



## Berry development – An overview. Part 1. Morphology and anatomy of a berry

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### Anatomy

The grape is a fleshy berry which comes in many shapes depending on the cultivar (Figure 1a). It consists of a pericarp and seeds (Figure 1b); most cultivars have a maximum of four seeds per berry. The pericarp consists of the following tissues, from exterior to interior (Figure 2):

- An exocarp (called skin), which comprises the cuticle (formed by lipid waxes), epidermal cells (isodiametric cells measuring 6,5 to 10 µm in width) and hypodermal cells (10 to 12 cellular layers measuring 100 to 250 µm in width) (Alleweldt *et al.*, 1981; Fougère-Rifot *et al.*, 1996). The skin contains stomata that are only functional until véraison (Bessis, 1972). The cellular vacuoles are extremely rich in polyphenols.
- A mesocarp (called pulp) composed of 25 to 30 layers of cells. During berry growth these layers expand to reach a size of 400 µm at the end of the second growth stage, versus maturation. The vacuoles of these cells represent 99% of their volume (Diakou & Carde, 2001). Their walls are fine and undergo important structural changes from véraison onwards, which are responsible for the berry's softening and for its increased plasticity (Barnavon *et al.*, 2000; Nunan *et al.*, 2001). The mesocarp also contains an endocarp, which is the innermost

part of the fruit: this consists of a fine layer of cells which demarcates the carpels containing the seeds. The seeds consist of an embryo (2n), an albumen (3n) and are rich in oils and phenols (Winkler *et al.*, 1974).

The vascular bundles, which are linked to the pedicel of the berry, derive from the ovary (Pratt, 1971) and are situated:

- in a central position to nourish the seed(s) and the columella (central part of the berry),
- and in a peripheral position, forming a network around the berry. The vascular bundles are situated 15 to 18 cellular layers below the cuticle (Coombe, 1987; Fougère-Rifot *et al.*, 1996; Ollat *et al.*, 2002). Depending on the cultivar they may number 20 to 30 (Fillon, 1997; Park, 1995). The peripheral vascular bundles of the berry contain little xylem. The pole phloem of these vascular bundles contain several groups of sieve tubes associated with their companion cells. Transportation of the phloem to the receiving cells takes place in an apoplastic (through active hexose carriers) and/or symplastic manner (through plasmodesmata). The apoplastic space of young green berries is less important than that of ripe berries. What happens is an adaptation depending on the important influx of sucrose that the berry receives from véraison onwards (Fillon, 1996).

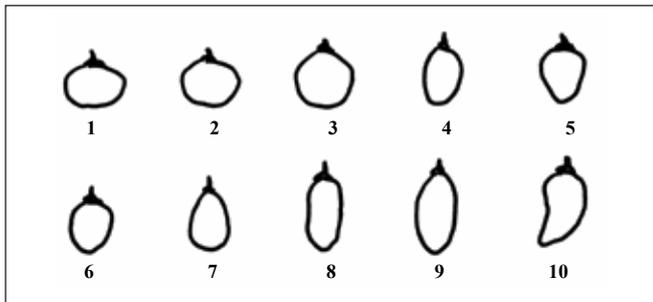


FIGURE 1a. Different berry shapes (OIV 223):  
1) flattened; 2) slightly flattened; 3) rounded; 4) short elliptical; 5) ovoid;  
6) troncoïd; 7) obovoid; 8) cylindrical; 9) long elliptical; 10) curved.

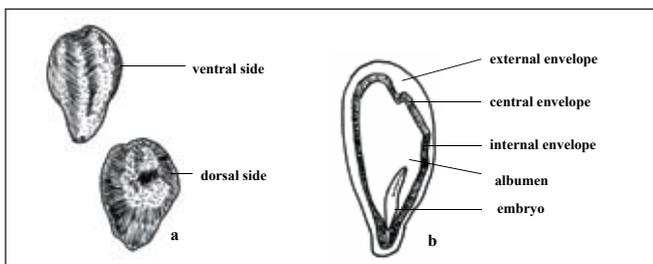


FIGURE 1b. The seed of the vine (*Vitis vinifera sativa*).  
a) example of the shape of a seed; b) longitudinal section of a seed.

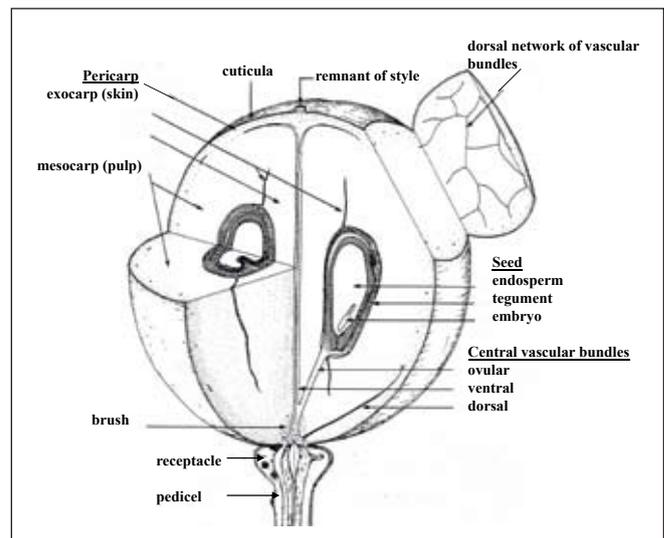


FIGURE 2. Diagram of a berry (from Coombe, 1987).

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## Berry development – An overview. Part 2.

### Berry growth – The four main stages

#### Growth of the fruit

Berry growth is characterised by an evolution of weight and volume following a curve in two growth phases (each of these may form a sigmoidal curve) separated by a stationary phase (Figure 3a). The two principal phases are herbaceous growth and maturation of the grape, the phases being separated by an arrest phase or by a slow-down in the growth followed by véraison (Staudt *et al.*, 1986; Coombe, 1976, 1992).

#### Stage 1 – Herbaceous growth or green phase

This is the first growth phase which follows immediately after fertilisation and starts with fruit set. This stage of the development ends before véraison, through an arrest or slow-down of growth, called “herbaceous plateau”. Herbaceous growth is subdivided into two sub-stages described by Ollat *et al.*, 2002; Fougère-Rifot *et al.*, 1997:

1. The start of the swelling of the ovary which follows immediately after fertilisations of the ovules. The ovary may become rounded, it encloses four ovules, each of which yields an average of one to four seeds. The carpillary wall then measures 300 µm and the cells are abundantly rich in vacuolar tannins. The peripheral (i.e. dorsal) vascular bundles demarcate the boundary between the hypodermis and the internal parenchyma.
2. Fruit set: the ovary becomes a small spherical berry which is properly attached to its pedicel. At this growth stage the abscission zone of the pedicel is no longer functional. The dorsal vascular bundles in turn demarcate the boundary between the hypodermis and the internal parenchyma. The total number of cells of the berry is fixed at a very early stage, which is to say eight to ten days after the start of the swelling of the ovary: 90% of the cellular multiplication takes place essentially during the phases of ovary swelling and fruit set (Ojeda *et al.*, 2001).

After fruit set, the growth of the berry is essentially due to cellular growth. Some cellular multiplications still occur, but in the hypodermis only. The most external cells of the hypodermis contain polyphenols. The duration of cellular growth before the herbaceous plateau (sudden growth arrest) is approximately 40 to 50 days after anthesis (flowering).

#### Stage 2 – The Herbaceous Plateau

During this phase growth slows down or ceases, thus separating the herbaceous growth from the maturation of the fruit. The

duration of this stage depends on climatic and cultivation conditions and on the cultivar (Ollat *et al.*, 2002). This is a preparatory stage for the onset of véraison.

#### Stage 3 – Véraison

This stage corresponds to the start of maturation in the fruit. In the grapevine it is characterised by an abrupt softening of the berry (in 24 hours) which then becomes transparent. This softening is accompanied by an active entry of sugars in the berry (sucrose rapidly hydrolysed into hexoses: glucose and fructose). In black

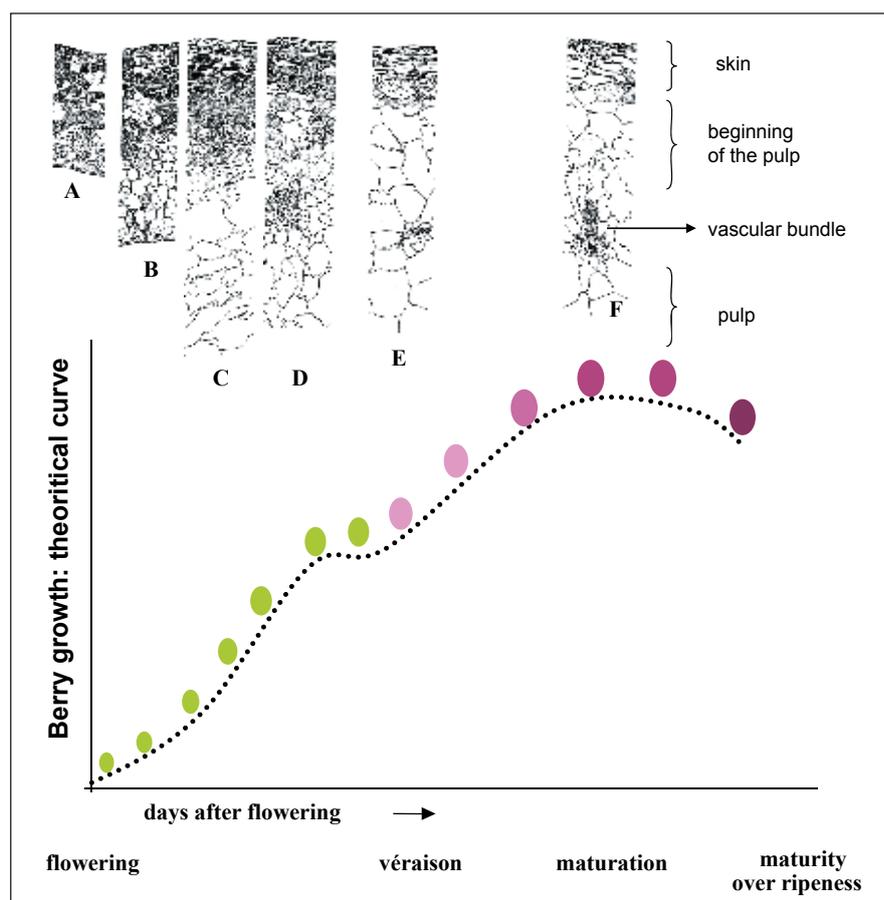


FIGURE 3a. Growth and anatomical evolution of the berry. Berry growth occurs in two stages: herbaceous growth and maturation. Véraison marks the onset of maturation, following a period of growth arrest of which the duration is variable.

