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Ultraviolet-B radiation or heat cause changes in photosynthesis, antioxidant enzyme activities and pollen performance in olive tree

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Abstract

The present study attempts to determine how some physiological and reproductive functions of olive tree (*Olea europaea* L., cv. Koroneiki) respond to enhanced UV-B radiation or heat. Enhanced UV-B radiation was applied to (*1*) three-year-old potted plants in an open nursery (corresponded to *ca*. 16% ozone depletion), and (*2*) *in vitro* cultured pollen samples (220 μmol m^{-2} s^{-1}, PAR = 400−700 nm + UV-B at 7.5, 15.0, or 22.5 kJ m^{-2} d^{-1}). Potted olive plants were also subjected to high temperature (38 ± 4°C) for 28 h to mimic heat levels regularly measured in olive growing areas. A significant effect of UV-B on photosynthetic rate was observed. However, enhanced UV-B radiation did affect neither chlorophyll nor carotenoid content, supporting previous reports on hardiness of the photosynthetic apparatus in olive. Increased superoxide dismutase activity was observed in UV-B-treated olive plants (+ 225%), whereas no effect was found in the plants under heat stress. Neither UV-B and nor heat did affect H_2O_2 accumulation in the plant tissues. However, the same treatments resulted in enhanced lipid peroxidation (+ 18% for UV-B and + 15% for heat), which is likely linked to other reactive oxygen species. The increased guaiacol peroxidase activity observed in both treatments (+ 32% for UV-B and + 49% for heat) is related to the defense against oxidative membrane damage. The observed reduction in pollen germination (20–39%) and tube length (11–44%) could have serious implications on olive yields, especially for low fruit–setting cultivars or in years and environments with additional unfavorable conditions. UV-B and heat effects described here support the hypothesis that plant response to a given stressor is affected by the overall context and that a holistic approach is necessary to determine plant strategies for climate change adaptation.

Aditional key words: abiotic stress; climate change; lipid peroxidation; ozone; pollen germination.

Introduction

The effects of climate change on plant physiology are classified as major research priorities worldwide (European Community 2009). UV radiation is a component of the solar light that naturally reaches the Earth and, depending on its wavelength, can be divided into three different ranges: UV-A (315−390 nm), UV-B (280−315 nm), and UV-C (100−280 nm). UV-A influences plant morphology and stomatal opening, while UV-C has no direct role in plants but artificial UV-C sources cause severe damage to exposed tissues (Paul and Gwynn-Jones 2003). Fortunately, UV-B is strongly affected by the ozone layer in the stratosphere, thus the amount of this radiation reaching the Earth's surface is extremely low, but it is increasing due to ozone depletion. The balance among the different wavebands, UV-B, UV-A, and PAR, influences the effect of UV-B radiation on plants (Aphalo *et al*. 2012).

In plants, damage to photosynthetic apparatus by UV-B radiation can induce oxidative stress and genetic mutations in plants that in turn result in a lower photosynthetic capacity (Heisler *et al.* 2003) and alterations in plant growth and biomass allocation (Rozema *et al.* 2001). Regarding the reproductive functions, pollen germination and tube growth are generally reduced by enhanced UV-B radiation (Koti *et al.* 2005). Morphological alterations were also observed in flowers and pollen grains of cotton (Kakani *et al.* 2003) and soybean (Koti *et al.* 2005) exposed to high

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Abbreviations: ANOVA – analysis of variance; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; DM – dry mass; FM – fresh mass; GPOX – guaiacol peroxidase; g_s – stomatal conductance; MDA – malondialdehyde; P_N – net photosynthetic rate; ROS – reactive oxygen species; SOD – superoxide dismutase; TBARS – thiobarbituric acid reactive substances; UV-B – ultraviolet-B radiation.
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doses of UV-B radiation.

Mediterranean plants, such as olive tree (*Olea europaea* L.), are considered to be highly tolerant to elevated UV-B radiation (Nogues and Baker 2000). The traditional olive cultivation countries around the Mediterranean basin are exposed to high daily UV doses that may reach or exceed 5−6 kJ m^{-2} (World Meteorological Organization 2011). This exposure is more prominent mainly during the summer on cloudless, clear-sky days and coincides with high temperature and drought, forming a complicated multistress environment for plants. Furthermore, olive cultivation has expanded during the recent decades into many nontraditional olive-producing areas around the globe that are characterized by a wide range of climatic limitations, including unfavourable temperatures, changes in winds and regional precipitation, and increased UV-B radiation in the atmosphere driven by ozone depletion (World Meteorological Organization 2011). Olive tree shows also genotype-differential sensitivity to atmospheric pollutants and UV-B radiation (Sebastiani *et al.* 2002). Although there is evidence for reduced olive pollen performance under other abiotic stresses, such as heat (Koubouris *et al.* 2009), the reproductive physiology of olive tree has not been sufficiently investigated in response to elevated UV-B radiation exposure.

