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Different responses of *Arbutus unedo* and *Vitis vinifera* leaves to UV filtration and subsequent exposure to solar radiation

Daniele Grifoni[a,e,*], Giovanni Agati[b], Filippo Bussotti[c], Marco Michelozzi[d], Martina Pollastrini[c], Gaetano Zipoli[a]

[a] Institute of Biometeorology (IBIMET), National Research Council of Italy (CNR), Via Madonna del Piano 10, Sesto Fiorentino, 50019 (FI), Italy
[b] Institute of Applied Physics Nello Carrara (IFAC), National Research Council of Italy (CNR), Via Madonna del Piano 10, Sesto Fiorentino, 50019 (FI), Italy
[c] University of Firenze, Department of Agri-Food Production and Environmental Science, Piazzale delle Cascine 28, 50144 Firenze, Italy
[d] Institute of Biosciences and Bioresources (IBBR), National Research Council of Italy (CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino, (FI), Italy
[e] LaMMA Consortium, Via Madonna del Piano 10, Sesto Fiorentino, 50019 (FI), Italy

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ABSTRACT

An UV-exclusion experiment was conducted on Mediterranean plant species grown in pots: the evergreen sclerophyllous shrub *Arbutus unedo* and the deciduous woody crop *Vitis vinifera*. After 130 days, of exclusion of UVB and both UVA and UVB radiations, during leaf unfolding and development, the plants were exposed to the ambient solar radiation (about 80% of the whole solar spectrum). The different radiative treatments were obtained with tunnels, covered with appropriate filters (cutting UV radiation), where the plants grown. The leaf UV absorbing compounds were analysed, during the growing season, in both species, in plants under the different radiative regimes. The flavonols content in leaf epidermis was, also periodically assessed, in both species, by means of a non-destructive optical index (the FLAV index of the Multiplex sensor). Structural characteristics and functionality of the photosynthetic apparatus of the plants were assessed by means of chlorophyll *a* fluorescence measurements.

The UV-exclusion provoked a reduction of UVA-absorbing compounds, mainly flavonols, in the leaves of both species. The results evidenced an increase of these compounds over time in plants previously grown under both UV-exclusion treatments, especially in *V. vinifera*. Chlorophyll *a* fluorescence analysis showed a delay in the development of the photosynthetic apparatus in *V. vinifera* leaves previously grown under total UV exclusion (both UVA and UVB) and damage to the oxygen evolving complex. No significant effects of the UV treatments on the leaf photochemical properties were detected in *A. unedo*. Overall the results highlighted the relevance of UV radiation on the leaf physiology of the species considered and its essential role in the photomorphogenesis of protective substances in the leaves.

1. Introduction

Ultraviolet radiation (UV total radiation 280–400 nm, UVA 315–400 nm, UVB 280–315 nm), although represents a small part of the solar radiation reaching the ground, has relevant biological effects on humans and ecosystems.

The discovery of the depletion of the stratospheric ozone and the consequent expected increase in UVB radiation at ground level drove a big concern for its possible impacts on terrestrial ecosystems (Brown et al., 1994; Caldwell and Flint, 1994; Manning and Tiedemann, 1995; Caldwell et al., 1998; Paul, 2001). Several experiments were conducted to investigate such impacts on vegetation trying to identify and quantify the effects induced on growth and physiology of plants grown under enhanced UVB radiation emitted by lamps. As documented by Caldwell and Flint (1994) many of the first experiments were conducted under high unrealistic UVB enhancement and under limited environmental conditions (greenhouses and/or growth chambers experiments with reduced PAR and other solar radiation components active in the photo-repair processes). These experimental results led to overestimate the possible harmful effects of UVB radiation on natural vegetation. In fact in the early 2000s, a first quantitative

* Corresponding author at: Institute of Biometeorology (IBIMET), National Research Council of Italy (CNR), Via Madonna del Piano 10, Sesto Fiorentino, 50019 (FI), Italy.
E-mail address: d.grifoni@ibimet.cnr.it (D. Grifoni).

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analysis (Searles et al., 2001), considering only the field experiments conducted up to 1999, concluded that beside a general increase in the so-called UVB-absorbing compounds little or no effect was found for what concerned other parameters of plant morphology and no effect on leaf photosynthesis.

In the late 90's a new vision of the interaction between UV and plant life focused the attention on the multiple effects in terms of morphogenesis, damage, repair and acclimation (Jansen et al., 1998). Consequently UV radiation was considered as an essential ecological factor rather than a simple environmental stressor (Hideg et al., 2013).

This new vision has driven a renewed interest, for what concern field experiments, to the exclusion approach, where the effects of a specific UV band (typically the UVB band) on plant development were studied by means of filters excluding specific wavelengths of solar radiation. This approach was particularly appropriate when considering species naturally growing in high radiative environments or well adapted to them. In the Mediterranean region, with high solar irradiation, native vegetation is characterized at foliar level by sclerophylly and high content of UV screening pigments (Tattini et al., 2005). These features are evolutionary characteristics connected to the resistance of leaves to oxidative stress, of both natural (high light, drought, high temperature) and anthropogenic origin (enhanced levels of tropospheric ozone) (Salleo and Nardini, 2000; Jordan et al., 2005). Very few data are available on Mediterranean species, but generally no interactive effect between UVB and drought on plant growth have been reported (Nogués and Baker, 2000; Kyparissis et al., 2001). Recently in *Laurus nobilis* L. an increase in UV radiation in combination with low watering has found to ameliorate plant photosynthesis and plant growth (Bernal et al., 2015).

