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Photoprotective responses of Mediterranean and Atlantic trees to the extreme heat-wave of summer 2003 in Southwestern Europe

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Abstract Summer 2003 was extremely hot in Europe. High light in combination with heat and drought exacerbates the generation of photo-oxidative stress. Under these conditions photoprotective responses can be critical for plant survival. Photoprotection was analysed in 2003 in several Mediterranean and Atlantic woody species. These data were compared with previous summers (1998, 1999 and 2001) to evaluate the potential acclimation for each species. A pattern of changes consisting on a decrease in chlorophyll, ascorbate and Fv/Fm and an increase in tocopherol, xanthophyll cycle pigments (VAZ) and de-epoxidation index was regularly observed. Acclimation potential was measured by the use of the plasticity index for each parameter. Mediterranean species were more plastic than Atlantic ones. The latter were unable to increase antioxidant pools to the same extent or to down-regulate the efficiency of light energy conversion. These results indicate that most Mediterranean species are able to perform an efficient acclimation to heat stress, whilst Atlantic species will be more affected by climate warming.

Keywords Antioxidants · Heat-wave · Mediterranean species · Photoprotection · Summer 2003

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Introduction

Summer 2003 was the hottest in central and western Europe in the last 180 years, and probably at least since the 1500 AC (Luterbarcher et al. 2004) with temperatures up to 6°C above long-term means and annual precipitation 50% below average (Stott et al. 2004). Current climatic models suggest that this episode was almost certainly a result of climatic changes that are being observed in the last decades, and the occurrence of such heat-waves is expected to increase in the next future as a consequence of an increase in temperature variability (IPCC 2007; Schär et al. 2004). The influence of such temperature oscillations in ecosystems cannot be predicted only in the basis of mean temperatures, since the impact of such unusual, but recurrent, extreme heat episodes can be more critical for plant survival. Furthermore, leaf overheating caused by the lack of evaporative cooling under water stress (as occurred in 2003) can exacerbate leaf damage at temperatures far below lethal values.

Several long-term field studies on plant ecophysiology that were in course during that summer in several locations of Europe, took advantage of these unexpected weather conditions to study the plants responses to climatic alterations. Thus, these studies, partly summarised in Rennenberg et al. (2006), have shown the alteration of several physiological processes during the heat-wave: water relations in trees and forest stands (Bréda et al. 2006), nitrogen balance (Nahm et al. 2006a); pollen production (Gehrig 2006); photoprotection mechanisms (Hormaetxe et al. 2007) or natural regeneration (Czajkowski et al. 2005). Interactive effects with other factors such as ozone exposition (Low et al. 2006); insect pests (Rouault et et al 2006) or drought stress and CO_2 (Leuzinger et al. 2005) were also studied during the 2003 heat wave. As a

consequence of such adverse effects, a global reduction in forest primary production occurred that year (Ciais et al. 2005). In a longer term, the physiological effects of the progressive warming and recurrent heat waves may lead to a progressive decline in growth and an accelerated rate of mortality and/or pathogen infections in some tree species (Bréda et al. 2006; Desprez-Loustou et al. 2006; Jump et al. 2006; Rebetez et al. 2006).

At cellular level, supraoptimal non-lethal temperatures are potentially damaging because of the temperature dependence of the activation and oxigenase activity of Rubisco, which implies a reduction in photosynthesis. Exposure to leaf temperatures above 42°C causes direct damage to photosynthetic apparatus (Ludlow and Björkman 1984) because it increases thylakoid fluidity and protein denaturation leading to the damage of PSII (Berry and Björkman 1980). This process can be exacerbated by drought due to the reduced evaporative cooling, which leads to a leaf temperature several degrees higher than air temperature, exacerbating photoinhibitory damage (Larcher 2000). These conditions may cause the overproduction of reactive oxygen species (ROS), even at non-lethal temperatures (Rennenberg et al. 2006). As a consequence, the combination of light, drought and heat generates photo-oxidative stress, provoking the induction of photoprotection mechanisms (Badiani et al. 1997; Paolacci et al. 1997). Photoprotection mechanisms are able to counteract the increased ROS production being one of the critical keys for plant acclimation and survival. These mechanisms are composed by a set of strategies, reviewed by Demming-Adams et al. (1999), Mittler (2002), Müller et al. (2001), Niyogi (2000), that basically avoid ROS generation (by reducing light absorption or increasing energy dissipation) or directly detoxify ROS (by enzymatic and non-enzymatic antioxidants). Heat also affects negatively enzymatic defences leading to the compensation of the reduction in antioxidative enzymatic activity by an increased pool of free antioxidants (Rennenberg et al. 2006).