- A) Onset of the swelling of the ovary after fertilisation.  
 B) Set, principal stage of cellular multiplication (90% of total mitoses).  
 C & D) Berry growth by means of cellular growth, end of the last mitoses at the level of the epidermal zone. Biosynthesis of organic acids and accumulation in the vacuoles.  
 E) Véraison, when cellular growth resumes (accumulation of sugar and water in the vacuoles).  
 F) Fruit maturation ends, cellular growth ends. Possible loss of volume in the event of overripening.

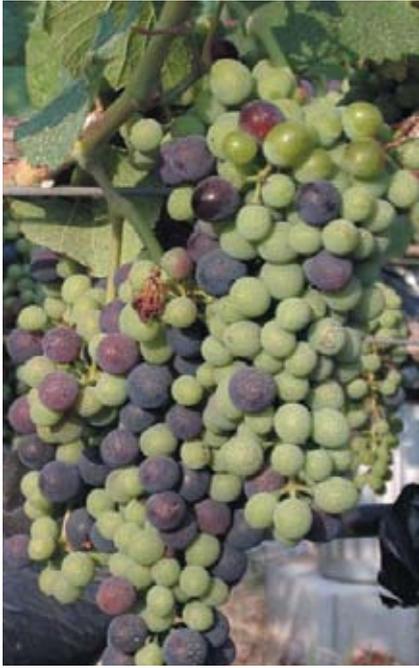


FIGURE 3b. The cultivar Syrah at the onset of véraison. Berry colouration occurs as a result of the biosynthesis of anthocyanins and is not dependent on a gradient at the level of the grape. Each berry follows its own maturation dynamic in the context of the bunch microclimate. At this stage there are several berry populations: green and hard berries; green and soft berries; and berries at different stages of colouration.

cultivars véraison is characterised, after softening, by the colouration of the skin in “red-blue” as a result of the biosynthesis of anthocyanins (Figures 3b, c).

Cellular growth resumes because water and sugars enter into the vacuoles, as well as potassium. At the end of véraison, the distance between the dorsal vascular bundles and the epidermis is approximately 700  $\mu\text{m}$ . The cells of the hypoder-



FIGURE 3c. The cultivar Syrah at the end of véraison. Generally speaking there is no berry biochemical gradient within a bunch, but there is a microclimatic gradient, related to light and temperature. This gradient has an effect on the dynamic of each berry maturation.

mis increasingly resemble the cells of the pulp with their fine walls.

Véraison does not occur simultaneously in all the berries: the same bunch may contain berries that are still green and hard, while others are green and soft, and yet others are beginning to change colour. There doesn't seem to be an obvious gradient of véraison for any particular bunch of grapes.

It has been shown that at the start of véraison, the influx of crude sap (water, mineral elements, ...) and elaborated sap (sugars, water, amino acids, ...) into the berry occurs mostly through the phloem. It is not clear yet why the cessation of xylem functioning occurs at the level of the fruit (and not its pedicel).

#### Stage 4 – Maturation

The cells of the hypodermis above the dorsal vascular bundles grow and partially form the future pulp. The cells of the pulp, below the dorsal vascular bundles, continue their growth mainly as an influx of sugars and water into the vacuole. The pellicular zone (eight to ten layers of cells approximately 10  $\mu\text{m}$  in width) measures approximately 100  $\mu\text{m}$ . The width of these cells has been decreased by the “compression” of the growth of the cells subjacent to the hypodermis and the pulp.

The main phenolic components of the berry (tannins, anthocyanins, some of the flavonols) are found in this tissue zone (i.e. the skin), as well as certain mineral elements (potassium, calcium) and some aromatic compounds. The hypodermis measures approximately 700  $\mu\text{m}$ , it consists of 14 to 18 layers of cells with an average thickness of 50  $\mu\text{m}$ .

The section below the dorsal vascular bundles constitutes the essence of the pulp. The pulp contains mainly sugars, organic and amino acids, the major mineral elements (potassium, calcium), some phenols (hydroxycinnamic derivatives, tannins), aromatic compounds and proteins.

The swelling of the berry “stretches” the pellicular zone and if the inflow of water is too substantial and rapid, the skin could burst, especially towards the end of maturation.

## Berry development – An overview. Part 3. Berry and water

### The flow of water (plant – fruit – atmosphere)

The loss of water in the course of maturation may occur as a result of four possible mechanisms (Figure 4a, b):

- Transpiration:** Even though the berry possesses few stomata, the latter being “sealed off” by waxes from the onset of maturation. At the end of maturation, if berry shrinking occurs, the fruit may suffer a daily weight loss of 15 mg as a result of transpiration through the cuticle: the loss of water does not result from cracks in the cuticle (Rogiers *et al.*, 2004);
- Water no longer enters into the fruit for the following reasons:** i) trophic (the grapevine experiences water deficiency); ii) or anatomical (interruption of the functioning of phloem or rupture of the berry’s entire endogenous conductor system) (Hunter & Ruffner, 2001; Rogiers *et al.*, 2004);
- A reverse flow of water towards the plant, called “back flow”** by Tyerman *et al.*, 2004. In fact, the source-sink ratio between the organs of the vine has shown that berries may become a source of water for the plant under dry conditions. Moreover, during maturation xylem may recycle excess water in the berry towards the stem (Keller *et al.*, 2006).
- Overripening:** At the end of fruit maturation, approximately 120 days after flowering, an interruption or a restriction of phloem flow may occur. This situation, especially in an area where water is restricted, may give rise to a very important reduction in the volume of the berry (as much as 40% of the initial volume) and to berry shrinking, which may be seen from the wrinkled appearance of the skin. Berry shrinking occurs when the inflow of water does not compensate for the outflow, or when the “return” of water towards the plant

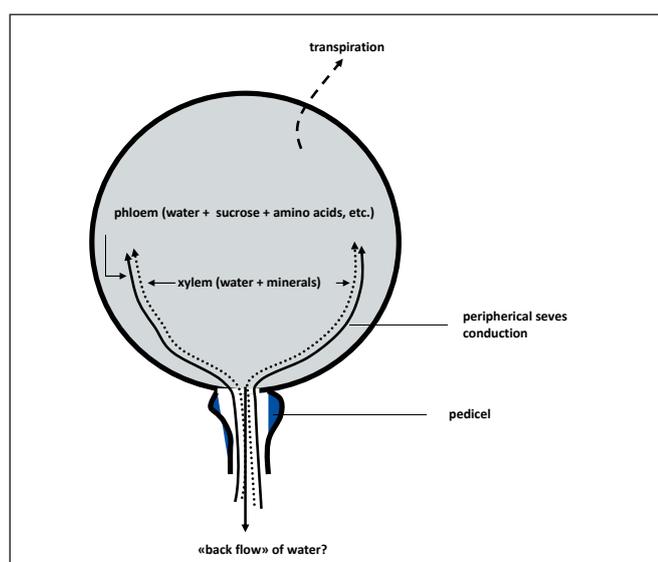


FIGURE 4a. Representation of the flow from the plant to the berry. The berry receives the water through the xylem until véraison and through the phloem during maturation. Water loss occurs: a) through transpiration; b) when water no longer flows into the berry; c) and/or by a possible return of water to the plant, called “back flow” (according to Coombe, 1992; Rogiers *et al.*, 2004).

becomes significant. Shrinking may occur from véraison to maturity, in fact as soon as the fruit softens.

The vine water status (which depends on the soil moisture (precipitation and/or irrigation), soil depth and on the root system morphology, development and functioning), will impact on the growth and the volume of the fruit, in other words on the yield per vine and per hectare (Figure 5).

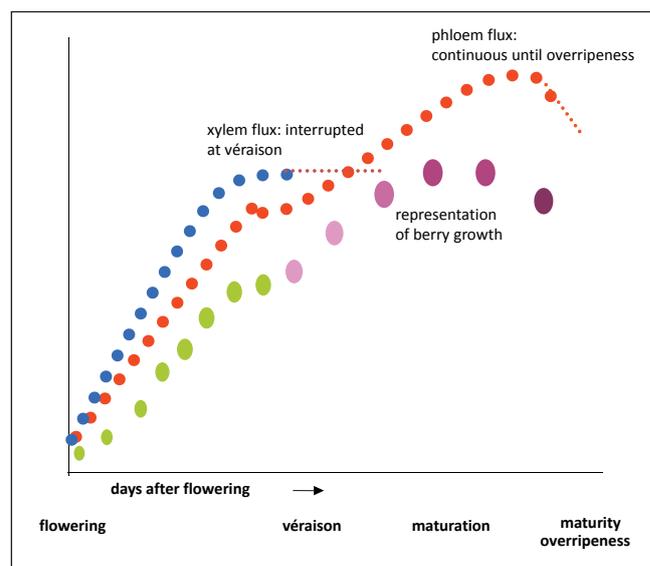


FIGURE 4b. Evolution of xylem and phloem flow during the growth of the berry. The functioning of xylem is interrupted at véraison. The phloem flow depends on the growth stage of the berry. When the fruit becomes overripe, phloem flow may be delayed or interrupted.

