Effects of nitrogen supply on must quality and anthocyanin accumulation in berries of cv. Merlot

G. HILBERT, J. P. SOYER, C. MOLOT, J. GIRAUDON, S. MILIN and J. P. GAUDILLERE

INRA, Station d’Agronomie, Equipe Ecophysiologie et Agronomie Viticole, Villenave d’Ornon, France

Summary

Nitrogen supply to Merlot vines (*Vitis vinifera* L.), grown under controlled conditions, affected must quality and the anthocyanin content in berry skins irrespective of vegetative growth. High N supply delayed fruit maturation; berries had a higher arginine and a lower anthocyanin content with relatively more abundant acylated anthocyanins compared to berries of vines supplied with low N. During maturation the anthocyanin content in the skin of berries decreased; this was more significant in high-N vines. It is concluded that high nitrogen supply affects the metabolic pathway of anthocyanins in different ways, e.g. it delays the quantitative and qualitative biosynthesis and enhances their degradation during the final steps of berry maturation.

Key words: nitrogen supply, grape maturity, anthocyanins, amino acids.

Introduction

Red berries of grapevine (*V. vinifera*) contain 3-mono-glucoside (3-gl), 3-acetylglucoside (3-gl-ac), 3-p-coumarylglucoside (3-gl-cou) and 3-cafeylglucoside (3-gl-caf) derivatives of the aglycones delphinidin (Dp), cyanidin (Cy), peonidin (Pn), petunidin (Pt) and malvidin (Mv). Malvidin derivatives are predominant at maturity (WULF and NAGEL 1978). Nevertheless, the 3-mono-glucoside derivatives are the only anthocyanins found in Pinot noir. Anthocyanins acylated by acetic, caffeic and coumaric acids are found in Cabernet Sauvignon, Syrah (BOSS et al. 1996), Cabernet franc and Merlot (MAZZA et al. 1999). Muscat and red Chardonnay contain relatively small amounts of malvidin derivatives (BOSS et al. 1996). The anthocyanin content is also influenced by environmental factors and viticultural management (e.g. HUNTER et al. 1995; CARMO-VASCONCELOS and CASTAGNOLI 2000).

Merlot, the major early red variety in the Bordeaux area, has a high anthocyanin content which is important for winemaking. The purpose of our study was to investigate the effects of nitrogen supply on anthocyanin content and on the composition of Merlot berries from veraison to maturity.

Material and Methods

Plant material: Two-year-old grapevines (*Vitis vinifera* L. cv. Merlot) grafted on Gravesac clone 343 were used. In 1999, vines were planted in 45 l plastic pots containing vermiculite, perlite and sand (1:1:1, v:v:v) and kept in a glasshouse for one season. At the end of the first growing season, 45 vines were selected for uniformity of shoot growth and fertility. Vines were trained in Guyot, two-year-old shoots bearing 6 buds; they were spaced 1m x 1m and had developed 4 canes on average. Vertically trained shoots were pruned above the 15th leaf. Lateral shoots were removed regularly. Leaf area was determined as described by CASTELAN-ETRADA (2001). Ten vines per treatment were randomly distributed in the glasshouse.

Water and nutrients were supplied 4 times per day by drip irrigation with complete nutrient solutions. In 2000 and 2001, three nitrogen treatments were applied from fruit set to leaf fall: 1.4 mM N, 3.6 mM N and 7.2 mM N (denominated N1, N2, N3, respectively). According to RODRIGUEZ-LOVELLE and GAUDILLERE (2002), N1, N2 and N3 are considered as limited, mean and excessive nitrogen levels. Nitrogen was supplied as ammonium nitrate, potassium nitrate, ammonium phosphate, calcium nitrate and ammonium sulfate. Except for nitrogen, all solutions had the same non-limiting concentrations of other mineral elements. In 2001, day temperature varied between 26 and 35 °C during ripening. For all treatments, the leaf area to berry weight ratio was ≥1.5 m² kg⁻¹ at harvest, a value recommended for normal berry development in vineyards (SMART et al. 1990).