Our study aimed to determine how olive physiological, biochemical, and reproductive functions respond to enhanced UV-B radiation. We hypothesised that (*1*) UV-B induces biochemical alterations that could act as indicators of long-term plant responses, and (*2*) reproductive functions of olive plants are more sensitive to environmental stresses compared to other physiological processes. The olive cultivar Koroneiki was selected due to its suitability for modern high-density plantations (Koubouris *et al.* 2009). Enhanced UV-B radiation was applied to (*1*) potted plants in an open nursery under ambient sunlight, and (*2*) pollen cultured *in vitro* under artificial growth chamber light. Considering that under Mediterranean climate, enhanced radiation and high temperatures are major environmental concerns for olive growing, the heat-stress experiment was conducted in order to simulate heat incidents regularly occurring in Mediterranean olive growing areas.

Materials and methods

Plant material and culture conditions: Ten three-year-old plants of *Olea europaea* L., cv. Koroneiki, were grown during the spring in the open nursery at the Subtropical Plants and Olive Tree Institute in Chania, Crete, Greece. The plants were grown in 12-L pots containing loamy-sandy soil/perlite mixture (3:1), and they were kept well-watered throughout the experiment to minimize any effects associated with drought stress (Sofo *et al.* 2009). Each plant was fertilized once in early March with 500 mL of 10 g L^{-1} of 20:20:20 (N:P:K) fertilizer, which was applied to the soil. Daily mean air temperature and relative humidity during the experiment are presented in Fig. 1. The experiment started on 7 April, at the flower cluster growing stage, and ended on 17 May, at the end of flowering.

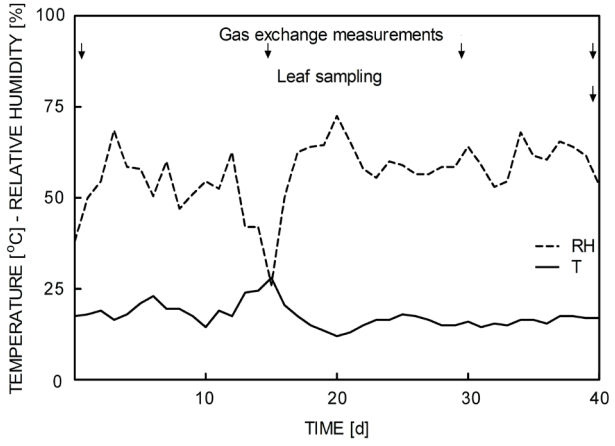

Fig. 1. Mean daily air temperature (T, *black line*) and relative humidity (RH, *dashed line*) during the experiments. Major time points of the experiment (gas-exchange measurements at 0, 12, 29, and 40 d of treatment and leaf sampling for biochemical analyses at 40 d of treatment) are also depicted.

UV-B radiation treatments: Half of the plants ($n = 5$) were maintained in control conditions (ambient UV-B radiation), whereas the other five were UV-B-treated (ambient + supplemental UV-B radiation). Enhanced UV-B radiation was provided as described by Doupis *et al*. (2011). Briefly, enhanced UV-B radiation was provided by UV-emitting fluorescent tubes (*Ultraviolet-B, TL40W/12RS*, *Philips,* Eindhoven, The Netherlands), which were filtered with preheated cellulose acetate (CA) film (*Clarifoil*, Coventry, UK) to exclude UV-C transmission. The CA film was regularly replaced every 20−25 h, to avoid ageing effects of the filters. The lamps were placed under mobile, adjustable frames at a distance of 40 cm above the plant canopy. The irradiance in the UV-B band was checked daily with a broadband radiometer (*SKU 430*, *Skye Instruments Ltd.*, Powys, UK). The supplemental biological effective UV-B dose (UV-BBE) estimated according to the generalised plant action spectrum (Caldwell 1971) was 6.04 kJ m^{-2} d^{-1}, which corresponded to + 15% environmental UV-B radiation and to 16% ozone depletion (Green 1983). UV-B radiation was supplemented for 105 min daily at solar noon (from 11:00 to 13:00 h), excluding cloudy or rainy days (totally, 30 d throughout the 40-d experiment, DAE).

Gas-exchange measurements were made four times during the experiment at two-week intervals (7 April, 21 April, 6 May, and 17 May), between 09:00 and 11:00 h. Ten leaves (two leaves from each of five plants) from each treatment and at every sampling date were used to measure photosynthetic rate (P_N), leaf conductance (g_s), and intercellular CO_2 concentration (C_i) using a portable gas-exchange system (*LI-6400, Li-Cor Biosciences*, Lincoln, NE, USA). The measurements were made on fully expanded leaves, selected along the median segment of new-growth shoots, at a constant 400 μmol(CO_2) mol^{-1}. For each date, all measurements were carried out under

fixed PAR (1,000; 1,300; 1,100; and 1,300 μmol m^{-2} s^{-1}), temperature (23, 28, 26, and 29ºC) and relative humidity (RH) (68, 55, 50, and 45%) according to the daily weather conditions.