In the present paper, we expanded data collected during the summer 2003 heat wave by describing changes in photoprotective compounds observed during that period in several Mediterranean (evergreen and deciduous) and Atlantic (deciduous) tree species growing in the field in the Basque Country. Since this European region lies between the Atlantic and Mediterranean bioclimatic regions, and it is supposed that edge areas will suffer more dramatic effects of climate changes, it represents an excellent site for the comparative study of species belonging to both climatic conditions. The study of trees physiological responses to changes in environmental conditions is specially important because adult trees may survive for decades after climate has become unsuitable for their physiological requirements, and on the other hand, trees are more vulnerable to rapid climatic changes, that do not allow the evolution of adaptive mechanisms or geographical range changes. The conclusions of these studies are essential for forest policy and management future decision (Czajkowski et al. 2005; Geßler et al. 2007)

Methods

Sampling strategies and species

Samples were taken from tree leaves in three different sites in which the summer composition of photoprotective molecules had previously been studied. These sites were: Unzá (lat. 42°58′N; long. 2°57′W; alt. 690 m), Izki Natural Park (lat. 42°42′N; long. 2°27′W; alt. 750 m) and Sobrón (lat. 42°46′N; long. 3°15′W; alt. 550 m). All sites were in a range of less than 50 km, with altitudes ranging between 550 and 850 m. Eighteen tree species were studied. Seven were Mediterranean evergreens: *Arbutus unedo* L., *Buxus sempervirens* L., *Phyllirea latifolia* L., *Quercus coccifera* L., *Quercus rotundifolia* Lam, *Rhamnus alaternus* L., *Viburnum tinus* L. Two were Mediterranean semi-deciduous *Cistus salvifolius* L. and *Lonicera implexa* Aiton. Three were Mediterranean deciduous: *Acer monspessulanum* L., *Pistacea terebinthus* L. and *Viburnum lantana* L. Six were Atlantic deciduous: *Coryllus avellana* L., *Betula alba* L., *Alnus glutinosa* L., *Populus tremula* L., *Cornus sanguinea* L. and *Fagus sylvatica* L. For some species (see Table 1) control values for each compound (those measured in non stressful summers) were obtained from data previously published by our group (García-Plazaola and Becerril 2001; García-Plazaola et al. 2003a) while data for other species were obtained from unpublished results obtained in 1998. This set of data was compared with those measured in same species in 2003 during the peak of the heat-wave.

Sampling was performed as described previously (García-Plazaola and Becerril 2001; García-Plazaola et al. 2003a). Basically, sun twigs were cut and kept in the dark for 12 h at room temperature (20–22°C) to reduce the effects of diurnal variations in antioxidants and pigments. Samples for biochemical analysis were collected after dark incubation, and immediately frozen in liquid nitrogen and stored at −80°C until biochemical analysis. Six parameters, indicative of temperature response and acclimation were studied: chlorophyll content (Chl $a + b$), xanthophyll cycle pigments to chlorophyll ratio (VAZ), tocopherol to chlorophyll ratio (Toc), ascorbate to chlorophyll ratio (Asc), de-epoxidation index $(A + Z)$/VAZ and maximal photochemical efficiency of PSII (F_v/F_m). VAZ, $(A + Z)$/VAZ and Fv/Fm(are indicative of the level of photoprotection and energy dissipation and they are usually proportional to the level of light stress to which leaves are

Table 1 Control values of photoprotective parameters of the 18 species analysed during July/August in years previous to the heatwave of summer 2003

