Viticultura

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ORIGINAL PAPER

Solar UV-B radiation modifies the proportion of volatile organic compounds in flowers of field-grown grapevine (*Vitis vinifera* L.) cv. Malbec

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Abstract Ultraviolet-B solar radiation (UV-B) is an environmental signal with biological effects in different plant tissues. Recent investigations reported dramatic changes of terpenes with a protective role in plant tissues submitted to biotic and abiotic stresses. This study examined the volatile organic compounds (VOCs) profile in flowers of *Vitis vinifera* L. cv. Malbec under filtered UV-B (or not). Gas chromatography–electron impact mass spectrometry analysis of flowers resulted in the identification of 12 VOCs, including eight sesquiterpenes, two aldehydes, and two ketones, being the oxygenated sesquiterpene farnesol the most abundant. The total amount of VOCs in flowers did not change irrespective UV-B had been filtered or not, suggesting those compounds have a protective role that is constitutive of the reproductive tissues. However UV-B increases the proportion of valencene, β-farnesene, α-panasinsene and hepatriacontanedione which would require further investigation.

Keywords Abiotic stress · Grape · Ultraviolet-B · Terpenes · VOCs

Introduction

Though ultraviolet-B radiation (UV-B; wavelength 280–315 nm) represents only a small portion of the total solar spectrum reaching the Earth's surface, it has large biological effects since it can activate the plant defense system leading to accumulation of secondary metabolites in different plant tissues (Teramura 2006), including grapevine leaves (Berli et al. 2010; Gil et al. 2012) and berries (Berli et al. 2011; Gil et al. 2013). Environmental UV-B levels are mainly regulated by season, latitude, altitude, time of day and cloudiness (McKenzie et al. 2003).

Plants synthesize and emit a large variety of volatile organic compounds (VOCs) with terpenoids and fatty-acid derivatives as the dominant classes (Pichersky and Gershenzon 2002). In grapevine, the evolution of these aromatic metabolites at the level of reproductive tissues has been explored mainly in grape berries of "floral" varieties like Muscat (Park et al. 1991; Coelho et al. 2006; Palomo et al. 2007). This is a biologically important aspect since VOCs can attract pollinators and may also protect reproductive tissues against pathogens (Pichersky and Gershenzon 2002; Lücker et al. 2004; Kegger and Pierik 2009). Different VOCs (especially monoterpenes and sesquiterpenes) with antimicrobial activity have been characterized in in vitro cultured grapevine (Escoriaza et al. 2013). VOCs production in flowers of Arabidopsis may contribute to control bacterial infection and/or fungal infestations (Tholl et al. 2005). VOCs may also have a plant protective function towards abiotic stresses due to

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their antioxidant activity that sequestrate free radicals so reducing oxidative damage (Aharoni et al. 2003; Wei and Shibamoto 2007) and inducing integrity and stability of membranes (Beckett et al. 2012). Therefore, one suspects that VOCs may reduce oxidative damage caused by UV-B in high altitude vineyard. If that occurs, VOCs will show considerably superior concentration in reproductive organs of grapes submitted to high UV-B.

In grapevine the pollen grains are site of terpene synthesis, which has been related with an increase in the activity of valencene synthase, enzyme responsible to produce sesquiterpenes in flowers of the cv. Cabernet Sauvignon (Martin et al. 2009). In the present work it was hypothesized that grapevine flowers increase the synthesis and/or accumulation of VOCs in response to UV-B in high altitude vineyards. The profile of VOCs in flowers of *Vitis vinifera* L. cv. Malbec field-grown plants from a high altitude vineyard exposed or not to relatively high ambient UV-B levels were monitored by gas chromatography–electron impact mass spectrometry (GC–EIMS).

