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Research Note

Is grape composition affected by current levels of UV-B radiation?

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Introduction: The depletion of the stratospheric ozone layer in central Europe is increasing the level of ultraviolet-B radiation (UV-B: 280-320 nm) reaching the earth's surface at the rate of about 8 % per decade (Blumthaler and Ambach 1990). The impact of UV-B on the morphological and physiological features of plants has been extensively studied and decreases of leaf expansion (Tevini and Teramura 1989), fresh and dry weight, total biomass and photosynthetic capacity have been noted (see review by Krupa and Jäger 1996). While the response of plants with respect to the above cited alterations has not been unanimous depending on species or cultivar sensitivity or the experimental system used (Krupa and Jäger 1996), increases in UV-absorbing compounds seem to be a more general reaction to increased UV-B radiation (Tevini 1996, Jansen *et al.* 1998). Some key-enzymes involved in flavonoid biosynthesis (chalcone synthase) and the phenylpropanoid pathway (phenylalanine ammonialyase) have been shown to be upregulated by UV-radiation, as are levels of key antioxidants glutathione and ascorbate (Jansen *et al.* 1998), whereas carotenoid pigment formation and the incorporation of nitrogen into amino acids can be inhibited (Döhler *et al.* 1995, Jansen *et al.* 1998). Since components such as flavonoids, amino acids and carotenoids are important constituents of grapes with a marked effect on flavour development, some influence of UV-B radiation on grape composition can be expected. Therefore, the objective of this study was to investigate the effect of UV-B radiation on: (i) amino acid formation and composition and (ii) carotenoid pigments in grape berries under field conditions.

Materials and methods: Growth conditions: Experiments were conducted in a 19-year-old vineyard of Riesling grapevines (*Vitis vinifera* L.) clone 198 on 5 C rootstock at the State Research Institute at Geisenheim, Germany (50° N, 8° E) in 1996 and 1997. The vines were pruned to 8 buds m^{-2}, shoots were positioned vertically, row orientation was East-West and row distance 2 m.

UV-treatment: Three weeks after bloom, UV-B absorbing polyester film (1.1 m width, 0.1 mm thickness, Schleußner KG, Dreieich, Germany) was installed on the south facing side of the canopy at an angle of 35 ° with respect to the foliage to cover the fruit zone (lower 75 cm of the canopy) in which approximately 20 % of the leaves had been removed (a common practice in many vineyards). The rest of the canopy remained unshielded. In 1996, 20 vines were covered by the film in 4 replicates of 4.8m length to allow free air circulation. In 1997 several hundred m^2 were used. The spectral characteristics of the film (bandwidth 300-1100 nm) were measured with a portable spectro-photometer (Li-Cor 1800, Lincoln, Nebrasca, USA); there was only a small fraction of UV-B light transmitted in the waveband near 320 nm. Light attenuation in the photosynthetic active region of the spectrum (400-700 nm) was 3-4 %. Maximum and minimum air temperatures under the cover differed by less than 1 °C from ambient.

Amino acid determination: Replicated samples (n = 4, 1996) of 200 berries were pressed, the juice was filtered and frozen at -20 °C until analysis. Thawed juice samples were extracted with sulfo-salicylic acid (4 %) (internal standard L-Norleucine, 0.3523mM), and NaOH added (2M). Dansyl chloride solution (1.5 mg ml^{-1} acetonitril) was added and heated for 1 h at 37 °C. Analyses were performed with HPLC on a Lichrosphere 100 RP-18 (5 µm, 250 x 3 mm) column.

Carotenoid pigment analysis: Sun-exposed berries were sampled at midday for skin pigment analysis by reverse-phase HPLC using gradient elution (Váradi *et al.* 1992). Five replicates of 4 berries each were sampled, the berry skin was peeled off, disks of 1cm diameter were cut, immediately fixed in liquid nitrogen (N_2) and stored at -20 °C until analysis. Samples were ground in liquid N_2 and extracted under dim light at 0 °C with 1 ml of 85:15 acetone-water, centrifuged at 10,000 rpm and re-extracted twice with 1ml acetone. Collected extracts were evaporated under a N_2 gas stream and then diluted to 1 ml with acetone. Sample solutions were directly injected into a Nucleosil-120 C18 (5 µm, 250 x 4.6 mm) column. For peak identification and quantification of main carotenoid pigments either pure carotenoid standards were used (e.g. zeaxanthin from Roche, Switzerland) or prepared according to Váradi *et al.* (1992).