Photosynthetic pigments: Ten leaf samples (two for each of five plants) from each treatment were collected at 40 DAE. The samples consisted of fully expanded leaves selected along the median segment of newly grown shoots. Leaf samples were picked, washed with distilled water, dried with filter paper, immediately frozen in liquid nitrogen, and then stored at –80ºC. Samples were separated into two subsamples: one was used for the determination of pigment contents, H_2O_2, and TBARS [calculated per fresh mass (FM)], while another one was used for the determination of dry mass (DM) after desiccation of leaves at 105°C for 24 h. Subsequently, contents were expressed on a DM basis to normalize the eventual differences in water content among the treatments. Frozen tissues were finely ground in liquid nitrogen using a mortar and pestle previously chilled with liquid N_2 and the frozen powder was used for the extraction. The determination of total chlorophyll (Chl), Chl *a*/*b* ratio, total carotenoids (Car), and Chl/Car ratio was carried out according to Bacelar *et al*. (2007). Briefly, 0.1 g of leaf samples were placed in 10 ml of cold 80% (w/w) acetone, homogenized and centrifuged at 6,000 × *g* for 15 min. Absorbance of leaf extract was measured on a *UV-Vis* spectrophotometer (*Helios Aquamate*, *Spectronic Unicam,* Cambridge, UK) at 470, 646, and 663 nm.

Antioxidant enzyme activities: Total superoxide dismutase (SOD, E.C. 1.15.1.1) and guaiacol peroxidase (GPOX, E.C. 1.11.1.7) activities were determined in ten leaf samples (two for each of five plants). The samples consisted of fully expanded leaves, selected along the median segment of new-growth shoots. Leaf sampling, storage, and freezing procedures were similar as those described in the previous paragraph. All procedures for enzyme extraction and determination of enzyme activities were carried out at 0°C in an ice bath unless stated otherwise. The extraction medium consisted of 0.1 M potassium phosphate buffer (pH 7.6), containing 1 mM ethylenediaminetetraacetic acid disodium salt, 0.5 mM ascorbate, and 1% polyvinylpolypyrrolidone. Tissue (0.2 g) was homogenised in 1.5 mL of the extraction buffer and the homogenate was centrifuged at 13,000 × *g* for 30 min. The supernatant was used for assaying the activities of the enzymes. The absorbance of the crude enzyme extract was measured with a *Hitachi U-1100* spectrophotometer (*Hitachi Ltd.*, Tokyo, Japan). Total SOD and GPOX activities were determined using the methodology described by Sofo *et al*. (2004). The volume of enzyme extract causing 50% inhibition in colour development at 560 nm was taken as one unit of SOD activity. One unit of GPOX activity was defined as the amount of enzyme that oxidizes 1 μmol of guaiacol per min at 20°C. Soluble protein content was measured according to the Bradford's method using *Micro-Total Protein Kit* (*TP0100, Sigma-Aldrich*, Saint Louis, Michigan, USA). The enzyme units were normalized per mg of total protein.

H_2O_2 and thiobarbituric acid reactive substances (TBARS): Ten leaf samples (two for each of five plants) from each treatment were collected at 40 DAE. The samples consisted of fully expanded leaves selected along the median segment of new-growth shoots. Leaf tissue (0.5 g), was homogenised with 5 mL trichloroacetic acid 0.1% (w/v) in an ice bath. The homogenate was centrifuged at 10,000 × *g* for 30 min and the supernatant was used. H_2O_2 content was measured spectrophotometrically after the reaction with potassium iodide. The values were expressed as μmol per g DM.

The degree of lipid peroxidation was measured by the amount of malondialdehyde (MDA), a product of membrane fatty acid peroxidation, which reacts with thiobarbituric acid forming red coloured complexes, known as thiobarbituric acid reactive substances (TBARS). These complexes were estimated by the method described previously (Sofo *et al.* 2005) by reading the absorbance of the supernatant at 532 nm, after subtracting the values for nonspecific absorption at 600 nm and 440 nm. The values were expressed as nmol per g of DM.

Effect of enhanced UV-B radiation on in vitro pollen performance: Thirty-year-old irrigated olive trees were used as pollen donors to investigate the impact of UV-B on germination and tube length. Ten inflorescences from each of five trees were collected at flower opening, and pollen was used during the same day.

Pollen samples were exposed to white light (220 μmol $m^{-2}s^{-1}$, PAR, 400–700 nm) supplemented with three UV-B radiation doses (7.5, 15.0, or 22.5 kJ m^{-2} d^{-1}) achieved through exposure for 50, 105, or 105 min to UV-B-enriched light. Control pollen was subjected to the same light conditions but without UV-B. The UV-B doses were chosen to test a mild, moderate, and severe UV-B stress, at similar levels of previous pollen studies (Conner and Neumeier 2002, Singh *et al.* 2008). Pollen was subsequently incubated at room temperature (22ºC) in the dark for 24 h, in a growth chamber (*Kottermann 2770, D3162*, Hanigsen, Germany) before counting pollen germination and pollen tube length. Throughout the experiment, pollen was cultured on solid medium consisting of 0.8% (w/v) agar, 15% (w/v) sucrose, 0.01% boric acid, and 0.006% tetracycline hydrochloride, according to Koubouris *et al*. (2009). Pollen germination was evaluated on five petri dishes containing over 50 pollen grains for each treatment. Pollen tube length was measured for approximately 80 pollen tubes for each treatment. The experiment was carried out twice.