Sample collection: In 2001, three berries of each plant were sampled at random every 10 d from veraison (11 July) to harvest (27 August for N1 treatment, 10 September for N2 and N3 treatments), when the sugar to titratable acid ratio was ≥50 (sugar g l⁻¹, titratable acidity g H₂SO₄ l⁻¹). Berries were weighed and the skins were separated from the pulp, frozen at -80 °C and freeze-dried. Fresh and dried skins, pulps and seeds were weighed. The dried skins were powdered in a ball grinder (Dangoumav, Prolabo, France) and extracted in methanol containing 0.1 % HCl (v/v). Extracts were filtered through a 0.45 µm polypropylene syringe filter (GHP Acrodisc, Gelman) for HPLC analysis of anthocyanins. Pulps were crushed to determine sugars, nitrogen and amino acids.

Harvest parameters: At harvest (47 d after veraison for N1, 61 d after veraison for N2 and N3), all grapes of each plant were harvested. The number and the weight of clusters per plant were used to determine yield and the average cluster weight. A sample of 100 berries per plant was collected at random and weighed to determine the average berry weight. The number of berries per cluster was calculated by dividing cluster weight by berry weight. The 100 ber-
ries were crushed and the must centrifuged. The supernatant was analyzed to determine soluble solids (°Brix), pH, titratable acidity, sugars, malate, tartrate and mineral nutrients.

**Cane and leaf analysis:** Pruning weight per plant was determined in 2000 and 2001. An aliquot was sampled, dried at 65 °C, then ground and passed through an 18-mesh screen for determination of mineral nutrients.

Leaves opposite to clusters were sampled at veraison to determine the status of mineral elements of vines during the growing season. Petioles and blades were separated, dried individually at 65 °C until the weight was constant, then ground and passed through a 40-mesh screen.

**Mineral analysis:** Total nitrogen and phosphorus in leaves, must and canes were determined after digestion by a modified Kjeldhal procedure (Hach et al. 1985) using sulfuric acid and hydrogen peroxide. NH₄⁺ and PO₄³⁻ were analyzed by an automated colorimetric method (TRAACS 800, Bran & Luebbe, Plaisir, France). The total S, K, Ca and Mg contents were determined, following digestion of leaves and canes in nitric acid and after dilution of must, by Inductively Coupled Plasma atomic emission spectrometer (Varian Vista, Varian, Mulgrave, Australia). All chemical reagents were of analytical grade (Mallinckrodt Baker France, Noisy-Le-Sec, France).

**Maturation parameters:** Soluble solids (°Brix) of berries were determined using a hand refractometer with an UltraspHERE ODS column 25 cm x 4.6 mm, 5 µm particle size with an UltraspHERE ODS guard column 4.5 cm x 4.6 mm obtained from Beckman Instruments Inc. (Fullerton, CA, USA), at ambient temperature. All reagents were of analytical grade. Water was purified (18 MΩ) with an ELGA (Bucks, UK) UHQ water purification system. Methanol (HPLC grade) was obtained from Baker (Mallinckrodt Baker France, Noisy-Le-Sec, France) and formic acid (99 %) from Merck (Merck Eurolab, Fontenay-sous-Bois, France). Binary gradient elution with a 1 ml min⁻¹ flow rate started with 100 % eluant A (5 % formic acid in water (v/v)) and ended with 100 % eluant B (5 % formic acid in methanol (v/v)). The injection volume was 20 µl for all samples. External standards were used for quantification on the basis of peak area. Millenium² software, version 3.05 (Waters, Milford, MA, USA) was used to calculate peak area and malvidin-3-glucoside was used as a common standard for all the quantified anthocyanins. Malvidin-3-glucoside (Oenin, HPLC grade) was purchased from Extrasynthese (Genay, France). Seventeen anthocyanins were identified and quantified.

**Statistics:** Data are presented as mean values ± standard error (S.E.). The Student’s t test at the 5 % level of probability was used to compare means. Statistical analyses were performed using Excel software.