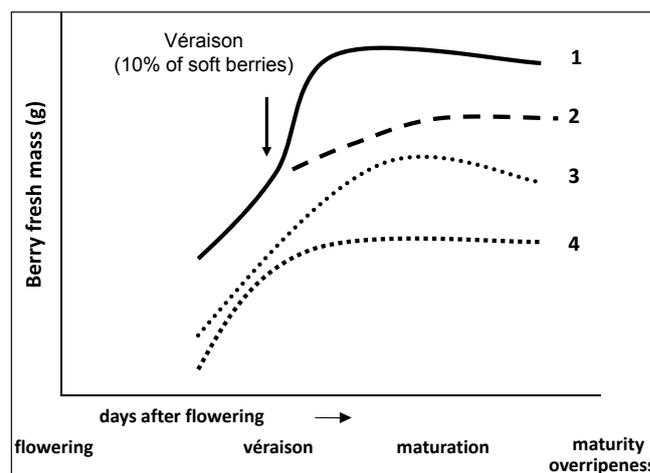


FIGURE 5. Example of the evolution of the average fresh mass of the berry of the grapevine that has been submitted to different levels of water restriction before and after véraison.

1) Example without water restriction. 2) Moderate water restriction at the beginning of véraison (arrow = 10% of softened berries) until maturity. 3) Moderate water restriction from flowering until véraison (suppression of restriction at véraison). 4) Severe water restriction from flowering to véraison (suppression of restriction at véraison). Water restriction (2) modifies the volume of the berry, although this is reversible. Premature water restrictions occurring before véraison (3 & 4) generally modify the volume of the berry in an irreversible manner (according to Ojeda *et al.*, 2001).

# Berry development – An overview. Part 4.

## Berry growth and seeds

### Growth of the berry – seeds and other factors

The development of the berry depends on the presence or absence of viable seeds. In fact, an absence of fertilisation or an aborted fertilisation provokes “coulure” (flower drop) and/or “millerandage” (abnormal development of the berry). For a berry to develop normally, the seed has to be well-formed at the very least. There is no direct relationship between the volume or the fresh mass of the fruit, or the number of seeds. The volume or the berry depends on the importation of carbon (source-sink ratio), on the vine water status, on the temperature (microclimate of the bunch and the vegetation), and on nitrogen nutrition.

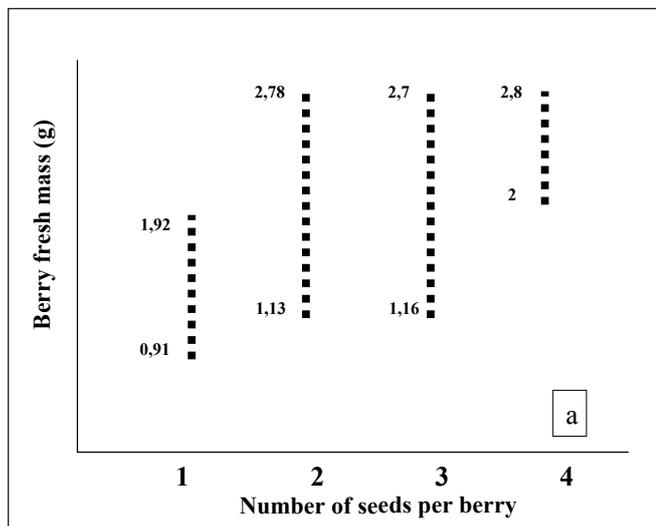


FIGURE 6a. Relation between the average fresh mass of a berry and the number of seeds (example taken from the Mourvèdre cultivar).

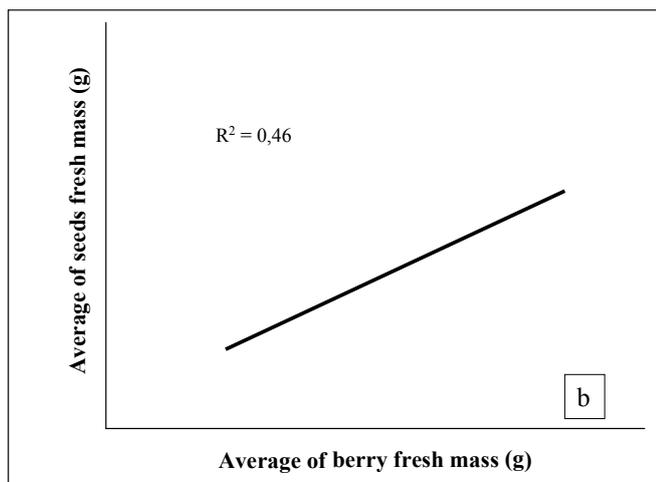


FIGURE 6b. There is a weak linear relation between the average fresh mass of the berry and the fresh mass of the average number of seeds contained in the berry.

Using Mourvèdre, the number of seeds per berry were counted and measured in relation to the mass of the fruit (Figure 6a, b). Taking the same berry population (from the same bunch), the results clearly show that the berries may have an identical fresh mass for a seed content ranging from 1 to 4. In other words, the variation in the fresh mass of berries containing 2 seeds for example ranges from 1,13 g to 2,78 g. The results nevertheless show the fresh mass of berries with 1 seed to be inferior to that of berries with 4 seeds. It is therefore a trend and not a direct correlation (number of seeds – fresh mass of the berry). According to May (2000) the stimulation of the growth of the pericarp induced by each seed decreases with the increase in the number of seeds.

One seed is therefore necessary and sufficient for the development of the fruit. The volume of the latter is firstly dependent on environmental factors (water and temperature) and on the relationship “vine – grape” (Figure 7), and equally on the number of seeds and more directly the mass of the seeds (Roby & Matthews, 2004). There will nevertheless be heterogeneous responses from berries possessing the same number of seeds, in a population of bunches taken from the same vine and from different vines in the same block. Berries from seedless cultivars have different growth curves (Nitsch *et al.*, 1960). Induction of fertilisation without the complete development of seeds suffices to trigger the growth of the fruit. For seedless varieties hormone gibberellins or the annular incision technique applied to the trunk is used to induce enlargement of the fruit.

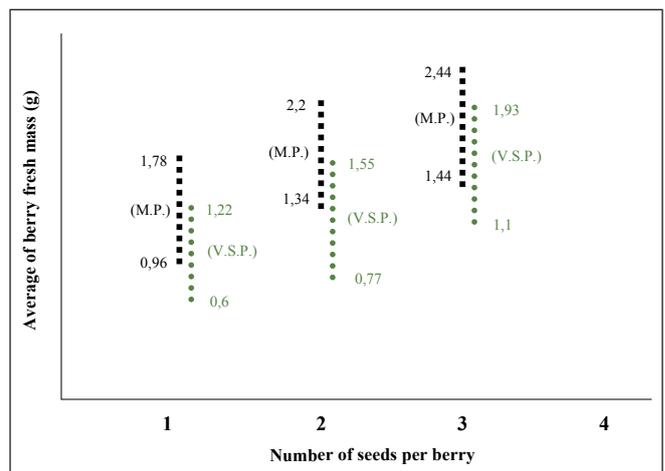


FIGURE 7. Comparison of the average fresh mass of Merlot berries, deriving from two different training systems: a) minimal pruning (M.P.) and b) vertical shoot positioning (V.S.P.). In determining the mass of the berry, the predominance of the training system with regard to the number of seeds per berry, is clearly shown. (Green dots: Minimal pruning (M.P.); black squares: V.S.P.).

## Berry development – An overview. Part 5. Berry and hormones

Hormones or phytohormones are molecules that are endogenous to vegetal tissue. In any organism, the hormone status depends on equilibrium between biosynthesis, degradation, importation and exportation. Hormones are molecules which act in a very weak molar concentration in the plant ( $10^{-9}$  à  $10^{-5}$  M).

The principal hormones which have been described are auxins, cytokinins, gibberellins, abscissic acid and ethylene (Figure 8). Another metabolic group which is considered to be a hormone has been encountered in the berry, namely polyamines. Little is known about hormonal control over the development of the berry and its maturation.

**Auxin**, cytokinins and gibberellins are found in maximum concentrations in the pulp before the herbaceous plateau, subsequently the concentration thereof decreases significantly during véraison. The concentration of abscissic acid (ABA) decreases during the herbaceous growth phase, then reaches a peak in concentration at véraison, and decreases rapidly at the onset of maturation (Coombe & Hale, 1973). ABA plays a role in the accumulation of sugars.

**Auxin** plays a role in berry growth during the herbaceous stage and intervenes in the regulation of cell division and differentiation. Molecular biology studies seem to point out that auxins delay véraison by acting on certain regulatory genes, by slowing down their induction. By dipping a Syrah bunch in a solution containing auxin, the benzothiazole-2-oxyacetic (BTOA), managed to delay maturation by two weeks. A similar study which used a salicylic acid solution succeeded in inhibiting and/or delaying véraison and berry maturation in Syrah by 2 to 3 weeks (Kraeva *et al.*, 1998; Davies *et al.*, 1997).

**Cytokinins** play a role in flowering and cellular multiplication (Weaver *et al.*, 1965; Srinivasan & Mullins, 1978).

**Gibberellins** are involved in cellular growth, as shown by the utilisation of gibberellins ( $GA_3$ ) to: a) increase berry size of seedless table grape cultivars; b) elongate the stalk to obtain loose clusters, to comply with the market demand for table grapes. For cultivars with seeds, gibberellins induce growth of the pericarp via cellular signals (Lavee *et al.*, 1967; Coombe & Hale, 1973).

**Abscissic acid** is involved in the onset on fruit maturation by controlling the regulation of certain genes that are linked to maturation (Robinson & Davies, 2000). The ABA was assayed in seeds, in the dormant winter buds (the concentration decreases when the buds are subjected to a cold period), in the roots and the leaves of grapevines subjected to water stress (Loveys & Düring, 1984; Koussa *et al.*, 1994; Antolin *et al.*, 2003). When vines are subject to water stress, the ABA of the leaves and the dosages of ABA in general cannot be used to indicate the vine water status.

**Ethylene** has been dosed in very small quantities in the grape, which is not a climacteric fruit such as citrus or cherries, compared to climacteric fruit such as the banana (where ripening depends on ethylene and can be artificially provoked by means of an exogenous application of this hormone), tomato, or melon.

In climacteric fruits ethylene provokes a sudden increase in respiration known as a respiratory crisis. Non-climacteric fruit produce little ethylene. In grapes concentrations of  $0,4 \text{ ml.l}^{-1}$  have been detected during véraison (Alleweldt & Koch, 1977), which is weak compared to concentrations of climacteric fruits which may go up to  $500 \text{ ml.l}^{-1}$ . No respiratory crisis has been observed during ripening of the berry (Kannellis & Roubelakis, 1993).