Exposure of plants to elevated temperature: A separate set of ten three-year-old irrigated olive trees was grown in the open nursery without any UV-B treatment. Throughout the experiment, plants were kept under optimal water conditions (Sofo *et al.* 2009) to minimize any effects associated with drought stress. Five plants were transferred to a glasshouse with air temperature of 38 ± 4°C for 28 h (08:00 h of the 1st day until noon of the 2nd day), in order

to simulate the air temperatures regularly occurring in Mediterranean olive growing areas (Hellenic National Meteorological Service 2013). The five plants belonging to the second group were grown at ambient conditions as controls.

Ten leaf samples (two for each of five plants) from each treatment were collected prior the heat treatment and at the end of the treatment. The samples consisted of fully expanded leaves, selected along the median segment of new-growth shoots. Gas-exchange measurements, photosynthetic pigments, antioxidant enzyme activities, H_2O_2, and TBARS were measured as described for the UV-B experiments. All the measurements were realised at a fixed PAR value (1,300 μmol m^{-2} s^{-1}), while mean air temperature and RH values were respectively 41ºC and 28% for treated plants and 29ºC and 45% for control plants, according to the daily weather conditions.

Statistical analysis: Data were analyzed using the *Sigmastat 3.1* software (*SPSS Inc.*, Chicago, IL, USA) and were subjected to one-way analysis of variance (*ANOVA*). Significantly different means between control and UV-B- or heat-treated plants were statistically analysed by *Fisher*'s LSD test at $P \leq 0.05$. The number of replicates (n) for each measured parameter was specified in the figure captions.

Results and discussion

Photosynthesis and photosynthetic pigments: In higher plants, net photosynthesis is inhibited by high temperatures (Koubouris *et al.* 2009, Ruelland and Zachowski 2010, Almeselmani *et al.* 2012), and usually subtly affected by UV-B radiation (Xiong and Day 2001). Previous studies suggested that photosynthetic productivity of olive is unlikely to be affected by the predicted UV-B increase (Nogues and Baker 2000). It was also shown that the existing protective morphological and chemical traits contribute to a protective ability of olive against abiotic stresses, such as plant exposure to enhanced UV-B radiation (Liakoura *et al.* 1999). In our study, UV-B radiation negatively affected the P_N of olive plants. Indeed, the UV-B-treated plants were markedly affected at the 12 and 40 DAE (Fig. 2*A*). However, it should be noted that P_N under enhanced UV-B, though decreased, was maintained relatively high compared with reference values reported for olive under normal conditions (Sofo *et al.* 2004, 2005, 2009). Moreover, no significant differences were observed at the 29 DAE (Fig. 2*A*), when temperature was lower (Fig. 1) implying a synergistic effect of UV-B and heat. It was demonstrated that UV-B radiation reduces photosynthetic rates through a stomatal inhibition (Grammatikopoulos *et al.* 1994). This mechanism was not observed in our study, where supplemental UV-B did not provoke any significant effect on either g_s or C_i (Fig. 2*B,C*). These findings support the idea that olive may cope well with UV-B in the absence of additional stressors.

By contrast, the impact of high temperature (38 ± 4ºC) on plants was more pronounced when compared to UV-B, resulting in significant decrease of P_N, g_s, and C_i (Fig. 3). In Mediterranean olive producing areas, high-temperature incidents around > 30ºC occur often and; at certain phenological stages or in multistress coexistence, implications can be serious (Koubouris *et al.* 2009, 2010). In the present study, the P_N of olive plants (Fig. 2*A*) was reduced under enhanced UV-B only at higher temperatures (28 and 29ºC during 2nd and 4th measurements) but not at lower temperatures (23 and 26ºC during 1st and 3rd measurements).

The results showed that enhanced UV-B radiation affected neither total Chl nor Car content (Fig. 4). Total Chl contents during UV-B exposure have been in some cases associated with UV-tolerance (Greenberg *et al.* 1997). However, the contents of total Chl did not correlate with plant sensitivity to abiotic stresses in a variety of vegetable crop plants studied, and it seems that the plant response to UV-B varies among species, varieties, seasons, and environments (Smith *et al.* 2000). Enhanced UV-B caused less damage to Chl in mung bean cultivars with higher content of Car (Choudhary and Agrawal 2014).