**Results and Discussion**

**Mineral content:** N treatments induced significant differences of the N content in leaves, petioles, must and pruning canes (Tab. 1). As expected, in vines supplied with high amounts of N (N3) the N content in all organs was highest while in N1 vines it was lowest. These differences were observed for the berry N content from veraison to harvest (Fig. 1 A). Leaves of N1 vines were light-green. The other mineral substances of the nutrient solution were not limiting, leading to high P, K and Mg contents compared to those observed in the vineyard (Reuter and Robinson 1997; unpubl. data).

In the N3 treatment, the P content was reduced in leaf blades but not affected in petioles (Tab. 1). The P content in must and canes was lower in the N1 vines but still in excess as compared to vineyard conditions (unpubl. data). As for nitrogen, differences in P content were observed in must from veraison to harvest (Fig. 1 B). These results are in contrast to those reported by Conradie (2001), where N supply reduced the P content in petioles but did not affect P in must. In must, the total S content was positively related to the N supply, the S content of N3 vines being highest. The K content was not affected by the N supply. The Ca²⁺ and Mg²⁺ contents were lower in N3 vines. N treatments induced different effects on the K⁺, Ca²⁺ and Mg²⁺ contents in petioles, blades and must, but the major cation contents of all vine organs were considered as not limiting according to Reuter and Robinson (1997).
Effects of nitrogen supply (N1=1.4 mM N; N2 = 3.6 mM N; N3 = 7.2 mM N) on the mineral status of petioles, blades, must and canes. Values are means ± S.E. (n=10). Must values are means of 10 replicates of 100 berries per vine sampled at harvest. Letters compare means of the three treatments, different letters representing statistically significant differences between means; student’s t test $P=0.05$

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>P</th>
<th>S</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
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<tbody>
<tr>
<td><strong>Petioles at veraison, % dw</strong></td>
<td></td>
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<tr>
<td>N1</td>
<td>0.40 ± 0.02 a</td>
<td>0.97 ± 0.04 a</td>
<td>3.07 ± 0.22 a</td>
<td>2.14 ± 0.12 a</td>
<td>0.88 ± 0.07 a</td>
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<tr>
<td>N2</td>
<td>0.91 ± 0.09 b</td>
<td>1.06 ± 0.04 a</td>
<td>3.42 ± 0.32 a</td>
<td>2.32 ± 0.25 a</td>
<td>1.29 ± 0.09 b</td>
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<tr>
<td>N3</td>
<td>2.69 ± 0.10 c</td>
<td>1.00 ± 0.04 a</td>
<td>1.81 ± 0.22 b</td>
<td>1.15 ± 0.04 b</td>
<td>1.58 ± 0.16 b</td>
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<tr>
<td><strong>Blades at veraison, % dw</strong></td>
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<tr>
<td>N1</td>
<td>2.20 ± 0.17 a</td>
<td>1.01 ± 0.08 a</td>
<td>1.70 ± 0.17 a</td>
<td>2.51 ± 0.14 a</td>
<td>0.41 ± 0.02 a</td>
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<td>N2</td>
<td>2.62 ± 0.09 a</td>
<td>1.11 ± 0.11 a</td>
<td>1.91 ± 0.13 a</td>
<td>2.24 ± 0.19 a</td>
<td>0.39 ± 0.03 a</td>
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<td>N3</td>
<td>3.41 ± 0.18 b</td>
<td>0.83 ± 0.03 a</td>
<td>2.00 ± 0.12 a</td>
<td>1.12 ± 0.08 b</td>
<td>0.34 ± 0.02 a</td>
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<td><strong>Must at harvest, mg l⁻¹</strong></td>
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<tr>
<td>N1</td>
<td>358 ± 47 a</td>
<td>272 ± 6 a</td>
<td>50.0 ± 4.9 a</td>
<td>2268 ± 36 a</td>
<td>77.4 ± 7.6 a</td>
<td>132.2 ± 5.2 a</td>
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<tr>
<td>N2</td>
<td>945 ± 39 b</td>
<td>327 ± 11 b</td>
<td>86.6 ± 3.2 b</td>
<td>2664 ± 73 b</td>
<td>66.5 ± 4.4 ab</td>
<td>129.6 ± 3.6 a</td>
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<tr>
<td>N3</td>
<td>1779 ± 59 c</td>
<td>354 ± 10 b</td>
<td>147.6 ± 6.1 c</td>
<td>2362 ± 71 ab</td>
<td>51.6 ± 3.9 b</td>
<td>119.2 ± 4.8 a</td>
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<tr>
<td><strong>Canes at pruning, % dw</strong></td>
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<td></td>
<td></td>
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<tr>
<td>N1</td>
<td>0.71 ± 0.09 a</td>
<td>0.20 ± 0.01 a</td>
<td>0.59 ± 0.01 ab</td>
<td>0.45 ± 0.02 a</td>
<td>0.10 ± 0.00 a</td>
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<tr>
<td>N2</td>
<td>0.93 ± 0.04 a</td>
<td>0.24 ± 0.01 b</td>
<td>0.63 ± 0.01 a</td>
<td>0.46 ± 0.01 a</td>
<td>0.11 ± 0.00 ab</td>
<td></td>
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<tr>
<td>N3</td>
<td>1.53 ± 0.06 b</td>
<td>0.26 ± 0.00 c</td>
<td>0.56 ± 0.01 b</td>
<td>0.28 ± 0.01 b</td>
<td>0.12 ± 0.00 b</td>
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</table>