Materials and methods

Plants of *V. vinifera* L. cv. Malbec from a high altitude vineyard at Gualtallary, Mendoza, Argentina (1450 m a.s.l., 69°15′W and 33°23′S) were exposed to two UV-B radiation regimens by covering the grapevines with specific plastic sheeting, from 15 days before flowering until sample collection. The experimental design and treatments were as previously reported in Berli et al. (2011). Solar UV-B radiation was cut-out to produce the minus UV-B treatment (−UV-B) using a clear polyester (100 μm), which absorbs more than 95 % of UV-B, and transmitted most of the solar radiation. Low density polyethylene (40 μm) transmitting most of the radiation from sunlight was used for the full UV-B treatment (+UV-B) and to minimize environmental differences between −UV-B and +UV-B treatments (no difference were found in air temperature). The plants in the +UV-B treatment were exposed to UV-B irradiances that reached up to 0.28 W m^{-2} during flowering (November). UV-B filtered and non-filtered plastics were positioned 2.5 m above ground level, covering the entire grapevine canopy. One inflorescence per experimental unit was sampled at flowering, stage 23 (Coombe 1995), collected at midday, immediately frozen with liquid nitrogen and kept at −80 °C until further analysis (n = 5).

Volatile organic compounds were determined according to Martin et al. (2009) with modifications. Ten flowers were randomly separated from inflorescences, weighed and immediately immersed in 0.5 mL of pentane in 1.5 mL glass vials with Teflon-coated screw caps, vortex-mixed vigorously and kept for 20 h at 4 °C in darkness. Then, aliquots of 1 μL of the pentane fractions were injected in split–splitless mode into a Perkin-Elmer Elite-5MS, cross-linked methyl silicone capillary column (30 m length, 0.25 mm inner diameter, and 0.25 μm film thickness) fitted in a GC–EIMS (Clarus 500, PerkinElmer, Shelton, CT, USA). The GC program was: initial temperature at 45 °C for 1 min, followed by an increase of 2 °C min^{-1}–130 °C, then from 130–250 °C at a rate of 20 °C min^{-1} and held for 10 min at 250 °C. The ionization potential was 70 eV and a range of 40–500 atomic mass units was scanned. The identities of compounds were confirmed by comparison of their retention times and full scan mass spectra with those of authentic standards, and with mass spectra of the National Institute of Standards and Technology (NIST) library. Peak areas were referred to *n*-hexadecane standard for quantification of compounds.

The statistical evaluation was performed using the software Statgraphics Centurion XVI version 15.0.10 (Statpoint Technologies Inc., Warrenton, VA, USA). One-way ANOVA was used to test the variation in concentration of VOCs from flowers exposed to two UV-B radiation regimens. Significant differences were considered at probability of $P \leq 0.1$. Results are reported as a mean of five independent replicated assays with their standard error (SE).

Results and discussion

The chemical structures of the volatile compounds characterized by GC–EIMS in flowers are presented in Fig. 1 (for full mass spectra see Figures 1S–3S of Supplementary Material). Flowers emitted a mixture of compounds belonging to four chemical classes: sesquiterpenes, oxygenated sesquiterpenes, aliphatic aldehydes and ketones. The GC–EIMS analysis resulted in the identification of 12 compounds: the hydrocarbons sesquiterpenes ($C_{15}H_{24}$) α-β-farnesene, α-β-caryophyllene, valencene, α-panasinsene, and the oxygenated sesquiterpenes ($C_{15}H_{26}O$) nerolidol and farnesol; the aldehydes pentadecanal and 7, 10, 13-hexadecatrienal; and the ketones pentadecanone and 6, 28-heptatriacontadien-2-one (Table 1).

Irrespective of the UV-B treatments, the sesquiterpenes were the most abundant with an average of 81 % of the total while ketones represented 14 % and aldehydes 5 %. The total amounts of VOCs were not noticeably affected by UV-B. Among, the sesquiterpene, the perception of UV-B reduced the proportion of the oxygenated farnesol, the most abundant sesquiterpene, wherein −UV-B was 94.5 % and +UV-B 72.6 %, respectively. UV-B increased specifically, β-farnesene (representing 17.3 % of the total sesquiterpene in +UV-B and 2.36 % in −UV-B), valencene (4.4 vs. 0.8 %) and α-panasinsene (3.9 vs. 0.9 %); the proportion of the other compounds identified (except for 6, 28-heptatriacontadien-2-one) did not change significantly in this treatment. That is,