Moreover, assays have shown the positive role of the exogenous application of ethylene (by using ethephon) or ethanol in the colouration of the grape, through the increase (stimulation?) of the biosynthesis of anthocyanins (Weaver & Montgomery, 1974; Chervin *et al.*, 2001; Nikolaou *et al.*, 2003). Ethephon, which is an analogue of ethylene, is used for the chemical thinning of flowers or very young fruits. It may result in partial or total necrosis of the inflorescens or the very young cluster.

In grapevines (wine cultivars), the exogenous usage of ethylene (Hirschfeld & Lavee, 1980), and hormones in general, is sensitive because it is necessary to find the exact dosage and the right application period (heterogeneity related to the developmental stage and temperature at the time of application), and care should be taken regarding the possible side effects on the plant (for example  $GA_3$  which may hamper the fertility of the inflorescence). Such an approach requires careful experimentation, repeated over several years; moreover it is necessary to measure the actual interest applied and the cost.

**Polyamines**, which have long been known in animals and microorganisms, have been studied in higher plants for 20 years. They are a source of carbon and nitrogen, but constitute a group of

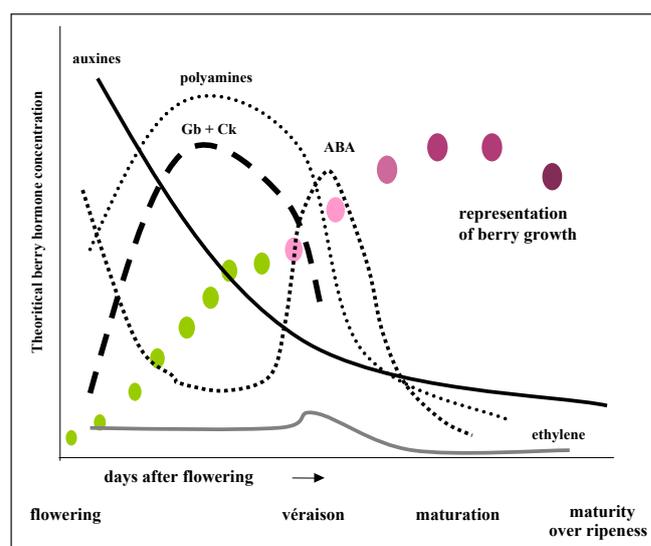


FIGURE 8. Evolution of hormones in the berry. Theoretical curves indicate major trends in respect of the growth curve of the berry. The proportions of the hormone contents (concentrations) are theoretical (from Coombe & Hale, 1973; Nitsch *et al.*, 1960; Broquedis *et al.*, 1995; Robinson & Davies, 2000).

Gb: gibberellines; Ck: cytokinins; ABA: abscissic acid.

growth hormones. In recent years several studies on the grapevine have enabled their identification in different cultivars. The principal polyamines that have been identified in *Vitis vinifera* L. are putrescine, spermidine and spermine. They have been measured mainly in the buds, the leaves, the stalks, the flowers and the berries (Geny *et al.*, 1997; Lespy-Labayette *et al.*, 1994).

Experiments with the application of putrescine during the flowering of Merlot (a cultivar that is sensitive to coulure) have enabled a significant increase (30 to 40%) in the setting rate (e.g. favourable concentration: 1 mM/l). However if the application of polyamines is performed in a climatic situation of non-coulure (in practice it is difficult to foresee climatic coulure), the result might well be excessive compaction of the clusters, even a rupture of the berries.

It is important to note that in practice polyamines are not used in viticulture today, such usage could entail more drawbacks than benefits.

The main uses of hormones in grapevines are:

- **Grafting:** auxins are used in the wax coating to stimulate callus and cambium differentiation, and to help binding of the graft/rootstock.

- **Rhizogenesis:** auxins are also used to stimulate rhizogenesis of the grafts that have been bound in the grapevine nursery.
- **Yields:** ethylene in its ethephon form is used to regulate yields through chemical thinning during flowering or at the very beginning of fruit set. On the other hand, polyamines may promote fruit set, at least on an experimental basis, if they are applied during flowering.
- **Véraison:** ethylene may be used to promote colouration of the berries during véraison (beware of side-effects such as premature ageing of the skin).
- **Table grapes:** gibberellins ( $GA_3$ ) are used to lengthen the stalk or increase the size of seedless berries of table grapes.
- **Research:** the majority of hormones are used *in vitro* to obtain callus, for micro-propagation and somatic embryogenesis.
- **Correlative inhibition:** hydrogenous cyanamide (which, although not a hormone, is interesting to mention here because of the role it plays) is commonly used to homogenise the start and growth of winter buds (lifting of correlative inhibition). This applies to table grapes or to wine grapes in Mediterranean and tropical climates where there is no cold period to break dormancy and where there are two harvests per annum (mainly in tropical climates).

## Berry development – An overview. Part 6. Berry and sugars

### Primary metabolism: sugars

#### Carbohydrates

Of the soluble sugars in the berry, glucose and fructose constitute 99%. Saccharose may be detected in very weak quantities and while technically invertase activity is inhibited, this enzyme has heightened activity in the berry. The berry does not contain starch (or in trace quantities) and the increase in hexoses is essentially due to the source (photosynthesis and reserves). Sugar, in the form of saccharose, is mainly transported by phloem (Stoeb, 1966; Glad, 1992a, b), some transportation nevertheless also occurs as glucose (Hunter & Ruffner, 2001). Grapes are some of the richest fruits in sugar. During alcoholic fermentation, an average of 17g.l<sup>-1</sup> sugar is required to obtain 1° of alcohol.

During herbaceous growth the grape contains little sugar. At this stage glucose is more important than fructose (Winkler *et al.*, 1974). The skin, not the flesh, might contain saccharose. The sugars that are imported by the berry are degraded by glycolytic enzymes and transformed into organic or respired acids. Respiration by the berry of oxygen consumed by fresh matter unit decreases during the development of the berry: the quantity which is estimated at 220  $\mu\text{moles O}_2 \cdot \text{min}^{-1} \cdot \text{kg}^{-1}$  at the start of the grape's growth is approximately 10  $\mu\text{moles O}_2$  at maturity (Coombe & Hale, 1973; Terrier, 1997). Ollat and Gaudillère (2000)

have shown that respiration in Cabernet Sauvignon berries during the day decreases significantly from berry set to maturity. The respiration rate of the berry 20 days after flowering is approximately 45  $\mu\text{moles g}^{-1}$  (fresh mass, FM)  $\text{h}^{-1}$  and reaches approximately 10  $\mu\text{moles g}^{-1}$  (FM)  $\text{h}^{-1}$  at véraison, then stabilises somewhere between this value and 5  $\mu\text{moles g}^{-1}$  FM  $\text{h}^{-1}$  at maturity. At night respiration in the berry is weaker, approximately 15  $\mu\text{moles g}^{-1}$  FM  $\text{h}^{-1}$  20 days after flowering.

The respiratory coefficient equal to 1 ( $\text{CO}_2$  emitted relative to  $\text{O}_2$  consumed) shows that respiration of carbohydrates is greater than malate synthesis (Hardy, 1968; Harris *et al.*, 1971). During the maturation phase, some aerobic fermentation may take place in the excised berries, which explains the increased respiratory coefficient (Terrier & Romieu, 2001). At the end of maturation, if the phloem conduction of the berries is interrupted, respiration of the berry may cause a reduction in the quantity of sugar per berry and of degree Balling.

The rate of imported carbohydrates is between 45 and 77  $\mu\text{moles per berry per day}$  during herbaceous growth. At the level of the berry, the onset of softening is characterised by an important sugar accumulation: namely véraison. The importation of carbohydrates is increased by factor 3-4 and reaches 190 to 266  $\mu\text{moles per berry per day}$ . The tempo of accumulation is 42  $\text{nmol} \cdot \text{min}^{-1} \cdot \text{baie}^{-1}$  or 18  $\text{nmol} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$  fresh mass to reach concentra-

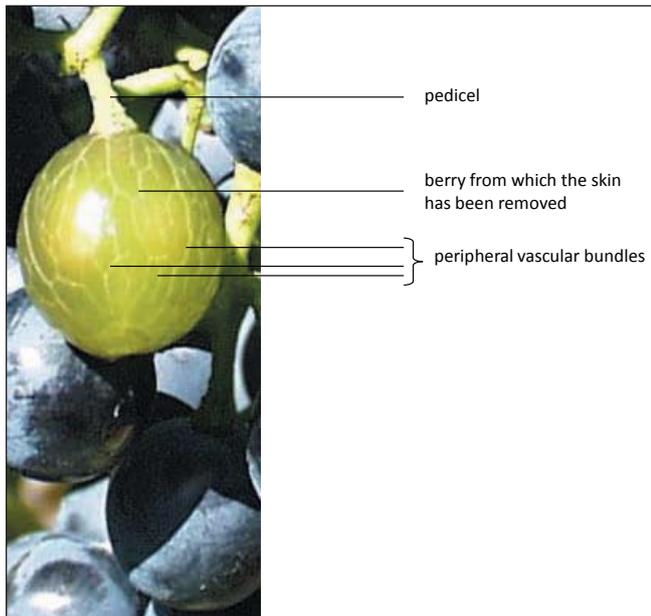


Figure 9. Peripheral vascular bundles of the berry. Essentially sugar phloem unloading into the berry, as derived from the plant (photosynthesis), takes place by means of the peripheral vascular bundles. The transportation of the sugars is essentially active, in other words apoplastic. In that regard, the bunch microclimate plays an essential role in berry functioning and composition.



Figure 10. Measurement of the temperature of berries using captors which are inserted between the skin and the pulp. Data loggers permit the automatic acquisition of temperature values. Cultivar Syrah (South Africa, vineyard in Stellenbosch, at the ARC-Infruitec-Nietvoorbij). The direction of the rows was north - south (Deloire & Hunter, 2005).

tions of 1 M at maturity (Ollat *et al.*, 2002; Terrier, 1997; Hawker, 1969). Sugars are accumulated 10 times quicker than respiration enables them to oxidise. The accumulation of sugars in the grape does not require much energy (Ollat & Gaudillère, 2000).