**Vegetative growth and yield:** Pruning weight, cane number and leaf area of the N1 vines tended to be lower than those of the N2 and N3 vines (Tab. 2). Although the differences were not significant for pruning weight and cane number, leaf area per vine significantly increased with nitrogen supply. N1 vines had a lower leaf area than the N3 ones and N2 vines had an intermediary leaf area.

For N3 vines, berry weight and number of berries per cluster were lowest (Tab. 3), while the number of clusters per vine was highest. This confirms results of DELAS et al. (1991) who showed that high nitrogen supply induces a significant yield decrease in the vineyard due to the high disposition of Merlot to coulure. Furthermore, RODRIGUEZ-LOVELLE and GAUDILLÈRE (2002) reported that berry growth is inhibited by high nitrogen supply before veraison. On the contrary, KELLER et al. (1998) showed that low nitrogen availability at bloom reduced fruit set due to necrotic inflorescences of Cabernet Sauvignon, the reduced number of clusters per vine and berries per cluster inducing a decrease in yield.

Berries of N1 vines had a higher sugar content and lower acidity (Tab. 4), while their yield was similar to that of N2 vines. For all treatments, the sugar content in berries increased rapidly until the fourth sampling date, and then increased slowly until harvest (Fig. 2). After the fourth date, the sugar content in N1 berries was significantly higher than in N2 and N3 berries, N2 and N3 berries having the same sugar content. However at the onset of maturation, N3 berries contained more sugar than N2 ones. No treatment significantly affected must pH. Nevertheless, titratable acidity was higher for the N3 treatment. The tartrate to malate ratio

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Fig. 1: Nitrogen (A) and phosphorus (B) content of berries (cv. Merlot). Vines were supplied with N1 = 1.4 mM N; N2 = 3.6 mM N; N3 = 7.2 mM. Values are means of 30 replicates (3 berries sampled from 10 plants for each treatment). Vertical bars represent standard error.
Table 2
Effects of nitrogen supply on growth parameters of Merlot vines in 2001. For details see Tab. 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pruning weight (g)</th>
<th>Cane number</th>
<th>Cane weight (g fw cane⁻¹)</th>
<th>Leaf area per vine (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>774 ± 28 a</td>
<td>4.1 ± 0.6 a</td>
<td>217 ± 26 a</td>
<td>2.01 ± 0.23 a</td>
</tr>
<tr>
<td>N2</td>
<td>822 ± 62 a</td>
<td>4.4 ± 0.3 a</td>
<td>194 ± 20 a</td>
<td>2.18 ± 0.23 a</td>
</tr>
<tr>
<td>N3</td>
<td>822 ± 32 a</td>
<td>4.8 ± 0.4 a</td>
<td>185 ± 19 a</td>
<td>2.80 ± 0.27 a</td>
</tr>
</tbody>
</table>

