GRAPE BERRY DEVELOPMENT : A REVIEW

REVUE BIBLIOGRAPHIQUE : DÉVELOPPEMENT DE LA BAIE DE RAISIN

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Abstract : Grape berry development is reviewed with special focus on berry growth, structure, substances imported, organic acid and sugar metabolism. Berry growth is divided into two growth periods. Berry structure and ultrastructure are adapted to sink function. Exocarp cells are characterized by intensive metabolic capacities, flesh cells by a storage role. Early growth is highly sensitive to internal and external parameters. Berry size is largely defined during the first growth period. After « véraison », the berry becomes a major storage sink. Many changes occur in berry metabolism and gene expression. Genomic researches are promising to elucidate the mechanisms of berry development.

Résumé : Cette synthèse bibliographique est relative au développement de la baie de raisin. Les principales caractéristiques de la croissance, de la structure, des importations, et du métabolisme des acides organiques et des sucres sont abordées en insistant sur les travaux récents qui ont permis une meilleure compréhension des mécanismes. La croissance de la baie suit une double sigmoide et peut être divisée en deux périodes. Le début de la maturation appelée véraison coïncide avec le début de la deuxième période de croissance. La structure et l'ultrastructure de la baie sont liées à sa fonction de puits. Les cellules de la pellicule sont caractérisées par la présence de plastes, de vacuoles contenant des composés phénoliques et de nombreuses mitochondries bien développées, ce qui révèle une intense activité métabolique. Les cellules de la pulpe ont une très grande vacuole, correspondant à leur rôle de stockage. Pendant la première période, la croissance est déterminée par le nombre de pépins, l'action des régulateurs de croissance, les relations source-puits et les paramètres climatiques. A ce stade, la baie présente une respiration élevée nécessaire à l'utilisation des métabolites. L'eau est principalement importée par le xylème. Le malate est synthétisé dans le cytoplasme des cellules et accumulé en grande quantité dans les vacuoles. Le saccharose, importé par la sève phloémienne, est métabolisé pour assurer le fonctionnement cellulaire. La taille potentielle des baies à maturité est largement déterminée pendant cette première période de croissance.
Après la véraison, la baie devient un puits de stockage. Les importations de carbone augmentent fortement. L'eau est alors principalement d'origine phloémienne. La continuité du tissu xylémien, notamment au niveau des faisceaux vasculaires périphériques, est apparemment interrompue. A ce stade, de nombreux changements métaboliques interviennent. Pendant la maturation, la dégradation du malate est forte. Une augmentation de la perméabilité du tonoplaste, ainsi que les mécanismes de régulation de la phosphoenolpyruvate carboxylase pourraient être responsables des variations de la teneur en malate après la véraison. Le saccharose est alors hydrolysé en hexoses qui s'accumulent en grande quantité dans le vacuole des cellules de la pulpe. Ces modifications du métabolisme du saccharose pourraient être liées à l'activité de transporteurs de sucres dont l'expression est stimulée à la véraison. Les bases moléculaires de nombreux aspects du développement de la baie commencent à être connues, même si le facteur déclanchant la maturation n'est pas encore identifié. Des recherches en génomique sont en cours et pourraient aboutir rapidement à une compréhension plus complète des mécanismes contrôlant le développement de la baie de raisin.

Key words : *Vitis vinifera*, berry, growth, anatomy, ultrastructure, composition, organic acids, sugars, phloem transport, gene expression.

Mots clefs : Vitis vinifera, baie, croissance, anatomie, ultrastructure, composition, acides organiques, sucres, transport phloémien, expression des gènes.

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J. Int. Sci. Vigne Vin, 2002, **36**, n°3, 109-131
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INTRODUCTION

The grape berry is a fleshy fruit developed from the various tissues of the ovary walls (ESAU, 1965). It is characterised by a large accumulation of water and organic compounds, leading to its juiciness and attractiveness (COOMBE, 1976). The berries are attached on a cluster. There is a large heterogeneity of developmental stages and sizes between the berries of the same cluster (MAY, 2000). The grape berry can be roughly divided into three parts: i.e. skin (exocarp), flesh (mesocarp) and seeds. Each of these parts has its cell structure and biochemical properties. Berry growth is characterised by two active periods that are distinguished by metabolism type. During the first period, the berry builds up structure, develops seeds, produces and stores a large amount of organic acids in the vacuole of the pericarp cells. These vacuoles are characterised by a low pH. Ripening occurs throughout the second growth period. The onset of ripening is named «véraison» and marked by several metabolic changes. «Véraison» is also characterised by a sequence of events (COOMBE, 1992). Softening and the onset of hexose, i.e. glucose and fructose, accumulation are the first events. Approximately one week later, berry growth resumes. Finally, skin colour changes in red varieties. Ripening leads to the accumulation of large amounts of sugars and secondary products as aromatic compounds and polyphenolics such as anthocyanins. These biochemical changes are linked to significant modifications in gene expression (ROBINSON and DAVIES, 2000). Gene regulation is clearly involved in the triggering of ripening.

The aim of this paper is to present some published and unpublished data and to review the most recent literature on berry development. Firstly, berry growth and the effects of several internal and external parameters are described. Then, berry structure and some aspects of ultrastructure are detailed. Berry sink activity is studied with a particular focus on carbon and water balance, and on mineral nutrient imports. Finally, the most recent data on organic acid and sugar metabolism is summarised, and some information about the identification of the genes involved in berry metabolism is presented.

BERRY GROWTH

The grape berry growth follows, as do many other fleshy fruits, a double sigmoid curve (COOMBE, 1992). After flowering, growth is by cell division and cell expansion. Cell division peaks approximately eight days after flowering and ends 30 to 40 days after flowering for the skin cells (JONA and BOTTA, 1988). During the same period, berry DNA content increases regularly. Based on DNA evaluation, cell division is the main factor of berry growth during the first fortnight after anthesis. Afterwards, berry growth is mainly related to cell enlargement (OJEDA *et al.*, 1999). Before «véraison», growth slows down. It resumes rapidly at the onset of ripening and then, is only supported by cell expansion. Growth is intense at the beginning of ripening and slows close to maturity. For some varieties like Shiraz, the volume and fresh weight decrease slightly before maturity (MCCARTHY, 1999). This double sigmoid curve is commonly described as three distinct developmental stages: stage I: first active growth period, stage II : slow growth phase, stage III : second active growth phase (COOMBE, 1973). However STAUDT *et al.* (1986) propose that berry growth is divided in only two phases, the lag phase being simply the final part of the first growth period.

These two phases clearly appear when single berry growth is registered on a Cabernet Sauvignon cluster developing on a fruiting cutting in a growth chamber (OLLAT *et al.*, 1998, figure 1). Non-destructive measurements of diameter were performed on 20 tagged berries every two days from anthesis to maturity. Volume, fresh and dry weight were then computed from mathematical relationships established between

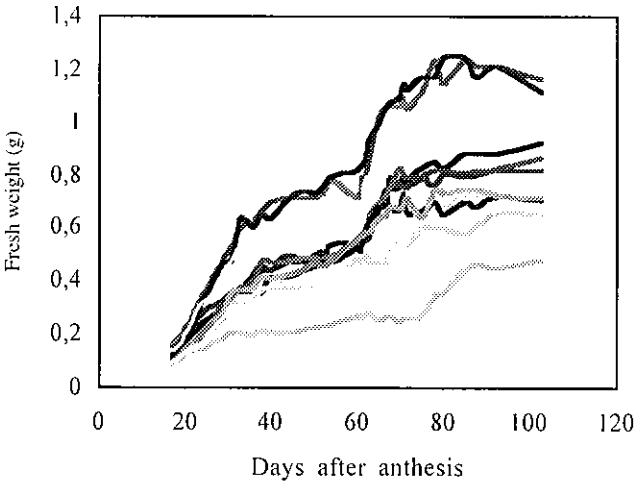


Fig. 1 - Single berry growth curves for ten berries of Cabernet Sauvignon growing on a single cluster on a fruiting cutting in a growth chamber (OLLAT *et al.*, 1998).

Fresh weights were computed from diameter measurements. «Véraison" occurred approximately 60 days after anthesis.

Courbes de croissance individuelles de 10 baies de Cabernet Sauvignon de la même grappe, développée sur une bouture fructifère en chambre climatisée (OLLAT *et al.*, 1998).

Le poids frais a été déduit de la mesure du diamètre. La véraison se situe environ 60 jours après la floraison.

that of two-seeded berries, 39.5 per cent, and that of three-seeded berries 8.3 per cent for both varieties (OLLAT, personal communication). The genetic inheritance of seedlessness is not clearly understood. BOUQUET and DANGLOT (1996) proposed that this trait is under the control of a major dominant gene, regulating several minor recessive genes of unknown function. Several RAPD (random amplified polymorfism detection) markers have been found to be correlated with seedlessness subtraits (STRIEM *et al.*, 1994). SCAR markers were identified (LAHOGUE *et al.*, 1998, ADAM-BLONDON *et al.*, 2001), however some evidence of quantitative inheritance remains (THIS *et al.*, 2000). Therefore, QTL (quantitative trait loci) analysis is in progress.

The seed effect on growth is mainly related to growth regulator supply (COOMBE, 1973). Gibberellins (GA) are considered to be the most important cell-enlarging hormones (LAVEE and NIR, 1986). The gibberellin-like substance concentration is correlated to the number of seeds and peaks during the first growth period (SCIENZA *et al.*, 1978). The practical use of GA to increase size of seedless berries is well known (WEAVER and MC CUNE, 1959). The size of seeded berries is usually not affected by GA treatments (WEAVER and POOL, 1965). SHIOZAKI *et al.* (1997) showed that pericarp cell development of a seedless berry after GA3 application follows a different course from that of a seeded berry. Treatments that modify source-sink relationships such as girdling and defoliation also affect GA concentration in grape berries (KANELLIS and ROUBELAKIS-ANGELAKIS, 1993). Finally, the growth retardant (2-Chloroethyl) trimethylammonium chloride (CCC) which was found to inhibit GA synthesis increases fruit set, but reduces berry growth and sugar accumulation (COOMBE, 1973; LOONEY, 1981).

The other groups of growth regulators appear to be more involved in development regulation rather than in growth control. Nevertheless, the application of cytokinins at bloom may cause an increase in berry size (LAVEE and NIR, 1986). The mode of action seems to be closely related to RNA and protein synthesis. In the berry, the concentration is low during most development stages and peaks just before the onset of ripening (KANELLIS and ROUBELAKIS-ANGELAKIS, 1993). Auxin concentration rises during the first growth phase, is maximal at the end of lag phase and drops during maturation. Exogeneous application of auxins at flowering has a positive effect on berry size, but a later spray mainly delays ripening. Recent work by DAVIES *et al.* (1997) showed that the delay of ripening after dipping clusters in benzothiazole-2-oxyacetic acid (an auxin like compound) six to eight weeks after flowering is associated with an abscissic acid concentration increase and some modifications in gene expression. Abscissic acid (ABA) concentration in the berries is low during most berry development but rises for a short period during the beginning of ripening and sugar accumulation. ABA may be involved in changing the cell wall permeability at «véraison», allowing carbohydrates and water to enter the exocarp cells more easily (LAVEE and NIR, 1986). Many analyses have showed that ABA may play a role in the ripening process of grape and phloem unloading (DURING and ALLEWELDT, 1984; COOMBE, 1992). It is likely that ABA in conjunction with auxins are crucial to the triggering and control of berry ripening by affecting the expression of genes involved in the ripening process (DAVIES *et al.*, 1997; ROBINSON and DAVIES, 2000).

