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Research Note

Effect of nitrogen supply on *trans*-resveratrol concentration in berries of *Vitis vinifera* L. cv. Cabernet Sauvignon

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K e y w o r d s : nitrogen, berry, resveratrol.

Introduction: Resveratrol belongs to the family of stilbenes, which are low molecular weight phenolics acting like phytoalexins; they are synthesized by plants in response to a number of biotic and abiotic elicitors.

Resveratrol is related to plant resistance against pathogens such as *Botrytis cinerea* and *Plasmopora viticola* and is synthesized in leaves and berries under stress conditions such as UV irradiation, aluminum chloride, ozone and other chemicals (Bavaresco and Fregoni 2001).

During alcoholic fermentation resveratrol is extracted from the berry skins into the wine and this process is affected by oenological practices (Jeandet *et al*. 1995; Mattivi *et al*. 1995).

Resveratrol is thought to be the active compound in red wines shown to reduce heart diseases (Renaud and De Lorgeril 1992) and to have chemopreventive activity with regard to cancer (Jang *et al*. 1977).

There is some evidence that the supply of fertilizer, such as nitrogen and potassium, affects resveratrol synthesis of elicited leaves (Bavaresco and Eibach 1987; Bavaresco *et al*. 1994), but no data are available for berries. Therefore the objective of this investigation was to study the effect of increasing fertilizer supply on berry resveratrol concentration, and other vegetative and qualitative parameters.

Material and Methods: S o i l a n d p l a n t m a t e r i a l : 4-year-old Cabernet Sauvignon, clone R5, vines grafted on *V. Berlandieri* Planch. x *V. riparia* Michx. 420 A rootstock, were grown in plastic pots one plant per pot (45 l) containing a mixture of soil, sand and peat (1:1:1). Before adding basic nutrients, the main soil characteristics were: pH 7.9; tot. N 0.9 g·kg^{-1}; P (Olsen) 4.4 mg·kg^{-1}; K (exch.) 163 mg·kg^{-1}; Mg (exch.) 235 mg·kg^{-1}. The vines were Guyot-trained with 8 shoots each and shoot positioning. The soil water content was maintained near field capacity by drip irrigation. Each treatment included 12 plants and the pots were placed outside on a platform under a hail-protection net. Nitrogen treatments were 0 g N·pot^{-1} (untreated vines), 4 g N·pot^{-1}, 8 g N·pot^{-1}, 16 g N·pot^{-1}. N was added as ammonium nitrate and applied at a shoot length of about 20 cm and 15 d later. P, K, Mg were added as follows: 1.7, 10, 1.2 g·pot^{-1}, respectively; moreover, the following trace-elements were added (mg·pot^{-1}): B 0.2; Mn 1.0; Zn 0.3; Cu 0.2; Co 0.2; Fe 0.3 (as Fe chelate).

T e s t s : Shoot length of one shoot per plant was measured every 10 d till the end of the experiment. At fruit set leaves opposite to the basal cluster were sampled and after wet digestion of the oven-dried material the N concentration was analyzed by the colorimetric method. During ripening (about 155 d after bud burst) two representative clusters per plant were sampled and the following parameters were analyzed: average cluster weight (g); average berry weight (g); total soluble solids (° Brix), pH, titratable acidity of must (g·l^{-1}), arginine (by the colorimetric method of Gilboe and Williams (1956), expressed as mg·l^{-1}) and *trans*-resveratrol of the must.

E x t r a c t i o n a n d i d e n t i f i c a t i o n o f *t r a n s*-r e s v e r a t r o l : After seed removal, fresh berries (about 25 g) were ground in a mortar with 30 ml methanol (95 %) and vigorously shaken for 20 min. A filtration by GF/A Whatman filters followed, the liquid was evaporated *in vacuo* at 40 °C and the water fraction was extracted twice with 5 ml ethylacetate and 5 ml $NaHCO_3$ (5 %) by phase partitioning. The organic phase was evaporated to dryness and resveratrol was recovered by 3 x 1 ml methanol (100 %). Resveratrol analysis was performed using *trans*-resveratrol (Sigma-Aldrich) as standard. A liquid chromatograph (Hewlett Packard 1090 L, Waldbronn, Germany) with an autosampler (50 µl injection volume) and DAD (diode array detector) set at 306 nm, was utilized. A C 18 Supelco column (Supelcosil ABZ plus 250 x 4.6 mm, 5 µm particle size) was used, eluting with a gradient of methanol (A) and 0.01 M potassium phosphate monobasic adjusted to pH 2.5 with phosphoric acid (B). The gradient was from 40 to 85 % of A at a flow rate of 0.7 ml·min^{-1}. Limit of detection was 0.1 mg·l^{-1}. Every value is the mean of three replicates.