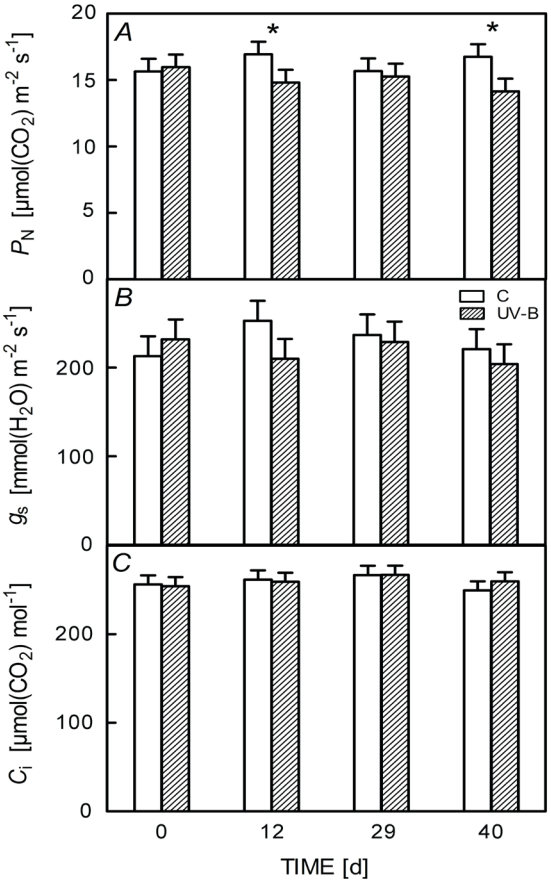

Fig. 2. Influence of supplemental UV-B radiation on (*A*) net photosynthesis (P_N), (*B*) stomatal conductance (g_s), and (*C*) intercellular CO_2 concentration (C_i) in olive leaves. Each bar is the mean ± SE for each treatment. * – significantly different at $P \leq 0.05$ (*Fisher*'s LSD test, n = 10). C – control plants; UV-B – UV-B-treated plants.

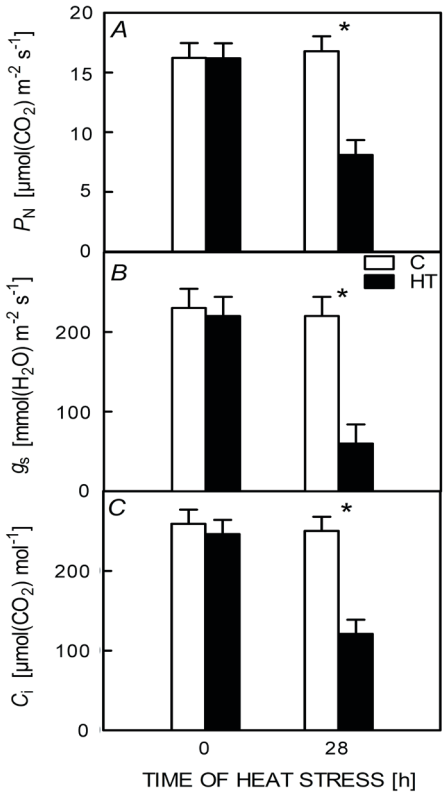

Fig. 3. Influence of temperature on (*A*) net photosynthesis (P_N), (*B*) stomatal conductance (g_s), and (*C*) intercellular CO_2 concentration (C_i) in olive leaves. Measurements were conducted at 28ºC in control plants and at 38 ± 4ºC in heat-treated plants. * – significantly different at $P \leq 0.05$ (*Fisher*'s LSD test, $n = 10$). C – control plants, HT – heat-treated plants.

Similar protective role for the photosynthetic machinery has been also suggested for the accumulation of flavonoids (Choudhary and Agrawal 2014). Apart from the protective role, accumulation of certain compounds in plant tissues exposed to UV-B has been suggested to have health promoting effects, such as flavonols in grape berries (Martínez-Lüscher *et al*. 2014). In our case, the Chl *a*/*b* ratio was altered by enhanced UV-B radiation (Fig. 4*B*) in agreement with Smith *et al*. (2000), who reported that a change of Chl *a*/*b* can be used as a good marker of a given stress. The insignificant differences in Chl/Car (Fig. 4*B*) were caused by the fact that neither total Chl nor Car contents were affected by UV-B stress (Fig. 4*A*). The impact of heat stress on the total Chl content is often genotype-dependent (Koti *et al.* 2007). In our case, high temperature resulted in increased leaf concentrations of both total Chl and Car (Fig. 5*A*). However, neither Chl *a*/*b* nor Chl/Car were affected in heated plants (Fig. 5*B*). The increase of both Chl and Car following short-term high-temperature stress (Fig. 5*A*) does not mean that heat was not a stress for olive plants, but could be attributed to biochemical changes following leaf desiccation through intense transpiration, as evidenced by Almeselmani *et al*. (2012).