II - SOURCE-SINK RELATIONSHIPS

Although the green berries are able to uptake carbon through photosynthesis and phosphoenolpyruvate carboxylase activity (PEPC, EC 4.1.1.31, BLANKE and LENZ, 1989), the carbon pool that supports berry growth and development is mainly translocated from the leaves and in some cases the woody parts of the vines (KOBLET and PERRET, 1982). There is strong competition between the different parts of the vines and it has been shown that the bunches are weak sinks until ripening commences (HALE and WEAVER, 1962). After «véraison», carbohydrates are imported into the berries with a higher rate and clusters become very competitive sinks. However, shoot growth during ripening may slow sugar accumulation in berries. At any stage, berry growth and development are affected by modification of carbohydrate supply. These modifications are commonly used to adjust the quantity grapes with leaf area in order to get the required grape composition. KAPS and CAHOON (1992) showed that berry weight follows source to sink ratio.

Berry set and size are mainly affected by carbon shortage during the early stages of development (COOMBE, 1962; BUTTROSE, 1966; KLIEWER, 1970). The effects of leaf removal on berry growth were studied at various stages on small fruiting cuttings (OLLAT, 1997). A large decrease in leaf area just after fruit set induces a reduction in berry growth. In this situation, KAPS and CAHOON (1992) showed that berry weight at maturity is related to the ratio leaf area/fruit weight. Berry size at maturity is proportional to berry size at «véraison» (figure 3) and it continues to be impaired, even if sufficient leaf area recovers during ripening. Mesocarp cell size is strongly reduced by early limitations of assimilate supply but the ripening process and hexose accumulation are not affec-

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the equatorial diameter and these parameters. OLLAT and GAUDILLÈRE (1998) have described these relationships. At ripening, Cabernet Sauvignon berry fresh weight lags in a range of 0.5 to 1.1 g. This observation illustrates the large heterogeneity between berries on a cluster.

Each growth parameter (fresh, dry weight, volume) follows a double sigmoid pattern. This double sigmoid curve can be described by two logistic equations, each of these equations as $W = Wf / [1 + (Wf - Wo) / Wo * \exp(-\mu * t)]$, where Wo and Wf are the initial and final values respectively, and μ the specific growth rate for each growth period (THORNLEY and JOHNSON, 1990). This mathematical expression enables the calculation of berry growth rate ($\mu * W * (1 - W/Wf)$) and relative berry growth rate ($\mu * (1 - W/Wf)$) (figure 2). The lag phase is not always characterised by a total cessation of growth, but often by a decrease in growth rate.

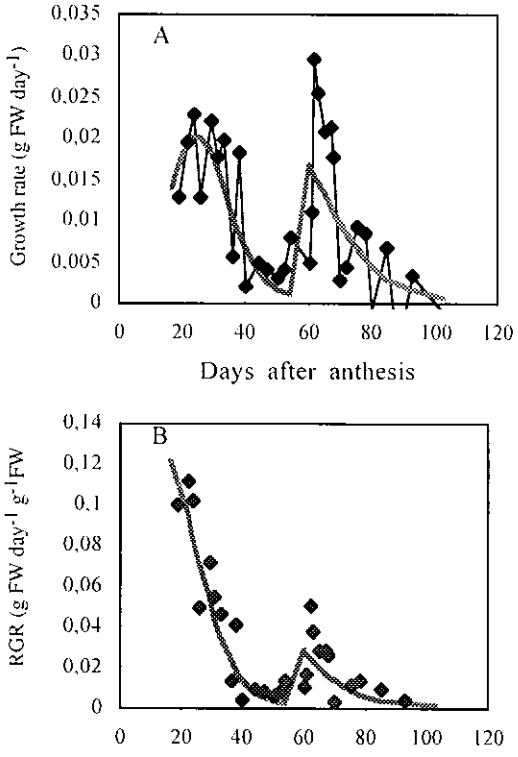


Fig. 2 - Berry growth rates (A) and relative growth rates (RGR, B) for Cabernet Sauvignon berries growing on a fruiting cutting in a growth chamber (OLLAT *et al.*, 1998).

These rates have been computed from experimental fresh weight data or deduced from mathematical logistic expression. "Véraison" occurred approximately 60 days after flowering.

Vitesse de croissance (A) et taux de croissance relative (RGR, B) pour des baies de Cabernet Sauvignon développée sur une bouture fructifère en chambre climatisée (OLLAT *et al.*, 1998).

Ces valeurs ont été calculées à partir des données expérimentales ou déduites du modèle logistique proposé. La véraison se situe environ 60 jours après la floraison.

The length of this lag phase is related to the triggering of maturation, but not to berry size. As PAVEL and DEJONG (1993) suggested for peach fruit, growth resuming at «véraison» is probably linked to the metabolic changes occurring at the onset of ripening. Recent work on differential gene expression confirms this hypothesis (ROBINSON and DAVIES, 2000). Berry growth rate is maximal at the beginning of the second growth phase. At «véraison», growth rates computed from the previously described equations underestimate experimental data. This sudden increase indicates that most of the berry cells start to enlarge at the same time at the onset of ripening. FANIZZA and COLONNA (1996) proposed another mathematical expression to describe growth curves for grape berries. However, these equations do not describe this maximal growth rate. «Post-véraison» berry enlargement is preceded by berry softening and sugar accumulation for several days (COOMBE and PHILLIPS, 1980). After «véraison», during the early phases of expansion, elasticity of the skin tissue drops, while berry deformability increases. Cell wall loosening may occur first in flesh tissues, allowing sugar accumulation, then in skin tissues, followed by berry expansion (HUANG and HUANG, 2001).

EFFECTS OF VARIOUS INTERNAL AND EXTERNAL PARAMETERS ON BERRY GROWTH

Potential berry size is a varietal feature and scion-rootstock interactions may slightly modify the potential berry size. Many other internal or external factors affect berry size including: number of seeds per berry; carbon nutrition; temperature; light; and, water supply.

I- SEED NUMBER AND GROWTH REGULATORS

The maximal seed number per berry is four, however the average seed number is less than two. It has been shown for some time that berry size is related to seed number (COOMBE, 1973). The relationship between berry size and seed number is not linear, but quadratic. The stimulation of pericarp enlargement induced by each seed decreases as the seed number increases (MAY, 2000). OJEDA *et al.* (1999) suggested that seed growth could have a positive effect on cell mitosis rather than on cell enlargement. However, seed number is not the only determinant of berry size variations between sites and years (MAY, 2000). The difference in berry size between varieties is also not strictly related to seed number. For example, seed number per berry for Merlot and Cabernet Sauvignon has been recorded in the Bordeaux area. Despite a difference in average berry fresh weight at maturity, of 2.0 and 1.6 g respectively, the percentage of one seeded berries is 52.5 per cent,

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without temperature modification, clusters grown without any light during the initial stages of berry growth present smaller berries and a delay in the onset of ripening (DOKOOZLIAN and KLIEWER, 1996). Normal berry development is not fully restored when the berries are exposed to light during maturation. Under controlled conditions, berry growth of Cabernet Sauvignon increases as cluster exposure to sunlight increases (DOKOOZLIAN (1990), cited by BERGQVIST *et al.*, 2001). However, in the vineyard, high light exposure is often related to high temperature and berry size is the result of opposite effects of both parameters (BERGQVIST *et al.*, 2001).

Water: Water budget is a main factor for organ enlargement, but it interacts largely with photosynthesis and berry carbon supply. Berry size is affected by water deficit, from anthesis to maturity. However, final berry size is more influenced by water deficits between flowering and «véraison» (BECKER and ZIMMERMANN, 1984, PONI *et al.*, 1994; OJEDA *et al.*, 2001). OJEDA *et al.* (2001) observed that water limitation does not affect cell division but decreases cell volume. This decrease is irreversible even if water is available during ripening.

Overall, it should be noted that berry growth between anthesis and «véraison» is more sensitive to internal and external parameters, and that the alterations of growth during this period are irreversible. It is also clear that berry potential size is highly defined at «véraison». Consequently, yield may be accurately predicted at this stage. Before «véraison», the grape berry is an utilising sink and berry growth is likely supported by cell general metabolism. After, this the berry becomes a storage sink and growth is most probably linked to sugar accumulation mechanisms. These differences might explain the varying sensitivity to external and internal parameters.

BERRY STRUCTURE

Histological and cytological studies of grape berries provide insights with respect to the contribution of different tissues to berry development and their metabolic role. Grape berries are representative of a number of fleshy fruits where the bulk of the organs consist of thin walled cells comprising a high volumetric proportion of vacuoles bounded by several layers of thick-walled collenchymatous cells (HARDIE *et al.*, 1996).

This structural organisation and the metabolic changes that occur during ripening do not favour an optimal preservation of cell ultrastructure when grape berry samples are immersed in a fixative mixture, especially at the later stage of development. This may help to explain why there is relatively little information on grape berry ultrastructure, especially «post-véraison». The anatomy and ultrastructure of the berry pericarp of Cabernet Sauvignon berries has been investigated during development by setting up an *in situ* fixation method that preserves the fine structure of both the thick walled cells of exocarp and the thin walled mesocarp tissues (DIAKOU and CARDE, 2001).

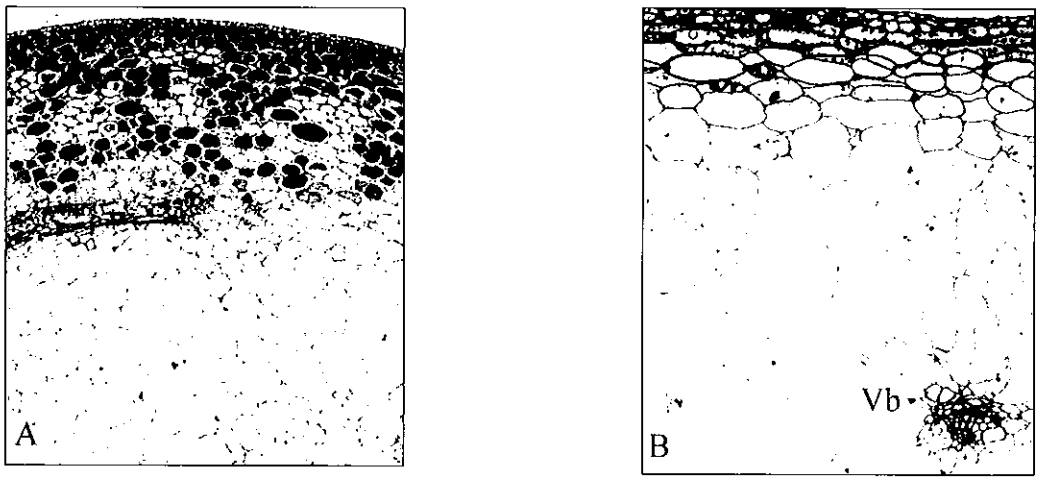


Fig. 4 - Evolution of grape berry structure during development. Semi thin section stained with toluidine blue.
A: «Pre-véraison» : pericarp cells are small and isodiametric.
B: «Véraison» : exocarp cells are tangentially elongated and the mesocarp cells are larger with an irregular shape. (from DIAKOU, 1999)

Évolution de la structure de la baie au cours du développement. Coupe semi-fine colorée au bleu de toluidine.
A : Pré-véraison : les cellules du péricarpe sont petites et isodiamétriques. **B** : Véraison : les cellules de l'epiderme sont allongées tangentiellement, et les cellules du mésocarpe sont plus grandes avec des formes irrégulières. (d'après DIAKOU, 1999).