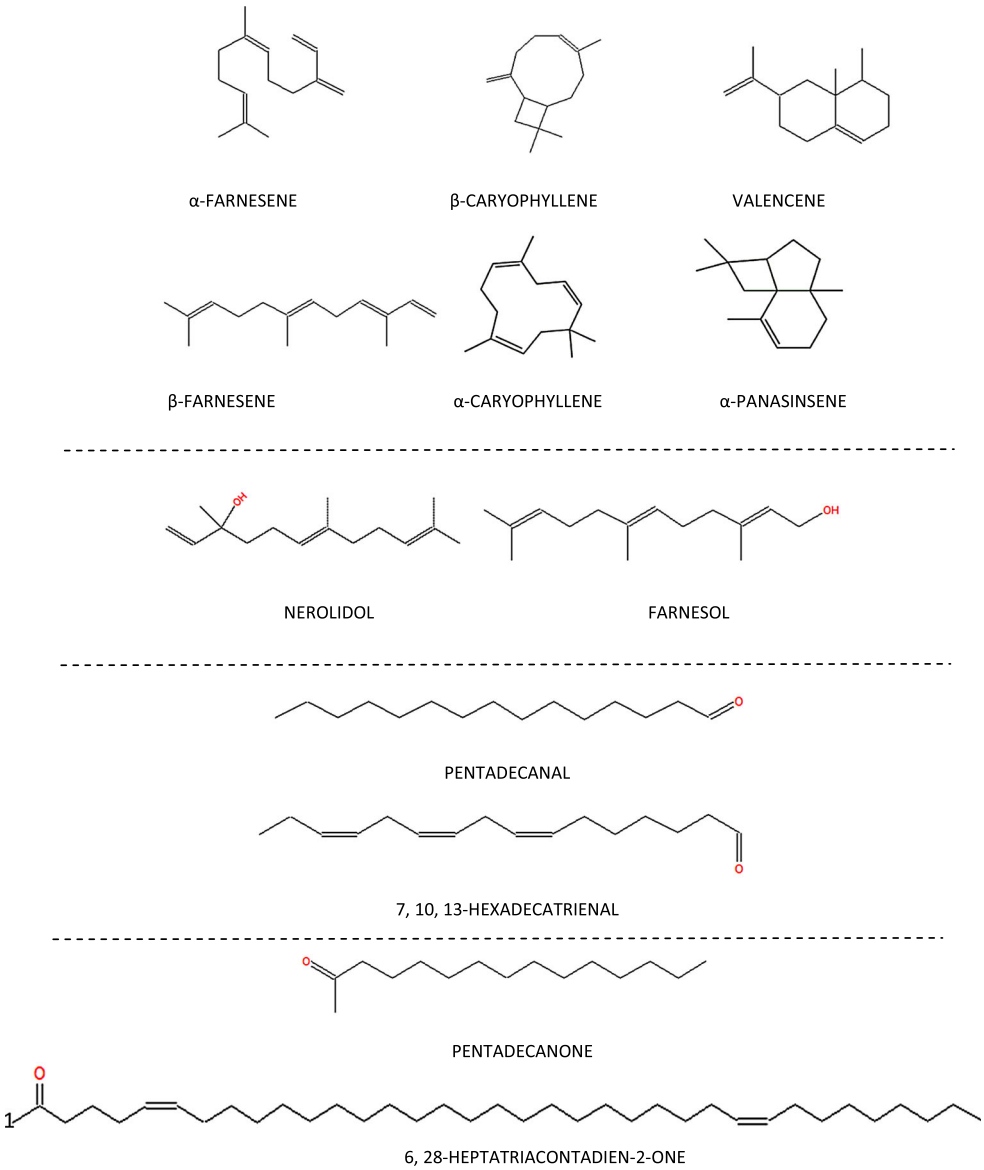

Fig. 1 Chemical structures of sesquiterpenes, oxygenated sesquiterpenes, aldehydes and ketones identified by GC–EIMS in grapevine flowers

+UV-B (as compared to −UV-B) increased β-farnesene (7.7-fold, $P_{(UV\text{-}B)} = 0.0557$), valencene (5.8-fold, $P_{(UV\text{-}B)} = 0.1236$), α-panasinsene (4.4-fold, $P_{(UV\text{-}B)} = 0.0872$), and 6, 28-heptatriacontadien-2-one (2.7-fold, $P_{(UV\text{-}B)} = 0.0356$).