At the end of the growing season, after leaf abscission, the pruning wood per plant was weighed.

Results and Discussion: In order to better understand the interaction between nitrogen supply and resveratrol concentration in berries some other parameters were also surveyed. As expected vegeative parameters such as shoot growth and pruning weight as well as N concentration in berries and arginine in must increased with increasing N supply. According to the reference values for foliar analysis only the two highest N rates were in a normal nutritional range, while with regard to arginine, only the highest N rate (16 g·pot^{-1}) provided an adequate nitrogen nutrition (Champagnol 1984); no nitrogen deficiency symptoms were anyway detected in the tested plants (Table). Cluster size was affected by the N supply in a positive way, while berry weight was not, meaning that nitrogen was effective in promoting fruit set, except for the highest N dose. The qualitative parameters of must were affected significantly way by the N supply, with an expected negative effect of high N supply on total soluble solids. Increasing N rates decreased *trans*-resveratrol concentration in berries confirming the

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Table

Effect of nitrogen supply on vegetative parameters and must quality checked at harvest

	N supply ($g \cdot pot^{-1}$)			
	0	4	8	16
Final shoot length (cm)	80.0 a	85.0 a	125.0 b	120.0 b
Pruning weight ($g \cdot plant^{-1}$)	158.0 a	211.0 a	344.0 b	436.0 c
Leaf N (%) [1)]	1.7 a	1.9 b	2.1 b	2.4 c
Cluster weight (g)	123.0 a	129.0 a	165.0 b	133.0 a
Berry weight (g)	1.1 a	1.0 a	1.0 a	1.0 a
Total soluble solids (°Brix)	20.2 a	20.4 a	19.6 a	17.1 b
pH	3.5 ab	3.6 ab	3.5 a	3.7 b
Titratable acidity ($g \cdot l^{-1}$)	4.4 a	4.2 a	4.6 a	4.8 a
Arginine ($mg \cdot l^{-1}$)	18.6 a	77.7 b	90.7 b	199.1 c
Trans-resveratrol ($mg \cdot kg^{-1}$)	0.4 a	0.2 a	n.d.*	n.d.*

* Not detectable (detection limit: 0.01 $mg \cdot kg^{-1}$ fresh berries)
[1)] Analysed at fruit set
Means followed by the same letter are not different at Tukey test ($p \leq 0.05$).

negative effect of high N supply already stated for leaves (BAVARESCO and EIBACH 1987; BAVARESCO *et al*. 1994). According to GRAHAM (1983), at low nitrogen supply the balance between primary and secondary pathways is probably shifted to the shikimate pathway, providing a large pool of phenolics and alkaloids; this is the basis for physical and chemical defence mechanisms of plants as well as for phytoalexins. The recorded values are much lower than those in literature for Muscadinia berries (ECTOR *et al*. 1996), but are in the same range as those of Cabernet Sauvignon berries grown in Japan (OKUDA and YOKOTSUKA 1996). We also expected to find different levels of resveratrol in red wines originating from differently fertilized grapes. The nitrogen supply in vineyards has to be carefully managed, in order to produce, on the one hand, grapes high in resveratrol, and, on the other hand, must with a sufficient nitrogen level suitable for proper yeast metabolism. According to the data of this investigation berries with a proper arginine level (highest N rate) showed no detectable resveratrol. High nitrogen rates in vineyard soils should be avoided not only because of the known effects on vegetative growth and fungus disease, but also because res-veratrol levels in must are reduced. Possibly, a better timing of N application during the vegetative growth might help to solve the antagonistic effects of increasing N supply on yield and resveratrol: High N supply at an early stage of development (*e.g.* as foliar application) and a lower N supply during fruit growth may guarantee both, sufficient yield and high resveratrol concentration. Field trials in commercial vineyards are in progress to confirm the present findings and to investigate the effect of nitrogen on resveratrol glucoside in berries.

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