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ted (OLLAT and GAUDILLÈRE, 1998, table I). When leaf removal occurs after «véraison», berry size is not significantly reduced, but sugar accumulation is impaired (BUTTROSE, 1966; KLIEWER, 1970; OLLAT, 1997; table III).

Cluster thinning is another way to modify source-sink ratio and is used as a management practice to decrease crop load. Cluster thinning tends to increase berry weight if performed early during the first growth period leading to a lower yield reduction (DOKOOZLIAN and HIRSCHFELT, 1995). After «véraison», cluster thinning has no effect on berry size.

III - CLIMATIC PARAMETERS

Temperature: as for other plant organs, temperature has a major effect on berry growth. HALE and BUTTROSE (1974) showed that a thermoperiod of 25/20°C (day/night) is optimal for berry growth and the last of berry development. Cooler temperatures do not impair final berry size but increases the length of the growth period because of a delay in the onset of ripening. Temperatures above 35°C during bloom-set period irreversibly reduce final berry size (KLIEVER, 1977). The first growth period is more sensitive to temperature than the ripening period. The berry size at the end of first growth period determines the potential for size increase during ripening.

Light: light and temperature are highly interactive because berry temperature in the field is largely determined by the flux density of absorbed radiation and convective heat loss (SMART and SINCLAIR, 1976) from the vines. If the light effect is studied artificially,

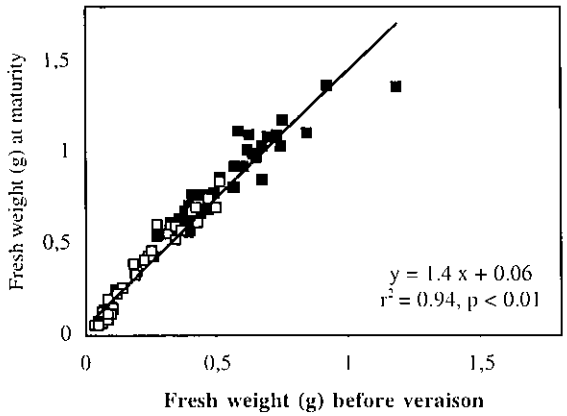


Fig. 3 - Relationship between single Cabernet Sauvignon berry fresh weight at the end of the first growth period and berry fresh weight at maturity.
The berries were grown on fruiting cuttings in a growth chamber (reprinted from OLLAT and GAUDILLÈRE, 1998; copyright 1998, with the permission of the American Journal of Enology and Viticulture).

Relation entre le poids frais d'une baie à la fin de la première période de croissance et à la maturité.
Les baies se sont développées sur des boutures fructifères en chambre climatisée (reproduit de OLLAT et GAUDILLÈRE, 1998 ; copyright 1998 avec la permission du Journal Américain d'Oenologie et de Viticulture).

Table I - Main analytical features at maturity for Cabernet Sauvignon berries submitted to various leaf to fruit ratio after fruit set or after "véraison" on fruiting cuttings in a growth chamber (OLLAT *et al.*, 1998).

Leaves were removed 17 days after anthesis for the first low leaf area treatment (low 1; 1 cm^2 per berry) or at the beginning of the second growth period for the second one (low 2.6 cm^2 per berry). In each experiment the results were compared to a sample of berries grown with a high leaf area (control, 19.5 cm^2 per berry during ripening).(n = 15, mean ± standard deviation, Student test, * : p<0.05; ** : p<0.01). Adapted from OLLAT and GAUDILLÈRE, 1998; copyright 1998 by the American Journal of Enology and Viticulture.

Principales caractéristiques analytiques des baies de Cabernet Sauvignon à maturité, soumises à différents rapports source-puits après la nouaison ou la véraison sur des boutures fructifères en chambre climatisée (OLLAT *et al.*, 1998).

Les feuilles ont été retirées 17 jours après l'anthèse pour le premier traitement (Low 1, 1 cm^2 par baie) ou au début de la deuxième période de croissance pour le second traitement (Low 2,6 cm^2 par baie). Pour chaque expérimentation, les résultats ont été comparés à ceux obtenus sur un échantillon de baies prélevées sur des plantes témoin (control, 19.5 cm^2 par baie pendant la maturation). (n = 15, moyenne ± écartype, test de Student, * : p<0.05; ** : p<0.01). Adapté de OLLAT et GAUDILLÈRE (1998) copyright 1998 du Journal Américain de d'Oenologie et de Viticulture.

	Low 1	Control 1		Low 2	Control 2	
Berry fresh weight (g)	0.54 ± 0.13	0.86 ± 0.16	**	0.76 ± 0.12	0.62 ± 0.22	*
Osmolarity (mosm L^{-1})	1662 ± 144	1653 ± 185		1412 ± 164	1845 ± 163	**
Titratable acidity (meq L^{-1})	119 ± 24.3	108 ± 30		102.5 ± 13.6	95 ± 11.7	
pH	3.51 ± 0.15	3.45 ± 0.09		3.45 ± 0.23	3.54 ± 0.16	
Malate (mmol L^{-1})	55.9 ± 18.5	39.4 ± 17.9	*	44.6 ± 11.6	35.8 ± 10.4	
Tartrate (mmol L^{-1})	42.4 ± 9.8	29.5 ± 4	*	26.1 ± 7.3	31.9 ± 6.1	*
Sucrose (mmol L^{-1})	14.3 ± 10.1	13.5 ± 6		14.5 ± 8.7	20.3 ± 10.8	
Glucose (mmol L^{-1})	509.1 ± 45	475.5 ± 43.3		400.5 ± 122.9	574.6 ± 41.3	**
Fructose (mmol L^{-1})	538.6 ± 50.9	472.7 ± 51.8	**	394.4 ± 121.6	580.4 ± 50	**
Potassium (mmoles L^{-1})	62.4 ± 12.2	43.4 ± 3.9	**	49.8 ± 15.9	63.8 ± 18.5	
Soluble nitrogen (mg L^{-1})	705.7 ± 120	617.2 ± 117		626.6 ± 163.6	710.1 ± 239	

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their cytoplasm contains fragmented vacuoles (figure 6a). In these vacuoles, the formation of new phenolic deposits is observed in contact with the tonoplast. The plastids contain an extensive system of parallel membrane thylakoids and a peripheral reticulum connected to the inner envelope membrane as well as a large array of plastoglobuli. The endoplasmic reticulum (ER) is less developed and the mitochondria are smaller (figure 6b). At the same stage the mesocarp cells consist mainly of a large vacuole (figure 6c). Some elongated chloroplasts are concentrated near the nucleus. The mitochondria are scattered, with a few cristae and a diluted matrix. The endomembranes are poorly developed. Cell walls are thin with local changes of texture related to the softening process. Some plasma membrane ingrowths are also evident as well as various membrane vesicles and small vacuoles enclosed in the cytoplasmic layer (figure 6d). They present a thin layer of cytoplasm (100-200nm thick) that is enclosed between tonoplast and plasma membrane. The presence of plasmodesmatal connections between adjacent mesocarp cells cannot be demonstrated.

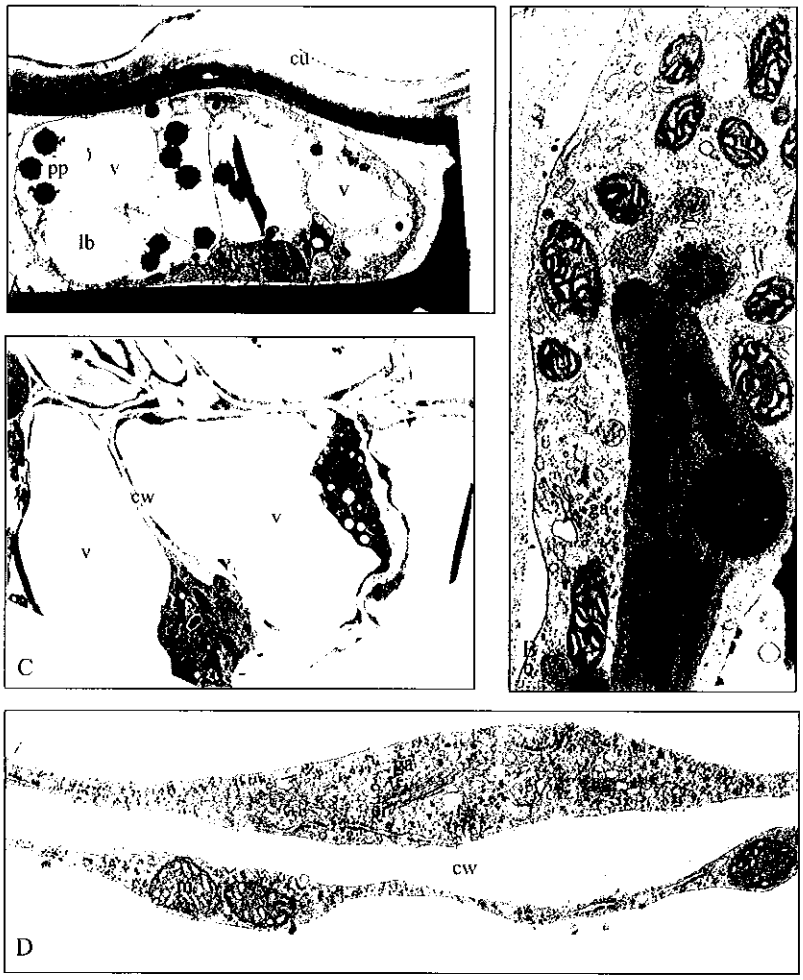


Fig. 5 - Ultrastructural organisation of grape berry cells at «pré-véraison» stage.

A: Epidermal cells with a fragmented vacuome. B: Details of epidermal cells showing dense mitochondria, plastids with large plastoglobuli and peroxysomes. C: Mesocarp cells with large clear vacuoles. D: Details of mesocarp cells showing with active dictyosomes and a well developed endoplasmic reticulum (A : x 7,3K, B: x 34K, C: x 4,6K, D: x 28,5K). (reproduced from «*In situ* fixation of grape berries», DIAKOU and CARDE, 2001, Protoplasma, 218, 225-235, figure 4; copyright notice of Springer Verlag).

Organisation ultrastructurale des cellules de la baie avant la véraison.

A : Cellules épidermiques avec un vacuome fragmenté. B : Détails des cellules épidermiques avec de nombreuses mitochondries, des plastes avec de grands plastoglobules et des peroxysomes. C : Cellules du mésocarpe avec de grandes vacuoles claires. D : Détails des cellules du mésocarpe avec des dictyosomes actifs et un réticulum endoplasmique bien développé (A : x 7,3K, B: x 34K, C: x 4,6K, D: x 28,5K). (reproduit de «*In situ* fixation of grape berries», DIAKOU et CARDE, 2001, Protoplasma, 218, 225-235, figure 4; copyright notice of Springer Verlag).