Species	Ref	Chl $a + b$	Asc	Toc	VAZ	$(A + Z)$/VAZ	F_v/F_m
Mediterranean deciduous							
A. monspessulanum	1	507 ± 37	7.2 ± 0.6	192 ± 35	60.0 ± 10.4	0.125 ± 0.051	–
P. terebinthus	1	428 ± 12	9.95 ± 0.66	241 ± 7	104 ± 2.1	0.239 ± 0.061	0.768 ± 0.023
V. lantana	1	283 ± 14	1.86 ± 0.10	187 ± 14	106 ± 10.6	0.161 ± 0.028	–
Mediterranean semi-deciduous							
C. salvifolius	1	370 ± 25	–	88.5 ± 13.8	46.7 ± 0.4	0.060 ± 0.005	0.833 ± 0.018
L. implexa	1	262 ± 1	6.51 ± 1.10	63.0 ± 5.1	66.1 ± 2.6	0.156 ± 0.011	0.838 ± 0.019
Mediterranean evergreens							
A. unedo	1	347 ± 2	12.3 ± 0.2	121 ± 4	61.8 ± 4.2	0.137 ± 0.014	0.774 ± 0.021
B. sempervirens	1	267 ± 3	11.1 ± 1.2	753 ± 46	104 ± 3	0.154 ± 0.011	0.632 ± 0.037
P. latifolia	1	381 ± 16	2.93 ± 0.96	108 ± 8	90.0 ± 6.1	0.097 ± 0.010	0.771 ± 0.035
Q. coccifera	1	223 ± 4	10.2 ± 1.4	212 ± 13	55.9 ± 7.7	0.093 ± 0.006	0.758 ± 0.043
Q. rotundifolia	1	339 ± 7	5.66 ± 0.41	124 ± 1	65.6 ± 0.1	0.100 ± 0.001	0.775 ± 0.029
R. alaternus	1	343 ± 11	11.0 ± 2.3	207 ± 12	75.3 ± 2.0	0.127 ± 0.011	0.768 ± 0.018
V. tinus	1	356 ± 8	1.36 ± 0.29	219 ± 10	73.3 ± 1.1	0.109 ± 0.003	0.759 ± 0.025
Atlantic deciduous							
C. avellana	2	574 ± 38	5.63 ± 0.487	983 ± 43	100.6 ± 43.3	0.145 ± 0.004	0.832 ± 0.005
B. alba	2	522 ± 53	10.2 ± 0.4	986 ± 27	111 ± 8	0.118 ± 0.004	0.818 ± 0.006
A. glutinosa	2	551 ± 30	5.24 ± 0.25	567 ± 3	114 ± 6	0.104 ± 0.006	0.832 ± 0.005
P. tremula	2	747 ± 55	6.84 ± 0.15	627 ± 5	64.6 ± 4.7	0.135 ± 0.006	0.832 ± 0.006
C. sanguinea	2	431 ± 13	–	859 ± 59	76.3 ± 2	0.161 ± 0.010	0.792 ± 0.004
F. sylvatica	3	653 ± 22	6.26 ± 0.20	286 ± 24	71.4 ± 4.5	0.182 ± 0.011	–

Origin of data is summarised as follows: (1) unpublished data collected in July 1998 in Sobrón, (2) data collected in Izki in August 2001 (García-Plazaola et al. 2003a) and (3) data collected in Unzá in August 1999 (García-Plazaola and Becerril 2001). Units are: chlorophyll $a + b$ (μmol m^{-2} leaf area), ascorbate (mol mol^{-1} chl $a + b$) and tocopherol and VAZ (mol mmol^{-1} chl $a + b$).

exposed (Logan et al. 1996; Niinemets et al. 2003; Tausz et al. 2004), α-Toc/Chl and Asc/Chl are indicators of the antioxidant potential and the level of photoprotection of the tissue (García-Plazaola et al. 2004), while total chlorophyll per unit of leaf area is proportional to the antena size and it decreases with light stress (Boardman 1977).