H_2O_2, TBARS and enzyme activities: In olive, the activity of SOD has been mainly studied under drought stress and the plant response seems to be genotype-depended (Guerfel *et al.* 2009). SOD activity increased in the cultivars Coratina and Koroneiki (Sofo *et al.* 2004, Doupis *et al.* 2013), while it was reduced in the cultivars Chemlali, Chetoui, and Cobrancosa (Bacelar *et al.* 2007, Guerfel *et al.* 2009). This work first reported SOD response in olive leaves exposed to enhanced UV-B radiation. Interestingly,

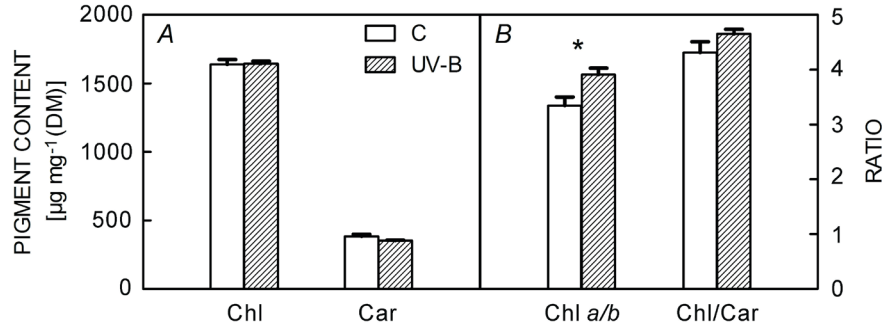

Fig. 4. Influence of supplemental UV-B radiation on (*A*) total chlorophyll (Chl) and total carotenoid (Car) content, and on (*B*) Chl *a*/*b* ratio and Chl/Car ratio in olive leaves. * – significantly different at $P \leq 0.05$ (*Fisher*'s LSD test, $n = 10$). C – control plants, UV-B – UV-B-treated plants.

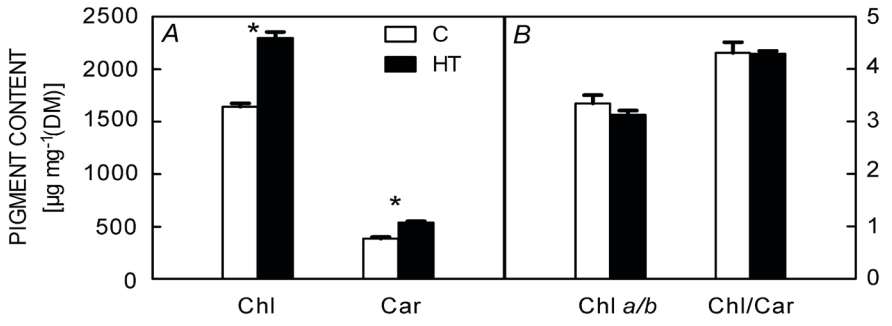

Fig. 5. Influence of high temperature (38 ± 4ºC) on (*A*) total chlorophyll (Chl) and total carotenoid (Car) content, and on (*B*) Chl *a*/*b* ratio and Chl/Car ratio in olive leaves. * – significantly different at $P \leq 0.05$ (*Fisher*'s LSD test, $n = 10$). C – control plants, HT – heat-treated plants.

UV-B excess provoked significant increases in SOD activity (Fig. 6*A*), whereas heat stress had no effects on the activity of this enzyme (Fig. 7*A*). SOD is the primary enzyme responsible for the dismutation of the superoxide anions and hence decreasing the risk of hydroxyl radicals from superoxide anions (Arora *et al*. 2002). Together with other antioxidant enzymes, GPOX is generally considered to play an important role in plant enzymatic defense against oxidative membrane damage (Kühn and Borchert 2002, Sofo *et al*. 2005). The isoforms of GPOX constitute a family of enzymes that are capable of reducing a variety of organic (lipid hydroperoxides) and inorganic hydroperoxides to the corresponding hydroxyl compounds by utilizing deducing equivalents. In this study, both heat stress and enhanced UV-B radiation resulted in increased GPOX activity compared with the control leaves (Figs. 6*B*, 7*B*), indicating the need of plant to defend against oxidative damage in agreement with previous findings in UV-B-treated grape (Doupis *et al.* 2011).

In olive, no relevant data are available for UV-B, however, GPOX activity usually increases in drought-stressed olive plants (Bacelar *et al.* 2007, Doupis *et al.* 2013).