Abbreviations (figures 5 and 6) : cu: cuticle, cw: cell wall, er: endoplasmic reticulum, ga: Golgi apparatus, lb: lipid body, m: mitochondrion, n: nucleus, pl: plastid, pg: plastoglobule, pm: plasma membrane, pp: polyphenolics, pr: peripheral reticulum, px: peroxysome, v: vacuole.
Abbréviations (figures 5 et 6): cu: cuticle, cw: paroi cellulaire, er: reticulum endoplasmique, ga: appareil de Golgi, lb: corps lipidique, m: mitochondrie, n: noyau, pl: plastide, pg: plastoglobule, pm: membrane plasmique, pp: polyphénols, pr: reticulum périphérique, px: peroxysome, v: vacuole.

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I- EVOLUTION OF GRAPE BERRY STRUCTURE

The pericarp originates in the development of the grape ovary wall. The pericarp is composed of three distinct tissues: the skin or exocarp including cuticle, epidermis and hypodermis, the mesocarp that is highly developed in the fully grown berry and represents the flesh, and the endocarp constituted by a thin layer of cells in contact with the seeds. The relative rate of development of exocarp and endocarp changes during the course of berry growth.

The epidermis consists of a single layer of first isodiametric, then flattened cells, 6.5 to 10.0 µm thick (ALLEWELDT *et al.*, 1981). The epidermal cells are covered by a thick cuticle (CASADO *et al.*, 2001). Stomata are present in the young epidermis but are no longer functional after «véraison» (BESSIS, 1972; BLANKE and LEYHE, 1987). They contain vacuolar polyphenolic compounds which precipitate under different forms after tissue fixation (figure 4a) (RAJAEI 1987, FOUGÈRE–RIFOT *et al.*, 1993). However these forms can hardly be interpreted as representative of distinct molecular species (HARDIE *et al.*, 1996).

The hypodermis is composed of 11 to 12 cell layers at the «pre-véraison» stage. During the first growth period, these cells are small, isodiametric, with thin walls. Most hypodermal cells contain polyphenolic compounds. However, the hypodermis also contains a number of clear areas corresponding to cell clusters lacking polyphenolic deposits. Other hypodermal cells are filled with raphids of calcium tartrate (HARDIE *et al.*, 1996). During ripening, the outer cell layers of the hypodermis become tangentially elongated, while the inner layers enlarge with an irregular shape, their walls remain thin, and they look like mesocarp cells (figure 4b).

The mesocarp is formed of 25-30 cell layers. During the first growth period, these cells are isodiametric. During berry development, the mesocarp cells enlarge considerably with a correlative expansion of the central vacuole, which represents up to 99 % of the cell volume (DIAKOU and CARDE, 2001). Cell volume within the inner mesocarp tissue of a single grape berry exhibits a 14-fold range. Cell shape is irregular and the planes of contact are rarely flat or simple (GRAY *et al.*, 1999). At the same time, the mesocarp cell walls remain thin, but undergo significant compositional changes (NUNAN *et al.*, 1998).

The observation of sections of fixed tissues suggests that large changes occur to the vacuolar polyphenolic contents of pericarp cells, in the course of growth and ripening (PARK, 1995; HARDIE *et al.*, 1996a). At the same time, the polyphenolic content of mesocarp cells decreases as berries develop to ripening and in ripe berries, mesocarp cells are nearly lacking visible polyphenolics (CONSIDINE and KNOX, 1979).

The vascular system, which supports berry growth, is derived from that of the ovary (PRATT, 1971). It is made up of a peripheral network in the outer part of the mesocarp and a larger central strand in the columella that supplies the seeds. The vascular strands are composed of spiral tracheids, sieve cells and elongated cells (PRATT, 1971). At «pre-véraison», the peripheral vascular bundles are situated at the border between hypoderm and mesoderm (figure 4a). During ripening, as the inner cell layers of the hypodermis evolve, the vascular bundles are surrounded by mesocarp type cells (figure 4b).

The endocarp surrounds the locules and has often been considered to be part of the mesocarp. The walls of the endocarp cells are three times thinner than that of epidermal cells (HARDIE *et al.*, 1996b). Seeds consist of embryo, albumen and tegument. They are rich in phenolics and oil (WINKLER *et al.*, 1974).

II- ULTRASTRUCTURAL STUDY

An extensive analysis of grape berry ultrastructure was carried out by HARDIE *et al.* (1996). Using a novel fixation method, DIAKOU (1999) and DIAKOU and CARDE (2001) have described the fine structure of the grape berry cells in the course of development. The comparison of characteristic developmental stages shows that cell organization is tissue-specific and closely related to the function of the various tissues and their changes during the growth and ripening stages.

At «pre-véraison», the walls of exocarp cells are 1.5 µm thick. The cuticle is well developed. The cells contain a large number of vacuoles, irregular in size and most of them contain polyphenolics as dense spherical inclusions. The smaller vacuoles near the cell wall are electron translucent (figure 5b). The cytoplasm is dense and the endomembrane system well developed. Amoeboid chloroplasts that contain large plastoglobuli, mitochondria with well developed cristae and dense matrices as well as crystal-containing peroxysomes and large lipid bodies are present (figure 5b). At the same stage of development, the mesocarp cells are filled with one or two large vacuoles. Most of the cytoplasm and organelles are concentrated around the nucleus (figure 5c). Many active dictyosomes are present in the dense cytoplasmic layer, especially near the cell junctions. However, the cell walls remain thin, as in the previous stage (figure 5d).

At the «véraison» and «post-véraison» stages, the exocarp cells have thick cell walls (up to 3.0 µm) and

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cells in sugar storage and of exocarp cells in polyphenolic and aromatic production and storage.

An extensive knowledge of ultrastructural organization is a preliminary step in understanding the role of each tissue and cell compartment in the synthesis and accumulation of various products associated with berry quality that are important for wine production, including sugars, organic acids, terpenes and phenolic compounds. The resolution of technical problems associated with berry fixation for analysis purposes (DIAKOU and CARDE, 2001) makes it possible to immunolocalize the enzymes associated with these different metabolic pathways in berry sections. For example, immunocytochemistry with specific antisera was used by FAMIANI *et al.* (2000) to determine the location of some enzymes in order to relate structure and function in grape berry. Many enzymes show an extremely heterogeneous distribution. Some of them, PEPC, NADP-dependent malic enzyme and glutamine synthetase are associated with the flesh vasculature. This suggests that extensive metabolism of assimilates occurs during their delivery to berry tissues.

BERRY IMPORTS

Berry growth is supported by imports of carbon, water and mineral nutrients. The description of these imports provides some information about the compounds required to support berry development. It also increases understanding of the physiological mechanisms underlying these imports.

I- CARBON BALANCE

The carbon balance of berries is an important factor determining their quality (HO *et al.*, 1987). The main carbon sources for grape berries are the current photosynthates from leaves or in some cases, stored carbohydrates. However, carbon fixation by the berries through photosynthesis or non-photosynthetic PEPC activity contributes a small part (BLANKE and

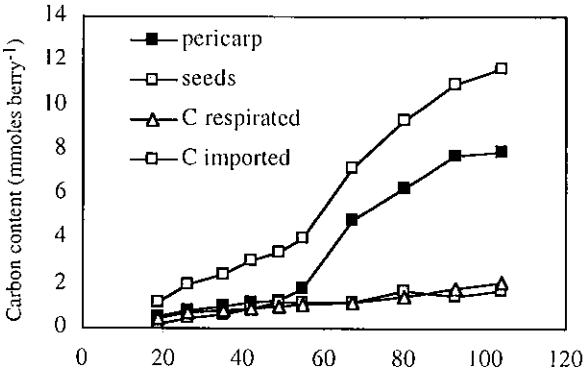


Fig. 7 - Carbon allocation to the main berry compartments and to respiration during Cabernet Sauvignon berry development.
The berries were grown on fruiting cuttings in a growth chamber. ■ Pericarp (skin and flesh), △ Carbon respirated by the whole berry, ❑ Carbon imported to the whole berry (reprinted from OLLAT and GAUDILLÈRE, 2000; with the permission of ISHS). «Véraison» occurred approximately 60 days after flowering.

Répartition du carbone entre les différents compartiments de la baie et la respiration pendant le développement.
Les baies de cabernet Sauvignon se sont développées sur des boutures fructifères en chambre climatisée. ■ Péricarpe (pellicule et pulpe), △ Carbone respiré par la baie entière, ❑ Carbone importé par la baie entière (reproduit de OLLAT et GAUDILLÈRE, 2000 ; avec la permission de la Société Internationale des Sciences de l'Horticulture). La véraison se situe environ 60 jours après la floraison.

Table II - Numeric data for xylem and phloem flow computed for individual berries of Cabernet Sauvignon growing on fruiting cuttings in a growth chamber (OLLAT *et al.*, 1998), measured at different stages of development (days after anthesis, DAA) with the technique described by GREENSPAN *et al.* (1996).
(mean ± standard deviation, n = 4 or 5). «Véraison» occurred at 60 days after anthesis.

Valeurs numériques des flux xylémiens et phloémiens déterminées pour des baies de Cabernet Sauvignon développées sur des boutures fructifères en chambre climatisée (OLLAT *et al.*, 1998), à différents stades de développement (DAA : jours après l'anthèse) selon la technique décrite par GREENSPAN *et al.* (1996).
(Moyenne et écartype, n = 4 ou 5). La véraison a eu lieu environ 60 jours après l'anthèse.

DAA	Xylem flow ($mm^3\ h^{-1}$ per berry)		Phloem flow ($mm^3\ h^{-1}$ per berry)	
	day	night	day	night
23	0.78 ± 0.35	0.60 ± 0.34	0.51 ± 0.3	0.37 ± 0.26
30	1.63 ± 0.58	1.21 ± 1.31	0.34 ± 0.11	0.35 ± 0.36
40	2 ± 0.58	1.8 ± 0.51	0.24 ± 0.14	0.31 ± 0.33
50	1.91 ± 0.8	2.27 ± 0.73	0.73 ± 0.38	0.22 ± 0.39
60	1.07 ± 1.3	0.75 ± 0.94	0.83 ± 0.7	1.27 ± 1.29
75	0.36 ± 0.5	-0.38 ± 0.15	2.09 ± 0.49	1.81 ± 1.2
80	0.38 ± 1.22	0.65 ± 0.5	2.71 ± 0.9	1.27 ± 0.49
100	0.47 ± 0.89	0.10 ± 0.06	3 ± 1.27	1.12 ± 0.29

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These observations show that the structural organization of exocarp and mesocarp tissue is different. In the exocarp cells, vacuoles with phenolics, plastids, numerous mitochondria and highly developed ER as well as dense cytoplasm suggest that a high metabolic activity takes place in these cells, especially in «prevéraison» stages. By contrast, in mesocarp cells, organelles are sparse and mitochondria crests degenerate more rapidly during fruit ontogeny while the vacuole becomes the major cell compartment. Moreover membrane tubules that may be involved in facilitating solute diffusion from cell to cell are more numerous during ripening at the plasma membrane level. These structures are consistent with the involvement of mesocarp