Analytical methods

Pigments, ascorbate, tocopherol and chlorophyll fluorescence were measured as described in García-Plazaola et al. (2003a) Briefly chlorophylls, carotenoids and tocopherols were were extracted with acetone and measured by reverse-phase HPLC following the method of García-Plazaola and Becerril (1999) with the modifications described in García-Plazaola and Becerril (2001). In the same extractions tocopherol detection was carried out with a fluorescence detector (Waters model 474) set to $\lambda_{exc} = 295$ nm and $\lambda_{em} = 340$ nm and calibrated with α-tocopherol standards (Calbiochem, San Diego, USA). Ascorbate was extracted with HClO$_4$ (2.5 kmol m^{-3}) as described in García-

Plazaola and Becerril (2001) and measured after neutralisation with a succinate buffer (200 mol m^{-3}, pH 12.7) to reach a final pH between 4 and 5. After neutralization was immediately measured spectrophotometrically by the change in A$_{265}$ after the addition of 10 μL ascorbate oxidase (100 kU L^{-1}). Maximal photochemical efficiency of PSII (F_v/F_m) was estimated by the ratio $F_v/F_m = (F_m - F_o)/F_m$ in dark adapted leaves using a portable modulated fluorimeter (OS 5-FL, Optisciences, Tyngsboro, USA).

Plasticity index

Phenotypic plasticity is the capacity of a given phenotype to render different phenotypes under different environmental conditions (Valladares et al. 2007). It represents a physiological solution to cope with changing environmental conditions in sessile organisms, and it is fundamental to predict species responses to global change. Several approaches have been done to quantify phenotypic plasticity (Valladares et al. 2006). Among them we have selected the plasticity index (P.I.) described in Valladares

et al. (2000), that it is defined and determined as the ratio between the range of variation of a parameter and the largest value measured for such parameter [PI = (maximum value − minimum)/maximum]. For these calculations, only the optimum (non stressful summer) and the extreme (summer 2003) values for each parameter were considered. Assuming that summer 2003 represented the hottest period in the last 150 years, and consequently the hardest that most European trees have ever suffered, this index would represent the potential increase from the basal level of photoprotection, in response to high temperature. Average PI for each species, parameter and functional group are shown in Table 2.

Results

The present study shows the composition of photoprotective compounds during the unusually hot summer of 2003 in several Mediterranean and Atlantic woody plant species growing in the Basque Country (South-western Europe). As a comparison these parameters were also analysed in the same trees in previous summers (1998, 1999 and 2001). Climatic conditions differed between years, so interspecific comparisons must be done, but they should be interpreted with care. In Fig. 1, some meteorological parameters from the period 1996–2005 are shown. Both summer 2003 and

the summers used as control were markedly dry (accumulated precipitation during the period February-July for the years 1998, 1999, 2001 and 2003 was respectively, 27, 3, 45 and 38% lower than the 30-years average). Contrasting with rainfall, mean August temperatures among these years were only slightly higher than the average (0.1–0.9°C), except the year 2003 when temperature exceeded the average for this month by 5°C. Thus, summers of control years and summer 2003, with the exception of summer 1999 that was not dry, differed mostly in average temperature. However, heat and drought are frequently linked and act synergistically in Mediterranean ecosystems, and it is not possible to evaluate separately the relative effect of each stress.

With the limitations imposed by the present experimental design, photoprotective composition during summer 2003, was compared with that of previous summers (Table 1) and the rates of response expressed as the plasticity index for each parameter (Table 2). Chlorophyll content during the heatwave was from 10 to 60% lower than control values, except in *Q. coccifera*. This general trend was also observed in ascorbate content (with the remarkable exception of the two *Viburnum* species and *R. alaternus*). Contrasting with chlorophyll and ascorbate, VAZ and tocopherol contents were much higher in all species during the heatwave (with the exception of *C. sanguinea*, *A. glutinosa*, *C. avellana* and *P. tremula* for

Table 2 Phenotypic plasticity index for each parameter and species analysed. Average value for each parameter, species and functional type are shown in bold type

For an explanation of the meaning and calculations of this index see Materials and Methods. The direction of change between 2003 and control years is included as a positive (increase) or negative (decrease) sign in *parenthesis*

