Beyschlag, W.: Two different strategies of Mediterranean macchia plants to avoid photoinhibitory damage by excessive radiation levels during summer drought. − Acta oecol. **20**: 15-23, 1999.

Zarco-Tejada, P.J., Miller, J.R., Mohammed, G.H., Noland, T.L.: Chlorophyll fluorescence effects on vegetation apparent reflectance: I. Leaf-level measurements and model simulation. − Remote Sensing Environ. **74**: 582-595, 2000.

Zhu, X.-G., Govindjee, Baker, N.R., deSturler, E., Ort, D.R., Long, S.P.: Chlorophyll *a* fluorescence induction kinetics in leaves predicted from a model describing each discrete step of excitation energy and electron transfer associated with Photosystem II. − Planta **223**: 114-133, 2005.