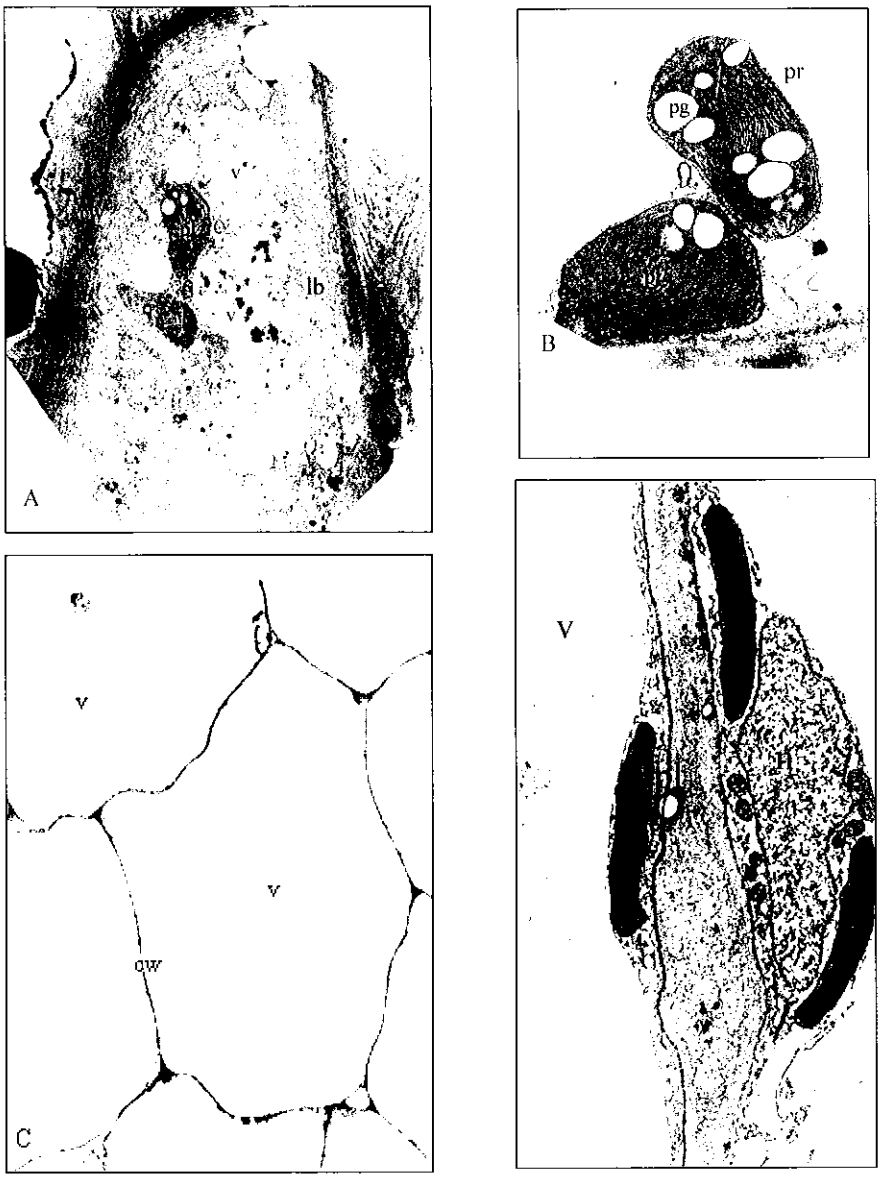


Fig. 6 - Ultrastructural organisation of grape berry cells at «post-véraison» stage.

A : Exocarp cells with very thick cell walls and fragmented vacuoles.
B : Plastids of hypodermal cell showing the presence of a peripheral reticulum as well as large plastoglobuli. **C:** Mesocarp cells: a single vacuole fills most of the cell volume. **D**: Details of the cell wall showing local changes of the wall texture (arrows) (A : x 5,7K, B: x 15,6K, C: x 1,25K, D: x 7,6K). (reproduced from «*In situ* fixation of grape berries», DIAKOU and CARDE, 2001, Protoplasma, 218, 225-235, figure 5; copyright notice of Springer Verlag).

Organisation ultrastructurale des cellules de la baie après la véraison.

A : Cellules de l'épiderme avec des parois très épaisses et des vacuoles fragmentées. **B** : Plastes des cellules hypodermiques avec du réticulum périphérique ainsi que des grands plastoglobules. **C** : Cellules du mésocarpe avec une vacuole unique occupant la majeure partie du volume cellulaire. **D** : Détails de la paroi cellulaire présentant par endroit des changements de texture (flèches). (A : x 5,7K, B: x 15,6K, C: x 1,25K, D: x 7,6K). (reproduit de «*In situ* fixation of grape berries», DIAKOU et CARDE, 2001, Protoplasma, 218, 225-235, figure 4; copyright notice of Springer Verlag).

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LENZ, 1989). Carbon loss occurs via respiration. The various components of the carbon balance have been estimated for berries of Cabernet Sauvignon (OLLAT and GAUDILLÈRE, 2000). Carbon content and dry matter allocation to the different berry parts, i.e. skin, flesh, and seeds have been determined. Gaseous exchanges of intact berries have also been measured to evaluate respiratory losses and carbon fixation. Carbon allocation to the different pools is presented on figure 7. The carbon import rate is 77 mmoles per berry per day during the first growth period. At « véraison », the carbon import rate increases 3.5 fold and reaches 266 mmoles per berry per day. During the first growth period, 30 per cent of this carbon is retained in the seeds and 43 per cent in the pericarp. Respiration accounts for 47 per cent of imported carbon. Because of recycling through photosynthesis, the net loss is only 27 per cent. As respiration accounts for a large part of imported carbon, the grape berry may be defined as an utilisation sink. During maturation, respiration accounts for 13 per cent of imported carbon, the remaining being stored in the pericarp. Grape berries are non-climacteric fruits, no increase in respiratory rate is observed at "véraison". Per unit of berry weight, respiratory rates are lower during ripening (figure 8). This decrease may be associated with mitochondria degeneration observed in the mesocarp cells. Then, it can be suggested that berry enlargement, sugar import and accumulation during ripening are supported by mechanisms that do not require significant amounts of energy (OLLAT and GAUDILLÈRE, 2000). However, TERRIER *et al.* (1996) and TERRIER and ROMIEU (2001) consider that the energy production of such low respiration rates may be limiting in order to face the increasing demand associated with new synthesis and the onset of a new growth period. It was established that Qr (CO_2 evolution rate over O_2 uptake rate ratio) was higher than one during ripening. This suggests oxidation of malate in place of imported sugars (KOCH and ALLEWELDT, 1978). TERRIER and ROMIEU (2001) reported a significant rate of aerobic fermentation in excised berries during ripening that could also explain why there is a high Qr. The induction of the fermentative pathway may be linked to energy limitation.

II- WATER BUDGET

As the grape berry is a fleshy fruit, water is a major component. Water is imported through xylem and phloem. Water balance is determined by growth in volume, transpiration and the return of water to the plant through the xylem (LANG and THORPE, 1986). In Cabernet Sauvignon, berry transpiration was measured by fresh weight loss in excised berries (figure 9). Per berry, the amount of water loss by transpiration increases slightly across berry development, depending on the variety (BLANKE and LEYHE, 1987). However, per unit of weight or skin area, transpiration rates decrease drastically; up to five fold between the beginning of the first growth period and ripening. Per unit of area, berry transpiration is 10 to 50 times lower than leaf transpiration. Between flowering and ripening, the amount of water entering the berry is nearly 10 times the volume of water stored in the berry at ripening (OLLAT, 1997). Among environmental parameters, berry transpiration is sensitive to temperature and light (FRIEDEN *et al.*, 1988). It also responds to the evaporative demand. This can be decreased by low

Table III - Mineral content (mg per berry) and ratio K/Ca (weight/weight) for berries of Cabernet Sauvignon growing on fruiting cuttings in a growth chamber (OLLAT *et al.*, 1998), determined at different stages of development

(days after anthesis, DAA, «véraison» : 60 DAA).

Composition minérale moyenne (mg par baie) et rapport K/Ca (poids/poids) de baies de Cabernet Sauvignon développées sur des boutures fructifères en chambre climatisée (OLLAT *et al.*, 1998), à différents stades de développement

(DAA : jours après l'anthèse, véraison à 60 DAA).

DAA	N	K	Ca	Mg	K/Ca
19	0.26	0.226	0.03	0.019	7.2 ± 1.3
26	0.37	0.31	0.04	0.027	7.7 ± 2.1
33	0.50	0.51	0.076	0.039	7.3 ± 2
44	0.55	0.57	0.083	0.043	6.9 ± 1.6
51	0.64	0.70	0.096	0.051	7.8 ± 1.9
59	0.645	0.72	0.10	0.057	7.2 ± 1.3
72	1.02	1.44	0.101	0.08	14.5 ± 7.6
84	1.23	1.71	0.109	0.093	15.2 ± 4.8
97	1.56	2.40	0.122	0.121	20.4 ± 3.5
103	1.73	2.81	0.138	0.137	20.5 ± 1.8

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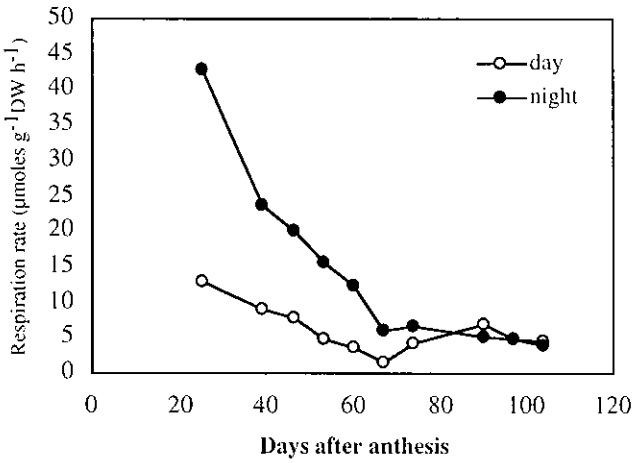


Fig. 8 - Cabernet Sauvignon berry respiration rates per unit of fresh weight during day or night, measured by gas exchanges of a whole cluster still attached to the fruiting cutting.

Plant culture and respiration measurements were performed in a growth chamber. «Véraison» occurred approximately 60 days after flowering (reprinted from OLLAT and GAUDILLÈRE, 2000, with the permission of ISHS.

Respiration des baies de Cabernet Sauvignon, exprimée par unité de poids frais, déterminée à la lumière ou à l'obscurité par des mesures d'échanges gazeux réalisées sur une grappe entière en place sur une bouture fructifère.

La culture des plantes et les mesures de respiration ont été réalisées en chambre de culture. La véraison se situe environ 60 jours après la floraison. (reproduit de OLLAT et GAUDILLÈRE, 2000 ; avec la permission de la Société Internationale des Sciences de l'Horticulture).

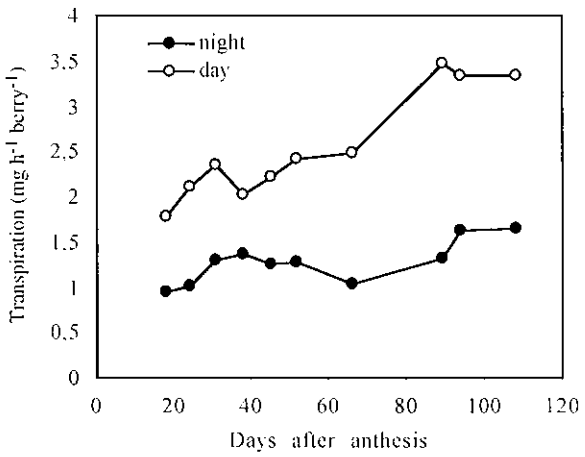


Fig. 9 - Transpiration rates of a berry of Cabernet Sauvignon growing on a fruiting cutting in a growth chamber, measured at different stages of development

Transpiration was measured from fresh weight losses of excised berries (n = 10). «Véraison» occurred approximately 60 days after flowering.

