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Interactive effects of UV radiation and reduced precipitation on the seasonal leaf phenolic content/composition and the antioxidant activity of naturally growing *Arbutus unedo* plants

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ABSTRACT

The effects of UV radiation and rainfall reduction on the seasonal leaf phenolic content/composition and antioxidant activity of the Mediterranean shrub *Arbutus unedo* were studied. Naturally growing plants of *A. unedo* were submitted to 97% UV-B reduction (UVA), 95% UV-A + UV-B reduction (UV0) or near-ambient UV levels (UVBA) under two precipitation regimes (natural rainfall or 10–30% rainfall reduction). Total phenol, flavonol and flavanol contents, levels of eight phenols and antioxidant activity [DPPH$^{\bullet}$ radical scavenging and Cu (II) reducing capacity] were measured in sun-exposed leaves at the end of four consecutive seasons. Results showed a significant seasonal variation in the leaf content of phenols of *A. unedo*, with the lowest values found in spring and the highest in autumn and/or winter. Leaf ontogenetic development and/or a possible effect of low temperatures in autumn/winter may account for such findings. Regardless of the watering regime and the sampling date, plant exposure to UV-B radiation decreased the total flavanol content of leaves, while it increased the leaf content in quercitrin (the most abundant quercetin derivative identified). By contrast, UV-A radiation increased the leaf content of theogallin, a gallic acid derivative. Other phenolic compounds (two quercetin derivatives, one of them being avicularin, and one kaempferol derivative, juglanin), as well as the antioxidant activity of the leaves, showed different responses to UV radiation depending on the precipitation regime. Surprisingly, reduced rainfall significantly decreased the total amount of quantified quercetin derivatives as well as the DPPH• scavenging activity in *A. unedo* leaves. To conclude, present findings indicate that leaves of *A. unedo* can be a good source of antioxidants throughout the year, but especially in autumn and winter.

1. Introduction

Climate models predict an increase in the levels of UV radiation reaching Mediterranean ecosystems in the coming decades, due to a decrease in cloud cover, which will also reduce precipitation, especially in summer [1–3]. Enhanced UV radiation, particularly UV-B (the most energetic part of the daylight spectrum), and water deficit can cause stress in naturally growing plants, which can be associated with cellular damage mainly due to the production of reactive oxygen species (ROS) [4]. In response, plants activate various protective mechanisms, including the modification of leaf morphology (e.g. development of smaller and/or thicker leaves) and/or the increase in the leaf content of phenolic compounds [5].

Phenols are multifunctional secondary metabolites ubiquitous in plant tissues [6]. Flavonoids are the largest group of phenols, and mainly those bearing a 3,4-dihydroxy group in B-ring (e.g., luteolin and quercetin derivatives) assist plants to cope with the stress induced by environmental factors, such as high levels of UV-B or drought [6]. This protective function has been associated with their higher antioxidant capacity and effectiveness in regulating morphogenic responses when compared to their monophenolic counterparts (apigenin and kaempferol) [6–9].

In the field, plants are often exposed concomitantly to different stress factors, which do not necessarily aggravate the negative symptoms of stress. On the contrary, owing to commonalities among stress responses, the primary exposure to a stress factor might limit collateral damage caused by others, a phenomenon known as cross-tolerance [10]. In particular, plant exposure to high levels of UV-B and water deficit has resulted in an additive increase of leaf phenolics in some species, whereas it has not affected or it has decreased the levels of these compounds in others [11–17]. In the case of Mediterranean sclerophyllous species, the information on this subject is scarce, with published studies

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reporting no interactive effects between UV-B radiation and water availability, either on the total leaf content of phenols [18–20], or on the leaf content of specific phenolic compounds [21]. However, these studies were carried out with one- or two-year-old seedlings growing in pots.

Since plant stress responses might be influenced by plant age and seasonal climate conditions, we considered essential to provide information on a seasonal basis about the interactive effects of UV radiation and reduced precipitation on the leaf content of phenols, as well as on the leaf antioxidant activity of mature naturally growing Mediterranean plants. To achieve this goal, a common sclerophyllous broad-leaved Mediterranean shrub, *Arbutus unedo*, which typically occurs in holm oak forests, was selected. This species was chosen due to its important ecological role as a source of food for many animals [22,23], its high capacity to survive by re-sprouting after a fire [24] and its economic interest for food and pharmaceutical industries [25]. The study was carried out over a period of one year in a natural Mediterranean shrubland, with samplings being performed at the end of each season. The overall plant community was experimentally exposed to three different UV radiation conditions (~97% UV-B reduction, ~95% UV-A + UV-B reduction and near-ambient UV levels) in combination with two precipitation regimes (natural rainfall or 10–30% rainfall reduction). Present findings are expected to contribute towards the understanding of the role of UV radiation and water availability in the modulation of the leaf phenolic pool in naturally growing *A. unedo* plants. In addition, the information obtained will be useful for the better exploitation of *A. unedo* leaves as a source of antioxidant compounds.

2. Material and Methods

2.1. Study Area, Experimental Design and Plant Material

The field experiment was established in the Mediterranean Gavarres Massif (41° 91′ N, 2° 91′ E) near Cassà de la Selva (Girona, NE of the Iberian Peninsula) in August 2011. The study site was situated at 250 m above sea level in a south-facing shrub community composed mainly by *Arbutus unedo*, *Erica scoparia* and *Phillyrea angustifolia*, with a few individuals of cork oak (*Quercus suber*) and pines (*Pinus pinaster* and *P. pinea*). The soil was mainly siliceous with very good drainage.