While Mediterranean natural vegetation has been proved to be tolerant even to an enhanced intensity of UVB radiations, cultivated tree species may have different reactions. Among typical Mediterranean woody crops, olive trees (*Olea europaea* L.) were demonstrated to be tolerant, whereas grapevine (*Vitis vinifera* L.) showed a more complex behavior. This important agricultural species was the subject of many researches addressing the physiological response to UV radiation (Schultz, 2000; Kolb et al., 2001; Nuñez-Olivera et al., 2006; Pollastrini et al., 2011; Jug and Rusjan, 2012; Martínez-Lüscher et al., 2013). These studies evidenced that UV can have both advantageous and disadvantageous effects on grapevine growth and on its secondary metabolism, especially enhancing the accumulation of phenolic compounds in the leaves.

Increased UVB levels were found to depress grapevine photosynthesis (Berli et al., 2010) and increase the overall antioxidant system in young leaves, with special reference to UVB absorbing pigments, anthocyanin and total phenolic content (Kolb et al., 2001; Jitareanu et al., 2011; Majer and Hideg, 2012), making leaves more resistant to biotic and abiotic stresses. UV absorbing compounds play an important role in the plant protection system. This role is not limited to UV attenuation but includes protection against herbivores and several other oxidative stresses (cross-resistance, Mittler, 2006). The morphogenetic importance of UV radiation includes other responses at foliar level, such as the formation of shorter petioles and shorter, narrower and/or thicker leaf blades (Robson et al., 2015), as well as stimulation of the synthesis of chlorophyll and the development of the photosynthetic apparatus (Salama et al., 2011). According to Carbonell-Bejerano et al. (2014), UVB plays an essential role in ripening the grapevine berries, enhancing biosynthesis and the accumulation of secondary metabolites, well appreciated in winemaking.

UV-filtration (or subtraction) experiments were conducted across Europe to determine the impact and/or morphogenetic role of the current levels of UV (UVA and UVB) radiations on vegetation (Comont et al., 2012). In boreal regions, the current levels of UV are harmful for photosynthesis (Albert et al., 2005, 2008, 2011) and plants beneficiate for their exclusion. In Southern Europe the exclusion of UV radiation was detrimental for the morphogenetic processes related to the adaptation of Mediterranean vegetation to an environment characterized by high oxidative pressure (Pollastrini et al., 2011).

This paper presents the results of an UV-filtration experiment comparing the different response of two woody species: a native Mediterranean shrub (*Arbutus unedo* L., strawberry tree, typical component of the Mediterranean maquis), and the woody crop *Vitis Vinifera*. The aim of the study was to assess the functional role of the leaf screening/antioxidant compounds developed under natural irradiation. The hypothesis to be tested is that plants with low amounts of screening/antioxidant compounds (plants grown under UV-filtration condition) suffer the impact of the full UV radiation when re-exposed to natural solar radiation. Moreover, the response may be different in sclerophyllous (*A. unedo*) and deciduous (*V. vinifera*) species, since higher resistance is expected in the former.

2. Material and methods

2.1. Experimental site, plant material and growth conditions

An experiment under natural sunlight radiation was conducted in the outdoors area of the National Research Council in Sesto Fiorentino (Florence, lat. 43°49′08″, lon. 11°12′07″, 40 m a.s.l.), Central Italy. The experiment was carried out during the period March–September 2012. Three iron cage tunnels ($4 \times 2 \times 2$ m) were mounted with the longer axis aligned in a north-south direction and at an appropriate distance to avoid any mutual shading. The tunnels were wrapped with three different types of plastic foil: 100 μm Teflon (NOWOFOL Kunststoffprodukte GmbH and Co., Siegsdorf, Germany) transparent to the entire region of natural UV–vis sunlight, 125 μm polyester (Folex GmbH, Germany) equivalent to Mylar, transparent to wavelengths above 312 nm (-UVB) and Lee 226 UV (Lee Filters, Andover, UK) transparent to wavelengths above 400 nm (-UVB -UVA). For wavelengths higher than 400 nm the spectral transmittance was almost the same for the three foils (Fig. 1)

Foils spectral transmittance was measured in the laboratory by means of a spectrophotometer (Jasco UV/VIS Spectrophotometer model V-560, Jasco International Co., Ltd., Tokyo, Japan), with an integrating sphere added to measure both direct and diffuse transmitted light. The transmittance of the coupled system foil/tunnel was also periodically measured (every two weeks), using a portable spectroradiometer (model SR9910-PC; Macam Photometrics Ltd., Livingstone, UK), monitoring in this way the solar radiation inside and outside the tunnels at solar noon. The

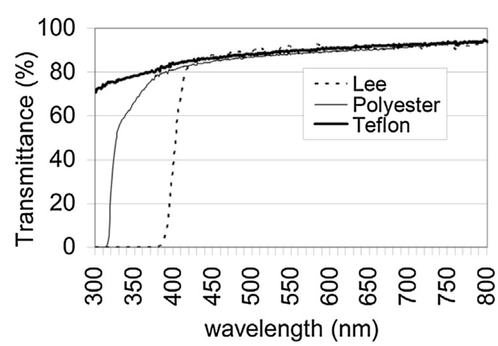

Fig. 1. Spectral transmittance of plastic foils.