Transpiration de baies de Cabernet Sauvignon se développant sur des boutures fructifères en chambre climatisée.

La transpiration a été déterminée par mesure de la perte de poids frais de baies excisées (n = 10). La véraison se situe environ 60 jours après la floraison.

vapour pressure deficit, but an increasing deficit has no effect on water loss (REBUCCI *et al.*, 1997).

The net water flow into a berry equals inflow through xylem and phloem minus loss by transpiration. It also equals berry volume change. This net flow can be deduced from continuous diameter recordings of attached berries using electronic callipers. The contribution from xylem can be calculated from similar measurements on attached berries with phloem that has been disrupted by heat girdling using a constantan wire heated (90°C) by electrical current (GREENSPAN *et al.*, 1994). The components of water balance were evaluated with this methodology (figure 10 and table II, OLLAT and GAUDILLÈRE, 1996). For Cabernet Sauvignon berries developing in a growth chamber, increase in berry volume is the same for the first and second growth phases. Consequently, net water flow is identical. Before «véraison», 75 per cent of the water is delivered to the berries through xylem. Xylem flow reaches 50 mm^3 per berry per day. Phloem inflow is lower and does not exceed 20 mm^3 per berry per day. From «véraison» to maturity, there is a total change in the main water import pathway. At this stage, phloem flow contributes to 80 per cent of water import to the berry. Consequently, carbon and the major part of water entering the berry during ripening both originate from the phloem sap. Their imports are regulated by the mechanisms that control phloem flow and unloading. According to REBUCCI *et al.* (1997) and DREIER *et al.* (2000), berry transpiration may contribute to the control of sugar import into grape berries during ripening. In contrast to xylem flow, which is driven by water potential gradients and closely related to transpiration, phloem sap is driven by a hydrostatic pressure gradient activated by active pumping of solutes into the phloem tissue (MUNCH, 1930). Phloem loading of sucrose in the leaves results in a high osmotic pressure in the sieve tubes. Water consequently enters the conductive complex, increasing the hydrostatic pressure. In turn, water influx is thought to drive phloem sap solutes by mass flow (DELROT *et al.*, 2001). In the berry, water loss by transpiration may contribute to increase the pressure gradient by lowering the osmotic pressure. This would favour bulk phloem flow in the cells (THORPE and MINCHIN, 1996).

This change in water import pathway during berry development is confirmed by data published for other varieties grown under different conditions (LANG and THORPE, 1989; GREENSPAN *et al.*, 1994, 1996). The decrease of water import through xylem is related to the anatomical observations of xylem breakage in maturing grape berries. Direct evidence for xylem flow interruption is provided by physical gaps observed in

aquaporins which are special proteins behaving as water channels. These aquaporins are supposed to facilitate transmembrane water movement (REIZER *et al.*, 1993). In grape berries, two genes encoding for putative plasma-membrane aquaporines (PIP) have been isolated (DELROT *et al.*, 2001). One of these genes is induced at the onset of ripening.

III- MINERAL NUTRIENTS

The amounts of nitrogen, potassium, calcium and magnesium in Cabernet Sauvignon berries developing in a growth chamber are given in table III. Calcium is mainly imported during the first growth phase whereas potassium, nitrogen, and magnesium are mainly accumulated during maturation. These results are in agreement with most published data (DONÈCHE and CHARDONNET, 1992; ESTEBAN *et al.*, 1999). Before « véraison », ammonium ions account for more than half of total nitrogen (KANELLIS and ROUBELAKIS, 1993). Active amino acid and protein synthesis takes place in developing berries. During maturation, the organic nitrogen in the berries increases while ammonia decreases. Fifty to 90 per cent of the nitrogen in berry juice is in the form of free amino acids with the remainder present as ammonium ions, nitrate, peptides and proteins (references cited in VAN HEESWICK *et al.*, 2001). Proline and arginine are the predominant amino acids, depending on variety, berries maturity, environmental conditions and viticultural practices. In the grape berry, potassium is mainly stored within the cell vacuole, and its concentration is higher in skin cells (STOREY, 1987; ILAND and COOMBE, 1988). Calcium is an important constituent of the cell wall. The skin is also characterised by the highest calcium content during the whole berry development (POSSNER and KLIEWER, 1985). During the first growth period, a large proportion of magnesium is retained in the cell walls of the flesh and of the skin. The flesh is richer than the skin. The parietal magnesium fraction disappears at «véraison» (DONÈCHE and CHARDONNET, 1992). Potassium and magnesium are considered highly phloem mobile whereas calcium has low phloem mobility (MARSCHNER, 1995). Consequently, the changes in the amounts of mineral nutrients during berry development may be analysed as the consequences of changes in the main pathway of imports as proposed by various authors (LANG and THORPE, 1989; GREENSPAN *et al.*, 1994, 1996; OLLAT and GAUDILLÈRE, 1996).-

BERRY METABOLISM

Grape metabolism and its effect on berry composition and wine quality, organic acids, sugars and anthocyanins has been studied in detail for many years (RIBÉREAU-GAYON, 1964; HARDY, 1968; HAWKER, 1968; 1969; COOMBE and PHILLIPS, 1980). Today, the mechanisms that support accumulation and degradation of these compounds are better understood even if the regulatory processes remain unknown. Because of the continuing evolution of analytical techniques, some studies are now performed on the secondary metabolites that are of low concentration. However, the metabolism of secondary metabolites is far from being elucidated.

ROUBELAKIS-ANGELAKIS (2001) reviewed many aspects of grape berry metabolism. Here only the more recent data on grape berry acidity and sugar accumulation is summarised. The grape berry is an extreme fruit as pericarp cells are able to accumulate and store a high concentration of organic acids and sugar. Before «véraison», organic acid accumulation contributes to the osmotic potential in the berry cells. After «véraison», hexoses are the main osmotic active compounds.

I- GRAPE BERRY ACIDITY

The acidity of grapes and wine depends on the ratio between organic acids and potassium concentration. The acid fraction consists mainly of tartaric and malic acid. The metabolism of organic acids was reviewed by RUFFNER (1982). The acid concentration of grapes is affected by exogenous factors. BLOUIN and GUIMBERTEAU (2000) reported an organic acid concentration range for Cabernet Sauvignon in a Bordeaux vineyard (Margaux) from 1984 to 1997 from 10^4 to 78 meq L^{-1} for tartrate and 26 to 65 meq L^{-1} for malate. Malate concentration is more affected by environmental factors than tartrate concentration (HUGLIN AND SCHNEIDER, 1998). The ratio between tartrate and malate concentration varies also considerably with grape variety (KLIEWER *et al.*, 1967). Classification of the varieties by precocity or time to ripeness for wine-making purposes is related to acid concentration at maturity. Varieties with berries of low acid concentration such as Chasselas and Cardinal are classified as early varieties. KLIEWER *et al.* (1967) reported a range of tartrate and malate concentration at maturity for 25 grapevine varieties growing in California from 106 meq L-1 (7.95 g L^{-1}) to 63 meq L^{-1} (4.7 g L^{-1}) for tartrate and 75 to 22 meq L^{-1}(5 to 1.6 g L^{-1}) for malate. BOUBALS *et al.* (1971) have described a variety Gora Chirine that stores only low amounts of malate and tartrate (figure 12). This variety, compared to Cabernet Sauvignon is characterised by a much higher juice pH, but an identical vacuolar pH at «véraison» (DIAKOU *et al.*, 1997). At this stage, the main contributors to berry osmotic potential are organic acids for Cabernet Sauvignon and glucose for Gora Chirine. The concen-

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xylem tracheids (figure 11A) of the berry peripheral vascular bundles and experiments with apoplastic dyes. Figure 11B shows clearly that a fluorescent dye circulating through the xylem is present in the whole vascular network before "véraison", but is restricted to the brush region afterwards. This phenomenon has also been reported for Muscat Gordo Blanco (FINDLAY *et al.*, 1987), Riesling (DURING *et al.*, 1987), Pinot Noir and Merlot (CREASY *et al.*, 1993), Shiraz (ROGIERS *et al.*, 2001). However, the latter authors suggested that berries continue to import water from xylem during ripening, because they observed a larger water loss for excised berries (xylem and phloem disrupted) in comparison to girdled berries (phloem disrupted only). Our data shows that xylem flow continues to contribute to 20 % of water import (table II, figure 5). This flow may be restricted to the brush region, as the peripheral network appears to be disconnected.

Trying to explain the shrinkage of Shiraz berries that occurs close to maturity, COOMBE and MC CARTHY (2000) proposed that phloem flow might also be disrupted at the end of ripening. Indeed, the amount of sugar per berry remains steady when shrinkage occurs. Some morphological observations using a fluorescent dye loaded into the phloem led to the same kind of hypothesis for phloem disruption (OLLAT, 1997). However, observations of the phloem pathway with fluorescent dyes should always be considered critically and confirmed using other techniques.

The main water pathway controls water relations between vegetative parts and berries. During the first growth period, when xylem is the main pathway, the berries are sensitive to water stress (MATTHEWS and ANDERSON, 1989). Depending on the plant water status, some water can flow back from the berries to the plant inducing daily berry shrinkage. After «véraison», berry shrinkage during water stress is small (GREENSPAN *et al.*, 1996). This observation is indirect evidence of the inability of xylem vessels to carry water from the berries to the plant during ripening.

The data presented above underline the importance of water flow for berry development and composition. Whatever the main pathway for water flows to the berry, water must cross the plasma membrane and the tonoplast of the cells. Both membranes contain abundant

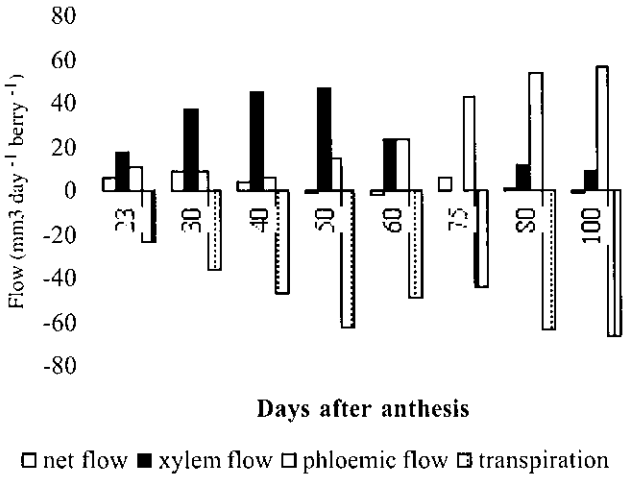


Fig. 10 - Various components of water budget for Cabernet Sauvignon berries growing on a fruiting cutting in a growth chamber, determined at different stages of development (n = 4 or 5) with the technique described by GREENSPAN *et al.* (1996).

«Véraison» occurred approximately 60 days after flowering.

Différentes composantes du bilan hydrique des baies de Cabernet Sauvignon se développant sur des boutures fructifères en chambre climatisée (n = 4 ou 5), déterminées à plusieurs stades de développement par la technique décrite par GREENSPAN *et al.* (1996).

La véraison se situe environ 60 jours après la floraison.