Table 3
Effects of nitrogen supply on yield parameters. Berry weights are means of 10 replicates of 100 berries per vine sampled at harvest. For details see Tab. 1

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Yield cluster (g vine⁻¹)</th>
<th>Berry weight (g fw)</th>
<th>Berries per cluster</th>
<th>Berries per vine</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>1024 ± 66 a</td>
<td>1.60 ± 0.06 a</td>
<td>90 ± 12 a</td>
<td>624 ± 85 a</td>
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<tr>
<td>N2</td>
<td>1052 ± 138 a</td>
<td>1.49 ± 0.05 a</td>
<td>106 ± 13 a</td>
<td>698 ± 87 a</td>
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<tr>
<td>N3</td>
<td>797 ± 77 a</td>
<td>1.14 ± 0.04 b</td>
<td>87 ± 5 a</td>
<td>705 ± 73 a</td>
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</table>

Table 4
Effects of nitrogen supply on quality parameters at harvest. For details see Tab. 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Soluble solids (° Brix)</th>
<th>Sugar (g l⁻¹)</th>
<th>pH</th>
<th>Titratable acidity (g H₂SO₄ l⁻¹)</th>
<th>Malic acid (g l⁻¹)</th>
<th>Tartaric acid (g l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>23.5 ± 0.2 a</td>
<td>240.7 ± 2.8 a</td>
<td>3.59 ± 0.02 a</td>
<td>3.57 ± 0.07 a</td>
<td>1.79 ± 0.07 ab</td>
<td>5.95 ± 0.05 a</td>
</tr>
<tr>
<td>N2</td>
<td>21.1 ± 0.4 b</td>
<td>215.7 ± 5.3 b</td>
<td>3.75 ± 0.04 b</td>
<td>3.58 ± 0.16 a</td>
<td>2.13 ± 0.15 a</td>
<td>6.37 ± 0.16 a</td>
</tr>
<tr>
<td>N3</td>
<td>20.7 ± 0.2 b</td>
<td>206.7 ± 2.5 b</td>
<td>3.69 ± 0.03 ab</td>
<td>3.90 ± 0.08 a</td>
<td>1.51 ± 0.09 b</td>
<td>7.07 ± 0.09 b</td>
</tr>
</tbody>
</table>

Fig. 2: Sugar concentration in berries during ripening. For details see Fig 1.

was higher for the N3 treatment than for N1 and N2 (4.85, 3.37 and 3.13 respectively). Moreover, at harvest, the sugar to titratable acidity ratio was 67 for N1, 60 for N2 and 53 for N3. These ratios suggest that maturation of N3 berries was delayed due to the high nitrogen supply. Similar results have been reported by Christensen et al. (1994) and Spayd et al. (1994).

The proportions of berry skin, must and seeds in relation to the nitrogen supply are presented in Fig. 3. There was no difference in berry weight between N1 and N2 vines (Fig. 3 A), but N3 vines had the lowest berry weight after veraison and this difference persisted until harvest (Tab. 3). Changes in skin weight were similar for all treatments during ripening (Fig. 3 B) with the highest values for N1 vines at harvest. However, neither the skin/pulp ratio nor skin thickness were clearly affected by the treatments (Fig. 3 C and 3 D). The number of seeds per berry and seed weight were not changed by the treatments (data not shown). Under our experimental conditions, berry morphology and fertility were not significantly modified by treatments, only berry size.

Amino acid content: Amino acid and total N in must increased during berry maturation (Figs 1 A and 4), must from N3 vines having both higher amino-N and total N contents. The amino acid composition however, changed according to the level of maturity and the treatment. At harvest, proline and arginine represented about 74, 70 and 78% of the total N amino acid in must for N1, N2 and N3, respec-
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During ripening, proline increased in berries of all treatments; it was higher in N3 vines while N1 and N2 vines had the same proline content (Fig. 5 A). On the contrary, arginine increased in the must of N2 and N3 vines but strongly decreased after the first sampling date in N1 vines to stay at a very low level until harvest. Histidine had a pattern like proline (data not shown). Alanine, asparagine, glutamine, serine and threonine accumulated similar to arginine (data not shown). Throughout fruit ripening, aspartic acid, α-amino butyric acid, glutamic acid, glycine, lysine, leucine, isoleucine and valine contents were only slightly or not affected by the treatments. Moreover, cysteine and methionine levels were not affected by treatments in spite of the highly different S content in must.