Species	Average	Chl	AZ/VAZ	VAZ	Toc	F_v/F_m	Asc
A. monspessulanum	0.35	0.27 (−)	0.13 (+)	0.36 (+)	0.68 (+)	–	0.32 (−)
P. terebinthus	0.48	0.31 (−)	0.52 (−)	0.04 (−)	0.84 (+)	0.01 (+)	0.73 (−)
V. lantana	0.68	0.56 (−)	0.64 (+)	0.59 (+)	0.93 (+)	–	0.70 (+)
Mediterranean deciduous	**0.50**	**0.38**	**0.43**	**0.33**	**0.82**	**0.01**	**0.58**
C. salvifolius	0.54	0.49 (−)	0.55 (+)	0.65 (+)	0.94 (+)	0.07 (−)	–
L. implexa	0.49	0.14 (−)	0.41 (+)	0.44 (+)	0.96 (+)	0.04 (−)	0.92 (−)
Mediterranean semi-deciduous	**0.53**	**0.32**	**0.49**	**0.54**	**0.95**	**0.06**	**0.92**
A. unedo	0.51	0.21 (−)	0.67 (+)	0.64 (+)	0.90 (+)	0.09 (−)	0.53 (−)
B. sempervirens	0.48	0.36 (−)	0.67 (+)	0.57 (+)	0.75 (+)	0.16 (−)	0.39 (−)
P. latifolia	0.48	0.01 (−)	0.63 (+)	0.49 (+)	0.83 (+)	0.02 (−)	0.86 (−)
Q. coccifera	0.41	0.32 (+)	0.14 (−)	0.38 (+)	0.83 (+)	0.09 (+)	0.71 (−)
Q. rotundifolia	0.32	0.07 (−)	0.29 (+)	0.36 (+)	0.79 (+)	0.03 (+)	0.41 (−)
R. alaternus	0.34	0.11 (−)	0.41 (+)	0.51 (+)	0.68 (+)	0.08 (−)	0.28 (+)
V. tinus	0.66	0.62 (−)	0.74 (+)	0.71 (+)	0.92 (+)	0.17 (−)	0.45 (+)
Mediterranean evergreens	**0.45**	**0.24**	**0.51**	**0.52**	**0.82**	**0.09**	**0.57**
C. avellana	0.21	0.49 (−)	0.16 (−)	0.01 (+)	0.17 (−)	0.05 (−)	0.40 (−)
B. alba	0.24	0.39 (−)	0.10 (−)	0.18 (+)	0.18 (−)	0.01 (+)	0.57 (−)
A. glutinosa	0.29	0.28 (−)	0.23 (−)	0.09 (−)	0.41 (+)	0.01 (−)	0.19 (−)
P. tremula	0.20	0.47 (−)	0.18 (+)	0.14 (+)	0.24 (−)	0.01 (+)	0.19 (−)
C. sanguinea	0.02	0.03 (+)	0.01 (+)	0.03 (−)	0.02 (+)	0.01 (−)	–
F. sylvatica	0.38	0.49 (−)	0.01 (+)	0.39 (+)	0.84 (+)	–	0.22 (−)
Atlantic deciduous	**0.22**	**0.36**	**0.12**	**0.14**	**0.31**	**0.01**	**0.31**