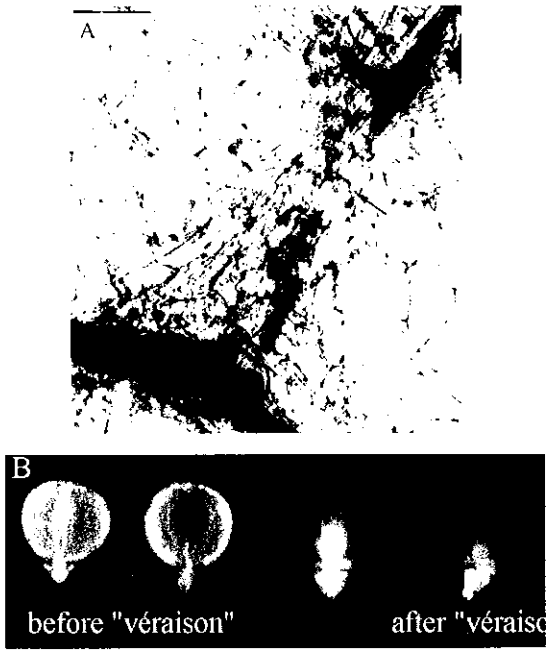


Fig. 11 - Structural changes supporting berry water supply.

A: Tracheids of peripherical vascular network in a Cabernet Sauvignon berry after veraison. Fresh slice under white light (bar = 50 µm). Arrow indicates a discontinuity.

B: Longitudinal cut in Cabernet Sauvignon berries after soaking the stem part carrying the cluster in a fluorescent dye (HPTS acetate). Observations under UV light before and after «véraison».

Changements structuraux accompagnant les modifications d'alimentation en eau de la baie.

A : Trachéides du réseau vasculaire périphérique dans une baie de cabernet sauvignon après la véraison. Coupe fraîche observée en lumière blanche (barre = 50 µm). La flèche indique une discontinuité.

B : Coupe longitudinale de baies de Cabernet Sauvignon après trempage de la tige portant la grappe dans un colorant fluorescent (HPTS acetate). Observations sous lumière ultra-violette avant et après la véraison.

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tration of fructose, amino acids and potassium is also higher at «véraison» for Gora Chirine.

Malic and tartaric acids are mainly synthesised in the grape berry and accumulate during the first growth period (figure 12, RIBÉREAU-GAYON, 1968). Tartaric acid is accumulated shortly after anthesis, during cell division. Ascorbic acid seems to be the main precursor of tartaric acid even if the biosynthetic pathway is not fully elucidated (RUFFNER, 1982). During ripening, tartaric acid concentration decreases, mostly through dilution.

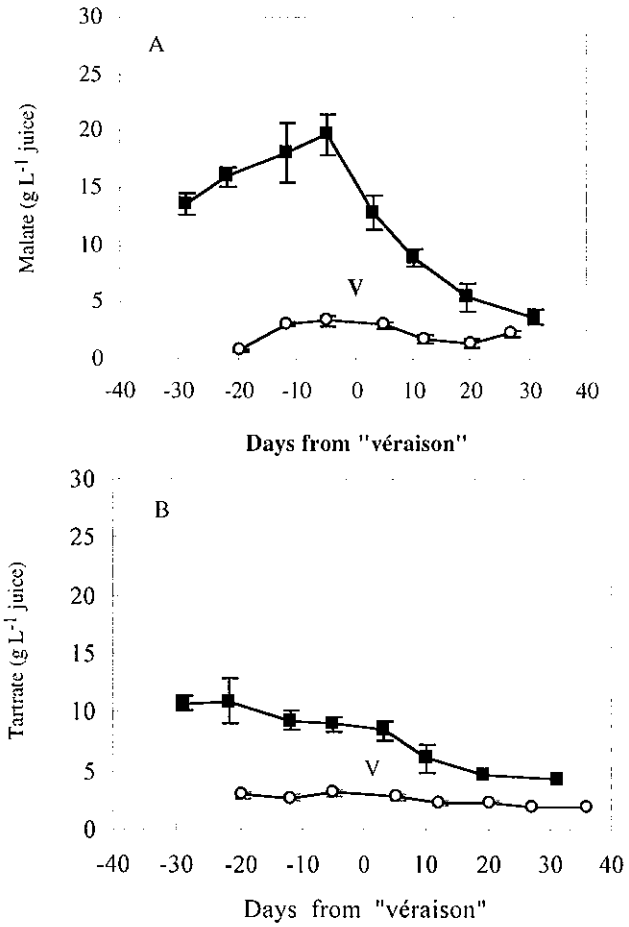


Fig. 12 - Changes in juice organic acid concentration during the development of Cabernet Sauvignon (■) and Gora Chirine (○) berries, grown under greenhouse conditions.

A : malic acid; **B**: tartaric acid. V indicates veraison. (reprinted from DIAKOU *et al.*, 1997 with the permission of the Australian Society of Viticulture and Enology).

Variation de la concentration en acides organiques au cours du développement des baies de Cabernet Sauvignon (■) et Gora Chririne (○), poussant sous serre.

A : acide malique; **B**: acide tartrique. V indique la véraison. (Reproduit de DIAKOU *et al.*, 1997 ; avec la permission de la Société Australienne de Viticulture et d'Œnologie).

Malate accumulates in the cell vacuole during the first growth period. Malate content and concentration reach maximum just before « véraison », and decreases throughout ripening. Malate concentration is higher in the flesh during the first growth period and in the skin during ripening (ILAND and COOMBE, 1988). Moreover in the flesh during ripening malate concentration is the lowest near the vascular bundles (COOMBE, 1987).

Malic acid is synthesised in the cytosol from the β-carboxylation of phosphoenolpyruvate (PEP) by the enzyme phosphoenolpyruvate carboxylase (PEPC). The oxaloacetate primarily formed is reduced by NAD-dependent malate dehydrogenase to give malate. Malate can be degraded by malic enzyme to produce pyruvate or by PEP carboxykinase to produce PEP and then glucose by neoglucogenesis. There is no correlation between PEPC *in vitro* activity and malate accumulation, or malic enzyme and PEP carboxykinase *in vitro* and acid decrease (HAWKER, 1969; RUFFNER, 1982). However, DIAKOU *et al.* (2000) showed that regulation of PEPC by phosphorylation could contribute to the control of malic acid accumulation. Indeed the enzyme is more sensitive to inhibition by malate, and low pH, during ripening than during the first growth period. However, varietal differences such as those described for Gora Chirine do not seem to be related to the regulation of this enzyme. Compartmentation control may regulate malic acid concentration during ripening (RUFFNER, 1982; TERRIER and ROMIEU, 2001a). TERRIER and ROMIEU (2001a) describe a scheme for malate compartmentation in grape berry cells. In the tonoplast, two primary electrogenic pumps, a V-ATPase and a pyrophosphatase (V-PPase) transduce the chemical energy of ATP or inorganic phosphate in an electrochemical proton gradient, enabling the secondary transport of many solutes as organic acids against their electrochemical gradient by this transport system. Vacuolar organic acid transport was studied in grapes by TERRIER *et al.* (1998). However, the molecular structure of the transporter remains unknown. Neither vacuolar H^+ pump quantity nor *in vitro* activity explains the high rates of organic acid accumulation during the first growth period and the metabolic changes that occur at «véraison». A higher level of V-PPase activity in comparison to V-ATPase activity could explain the increase of vacuolar pH during ripening. However this ratio is always high, even before «véraison» when the vacuolar pH is low (TERRIER *et al.*, 2001a). The passive permeability of the tonoplast increases during ripening (DREIER *et al.*, 1998; TERRIER *et al.*, 2001a). The regulation of vacuolar H^+ pump activity and the occurrence of tonoplast leakage may be of critical importance to the control of acidity in grape berry cells.

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II- SUGAR ACCUMULATION

Up until « véraison », most of the sugar imported into the berry is metabolised and there is little storage. At « véraison », sugar accumulation begins precipitously in pericarp cells (figure 13). While sucrose is imported from the sources, glucose and fructose reach high concentrations in the berry at maturity. In comparison with other fruit such as peaches, the grape berry does not store starch. The rate of hexose accumulation

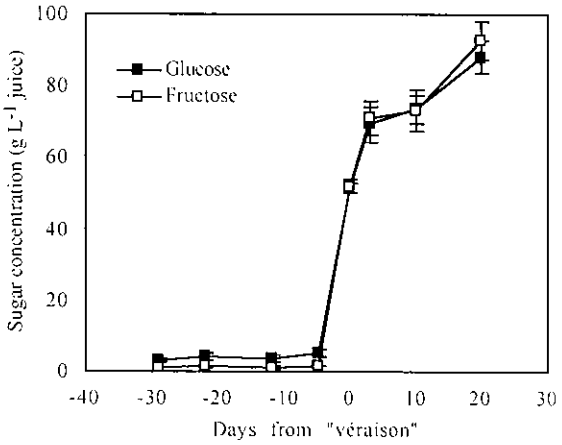


Fig. 13 - Changes in juice hexose concentration during the development of Cabernet Sauvignon berries, grown under greenhouse conditions.
V indicates «véraison» (adapted from DIAKOU *et al.*, 1997, with the permission of the Australian Society of Viticulture and Enology).

Variations de la concentration en hexoses du jus pendant le développement de baies de Cabernet Sauvignon sous serre.
V indique la véraison. (Adapté de DIAKOU *et al.*, 1997 ; avec la permission de la Société Australienne de Viticulture et d'Œnologie).

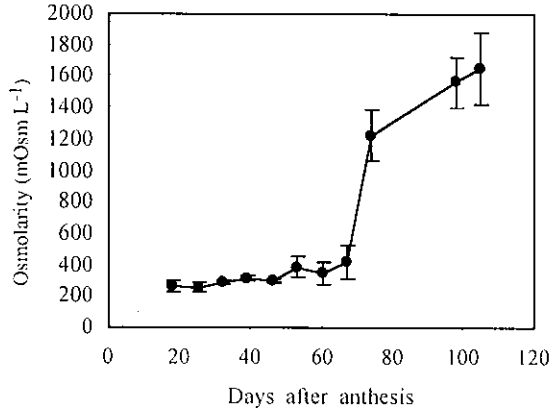


Fig. 14 - Changes in juice osmolarity during Cabernet Sauvignon berry development (n = 10).
The berries were grown on fruiting cuttings in a growth chamber. "Véraison" occurred approximately 60 days after flowering.

Variations de l'osmolarité du jus au cours du développement des baies de Cabernet Sauvignon (n = 10).
Les baies ont été produites sur des boutures fructifères en chambre climatisée. La véraison se situe environ 60 jours après la floraison.

parallels that of softening, and this is before colour changes and growth resumes (COOMBE and PHILLIPS, 1980; HUANG and HUANG, 2001). The composition of individual berries from a single cluster is determined seven days after the first berry starts to colour (table IV). At this stage, the green berries have already started to accumulate sugar because their osmolarity is 703 mOsm L^{-1} whereas during the lag phase, before any sign of « véraison », the osmolarity of the juice is on average 470 mOsm L^{-1} (figure 14). At the onset of ripening, hexose accumulation occurs at a high rate. Indeed, the red berries that started to colour seven days previously have reached 1.12 Osm L^{-1}. At maturity, for Cabernet Sauvignon, sugar represents 60 per cent of the solutes in the berry juice and the osmolarity is on average 1.6 Osm L^{-1} (DIAKOU *et al.*, 1997; OLLAT, 1997). Once triggered, hexose concentration increases at a steady rate. This means that the amount of sugar increment matches that of water over time to result in a steady increase in concentration (COOMBE, 1992). This result shows that sugar and water accumulation, or berry growth are linked. It also supports the conclusion that most of water is supplied through the phloem. Sugar accumulation and berry growth (sink strength) during maturation are probably driven by the general osmotic balance in the berry. This balance is related to solute import, metabolism and storage mechanisms, intracellular water trafficking and global berry water balance (phloem import, growth and transpiration).