Twelve VOCs were identified and quantified in grapevine flowers cv. Malbec in which sesquiterpenes predominated. These terpenoids have been previously reported in flowers and berries of *V. vinifera* L. (Lücker et al. 2004; Martin et al. 2009); they are synthesized by plants mainly as a defense mechanism against fungi (Moreira et al. 2003; Escoriaza et al. 2013) and as insect pollinator attractants (Stranden et al. 2003). Previously, Gil et al. (2013) analyzed VOCs at different developmental stages in grape berries (at veraison, pre-harvest and harvest) and compounds differ from those identified in grape flowers. Four monoterpenes (limonene, pinene, geraniol and eucalyptol), four aldehydes (hexanal, 2-heptenal, 2-pentenal and octanal), two alcohols (3-hexen-1-ol and 3-methyl pentanol) and one ketone (5-hepten-2-one) were detected. In the present study, and contrary to the hypothesis, UV-B did not stimulate the overall contents of VOCs in grapevine flowers. However, a differential synthesis of VOCs was detected; where the proportion of farnesol decreases while β-farnesene, valencene α-panasinsene, and 6, 28-heptatriacontadien-2-one increased. Anthers have been previously identified as the floral organs with the highest level of sesquiterpene volatiles in flowers of the cv. Cabernet Sauvignon (Martin et al. 2009). After flower opening, pollen is still well protected in the anther sacs and their walls attenuate UV-B radiation by at least 98 % (Flint and Caldwell 1983), and even the pollen grain wall is effective in attenuating

Table 1 VOCs assessed by GC–EIMS (ng mg^{-1} flower FW) in grapevine flowers in field experiments (+UV-B and −UV-B). $P_{(UV-B)}$: UV-B effect

	−UV-B	+UV-B	$P_{(UV-B)}$
Sesquiterpenes			
α-Farnesene	1.82 ± 2.06	3.30 ± 3.40	0.4283
β-Caryophyllene	1.19 ± 0.56	0.92 ± 0.35	0.3920
Valencene	2.03 ± 1.53	11.85 ± 8.6	0.1236
β-Farnesene	6.07 ± 6.88	46.94 ± 25.57	0.0557
α-Caryophyllene	0.31 ± 0.18	0.30 ± 0.22	0.8910
α-Panasinsene	2.41 ± 1.52	10.5 ± 7.66	0.0872
Nerolidol	0.37 ± 0.12	0.49 ± 0.61	0.7067
Farnesol	243.37 ± 55.03	197.28 ± 71.01	0.2844
Aldehydes			
Pentadecanal	0.61 ± 0.20	0.54 ± 0.06	0.4797
Hexadecatrienal	17.25 ± 9.10	17.85 ± 3.61	0.8951
Ketones			
Pentadecanone	46.63 ± 15.86	43.71 ± 16.08	0.7803
Heptatriacontadienone	0.275 ± 0.09	0.755 ± 0.34	0.0356

Values are mean ± SE

UV-B radiation (Uber 1939). Also, it has been reported a low responsiveness of grapevine flowers to UV-C, the most energetic type of UV radiation (Petit et al. 2009). Contrary to these reports, and in agreement with the study of Martin et al. (2009) in which sesquiterpene emission is light-dependent, we found differential responses by UV-B in some specific VOCs in flowers of grapevine cv. Malbec. Probably, the signal of UV-B is perceived directly by the flower and/or transmitted by other tissues. Gil et al. (2013) also found that in grapevine berries, monoterpenes associated with defense increased in response to +UV-B. The increase of β-farnesene, α-panasinsene and valencene may reduce oxidative damage in high altitude vineyards by UV-B and so requires further investigations. Especially because the augment of terpene synthesis in response to stressful environmental conditions seems to be crucial for the plant′s tissues resistance to adverse environmental conditions (Berli et al. 2010; Beckett et al. 2012), defense towards pests (Moreira et al. 2003; Escoriaza et al. 2013), and/or as pollinator attractants (Pichersky and Gershenzon 2002).

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