Among the ROS compounds, H_2O_2 is the one that received more attention by the scientific community in the last decade (Petrov and Van Breusegem 2012). It is involved in virtually all major areas of aerobic biochemistry and is produced in copious quantities by several enzymatic systems, even under optimal environmental conditions (Gechev and Hille 2005, Petrov and Van Breusegem 2012). Furthermore, depending on its concentration in plant tissues, H_2O_2 may either act as a signaling molecule or as a harmful component of oxidative stress (Gechev and Hille 2005). In this study, H_2O_2 content was unaffected by both UV-B and heat stresses (Figs. 6*C*, 7*C*). Particularly, we hypothesize that the increased SOD (H_2O_2-producing) activity, observed in UV-B treated olive plants (Fig. 6*A*), together with the increase in GPOX (H_2O_2-scavenging) activity (Fig. 6*B*) determined a steady concentration of H_2O_2, as also observed by Xu *et al*. (2008) in soybean. On the other hand, the situation in heat stressed olive plants was markedly different, as GPOX scavenging action increased (Fig. 7*B*), whereas SOD activity remained stable (Fig. 7*A*). This means that the H_2O_2 production rate under heat was likely higher and/or not only SOD-dependent

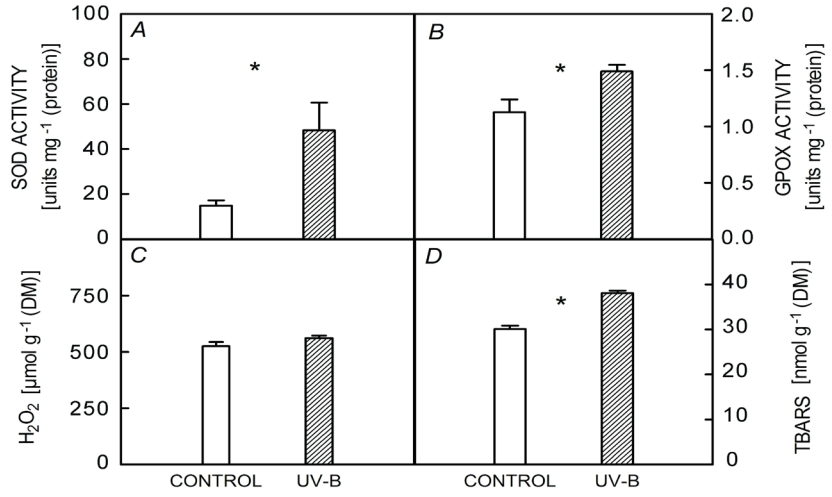

Fig. 6. Influence of supplemental UV-B radiation on (*A*) superoxide dismutase (SOD) activity, (*B*) guaiacol peroxidase (GPOX) activity, (*C*) H_2O_2 content, and (*D*) total thiobarbituric acid reactive substances concentration (TBARS) in olive leaves. Each bar is the mean ± SE for each treatment. * – significantly different at $P \leq 0.05$ (*Fisher*'s LSD test, $n = 10$).

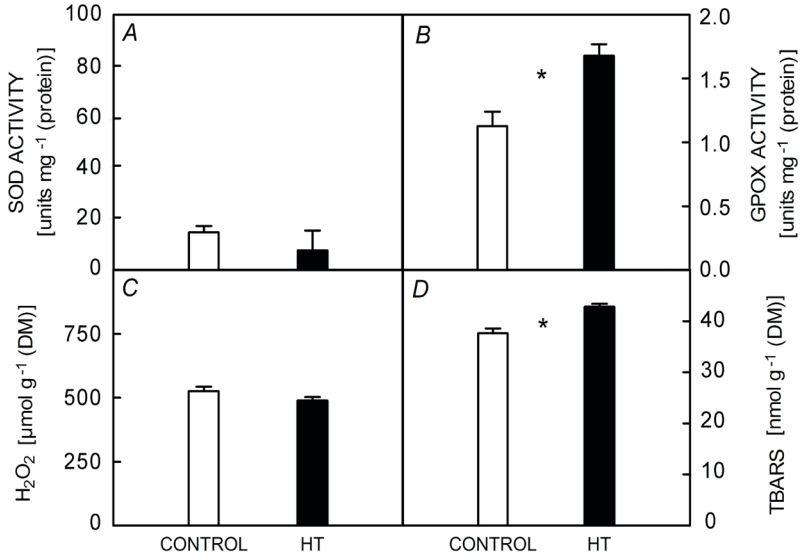

Fig. 7. Influence of high temperature (38 ± 4°C) on (*A*) superoxide dismutase (SOD) activity, (*B*) guaiacol peroxidase (GPOX) activity, (*C*) H_2O_2 content, and (*D*) total thiobarbituric acid reactive substances concentration (TBARS) in olive leaves. Each bar is the mean ± SE for each treatment. * – significantly different at $P \leq 0.05$ (*Fisher*'s LSD test, $n = 10$).

compared to UV-B-treated plants. Reduction of ethylene content has been suggested to play an important role in decreasing the ROS content in UV-B treated plants and alleviating damage (Yang *et al.* 2014).