The importance of proline and arginine in must has already been reported (Treeby et al. 1998; Stines et al. 2000; Rodríguez-Lovel and Gaudillère 2002). The amino acid profile of must was significantly changed by N supply. During ripening, the amount of arginine related to the total amino acid content was very low in N1 vines (Fig. 5 B). In N2 and N3 vines, nitrogen of proline and arginine accounted for approximately 40 and 33 % of the total soluble nitrogen of must. These ratios are similar to those reported by Rodríguez-Lovel and Gaudillère (2002). The proline/arginine N ratio was largely changed by N nutrition.

During maturation, the content of phenylalanine in must was low for N1 vines and high for N2 and N3 vines (Fig. 6). In must of N2 vines, the phenylalanine increase was delayed compared to N3 vines. For all the treatments phenylalanine, a precursor in the biosynthetic pathway of phenols, never contributed more than 0.2 % of the soluble nitrogen in must.

Anthocyanin content: The anthocyanin content of berry skin increased rapidly until the third sampling date (20 d after veraison) and then decreased (Fig. 7). This pattern has been reported for grapes grown in South Australia, Côtes du Rhône and British Columbia by Somers (1976), Roggero et al. (1986) and Mazza et al. (1999) respectively. The highest anthocyanin content in berry skin was observed in N1 vines. The differences between N1, N2 and

Fig. 3: Effect of nitrogen supply on berry growth during ripening. A: berry fresh weight; B: skin fresh weight; C: skin to pulp ratio; D: skin thickness, expressed by the weight to area ratio. For details see Fig 1.

Fig. 4: Amino acid concentration in berries during ripening. For details see Fig 1.

Fig. 5: Proline and arginine in berries during ripening. A: Concentration in the must; B: Percentage of total amino acids in the must. For details see Fig 1.

Fig. 6: The phenylalanine concentration in berries during ripening. For details see Fig 1.

Fig. 7: The anthocyanin content in berries during ripening. For details see Fig 1.
N3 were significant shortly after veraison. The difference in anthocyanin accumulation rates could not be attributed to a competition between fruit and shoot growth, since vegetative growth parameters and grape yield were very similar for the different treatments in this experiment (Tab. 2 and 3).

The anthocyanin concentration peaked as sugar continued to increase in berries (Fig. 2 and 7) as was reported by ROGGERO (1986). LARRONDE et al. (1998) showed that anthocyanin synthesis in *Vitis vinifera* cell suspension cultures increased when intracellular sugar levels were high. DO and CORMIER (1990) concluded that high sucrose concentrations can act osmotically when promoting the anthocyanin production. However, in N1 vines the anthocyanin content of skins increased before significant changes of sugars occurred in the 3 treatments. KELLER and HRAZDINA (1998) suggested a relation between the sugar and anthocyanin contents of berries, but this relation was not observed in our experiment (Figs 2 and 7). PRIE and MULLINS (1977) and GONZALEZ-SAN JOSÉ and DIEZ (1992) stated also the absence of a correlation between sugar and anthocyanin concentrations in berries.

High nitrogen supply inhibited the synthesis of anthocyanins (Fig. 7) in Merlot and in Cabernet Sauvignon (KELLER and HRAZDINA 1998). This inhibitory effect is already effective in N2 vines with a berry N content of > 900 mg l⁻¹. Unlike AWAD and JAGER (2002) for apple, no direct correlation was found between the plant nitrogen content and the total anthocyanin content in skins during ripening.