Saccharose, which enters the berry through the phloem of the peripheral vascular bundles, is broken down into glucose and fructose through parietal (apoplasma) and vacuolar invertase. Molecular biological studies (Davies & Robinson, 1996) have shown that the induction of the expression of ARNm code for the invertases takes place before véraison and that the activity of these enzymes is constant during maturation. These enzymes do not restrict the accumulation of sugars.

After véraison neoglucogenesis from malate onwards only accounts for 5% of the accumulation of hexoses (Hardy, 1968). The accumulation of sugars depends on the source (photosynthesis) and sinks activity of the berry. The microclimate of the grapes is an essential factor in the functioning of the berry which is able to regulate its physiology (Figures 10 & 11).

### Transportation of the sugars

The accumulation of sugars during maturation may occur through symplastic or apoplastic pathways (Fillion, 1997; Delrot & Bonnemain, 1989; Delrot, 1987). Little is known, however, about the organisation of these apoplastic and symplastic processes of the sugars, from the sieve tubes (phloem of the peripheral vascular bundles) to the skin and pulp cells. The xylem from the peripheral vascular bundles of the fruit loses its functionality after véraison (Düring *et al.*, 1987; Ollat, 1997) and that the phloem ensures transportation of essentials during maturation of the grape (Figure 9). Several studies have shown the predominance of apoplastic transportation of sugars to the berry's cells (Fillon, 1997).

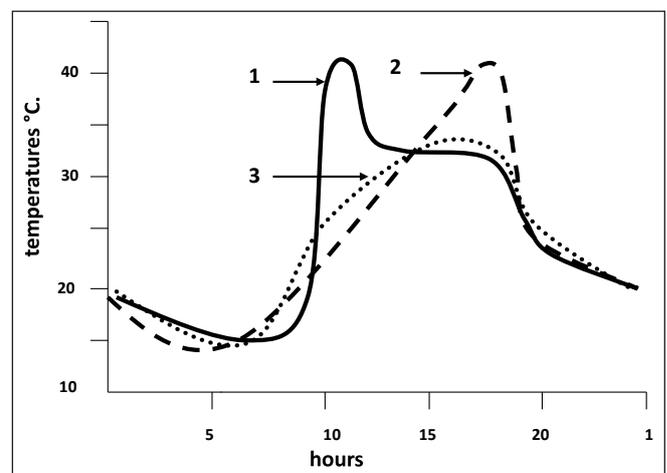


Figure 11. Example of the evolution of temperature in berries (measured between the skin and the pulp). Cultivar Syrah (South Africa, vineyard in Stellenbosch, at the ARC-Infruitec-Nietvoorbij). The direction of the rows was north - south. 1 - East side of the canopy; exposed bunches. 2 - West side of the canopy; exposed bunches. 3 - Inside the canopy; fully shaded bunches. The temperature of the exposed berries may increase from 20°C to reach 40 to 45°C in 30 mn. After midday the decrease in temperature is slow and related to the air temperature and humidity (Deloire & Hunter, 2005). In a cool or temperate climate, or for vineyards under the influence of the sea breeze effect in the Western Cape area, it should be possible to open the canopy at the bunch level, to get more exposed bunches, as the wind will cool down the bunch temperature, thus the light effect will prevail. This could allow to create diversity in canopy management, choice of training system and could allow to increase the diversity in wine style (Deloire *et al.* 2010, unpublished).

## Berry development – An overview. Part 7. Berry and organic acids

### Primary Metabolism: Organic Acids

#### Acidity

The grape is known to be an acidic fruit. At *véraison*, the acidity level is approximately 450 mEq and the pH 2,5 to 2,7. At maturity – harvest, the pH is 3,5, or more, depending on cultivar and climatic conditions. Malic acid and tartaric acid and their acid-base balance with the predominant cation of the grape, potassium ( $K^+$ ), are responsible for 90% of the acidity. The two acids are mostly biosynthesised in the berry, they are not mainly imported from the stem-leaves complex (Ruffner, 1982a, b). The final concentrations of tartrate and malate generally amount to between 30 and 50 mM. Citric acid, the third major acid, occurs in a comparatively weaker concentration, at about 10 mM. The other organic acids (succinic, ...) occur at a concentration below 1 mM (Terrier, 1997). At the sub-cellular level, these acids are stored in vacuoles.

At a technological level, the acid/sugar relationship and the malic/tartaric/ $K^+$  balance, are important parameters of the quality of the harvest and the wines. The berry acids impact on the acidity of the wine, the colour, the sensorial qualities and the hygienic condition of the wines. An excess of  $K^+$  and malate in relation to the tartrate content results in the alkalisation of wine after malolactic fermentation. This in turn leads to poor physical and biological stability in wine. Technical solutions include: sulphiting for microbiological quality, ultra filtration, electrodialysis, or the addition of tartaric acid. The addition of tartaric acid occurs frequently in warm climates in red as well as white wines.

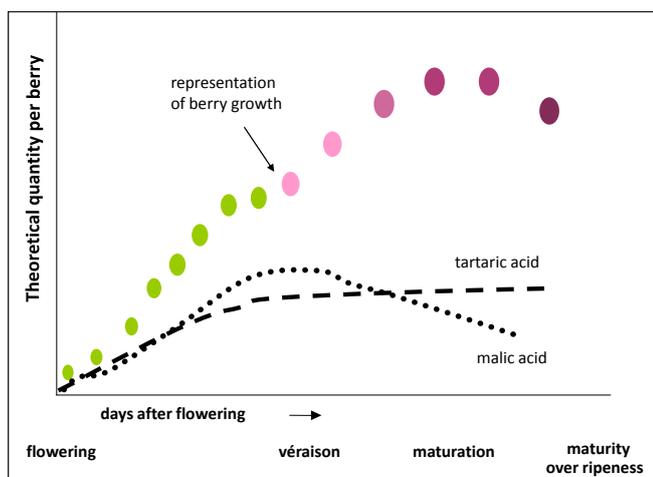


FIGURE 12. Evolution of the principal organic acids per berry (theoretical quantity). Tartaric acid is biosynthesised before *véraison* and, during maturation, the quantity thereof per berry remains stable. However, this organic acid could be catabolised for temperature  $\geq 35^{\circ}C$ . Malic acid is biosynthesised before *véraison* and, during maturation, it is degraded through respiration, consequently the quantity thereof per berry diminishes.

The malic/tartaric relationship depends on cultivar, climatic conditions and cultural practices (microclimate of the bunches and the canopy). The amount of potassium in the berry depends on the fertilisation conditions of the vine in relation to the rootstock (vs. root functioning), the soil water content and vine water status (Etchebarne *et al.*, 2009), even though the berry's reaction to potassium fertilisation of the soil is not immediate, unlike foliar fertilisation. Titratable acidity in the berry and its pH at the herbaceous stage are physiologically constant in the vine according to Peynaud and Ribéreau-Gayon (1971). There are nevertheless some exceptions, such as Gora Chirine, which has weak acidity (Boubals *et al.*, 1971).

The origin of malic and tartaric acids in the berry is disputed: in situ biosynthesis and/or imported from the plant, especially the leaves? The malic acid concentration of adult leaves starts decreasing when the ovary begins to grow (stage 27 on the Lorenz scale, 1995: 3 mg/g fresh weight) until the "pea size" stage of the berry (stage 29: 1,5 mg/g fresh weight), then increases in the course of maturation (4 to 5 mg/g fresh weight). The tartaric acid concentration of leaves has an inverse evolution (stage 27: 2 to 3 mg/g fresh weight; stage 29: 8 mg/g fresh weight; maturation: 2,5 to 1,5 mg/g fresh weight). Tartaric acid is formed in young leaves from ascorbic acid. These observations were made in Gewürztraminer/101.14 in South Africa by Hunter and Ruffner (2001). The organic acids are not necessarily imported by the berry, which is capable of biosynthesising them in situ.

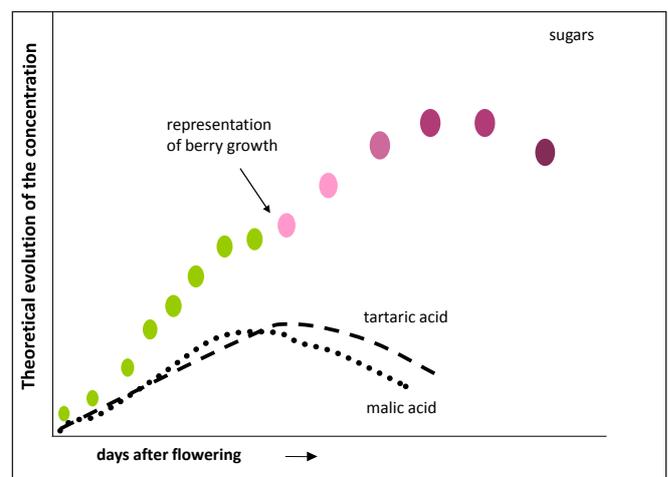


FIGURE 13. Evolution of the principal organic acids in concentration (theoretical quantity). Tartaric acid is biosynthesised before *véraison* and, during maturation, the quantity thereof in concentration decreases because of berry volume increases. Malic acid is biosynthesised before *véraison* and, during maturation, it is degraded through respiration, irrespective of the berry's volume.

## Tartaric acid

Tartaric acid is a strong di-acid, it accumulates through biosynthesis in the berry in the course of herbaceous growth until concentrations of 100 mM are reached (Figure 12 & 13). There is a relationship between the development (i.e. acquisition of new cells) of the young leaf and the young fruit and the accumulation of tartaric acid (Hale, 1962). From véraison onwards and during maturation of the grape, the amount of tartaric acid in the berry is stable, subsequently its concentration diminishes when the berry increases in volume (dilution effect). At maturity, the concentration may be relatively high, about 50 mM. This organic acid could be catabolised for temperature  $\geq 35^{\circ}\text{C}$ . In 2009, a comparison of tartaric acid evolution in Sauvignon blanc berry from warm (Paarl) vs. cool (Elgin) regions of the Western Cape area has shown a decrease, on a per berry basis and in concentration, for the warm regions (Deloire *et al.*, 2009). Hale (1977) noted in Sultanina that at maturity the concentration of tartrate in the skin was greater than in the flesh (practically double in % of the dry weight). The tartrate precursors are possibly glucose, glycolate and ascorbate (Hunter & Ruffner, 2001; Peynaud & Ribéreau-Gayon, 1971). The pathways of biosynthesis are not well-known, however, the first gene encoding an enzyme in the biosynthesis of tartaric acid was recently identified.