Precipitation and temperature were recorded from August 2011 to September 2012 at the meteorological station placed at Cassà de la Selva (177 m above sea level, 41° 87′ N, 2° 93'E), 3 km away from the study site (Table 1). Seasonal fluctuations in these variables during the experimental period were typical of the Mediterranean climate, with the highest temperatures being registered in summer and the lowest in winter (seasonal mean temperatures in °C were: 14.1 ± 0.45, 22.3 ± 0.22, 12.7 ± 0.45, and 5.5 ± 0.31 for spring, summer, autumn and winter, respectively), and with higher values of accumulated rainfall in autumn and spring (482.4 mm and 175.2 mm, respectively) than in winter (33.8 mm) and summer (91.1 mm). Ground-level ultraviolet irradiance was also recorded throughout the study period (Table 1).

The experiment was arranged in a randomized block design with treatments (UV radiation and precipitation regime) being replicated 3 times. Hence, plots were distributed in 3 blocks, each block having 6 plots, i.e. 2 plots of each one of the 3 UV radiation conditions, one for each precipitation level (see treatment's description below). Plots had an area of 9 m^2 (3 m × 3 m) and they were covered by transparent filters suspended on metal frames. The filters also functioned as roofs, having a 10° slope towards the south. At the centre of the plot, the distance from the roof to the soil was about 155 cm, which allowed wind circulation under the filters in the plots (Supplementary material, Fig. S1). At the south-face front of each plot, a 40 cm-wide fringe made from the same material as the roof was installed in order to reduce plant exposure to unfiltered direct solar radiation.

Samplings were performed at the end of four consecutive seasons: autumn 2011, and winter, spring and summer 2012 (sampling dates: 12 December 2011, 8 March 2012, 11 June 2012 and 18 September 2012). In each season, we sampled one foliar disc of 0.64 cm^2 (in some cases 0.79 cm^2) from four leaves from different stems centrally located of one or two *A. unedo* plants per plot. Individuals of *A. unedo* were multi-stemmed and their tallest stem ranged from 30 to 100 cm. The sampled leaves were always fully expanded, south oriented and located at the top of the plant canopy. Samples were taken at midday under clear sky conditions, and they were immediately frozen with liquid nitrogen. Once at the laboratory, samples were stored at −80 °C and freeze-dried before the analyses. Leaves sampled in autumn 2011 and winter 2012 had developed in spring 2011, while leaves sampled in spring and summer 2012 belonged to the cohort appeared in spring 2012.

2.2. UV Treatment

The UV treatment consisted of exposing the naturally growing plant community of the different experimental plots to three different UV conditions: environmental UV radiation (hereafter UVBA plots), UV-B exclusion (hereafter UVA plots) and UV-A and UV-B exclusion (hereafter UV0 plots) (Table 2). Therefore, in each of the 3 experimental blocks

Table 1
Monthly means of ground-level ultraviolet doses weighted with the erythemal irradiance algorithm (UVE), the generalized plant response action spectrum (GEN), and the plant growth weighting function (PG), as well as unweighted UV-B and UV-A irradiances and photosynthetically active photon flux density (PPFD) measured at the radiometric station of the University of Girona. Monthly mean air temperature and accumulated precipitation were obtained from Cassà de la Selva meteorological station, 3 km away from the field experiment. Missing values for August and September 2012 are due to the calibration of the EPG erythemal sensor at the manufacturer facilities.

Month	UVE[a]	GEN[b]	PG[c]	UV-B	UV-A	PPFD	Temperature	Precipitation
	(kJ/m^2 day)	(kJ/m^2 day)	(kJ/m^2 day)	(kJ/m^2 day)	(MJ/m^2 day)	(mol/m^2 day)	(°C)	(mm)
August 2011	3.96	5.36	28.2	30.3	1.04	38.1	22.7	10.6
September 2011	2.84	3.66	22.1	21.9	0.80	30.0	20.2	10.5
October 2011	1.68	1.95	15.1	13.0	0.56	21.9	15.5	171.0
November 2011	0.65	0.60	7.5	4.8	0.33	13.7	11.8	309.8
December 2011	0.59	0.49	7.3	4.2	0.37	15.5	6.5	2.0
January 2012	0.67	0.57	8.2	4.8	0.45	18.8	4.9	24.8
February 2012	1.10	0.99	13.0	8.0	0.63	25.0	4.0	3.5
March 2012	1.80	1.91	17.9	13.8	0.83	31.3	9.4	44.5
April 2012	2.54	3.05	21.7	19.7	0.93	33.9	10.3	65.8
May 2012	3.52	4.56	27.0	27.2	1.26	45.9	15.4	52.9
June 2012	4.50	6.12	31.7	34.4	1.29	46.8	21.0	16.8
July 2012	4.24	5.73	30.2	32.4	1.19	43.5	21.7	9.5
August 2012	–	–	–	–	1.07	39.4	23.9	7.2
September 2012	–	–	–	–	0.71	26.6	19.4	110.1

[a] UVE was calculated according to McKinlay and Diffey [26].
[b] GEN was calculated according to Caldwell [27].
[c] PG was calculated according to Flint and Caldwell [28].