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Photomorphogenic effects of UVB and UVA radiation on leaves of six Mediterranean sclerophyllous woody species subjected to two different watering regimes at the seedling stage

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ABSTRACT

We aimed to investigate the effects of UV radiation and drought on the leaf morphology and anatomy of six native Mediterranean species with different degrees of sclerophylly (*Phillyrea angustifolia*, *Pistacia lentiscus*, *Daphne gnidium*, *Ilex aquifolium*, *Laurus nobilis*, *Rosa sempervirens*). One-year-old seedlings of these species were grown in a glasshouse under three different UV conditions: UVB plus UVA radiation (UVBA), UVA radiation (UVA) and without UV radiation (UV0), and under two watering regimes (low- and well-watered). We observed a significant reduction in leaf area and thickness in response to drier conditions in all the species. The combination of UVB and UVA radiation resulted in leaves with a higher leaf mass area (LMA) and thickness, basically as a consequence of an increase in the thickness of the palisade parenchyma. UVA radiation specifically affected the adaxial epidermal cells, which were thicker and longer than those grown without UV. However, when UV radiation effects were analysed within each watering treatment, well-watered plants showed a higher sensitivity to UV whilst UV did not affect significantly the leaf parameters measured in low-watered plants. Lastly, the more sclerophyllous plants were the least sensitive to UV radiation which suggests that leaf sclerophylly would influence the UV plant response.

1. Introduction

About 5% of the sun's energy reaching terrestrial plants at the Earth's surface corresponds to UV radiation (280–400 nm). The levels of UVB (280–315 nm) have increased in recent decades due to the reduction of the stratospheric ozone layer (McKenzie et al., 2007). Despite the efforts made to reduce the quantity of ozone-depleting chemicals released into the atmosphere, the UVB flux reaching the Earth's surface is expected to remain high until at least halfway through the twenty-first century (McKenzie et al., 2007). Moreover, UVB fluxes can also be modified by other environmental factors, such as clouds, aerosols and air pollution (Munakata et al., 2009). In fact, the predicted decreases in the mean cloudiness of the Mediterranean Basin due to climate change may lead to increases in UVB radiation reaching Mediterranean ecosystems in the near future (Zerefos et al., 1997; Foyo-Moreno et al., 2003; Giorgi et al., 2004; WMO, 2010). A decrease in cloud frequency and cover will also increase UVA radiation fluxes (Calbó et al., 2005; Vernet et al., 2009), which can also have harmful effects on living cells (Flint and Calwell, 2003; Ridley et al., 2009). In addition, the expected temperature increases in the Mediterranean region, together with a decrease in water availability (IPCC, 2007), will force some species to migrate to higher altitudes (Peñuelas et al., 2004; Bertin, 2008), which could expose them to higher doses of both UVB and UVA radiation (Blumthaler et al., 1997).

In terrestrial plants, UVB and UVA radiation have great importance in photomorphogenesis, because UV radiation provides plants with information on the light conditions that they need to develop normally (Gitz and Liu-Gitz, 2003; Krizek, 2004). However, an excess of UV radiation (both UVB and UVA) can generate reactive oxygen species resulting in cellular damage (Jansen et al., 1998; Cadet et al., 2009), unless plants have effective protective mechanisms against this radiation. Since leaves are considered to be the most important target organs of UV radiation in plants, they have been the focus of numerous studies in the last 20–30 years. Recent reviews (Jansen, 2002; Caldwell et al., 2003, 2007;

Abbreviations: Dg, *Daphne gnidium* L.; Ia, *Ilex aquifolium* L.; Ln, *Laurus nobilis* L.; Pl, *Pistacia lentiscus* L.; Pha, *Phillyrea angustifolia* L.; Rs, *Rosa sempervirens* L.; UVA, UVA radiation; UVBA, UVB plus UVA radiation; UV0, no UV radiation.

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Table 1
Montly UVE (J/m^2/day), PAR (J/m^2/day) and daily UVE/PAR ratio inside the glasshouse and outdoors (environment).