Fig. 1 Climatic conditions (temperature and precipitation) during the period 1996–2005. *Solid line* represent mean temperature for each month, dotted line absolute maximum temperature for each month (in °C) and *black areas* anomalies from the average precipitation of the last 30 years (in mm). When maximum temperatures were higher than 35°C (and potentially damaging to vegetation) *black areas* were drawn. *Arrows* indicate sampling periods
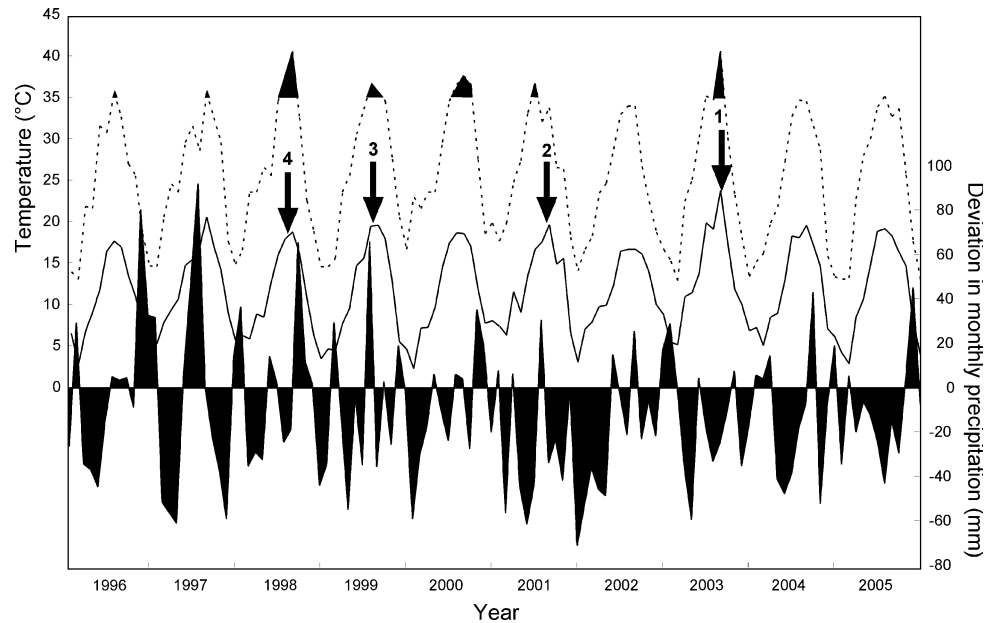
the first and *C. sanguinea* for the latter). The difference between control years and 2003 was especially dramatic in the case of tocopherol. The ratios F_v/F_m and $[(A + Z)/$VAZ] also varied in a coherent direction. It was observed during 2003 an increase in $(A + Z)$/VAZ and a decrease in F_v/F_m, especially in evergreen and semi-deciduous Mediterranean species.

The proportion of de-epoxidised xanthophylls and F_v/F_m was proportional among different species in 2003 (Fig. 2), indicating that the sustained reduction of the photochemical efficiency was proportional to the retention of $A + Z$, and it was consequently the result of the process photosynthesis down-regulation. Taken all species in consideration, not only did these two indexes correlate, but also all photoprotective parameters analysed in this study (except ascorbate) were correlated among them (Table 3). This suggests that all species share an integrate set of photoprotective strategies, which are induced in a coordinated way under similar stress conditions, but the main difference between each functional group is their potential to generate such responses.

Considering that summer 2003 was probably the most stressful summer (in terms of temperature) that these plants have ever suffered in their life cycle, plasticity index calculated for each photoprotective parameter (Table 2) was a measurement of maximum potential to modify phenotypic characteristics in response to summer stress. On average responses were more plastic in Mediterranean than in Atlantic species, irrespective of the deciduous or evergreen nature of leaves. The most plastic response was observed in the species of the genus *Viburnum*. Among all parameters analysed, the highest variation was observed in tocopherol, with a marked positive response in most species.

Discussion

Summer 2003 was exceptionally hot in Western and Central Europe, with scarce precipitation and temperatures up to 6°C above long-term means (Rebetez et al 2006). Maximum air temperature anomalies were higher than minimum temperature anomalies, reaching in some localities values higher than damaging limits for temperate plants (Ludlow and Björkman 1984). Drought conditions prevailing in summer 2003 could exacerbate the symptoms
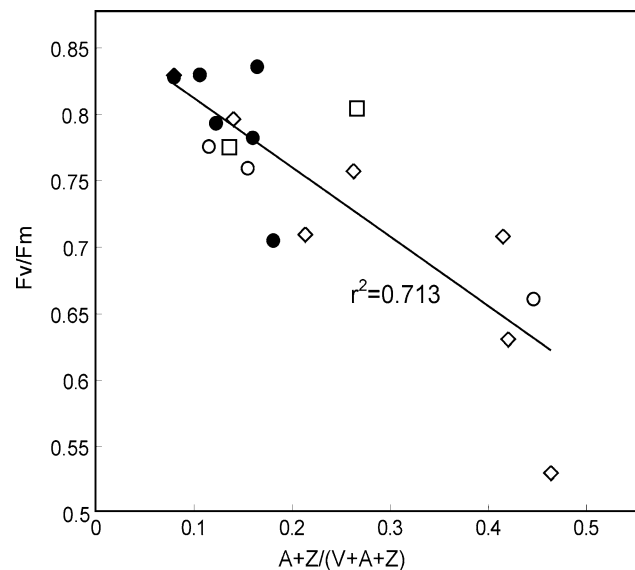