The TBARS assay, which estimates the amount of MDA, a secondary end product of polyunsaturated fatty acid oxidation, has been extensively used as an index of general lipid peroxidation. MDA has been extensively studied in olive as a stress indicator for drought (Bacelar *et al.* 2007, Doupis *et al.* 2013, Guerfel *et al.* 2009, Sofo *et al.* 2004), however, relevant data on either UV-B or heat stresses are missing. In agreement with previous reports on other species (Doupis *et al.* 2011), enhanced UV-B radiation resulted in increased lipid peroxidation likely due to the oxidative damage (Fig. 6*D*). However, this effect is not universal, as no lipid peroxidation was observed after the exposure to UV-B radiation in some plant species (Giordano *et al.* 2004). It was also suggested that the occurrence of lipid peroxidation in plants depends on UV-B dosage applied in conjunction with PAR level (Giordano *et al.* 2004). Heat stress enhanced also TBARS accumulation (Fig. 7*D*), thus indicating a stress effect on the plant physiological state. Lipid peroxidation has also been reported for other plant species exposed to high temperatures for weeks (Djanaguiraman *et al.* 2010) or even for few hours (Ali *et al.* 2005), but this is the first report for olive plants exposed to these conditions. As in this study, the H_2O_2 content was unaffected by both UV-B and heat stresses (Figs. 6*C*, 7*C*), the increased lipid peroxidation was likely linked to other ROS, as demonstrated in soybean (Xu *et al.* 2008). However, in olive, no other bibliographic data are available for a comparison.

Pollen germination: The magnitude of UV-B effects on plants under experimental conditions has been linked to PAR irradiance and scattering, as well as temperature, which can be affected by cloud cover, surface reflectance, and canopy shading (Paul and Gwynn-Jones 2003). *In vitro* studies exclude other factors that might interfere with the experiment, such as pollen-pistil interaction and various environmental components (Koubouris *et al.* 2009). In this study, reproductive processes were drastically downregulated after the exposure of pollen to supplemental UV-B radiation (Fig. 8). Indeed, enhanced UV-B radiation significantly affected *in vitro* pollen germination at all three dosages applied (Fig. 8*A*). Low and intermediate UV-B treatments (7.5 and 15 kJ m^{-2} d^{-1}) were similarly harmful, while the most extreme UV-B dose (22.5 kJ m^{-2} d^{-1}) resulted in the lowest germination percentage. Pollen tube length was also significantly diminished by supplemental UV-B radiation in a dose-specific manner (Fig. 8*B*).

Reduced pollen performance after enhanced UV-B has also been reported for soybean (Koti *et al.* 2005), where various agronomical, biochemical, or ultrastructural changes in reproductive organs have been linked to UV-B radiation (Koti *et al.* 2005). The reduced pollen

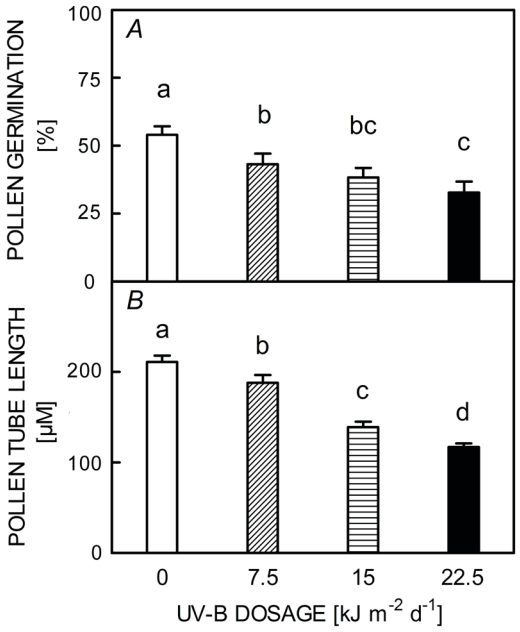

Fig. 8. Influence of supplemental UV-B radiation on (*A*) *in vitro* pollen germination ($n = 500$) and (*B*) tube length ($n = 160$). Each bar is the mean ± SE for each treatment. *Different letters* indicate statistical differences between treatments $P \leq 0.05$ (*Fisher*'s LSD test, $n = 10$).

germination and tube length observed in this study (Fig. 8), the first report on olive pollen performance under enhanced UV-B radiation, could have serious implications on the olive yield, especially, for low fruit-setting cultivars or in years and environments with additional unfavorable conditions. In the short term, adult trees could present reduced sensitivity and greater alleviation potential, for instance by the activation of efficacious antioxidative responses. However, long-term exposure of olive trees to enhanced UV-B radiation could result in cumulative changes affecting reproductive success, as observed in Mediterranean forest species (Paoletti 2005).

Conclusion: The results of this study indicate olive resilience to UV-B radiation expressed as satisfactory photosynthetic activity. However, other biochemical stress indicators were variably affected. Furthermore, reproductive efficiency seems to be more sensitive compared with physiological functions. Such a contrasting response of gas exchange and antioxidant performance suggested the different effects of heat and UV-B on plants; the former is a normally occurring stressor and some plant species, such as olive, can tolerate it; the latter one is likely much more harmful and able to induce a stronger antioxidant response. UV-B- and heat-induced effects support the hypothesis that plant response to a given stressor is affected by the overall environment, and that a holistic approach is needed to determine plant strategies for climate change adaptation.

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