## Malic acid

Malic acid is accumulated through biosynthesis during herbaceous growth; subsequently its quantity per berry as well as its concentration diminishes in the course of maturation (Figure 12 & 13). The malate is formed from the start of b-carboxylation of phosphoenolpyruvate (PEP) derived from glycolysis, in other words glucose requirement. Oxaloacetic acid formed by PEP carboxylase is reduced to malate by malate dehydrogenase enzyme. The principal malate degradation route during the grape's maturation is through respiration. The degradation of malate from véraison onwards is associated with a relative loss of tightness in the vacuolar membrane from véraison (Terrier & Romieu, 2001). Malate thus released to the cytoplasm is degraded to pyruvate by means of malic enzyme (Ruffner, 1982). The pyruvate is incorporated into the Krebs cycle or converted into acetaldehyde through decarboxylase pyruvate. The acetaldehyde is converted into ethanol by alcohol dehydrogenase (Tesnière & Verries, 2000). Hale (1977) does not show the difference in malate concentration in the skin and flesh respectively at maturity in Sultanina.

# Berry development – An overview. Part 8. Berry and phenolic compounds

## Secondary metabolisms: Phenolic compounds

Even though much has been written about the phenolic compounds of the grape, a fair amount remains unknown (Ribéreau-Gayon, 1964; Cheynier *et al.*, 1998; Cheynier, 2006). Table 1 summarises the principal phenolic compounds of the berry. The

two best known groups are anthocyanins which are responsible for colouration of grapes and wines, and flavan-3-ols (tannins) which impart structure and astringency to wines. The evolution of the anthocyanins and the tannins, both in concentration and per berry, is described in Figures 14 and 15.

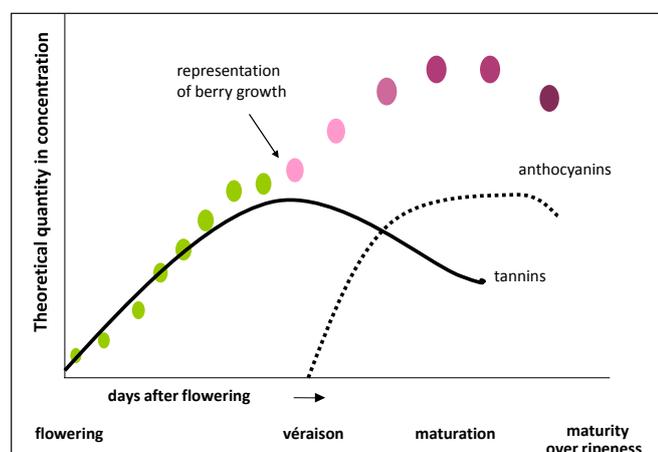


FIGURE 14. Evolution of the concentration of principal tannins and anthocyanins. The tannins are biosynthesised before véraison and the concentration thereof diminished during maturation because the volume of the fruit increases. The anthocyanins are biosynthesised from the onset of véraison.

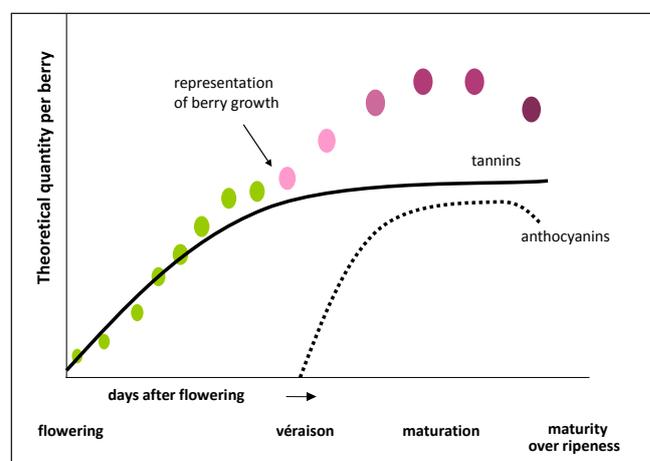


FIGURE 15. Evolution of the principal tannins and anthocyanins per berry. The tannins are biosynthesised before véraison and the quantity thereof per berry is stable during maturation, independently of the volume of the fruit. The anthocyanins are biosynthesised from the onset of véraison.

TABLE 1. The principal phenolic compounds of the berry, according to Cheynier *et al.*, 1998.

Compounds	Phenolic		Localisation in the tissue
Non-flavonoid compounds	Phenol acids	Hydroxycinnamic acids present as tartaric esters (cafeoyl tartaric acids, p-coumaroyl tartaric and feruloyl tartaric)	Skin cell vacuoles and pulp
		Benzoic acids (mainly gallic acid)	
	Stilbenes	Resveratrol	
Flavonoids	Anthocyanins	Anthocyanidins: cyanidin, peonidin, delphinidin, petunidin, malvidin, (in the form of glucosylated and eventually acylated derivatives)	Skin cell vacuoles and pulp for red cultivars
	Flavan-3-ols (3-flavanols) = tannins	Monomers (catechins, gallicocatechin, epigallocatechin)	Seeds, skin and pulp
		Oligomers and polymers or proanthocyanidins (procyanidins, prodelphinidins)	Seeds, skin and pulp
	Flavonols	Campherol, quercetol, myricetol, isorhamnetol	Skin
Flavanonols	Engeletin, astilbin	Skin	

Phenolic compounds play a major role in wines as well as intervening in the development and defense of plants. Their biosynthesis in the berry depends on the environmental conditions, notably water (hydric constraint or stress) (Figure 16) and the bunch microclimate (temperature and light). A study on Pinot noir has shown that 11% of the grape tannins are in the skin and 89% in the seeds. A small portion of the grape tannins are extracted during maceration (9% in this example) and the maceration favoured the skin tannins extraction (29%) over seed tannins (6%). Therefore, in this example on Pinot noir, the wine tannin proportion was 36% from skin and 64% from seeds (Kennedy, 2008).

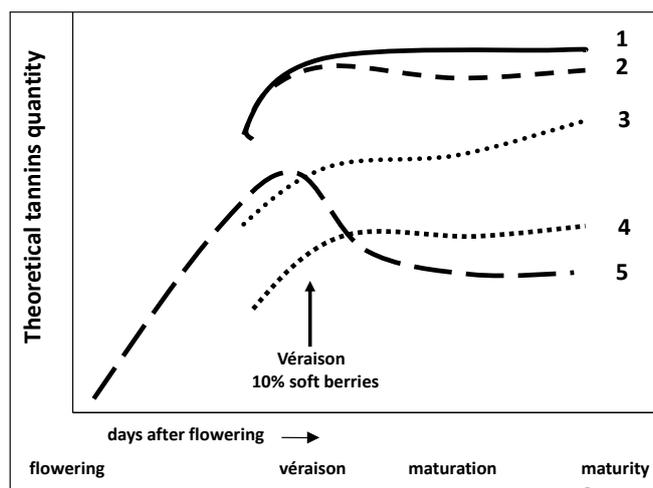


FIGURE 16. Example of the evolution of tannins per berry (1, 2, 3 and 4) and in concentration (5) of vines submitted to different levels of water constraint before or after véraison. The experimentation was done on Shyras (Ojeda *et al.*, 2002).

- 1 – Control without water constraint.
- 2 – Moderate water constraint from the beginning of véraison (arrow) to maturity.
- 3 – Moderate water constraint from flowering to véraison (suppression of the constraint at véraison).
- 4 – Severe water constraint (even water stress) from flowering to véraison (suppression of the constraint at véraison).
- 5 – The results showed a comparable evolution of the tannin concentration for the treatments 1, 2, 3 and 4, because the tannin concentration is related to the quantity of tannins per berry and the volume of the berry. Only the quantity of tannins per berry has been affected by the treatments and possibly the structure of the tannins (polymerisation or galloylation), which could result in wine astringency.
- Water constraint after véraison (treatment 2) does not modify the biosynthesis of tannins, as they are mostly already biosynthesised before véraison. Water constraint and stress (treatments 3 and 4) applied before véraison disturbed the biosynthesis of tannins.

## Berry development – An overview. Part 9. Berry and aromatic compounds

### Secondary Metabolisms: Aromas

Wine aromas have been described by Bayonove *et al.*, 1998. They derive from a complex association of volatile compounds which interact with other components of wine, such as phenols, proteins, ethyl alcohol, organic acids and polysaccharides. Of the 900 volatile compounds that have been identified in wine, only 10% contribute to aroma. The aromatic potential of the grape consists of 2 groups of compounds depending on the cultivar: i) non-volatile aroma precursors, non-odourants (glycosides, fatty acids, phenolic acids, ...); ii) volatile odourant compounds (terpenols, C<sub>13</sub> norisoprenoids, ...).

### Volatile odourant compounds

Volatile odourant compounds deriving from cultivar-specific aromas belong to two chemical families: pyrazines and terpenols (Table 2).

#### Pyrazines

Specifically 2-alkyl-3-methoxypyrazines (so-called “green pepper” aroma). Pyrazine concentration in the berry varies depending on the degree of maturation; from véraison onwards it decreases. Climate determines its concentration in the berry; generally the grape has a greater pyrazine concentration in colder climates and less concentration in warm climates. The microclimate of the grapes likewise plays a determining role: shaded grapes have more pyrazines than exposed grapes (light and temperature). The practice of breaking out leaves may impact largely on the concentration of pyrazines responsible for the green pepper aroma in grapes (Marais *et al.*, 1996 & 1999; Hunter *et al.*, 2004; Schneider *et al.*, 2004). Such climatic factors may result in important variations in the same cultivar. Yield does not appear to impact on pyrazine levels (Allen & Lacey, 1993). Its

biogenesis in the plant is associated with the metabolism of amino acids: leucine, isoleucine, valine and glyoxal (Murrey & Whitfield, 1975).