Month	Glasshouse			Environment		
	UVER glass (J/m^2/day)	PAR glass (J/m^2/day)	UVER/PAR glass (daily)	UVER envir (J/m^2/day)	PAR envir (J/m^2/day)	UVER/PAR envir (daily)
June	1.47E+03 ± 5.92E+01	4.61E+06 ± 2.63E+05	3.20E−04 ± 8.87E−06	5.10E+03 ± 1.61E+02	1.49E+07 ± 6.74E+05	3.42E−04 ± 7.53E−06
July	1.79E+03 ± 5.48E+01	3.46E+06 ± 2.19E+05	5.19E−04 ± 6.94E−05	4.25E+03 ± 1.69E+02	1.09E+07 ± 5.09E+05	3.90E−04 ± 5.82E−06
August	1.80E+03 ± 5.92E+01	1.98E+06 ± 2.48E+05	9.08E−04 ± 1.20E−04	3.75E+03 ± 1.51E+02	9.62E+06 ± 4.24E+05	3.89E−04 ± 4.54E−06
September	2.12E+03 ± 4.99E+01	1.79E+06 ± 1.44E+05	1.18E−03 ± 1.53E−04	2.46E+03 ± 1.20E+02	6.72E+06 ± 3.55E+05	3.66E−04 ± 6.06E−06
October	2.18E+03 ± 7.28E+01	9.57E+05 ± 1.21E+05	2.28E−03 ± 6.86E−04	1.52E+03 ± 1.05E+02	4.92E+06 ± 3.94E+05	3.09E−04 ± 1.06E−05
November	2.74E+03 ± 8.73E+01	5.97E+05 ± 3.41E+04	4.60E−03 ± 4.82E−04	8.27E+02 ± 4.93E+01	4.07E+06 ± 2.99E+05	2.03E−04 ± 1.21E−05

Barnes et al., 2005; Paoletti, 2005) and meta-analyses (Searles et al., 2001; Newsham and Robinson, 2009; Li et al., 2010) show that plant responses to enhanced UVB radiation, independently of plant life form and habitat, include biochemical, morphological and anatomical changes in leaves. Most researchers agree that UVB radiation promotes leaf thickening and negatively affects leaf area, and that UV-absorbing compounds (hereafter UACs) in the epidermis layer (i.e. in trichomes, surface waxes, cuticle and/or epidermal cells) constitute the leaf's first barrier to UVB penetration. However, despite the epidermis appearing to be an efficient UVB filter, UVB radiation reaching deeper leaf tissues has been found to be significant in many species (Day et al., 1992, 1993; Day, 1993; Àlenius et al., 1995). If this happens, UVB radiation is expected to promote changes at the mesophyll level aimed at protecting photosynthetic machinery or nucleic acids from damage. Nevertheless, few data about UVB photomorphogenic effects on the leaf parenchyma structure are available (Schumaker et al., 1997; Nagel et al., 1998; Nogués et al., 1998; Olsson et al., 1999; Wulff et al., 1999; Laakso et al., 2000; Kostina et al., 2001; Kakani et al., 2003; Heijari et al., 2006) and to our knowledge there is no information on Mediterranean woody species. Most studies investigating UVB photomorphogenic effects at the leaf morpho-anatomical level have focussed only on leaf thickness or leaf mass per area (LMA) and leaf area (see reviews of Searles et al., 2001; Caldwell et al., 2003; Barnes et al., 2005; Paoletti, 2005; Newsham and Robinson, 2009; Li et al., 2010), despite the fact that changes in the structure of the photosynthetic parenchymas can markedly affect leaf photosynthesis and water relations and, consequently, plant productivity (Flexas et al., 2008). With regard to UVA radiation, as far as we know, only some studies, most of them using agricultural species, have provided information about the effects of UVA on plant growth (Tezuka et al., 1994; Antonelli et al., 1998; Newsham et al., 1999; Hollósy, 2002; Jayakumar et al., 2003), and very few have included data on leaf morpho-anatomy (Heijari et al., 2006; Fagerberg, 2007; Victório et al., 2011).