Fig. 2 Relationship between $(A + Z)$/VAZ and F_v/F_m in summer 2003. *Solid circles* represent Atlantic deciduous, *open circles* Mediterranean deciduous, *open squares* Mediterranean semi-deciduous and *open diamonds* Mediterranean evergreens

Table 3 Correlation matrix of the photoprotective parameters analysed in 2003

Values correspond to r^2

Asterisks denote statistical significance of the correlation at * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Parameter	Chlorophyll	$(A + Z)$/VAZ	VAZ	Tocopherol	F_v/F_m
Ascorbate	0.052 NS	0.107 NS	0.127 NS	0.018 NS	0.241 NS
F_v/F_m	0.412*	0.713***	0.693***	0.573***	
Tocopherol	0.679***	0.492**	0.576***		
VAZ	0.606***	0.776***			
$(A + Z)$/VAZ	0.461*				

of damage because of the overheating caused by the low evaporational cooling (Ghoul et al. 2003; Ladjal et al. 2000). These conditions (heat and drought) produced stress symptoms such as defoliation, yellowing and shedding in many trees (Bréda et al. 2006), that in an ecosystem scale resulted in an overall primary production reduction (Ciais et al. 2005) and in a longer-term a process of forest decline and death (Bréda et al. 2006). Because of the global warming, during the present century, temperatures are likely to increase by 2–4.5°C in Europe (IPCC 2007), and at the same time the increasing temperature variability will favour the occurrence of heat-waves (Schär et al. 2004). In this scenario, the ecophysiological responses of long-lived tree species will be essential for the acclimation and survival of forest stands. These changes will be most noticeable in the border areas, mostly in the transition between Mediterranean and Atlantic regions, where a northward shift in bioclimatic limits is expected. These areas, such as the Basque Country, also represent an excellent site to study and model the comparative responses of species with different ecological requirements.

Supra-optimal (but sub-lethal) temperatures may not directly injure photosynthetic tissues, but could interfere with normal physiological performance of plants by affecting carbon balance, nutrient uptake and cycling and stress compensation mechanisms (Rennenberg et al. 2006). One of these effects is the drop in carbon assimilation that in some species, such as in the case of the Mediterranean evergreen *Quercus ilex* L., leads to a decrease of more than 50% of maximum rates at temperature higher than 35°C (Gratani 2000), even when the lethal temperature for this species is higher than 50°C (Munné-Bosch et al. 2004). Water stress exacerbates these effects by inducing stomatal closure and a reduction in evaporative cooling (Ludlow and Björkman 1984). Under these supraoptimal temperatures, energy utilisation and capture are not coupled, and this situation, may potentially generate damaging ROS (Larkindale and Knight 2002). The ability to overcome such ROS production, by the prevention of their generation or by direct reaction, can be one of the mechanisms involved in heat tolerance. A previous report from our group (Hormaetxe et al. 2007) showed that an evergreen Mediterranean species (*Buxus sempervirens* L.) responded to the heat-wave of 2003 with the accumulation of tocopherols and some specific carotenoids together with the down-regulation of photochemical efficiency. The induction of such mechanisms seemed to be enough to compensate photo-oxidative damage since a complete recovery of the physiological condition was observed after the heat-wave. However, this observation must not be generalised to all Mediterranean evergreens, since *B. sempervirens* is an extremely stress-tolerant species (García-Plazaola et al. 2000, 2003b).