#### Monoterpenols

Monoterpenols (terpene alcohol at 10 carbon atoms) are characteristic of very specific cultivars such as the Muscats or Gewürztraminer, but also occur in the aromas of wines made from several cultivars. Terpenoids are formed by the condensation of 2 units with an isoprenic base of 5 carbon atoms. In Muscats, the 3 major monoterpenols (geraniol, nerol, linalool) represent 40 to 50% of the dosable volatile substances (quantities of 500 to 1700 µg/L of juice), (Gunata *et al.*, 1985a). In Muscat berries, the free monoterpenols are situated in the pericarp, and reappear in the musts: for example 90% of free geraniol and nerol occurs in the skin and 50% of linalool occurs in the flesh (Gunata *et al.*, 1985b). It follows that the maceration period for certain white cultivars depends on the type of wine objective. The concentration of free monoterpenols is weak before véraison, 30 to 90 mg/Kg (Wilson *et al.*, 1984). Linalool for example only appears during véraison. The concentration of monoterpenols increases during maturation but until the stage of over ripeness is reached, its evolution is variable (for example Muscat de Frontignan). One should emphasise that the concentration of certain important monoterpenols (linalol, geraniol, nerol) diminishes in relative proportions in the juice of grapes that have been attacked by *Botrytis cinerea* (Cordonnier, 1987).

#### Aroma precursors

Non-aromatic cultivars may make wines that are high in aromatic quality. Such aromatic qualities probably derive from non-odorous precursors which are characteristic of the cultivar, namely aroma precursors (Table 3).

TABLE 2. Volatile compounds of cultivar-specific aromas belong to two chemical families: pyrazines and monoterpenols.

Volatile compounds	Molecules	Cultivars	Tissue localisation	Aromatic notes
Pyrazines	2-methoxy-3-isobutylpyrazine, 2-methoxy-3-isopropylpyrazine, 2-methoxy-3-isopropylpyrazine	Cabernet Sauvignon and Sauvignon family, Merlot ...	Pericarp	Green, herbaceous notes, green pepper, vegetal notes
Monoterpenols	Linalool, nerol, geraniol	Muscats, Gewürztraminer, ... Numerous red and white cultivars	Pericarp	Floral notes

## Glycosides

Glycosidic precursors constitute a widely divergent group of non-odorous compounds that have been identified in the grape (Cordonnier & Bayonove, 1974). They form the basis of a group of volatile compounds that are known for their overall contribution to wine aroma (Francis *et al.*, 1992; Francis *et al.*, 1996; Kotseridis, 1999; Schneider, 2001). These compounds consist of a glycosidic bond linked by a  $\beta$ -glycosidic volatile compound called aglycone. The sugar may consist of one or two sugar units (mono and diglycoside). In the grape glycosidic precursors are diglycosides, except for the  $C_{13}$ -norisoprenoids that have been identified. The aglycone section consists of volatile compounds belonging to chemical classes of non-terpenic alcohols, C6 compounds, volatile phenols, monoterpeneols and  $C_{13}$ -norisoprenoids. These chemical classes rearrange the compounds thereby resulting in an alcoholic, phenolic or acidic function to establish the glycosidic link.

The concentration in the grape amounts to a few mg/L for the so-called neutral cultivars (Schneider *et al.*, 2002; Ségurel, 2005), but may reach several dozen mg/L in the juice of Muscat, in which the monoterpeneol class predominates (Voirin *et al.*, 1990). In red or white cultivars distribution in the different parts of the berry varies, although the skin contains the biggest amount, between 60 and 75% (Wilson *et al.*, 1984; Gunata *et al.*, 1985a; Gomez *et al.*, 1994), hence the importance of pre-fermentary stages and of maceration on the extraction of these precursors (Mc Mahon *et al.*, 1999).

Glycosides appear in the berry at the onset of véraison and accumulate during maturation (William *et al.*, 1984; Wilson *et al.*, 1986; Marais, 1987; Park *et al.*, 1991). The hydrolysis of glycosides, resulting in the formation of odourant compounds, may be enzymatic or chemical. In the course of vinification enzymatic activities may be restricted by weak enzyme activity depending on winemaking conditions. On the other hand, pH acid of musts and wines allows chemical hydrolysis of glycosides. This process, which is mostly slow and depends on temperature, essentially takes place during bottle-ageing of wine

(Marais, 1983; Voirin *et al.*, 1990; Winterhalter, 1993). The aglycones which are thus liberated contribute either directly or following chemical transformation to the aroma of the wine (Francis *et al.*, 1992; Schneider, 2001; Ségurel, 2005).

## Cysteinic precursors

During fermentation yeast, assisted by enzymes of the S-b-lyase kind, liberates odourant thiols from the cysteine component by breaking the C-S link of the precursors (Tominaga *et al.*, 1998b). The transformation yields of the cysteinylated precursors (cf description below) by the yeast are poor: 0,06% to 0,6% for the P-4MMP (Murat *et al.*, 2001a), 0,7 to 2,5 % for the P-3MH (Tominaga & Dubourdieu, 2000). Yeast is likewise responsible for the formation of ac3MH through acetylation of 3MH.

4-methyl-4-sulfanylpentan-2-one (4MMP), 3-sulfanylhexan-1-ol (3MH) and acetate of 3-sulfanylhexyl (ac3MH), with extremely low perception thresholds of 0,8 ng/L, 60 ng/L and 4,2 ng/L respectively in a hydro alcoholic solution (Tominaga *et al.*, 2000), develop particularly strong odours in certain wines. The concentrations being between a few ng/L and 50 ng/L in Sauvignon wines, 4MMP contributes notes of boxtree and blackcurrant buds to the aroma of these wines (Darriet *et al.*, 1995).

3MH which has notes of grapefruit and passion fruit, and ac3MH which imparts notes of exotic fruits, have been described in wines of various cultivars (Tominaga *et al.*, 1996; Kotseridis & Baumes, 2000; Tominaga *et al.*, 2000; Schneider *et al.*, 2003; Fretz *et al.*, 2005). In wine their concentration ranges between several dozen ng/L and several hundreds of ng/L for ac3MH and a few mg/L for 3MH (Tominaga *et al.*, 2000).

4-methyl-4-sulfanylpentan-2-ol (4MMPOH) and other thiols have also been identified in wine, but these are not very interesting from an aroma point of view (Tominaga *et al.*, 1998a).

4MMP, 3MH and 4MMPOH exist in the berry of the grape as S-conjugates to cysteine: S-3-(hexan-1-ol)-L-cystein (P3MH), S-4-(4-methylpentan-2-one)-L-cysteine (P4MMP) and S-4-(4-methylpentan-2-ol)-L-cysteine (P4MMPOH) (Tominaga *et al.*,

TABLE 3. Aroma precursors.

Aroma precursors	Aromatic compounds	Varieties	Tissue localisation	Aromas
Glycosides	Phenols (zingerone, ...), -monoterpenols (linalool etc.) -C13-norisoprenoids ( $\beta$ -damascenone)	All	60 - 75% in the skin	Variable according to the aglycon structure and its derivatives – exotic fruit, kerosene, camphor, eucalyptus, cut grass
Cystenyl precursors	4-methyl-4-sulphanylpentan-2-one (4MMP)	Sauvignon, Muscat of Alsace, Gewürztraminer, Sémillon etc.	Skin, flesh	Boxtree, blackberry bud
	3-sulfanylhexan-1-ol (3MH)	Most	Skin, flesh	Exotic fruit, grapefruit, passion fruit
	3-sulfanylhexyle (ac3MH) acetate			
Dimethyl sulphide precursors	Dimethyl sulphide	Petit and Gros Manseng, Syrah, Grenache noir etc.	Not known	Fruity notes, truffle (Jura truffle) and black olive (wines of Syrah and Grenache noir)
Carotenoids	- $\beta$ -ionones - $\beta$ -damascenone -TDN	All	Plasts of skin cells (principally)	Violet, exotic fruit, petrol, tobacco, eucalyptus

1995). In the berry, P4MMP and 4MMPOH are distributed equally in the skin (Murat *et al.*, 2001; Peyrot des Gachons *et al.*, 2002). Maceration on the skin essentially affects the P3MH, so that the quantities that are recovered in the juice are multiplied by 2,5 compared to a classic vinification.

### Precursors of DMS

The structure of the precursor(s) of DMS (dimethyl sulphide) has not been identified in grapes nor in wine. According to Segurel *et al.* (2005), SMM (monomethyl sulphide) is probably the precursor of DMS. SMM, which is present in the grape, is likely transmitted to the wine where it disengages from the DMS through a chemical degradation reaction during preservation. Thus DMS, which has long been known to exist in wine, may form in the course of different stages during the vinification process and the preservation of wine. During fermentation the DMS may be liberated by the yeasts as soon as amine acids are formed or as derivatives such as cystine, glutathion and S-adenosylmethionine (De Mora *et al.*, 1986). The reduction of DMSO (dimethyl sulfoxide) into DMS by yeast is possible under winemaking conditions (Anocibar Beloqui, 1998), but DMSO is either absent, or present in the musts in very weak concentrations (Ségurel, 2005). Certain studies have shown the DMS levels to be very weak in young wines directly after bottling, but they increase with time and temperature during bottle ageing (Marais, 1979). Grape analyses that have been conducted up to now indicate a very heterogeneous DMS potential which is sometimes high (5 mg/L). Potential levels are very high in the berries of overripe Petit and Gros Manseng (Dagan, 2006), compared to potential levels encountered in the berries of technologically ripe Grenache noir and Syrah (Ségurel *et al.*, 2004). In Petit and Gros Manseng the variation in DMS levels depends notably on the ripeness of the grape, the cultivar and the vintage (Dagan, 2006).