Results and Discussion: The total amino acid (AA) concentration in Riesling must at harvest (3.11. 96, 23.10.97) was significantly lower at ambient UV-B levels as compared to the low UV-B treatment (approx. 10 % of ambient UV-B level) (Table). Additionally, AA composition was altered under reduced UV-B, exhibiting higher levels of arginine and glutamine, the main sources of AA for yeast metabolism. These results confirm those obtained on algae by Döhler *et al.* (1995). They speculated that a reduction in AA biosynthesis may be due to a reduction of the supply with carbon skeletons via damage of the Calvin cycle, yet in the present case most of the photosynthesizing leaf surface area of both treatments was exposed to UV-B, and thus should have been equally affected. A more likely explanation would be a direct damaging effect on key enzymes of the nitrogen metabolism, but both inhibiting and stimulating effects have been reported (Döhler *et al.* 1995). The AA concentration was also markedly affected by the climatic conditions, 1996 being a cool year with sufficient moisture, and 1997 being very dry and warm with a marked water deficit towards the end of the season. These factors influence AA concentra-

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Table

Effect of current level UV-B radiation on the density of the total carotenoid pool and zeaxanthin levels in berry ($ng \cdot cm^{-2}$, ± SD, n=5) skins about 2 weeks after veraison and one week before harvest, and on the concentration of various amino acids in White Riesling must ($mg \cdot l^{-1}$, ± SD) at harvest. Must samples in 1997 were not replicated

		1996			1997		
		- UV-B	current level UV-B	change in %	- UV-B	current level UV-B	change in %
Berry skins	*Tot. carotenoids*						
	beginning of Sept.	1324 ± 39*	1403 ± 33	+6	601 ± 88*	831 ±102	+38
		↓ *-50 %*	↓ *-54 %*		↓ *-38 %*	↓ *-71 %*	
	end of October	656 ± 27*	640 ± 39	-2	375 ± 55**	245 ± 37	-35
	Zeaxanthin						
	beginning of Sept.	371 ± 13**	531 ± 4	+30	158 ± 20**	246 ± 22	+36
		↓ *-41 %*	↓ *-48 %*		↓ *+13 %*	↓ *-59 %*	
	end of October	218 ± 42 *ns*	279 ± 38	+22	179 ± 21*	102 ± 19	-35
Must	Tot. amino acids	663 ± 2**	420 ± 62	-37	111	76	-32
	Arginine	161 ± 3*	122 ± 2	-24	24	7	-71
	Glutamine	141 ± 2*	130 ± 3	-8	22	15	-22
	Proline	58 ± 2*	44 ± 5	-24	33	27	-18

** $P<0.01$, * $P<0.05$, and *ns*, not significant

tion in a similar manner as shown in the Table (KLIEWER and ROUBELAKIS-ANGELAKIS 1992).

The total carotenoid pigment content in the berry skins at harvest was reduced by exposure to ambient level UV-B radiation, especially in 1997. Since carotenoid levels at the onset of ripening were higher in UV-B exposed fruit, the results indicate a more rapid degradation induced by UV-B (Table). This reduction over time was also observed for zeaxanthin. Zeaxanthin formation from violaxanthin (which was not different between treatments and sampling times in the current study) plays a key role in photoprotection to excess light and UV-B radiation (ESKLING *et al.* 1997). The data indicate both a stimulation of zeaxanthin formation through UV-B earlier during ripening and a more rapid decrease resulting in no or only small differences between the treatments at harvest.

Both, the effects on amino acids and carotenoids have been reported for other plants (JANSEN *et al.* 1998) but never for fruits. However it seems to be important to study specifically the interactions between UV-B and fruit composition, since many UV-B responsive components of secondary metabolic pathways, such as carotenoids, are constituents and/or precursors of important fruit and wine flavours or ingredients (MARAIS *et al.* 1991). Carotenoid derivatives, such as the norisoprenoid compounds, have been linked to the aging flavour of wines (vitispirane, 1,1,6-trimethyl-1,2-dihydronaphthalene, TDN) and the fruity character of must and wine (damascenone) and can be influenced by climatic factors (MARAIS *et al.* 1991). Equally possible is a link between low AA concentrations in grapes and must and disturbed fermentation processes and off-flavour formation (RAPP *et al.* 1993). In the latter case, possible precursors of the off-flavour component 2-aminoaceto-phenone, such as tryptophan and indoleacetic acid (IAA), are sensitive to UV-B (JANSEN *et al.* 1998). If confirmed in future studies, the consequences of the present results in the light of possible changes in the global climate, may necessitate altered viticultural and/or enological practices.

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