Hexose accumulation from sucrose import implies the activity of a sucrose cleaving enzyme between the phloem unloading point and the pericarp cell vacuole, i.e. in apoplast, in the cytoplasm or in the vacuole itself. Invertase and sucrose synthase are commonly associated with sucrose conversion to glucose and fructose. Invertase is by far the more active sucrose cleaving enzyme in the grape pericarp (HAWKER, 1968; RUFFNER *et al.*, 1995). Some cultivars, in particular those derived from *Vitis labrusca*, store higher amounts of sucrose. TAKAYANGI and YOKOTSUKA (1997) measured a much lower invertase activity in the flesh of Steuben berries, a *Vitis labrusca* hybrid. Consequently, invertase is likely involved in the control of the composition of stored sugars. In the *Vitis vinifera* varieties, invertase activity per unit of weight increases during the first growth period, is maximal at « véraison » and decreases during hexose accumulation (HAWKER, 1968). Per berry, the level of invertase activity remains high and steady during ripening. Berry invertase seems to be highly soluble and more closely related to vacuolar types (RUFFNER *et al.*, 1995; DAVIES and ROBINSON, 1996). An immunohistochemical observation of cell wall invertase has been made in grape berries, however non-specific anti-

Table IV - Main physico-chemical features at «véraison» for individual berries of Cabernet Sauvignon growing on fruiting cuttings in a growth chamber (OLLAT *et al.*, 1998).

The berries were collected on a single cluster 7 days after the first berry turned red (n = 9, mean ± standard deviation, ANOVA analysis, ** : p<0.01).

Principales caractéristiques physico-chimiques à la véraison de baies de Cabernet Sauvignon développées sur des boutures fructifères en chambre climatisée (OLLAT *et al.*, 1998).

Les baies ont été récoltées sur une seule grappe 7 jours après le changement de couleur de la première baie, et analysées individuellement. (n = 9, moyenne ± écartype, Analyse de variance, p<0.01).

	Green berries	Pink beries	Red berries	
Berry fresh weight (g)	0.61 ± 0.11	0.78 ± 0.15	0.94 ± 0.45	
Seed fresh weight (g)	0.06 ± 0.008	0.067 ± 0.03	0.094 ± 0.05	
Juice osmolarity (mOsm L-1)	703 5 ± 107.9	933.3 ± 36.6	1123.1 ± 43.8	**
Osmotic pressure (MPa at 25°C)	1.7 ± 0.27	2.3 ± 0.09	2.8 ± 0.11	
Titratable acidity (meq L-1)	393.3 ± 18.71	325.4 ± 40.1	228 ± 17.9	**
pH	2.8 ± 0.04	2.9 ± 0.07	3.09 ± 0.03	**

bodies raised against cell wall invertase were used (FAMIANI *et al.*, 2000). Putative vacuolar invertase genes appear to be highly expressed during the first growth period and their mRNA expression declines to almost nothing after « véraison » when hexose accumulation is maximal. Therefore, the synthesis and the regulation of vacuolar invertase do not seem to be a controlling factor in sugar accumulation during ripening (BOSS and DAVIES, 2001). The location of invertase in the vacuoles also suggests that sucrose is imported into the vacuole where it is cleaved into glucose and fructose. Nevertheless DREIER *et al.* (1998) reported that invertase is leaking out of the vacuole during ripening because of cell decompartmentation. This would imply that, after « véraison », sucrose transport into the vacuole prior to hydrolysis is no more required.

The transport of sucrose from phloem to the location of hydrolysis in the pericarp cells implies the existence of sucrose transporters on the plasma membrane and/or tonoplast. In grapes, genes for putative sucrose transporters have been isolated from Shiraz berries (DAVIES *et al.*, 1999; MANNING *et al.*, 2001) and partially from Ugni Blanc berries (AGEORGES *et al.*, 2000). The expression in berries of some of these genes as vvsuc11 and vvsuc12 is upregulated at « véraison », indicating that these transporters may have a role in the import of sucrose into the ripening berries (DAVIES *et al.*, 1999). Putative hexose transporters have also been identified in grape berries (FILLION *et al.*, 1999) with an increased gene expression of vvht2 during sugar accumulation. One of the genes encoding for putative aquaporins is induced at the same time as the sucrose and hexose transporters isolated from Ugni Blanc berries (DELROT *et al.*, 2001). However, the functional assessment and the tissue and cellular location of sucrose and hexose transporters remain to be determined. The occurrence of both sucrose and hexose transporters implies that several mechanisms may support sugar accumulation during berry ripening. Further studies on gene regulation would explain the trigger and the controlling mechanism of ripening. COOMBE *et al.* (1987) suggested that the primary control of sugar accumulation during ripening was at the unloading step from the phloem in the apoplast of the vascular bundles of the berry. The pattern of expression of several of the sugar transporter genes supports this idea. LANG and THORPE (1986) proposed that the breakdown of apoplast/symplast compartmentation in pericarp cells at « véraison » could induce a much higher pressure gradient between phloem and berry cells in favour of movement of phloem sap into the berry. Evidence that leakiness develops in flesh cells at the beginning of ripening has been gathered (LANG and DURING, 1991; DREIER *et al.*, 1998; TERRIER *et al.*, 2001). However the occurrence of these leaks does not seem to prevent protein synthesis that is intense in grape berry cells during ripening (ROUBELAKIS-ANGELAKIS, 1993). Many of the ripening-associated genes in grapevine encode cell wall proteins, PR proteins or are induced by stress as in other plants (ROBINSON and DAVIES, 2000). The link between decompartmentation, the nature of the newly expressed genes and mesocarp cells metabolism during ripening should be investigated.

III- OTHER RIPENING RELATED GENES

A dramatic change in mRNA profiles at the onset of grape berry ripening is observed (ROBINSON and DAVIES, 2000). The putative proteins encoded by these genes are named GRIP (Grape Ripening Induced Proteins).

BOSS *et al.* (1996a, b, c) studied gene expression in the anthocyanin pathway in the berries of red and white grape cultivars. UDP glucose-flavonoid 3-o-glucosyl transferase (UFGT) appears to be a key enzyme for anthocyanin production. Expression of the gene

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encoding for this enzyme is detected only after « véraison», in the skin tissues of coloured varieties. Some genes involved in cell wall structure also have a ripening related expression (DAVIES and ROBINSON, 2000). BARNAVON *et al.*, (2000) and NUNAN *et al.*, (2001) studied the relationships between gene expression and enzyme activity for some of the proteins known to be involved in cell wall polysaccharide composition changes and berry softening, as β-galactosidase, pectin methylesterase, and polygalacturonase. Even if some transcription activity parallels enzyme activity, cell wall composition appears to be related to complex processes and regulation. Beside the large number of genes involved in cell wall structure, genes encoding for putative stress responses proteins are highly induced at « véraison » (ROBINSON and DAVIES, 2000; TERRIER *et al.*, 2001b). These include pathogenesis-related (PR) proteins, a metallothionein, a transcription factor, a cytochrome P450 enzyme and proteins induced by water, sugar or cold stress (ROBINSON *et al.*, 1997; TATTERSALL *et al.*, 1997; DAVIES and ROBINSON, 2000). These proteins may act to maintain cell wall integrity during rapid cellular expansion and prevent pathogen infection, or may help to ameliorate osmotic stress associated with the increase of osmotic potential in berry cells (ROBINSON and DAVIES, 2000).

Alcohol dehydrogenase (Adh) was the indicator of the onset of ripening first characterised. Adh activity increases sharply at « véraison » and parallels sugar accumulation (SAUVAGE *et al.*, 1991; TERRIER *et al.*, 1996). One of the three isolated genes encoding for Adh in grape berries is induced at « véraison » and its expression is correlated to Adh activity during ripening (SARNI-MANCHADO *et al.*; 1997; TESNIÈRE and VERRIÈS, 2000). According to TERRIER and ROMIEU (2001), the induction of Adh could be linked to respiratory pathway changes and to the impairment of acid compartmentation at the onset of ripening.

The use of large-scale analysis molecular biology techniques is now providing new insights for the understanding into berry growth and development. Generation and sequencing of grape Expressed Sequence Tags (ESTs) has already been initiated and will soon aid the identification of nearly all of the genes that are transcribed in the grape berry. Preliminary analysis of these EST databases has already provided some important information about genes expressed in developing berries (ABLETT *et al.*, 2000; TERRIER *et al.*, 2001b). The use of new technologies developed to enable the simultaneous analysis of the expression patterns of thousands of genes on a large scale and under different circumstances represents the next step to be performed. Transcriptome analysis of the grape berry at various developmental stages is currently under progress using cDNA or oligonucleotide microarrays. The resulting data will certainly lead to the identification of numerous genes or gene clusters potentially important to grape berry development. Identification of genes responsive to exogenous and/or endogenous signals, for example sugars and hormones, may also give some clues to the regulatory mechanisms of gene expression during berry growth and development (ROBINSON and DAVIES, 2000).

EST databases can also be used as an alternative source for the identification of genetic markers such as microsatellites (SCOTT *et al.*, 2000) and then contribute to the construction of a Vitis genetic map. However, it is obvious that genetic mapping is slow due to the long generation time of this species. On the other hand, the relatively small size of the Vitis vinifera genome (470 Mb) makes it an attractive species for genomic approaches including physical mapping. Grape bacterial artificial chromosome (BAC) libraries are already available (TOMKINS *et al.*, 2001) and a database of BAC clones anchored with ESTs and genetic markers such as microsatellites will soon be generated (ADAM-BLONDON, personal communication). Once completed, this work might represent the starting point of the complete sequencing of the Vitis genome.

CONCLUSION

In this review, we summarise some work on berry growth and development and report recent data on metabolism with special emphasis on acids and sugars. Most external and internal factors have a larger effect on berry growth before "véraison". Therefore berry size is largely determined at "véraison". Ripening is triggered rapidly but the regulating factor has yet to be identified. Auxins and abscisic acid are likely involved. After "véraison", growth and sugar accumulation are linked because water and sucrose are both imported through phloem sap. Ripening involves a deep change in gene expression and metabolism. Regulation mechanisms for organic acid and hexose accumulation are not fully understood even if considerable information has been gathered from molecular studies. The mechanisms supporting compartmentation and solute transport across plasma membrane and the tonoplast are possible controlling factors. Consequently berry structure and ultrastructure studies have a major importance in explaining berry metabolism, and some data presented in this review underline how structure and function could be linked.

In the future, the increasing availability of information resulting from genome studies, associated with sophisticated techniques to investigate metabolism,

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compartmentation and structure should enable a better understanding of the functioning of the grape berry. These techniques will also enable a focus on the mechanisms associated with the production of secondary metabolites that are important for quality, but as yet largely poorly understood.

Acknowledgment : The authors gratefully thank Russell JONHSTONE for his contribution to improve the english version of this work.

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Manuscrit reçu le 16 mai 2002 ; accepté pour publication le 17 juin 2002

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