The anthocyanins in the skin of Merlot berries consisted mainly of Mv-3-gl and the acylated forms (Mv-3-gl-ac, Mv-3-gl-cou) and Dp-3-gl, Cy 3-gl, Pt-3-gl, Pn-3-gl (Fig. 8). These substances peaked 20 d after veraison. Conversely the content of acylated anthocyanins, mainly due to Mv-3-gl-ac and Mv-3-gl-cou, reached a plateau and did not decrease at the end of maturation. No significant differences appeared to exist between N2 and N3 vines, except for Pn-3-gl which was higher in skins of N3 vines compared to N2 vines.

The contribution of individual anthocyanins to the total content during ripening was strongly affected by the N supply. Skins of N1 vines had relatively lower amounts of acylated anthocyanins, and a very high contribution of Dp-3-gl and Pn-3-gl (Fig. 9). In contrast, skins of N2 vines showed the lowest percentage of non-acylated anthocyanins and the highest contribution of acylated anthocyanins. On the other hand, the contribution of Mv-3-gl was only slightly affected by the treatments (Fig. 9).

Delphinidin and cyanidin are considered to be precursors in the biosynthetic pathway; during ripening they are transformed by methylation into peonidin, petunidin and malvidin (ROGGERO et al. 1986). It is concluded that the anthocyanin composition of the grape berry is changed by the N supply, as was already shown for the tomato (BONGUE-BARTELSMAN and PHILLIPS 1995). SATO et al. (1996) suggested that a limitation of mineral nutrients delays cell metabolism, increases the phenylalanine content in strawberry cell cultures and favours the phenolic biosynthetic pathway. Addition of phenylalanine to *Vitis vinifera* cell cultures stimulated anthocyanin accumulation (KASAI et al. 1999), the maximum phenylalanine accumulation in cells occurring just before the initiation of anthocyanin synthesis (KAKEGAWA et al. 1999).
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Fig. 9: Anthocyanins related to total anthocyanins in the berry skins. For details see Fig 1.

1995). However, our data suggested that the availability of phenylalanine is not the limiting factor of the anthocyanin synthesis in berries. In N3 vines, the amount of anthocyanins was reduced and the phenylalanine content was increased (Figs 6 and 7). Moreover, berries of N2 and N3 vines had the same anthocyanin content but different phenylalanine contents. Clearly, the control of the biosynthesis of anthocyanins by the nitrogen status occurred elsewhere in the biosynthetic pathway. Chalcone synthase and UDP glucose-flavonoid 3-o-glucosyl transferase (UFGT) are possible controlling points for anthocyanin synthesis in grapes (KAKEGAWA et al. 1995; BOSS et al. 1996).

Nitrogen supply affects the anthocyanin accumulation and degradation profile. Skins of berries of N1 vines had the highest anthocyanin content until harvest (Fig. 7); the anthocyanin decline was small and very progressive compared to the N2 and N3 treatments. The rapid decrease in the anthocyanin content suggests the establishment of a catabolic pathway activated by the high N supply during the late maturation phase. Very little is known about the in vivo enzymatic pathway of the anthocyanin degradation which may be induced by glycosidase and peroxidase activity in the vacuoles of the berry skin (KELLER and HRAZZINA 1998).

The N status can directly affect the control of the anthocyanin biosynthetic pathway, but also induces changes in the P status of grapevine organs (Tab. 1). Excess P may inhibit the induction of phenylalanine ammonia-lyase and chalcone synthase activity and may lower the anthocyanin content in grapevine cell cultures (KAKEGAWA et al. 1995). The P supply of the nutrient solution was not limited and the P status of grapevines was much higher than in the vineyard. Excess P probably lowered the general anthocyanin net accumulation in all the treatments.

In conclusion, N supply had a direct effect on the anthocyanin content in berries, which was independent of indirect effects linked to carbon partitioning between reproductive and vegetative growth or of the size of the berries (skin to must ratio). Nitrogen supply changed the anthocyanin composition. At low nitrogen concentration, berries contained relatively more Dp-3-gl and Pt-3-gl and less acylated Mv-3-gl. Degradation of anthocyanins was clearly observed in berry skins, excess nitrogen favouring this degradation. The enzymes involved in the N control of biosynthesis and degradation pathways of anthocyanin remain to be identified.

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