In the present study a common pattern of responses to stress was observed during summer 2003 in most Atlantic and Mediterranean species. Thus, chlorophyll, ascorbate and Fv/Fm decreased while tocopherol, VAZ and $(A + Z)$/VAZ increased. There were also some exceptions to this general pattern (19% of the observations), and most of them occurred in *C. sanguinea*, *P. tremula* and *Quercus*. However, the most dramatic changes of these parameters were observed in *Viburnum* species. These photoprotective responses to temperature stress have separately been described in several works. Thus, chlorophyll contents decrease under heat and drought stress as a consequence of antenna size readjustments, and this is a typical response in both Mediterranean (Munné-Bosch and Peñuelas 2004) and Atlantic (Peltzer et al. 2002) species. Similarly, sustained reductions in photochemical efficiency (F_v/F_m) coupled with an overnight retention of de-epoxidised xanthophylls, shown by the increment on $(A + Z)$/VAZ ratio, are regularly described in temperature stress responses (Munné-Bosch and Peñuelas 2004; Munné-Bosch et al. 2004; Streb et al. 2003) and are interpreted as a photo-acclimation process, more than a symptom of damage, as can be deduced from the correlation between both parameters (Fig. 2). The antioxidant responses to high temperature stress do not respond so clearly with a uniform pattern. Thus, some authors have described a decrease in the pools of ascorbate and tocopherol (Keles and Öncel 2002), while others show a general increase (Munné-Bosch and Peñuelas 2004) and even an opposite pattern in both antioxidants (Munné-Bosch et al. 2004) with a decrease in ascorbate and a large synthesis of tocopherol, similar to that observed in the present work. The dramatic increase in tocopherol (up to 20 fold higher during the heat-wave)

could be of great value for plant survival since this molecule contributes to thylakoid stabilization at high temperature (Havaux and Tardy 1996; Havaux et al. 2004), revealing its importance as stress biomarker. On the other hand, since summer 2003 lead to a general decrease in photosynthetic production (Ciais et al. 2005), ascorbate depletion could be due to a decrease in carbohydrates required for its synthesis.

In the present survey, all parameters analysed behave as photoprotective due to their induction or accumulation (tocopherol and xanthophylls cycle pool), or their decrease (chlorophyll) and their consumption (ascorbate). A remarkable exception was the huge amount of ascorbate accumulated by *Viburnum* species. All these photoprotective parameters were strongly correlated among species with different traits in this study (Table 3). The existence of such correlation between photoprotective parameters in response to temperature stress has been described in detail in the evergreen *B. sempervirens* (Hormaetxe et al. 2004, 2007). This is consistent with the idea that all these mechanisms constitute an integrated set of responses that operates in parallel, representing a continuous and rapid stress acclimation syndrome.

Assuming that the observed changes are the result of photoprotection mechanisms (induced as a response to the combination of light, heat and drought), and not symptoms of damage, relative rates of response, calculated as plasticity index for each parameter, were used to characterise the potential capacity of response for each functional group and process. In general, responsiveness was much higher in Mediterranean than in Atlantic species (Table 2), with tocopherol as the most sensitive parameter analysed. Common beech (*F. sylvatica*) behaved as the most plastic Atlantic species, as corresponds to its higher heat stress tolerance and metabolic plasticity (Nahm et al. 2006b). However its induction was lower than average level of all Mediterranean species (including deciduous and semi-deciduous), except *R. alaternus*, *A. monspessulanum* and *Q. rotundifolia*. As it is shown in this study, Atlantic species have a lower capacity to respond to acute summer stress by the simultaneous down-regulation of photosynthesis and the induction of photoprotective molecules, while most Mediterranean species have developed mechanisms that allow an efficient acclimation not only to stress during heat-waves, but also to episodic cold waves (García-Plazaola et al. 2003b). This could be of special relevance in the context of future climatic warming in which Atlantic species could be more severely affected. This has been recently observed in some species such as *F. sylvatica*, where a dramatic growth decline and mortality has recently been reported in the southern limit of its distribution area (Jump et al. 2006).

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