In the Mediterranean Basin, sclerophyllous evergreen broad-leaved woody species are highly abundant in shrub and forest communities (Balsamo et al., 2003; Paula and Pausas, 2006). Sclerophyllous leaves are hard, stiff and coriaceous (Read and Sanson, 2003) in part due to thick cuticles and cell walls (Groom and Lamont, 1999; Balsamo et al., 2003), which may give them a high tolerance to enhanced UV radiation (Day, 1993). In Mediterranean species, low water availability can also favour leaves with anatomical traits similar to those described for UV-irradiated plants, such as a thicker adaxial cuticle and epidermis (Bussotti et al., 2002; Turunen and Latola, 2005), reduced leaf area and increased leaf thickness – with a concomitant increase in LMA – (Nevo et al., 2000; Nogués and Baker, 2000; Wright et al., 2004). Thicker leaves could provide more photosynthetic cells, improving carbon assimilation without increasing water loss (Chaves et al., 2002; Gitz and Liu-Gitz, 2003). Therefore, some plant responses to drought stress at the leaf level might also help plants to cope with UV radiation, suggesting some kind of cross tolerance between the signalling pathways for drought and UV (Gitz and Liu-Gitz, 2003; Mittler, 2006; Caldwell et al., 2007). However, few data are available on the response of Mediterranean sclerophyllous species to UV under drought stress (Petropoulou et al., 1995; Drilias et al., 1997; Manetas et al., 1997; Nogués and Baker, 2000; Kyparissis et al., 2001) and even less on leaf morpho-anatomical traits.

The aim of this study was to assess the effects of UVB and UVA radiation and drought, as well as their interaction, on the leaf morphology and anatomy of six native Mediterranean sclerophyllous species (*Daphne gnidium*, *Ilex aquifolium*, *Laurus nobilis*, *Phillyrea angustifolia*, *Pistacia lentiscus*, *Rosa sempervirens*). Furthermore, we also aimed to determine how the degree of leaf sclerophylly can influence the response of these species to UV radiation and/or drought. To achieve this goal, we performed a controlled glasshouse experiment where seedlings from the six species were subjected to two watering regimes (low- or well-watered conditions) and to three UV radiation treatments (UVA plus UVB radiation, UVA radiation and without UV radiation). Our results contribute to improving our knowledge of the leaf-level adaptive strategies in response to UV of plants, and particularly of Mediterranean species, and on the potential capacity of Mediterranean species to acclimate to UV radiation (UVA and UVB) changes and drought stress, information that will help to elucidate their behaviour in a future scenario of climate change.

2. Materials and methods

2.1. Plant material

Six woody species typical of the Mediterranean Basin were selected for this study. Three of them, *P. lentiscus* L., *P. angustifolia* L. and *D. gnidium* L. (hereafter Pl, Pha and Dg, respectively), were considered xerophytes whilst the other three, *I. aquifolium* L., *L. nobilis* L. and *R. sempervirens* L. (hereafter Ia, Ln, and Rs, respectively) were considered mesophytes. The sclerophylly index, measured as the leaf mass per unit area (LMA; mg cm^{-2}) (Groom and Lamont, 1999), was similar for Pha and Ia, for Pl and Ln, and for Dg and Rs (Table 2). Hence, there were three pairs of species according to their sclerophylly index, being one species of each pair from xeric habitats and the other from mesic ones. All the studied species have simple leaves, except Pl which have compound leaves of 8–14 leaflets. In addition, leaves of all the species are glabrous and devoid of glands, except in the case of Pha, which show some glandular hairs on the abaxial leaf surface.

2.2. Experimental design and plant growth conditions

At least 90 one-year-old seedlings of each species were obtained from local nurseries (Vivers Aloma, Tarragona, Spain, for Ln, Ia, Pha and Dg, and Bioriza, Girona, Spain, for Pl, Rs) and transplanted into 2l pots (5 cm side × 20 cm depth) filled with 530 g (650 g for Dg and Pl) of a mixture containing composted bark of pine and Sphagnum peat (1:1 by volume). The growing media was fertilised with osmocote (4 kg/m^3), basal dressing (1 kg/m^3) and dolomite (4 kg/m^3) to avoid nutritional deficiencies during the experiment.