### Carotenoids

Carotenoids, which are photo-protector pigments resulting from photosynthesis, have the same origin as monoterpenols, but

they are more polymerised because they contain 40 carbon atoms. In grapes they are considered to be biogenetic precursors of glycosides of C<sub>13</sub>-norisoprenoids (Winterhalter, 1992; Baumes *et al.*, 2002). At véraison a coding ARNm has been shown to be an enzyme capable of cleaving carotenoids resulting in C<sub>13</sub>-norisoprenoids (Mathieu *et al.*, 2005). In wine these C<sub>13</sub>-norisoprenoids have been identified in glycosyded as well as in free form. The free compounds derive from glycoconjugates, such as β-damascenone (complex odour of exotic fruits), as well as from carotenoids such as β-ionone. These free forms represent great aromatic diversity: 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN) (kerosene aroma of old Riesling); isomers of vitispirane (camphor and eucalyptus aroma); (E)-1-(2,3,6-trimethylphenyl) buta-1,3-diene (aroma of freshly mowed grass); β-damascenones (flowers and exotic fruits, plum jam, ...); β-ionones (violet), (Simpson, 1979; Strauss *et al.*, 1986; Etievant, 1991).

Cultivar, terroir and vintage impact on the carotenoid concentration in the berry, which ranges from 1 to 2 mg/Kg at technological ripeness, and on the C13-norisoprenoid glycosyded derivatives, the total values of which are about 10 times less (Gross, 1984; Razungles *et al.*, 1988). The carotenoids are situated in the cell plasts of the flesh and essentially in the skin. They are not very soluble in an aqueous environment, being lipophilic, they are absent in the juice, hence the interest in maceration with fermentation (Razungles *et al.*, 1988). Sun exposure impacts on the initiation of carotenoids (β-carotene, lutein, neoxanthin, neochrome and flavoxanthin) in the berries of the Syrah cultivar (Bureau *et al.*, 1998), notably at the herbaceous growth stage of the berry. These carotenoids subsequently diminish between véraison and ripeness, notably because of sun exposure, while the values of their derived glycosyded C13-norisoprenoids increase.

Generally speaking, little is known about the effect of abiotic factors (light, temperature, water) on the initiation of aromas in the berry. Such studies are presently being conducted at Stellenbosch University (DVO-IWBT Department) on white and red cultivars.

# Berry development – An overview. Part 10. Minerals, nitrogen and lipids

## Mineral elements and Nitrogen

### Mineral elements

Throughout its growth (herbaceous and/or maturation phases) the berry accumulates large amounts of nitrogen, potassium, calcium, phosphorus and magnesium (Figure 17). Nitrogen and potassium accumulate before and after véraison, whereas calcium, phosphorus and magnesium accumulate preferably before véraison, as shown by Schaller (1999), whose research applies to the Riesling cultivar. A recent study showed (Figure 18) that berry uptake of cations was not affected by leaf:fruit ratio, but mainly by grapevine water status (Etchebarne *et al.*, 2009).

The distribution of mineral elements between the skin and the pulp varies depending on the cultivar and the circumstances (Tables 4 & 5).

### Nitrogen and proteins

During its growth the berry is an important sink for nitrogen. During the berry's herbaceous growth, nitrogen is consumed in the form of amino acids and polyamines (putrescine and spermidine), to support the multiplication and development of cells (Broquedis *et al.*, 1995). As soon as the bunch is formed, the total amount of polyamines per berry decreases, going from 300 - 400 µg/g of dry matter to less than 100 µg/g of dry matter at the onset of véraison. During ripening of the grape, the berry continues to consume nitrogen for the biosynthesis of amino acids. The total amount of nitrogen and the various amino acids depends on the nitrogen fertilisation of the vine and the viticultural practices

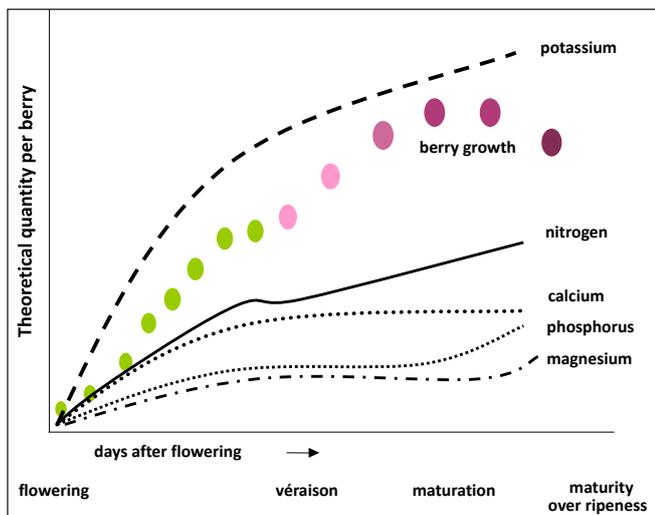


FIGURE 17. Example of the evolution of the principal mineral elements of the Riesling cultivar (according to Schaller, 1990). Theoretical curves indicate trends in evolution, before and after véraison, as well as the principal mineral elements per berry (potassium, nitrogen, calcium, phosphorus and magnesium).

(concurrency with cover crop, source-sink relation). The predominant amino acids are proline and arginine.

A study (Delas, 2000) conducted on Merlot which had been grafted onto SO4 made it possible to compare: 1) an “over-fertilised modality” at 100 Kg/N/ha/yr and 2) a modality at 0Kg/N/ha/yr. The results indicate for 1) and 2) respectively that the following amino acids are present in musts: aspartic acid

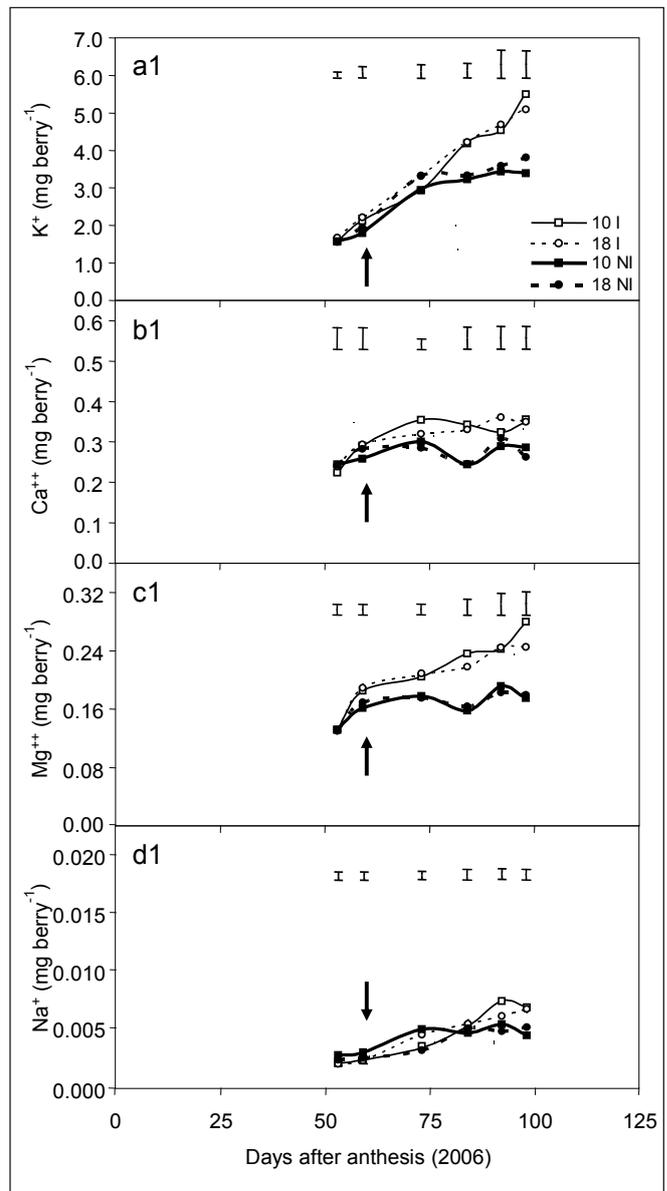


FIGURE 18. Evolution of cations (K+, Ca++, Mg++ and Na+) in the berry of Grenache noir, expressed in mg berry<sup>-1</sup>, during the post fruit set to ripening period. The interaction between the primary shoot leaf area (10 and 18 leaves per primary shoot – one bunch per primary shoot) and the vine water status (irrigated and non-irrigated) was compared. The results showed that berry uptake of cations was not affected by leaf:fruit ratio, but by grapevine water status (the arrow indicates véraison) (Etchebarne *et al.*, 2009).

(47 - 35 mg/l), threonine (71 - 36 mg/l), serine (39 - 40 mg/l), glutamic acid (114 - 97 mg/l), proline (1 574 - 881 mg/l), alanine (120 - 85 mg/l), valine (32 - 26 mg/l), acid  $\delta$ -aminobutyric (67 - 58 mg/l), arginine (731 - 330 mg/l). These studies confirm that the predominant amino acids are proline and arginine.

From the onset of véraison new proteins are synthesised in the berry. These are mostly defensive proteins (Pathogenesis proteins, PRs): chitinases, glucanases and thaumatine-like proteins (Robinson & Davies, 2000; Kraeva *et al.*, 1998).

### Lipids

According to Roufet (1986) the distribution of lipids in the bunch (stalk + berries) is: 75% in the seeds; 15% in the skin; 9% in the pulp and 1% in the stalk. The technological and organoleptic occurrence of lipids in wines is treated by Cabanis and Flanzy (1998). The cuticular wax of the grape, known as the bloom, consists 50% of D-oleanolic acid (Girncarevic & Radier, 1971). During véraison the surface content ( $\mu\text{g mm}^{-2}$ ) of the cuticular wax decreases and stabilises at the end of ripening (Rogiers *et al.*, 2004).

TABLE 4. Quantities of principal mineral elements per berry at maturity (mg/berry), example of Riesling in Germany according to Schaller, 1999.

Major mineral elements	mg/berry (pericarp at maturity)
Total nitrogen	1,5 - 2
Potassium	2 - 2,5
Calcium	0,4 - 0,5
Magnesium	0,14 - 0,16

TABLE 5. Example of the potassium concentration in the berry at maturity given in mg/g of dry matter, average of various cultivars, according to Mpelasoka *et al.*, 2003.

Organs of a berry	Potassium (mg/g fresh mass)
Skin	4.76 - 8.82
Pulp	1.29 - 2.88
Seeds	2.21 - 3.29