

FLOWERING NEWSLETTER REVIEW

## Sugars and flowering in the grapevine (*Vitis vinifera* L.)

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Received 11 February 2008; Revised 7 April 2008; Accepted 8 April 2008

### Abstract

Sugars play an important role in grapevine flowering. This complex process from inflorescence initiation to fruit maturity takes two growing seasons. Currently, most of the available data concern the involvement of sugars as energy sources during the formation of reproductive structures from initiation of inflorescences during the summer of the first year, until flower opening during the following spring. Sugars devoted to the development of reproductive structures are supplied either by wood reserves or by photosynthesis in leaves or inflorescences, depending on the stage of development. Female meiosis appears to be a key point in the success of flower formation because (i) flowers are vulnerable at this stage and (ii) it corresponds in the whole plant to the transition between reserve mobilization from perennial organs (roots, trunk, and canes) towards efficient leaf photosynthesis. The perturbation of reserve replenishment during the previous year provokes perturbation in the development of inflorescences, whereas altering the photosynthetic sources affects the formation of flowers during the same year. In particular, a lack of sugar availability in flowers at female meiosis caused by various environmental or physiological fluctuations may lead to drastic flower abortion. Apart from energy, sugars also play roles as regulators of gene expression and as signal molecules that may be involved in stress responses. In the future, these two topics should be further investigated in the grapevine considering the sensitivity of flowers to environmental stresses at meiosis.

Key words: flowers, grapevine, inflorescences, meiosis, photosynthesis, reserves, sugars.

### Introduction

Flowering in higher plants represents the process of sexual reproduction enabling genetic recombination and thus plant evolution. Also, flowers and derived organs such as fruits and seeds are the major components of yield in crops. The process of flowering has therefore been one of the most well studied phenomena in plant biology over the centuries. Nowadays, most of the phases are quite well understood although the detailed regulation of various steps has not been fully investigated.

Angiosperms are able to flower when they have reached their sexual maturity, varying from a few weeks in small plants such as *Arabidopsis thaliana* to several years in trees. At sexual maturity, both endogenous and exogenous parameters must be fulfilled so that the initiation of flowering occurs. The formation of reproductive organs in woody perennial plants such as grapevines, growing in temperate climates, extends over two successive seasons. The first one is devoted to the initiation of inflorescence primordia, whereas the second one is focused on inflorescence emergence, flower and then berry development. Sugars are important in the accomplishment of sexual reproduction in the grapevine because they are the main source of energy (Caspari et al., 1998), originating from reserve mobilization or photosynthesis performed in the leaves or inflorescences. In this species, sugar supply is important at various key stages of reproductive organ formation, from the initiation of inflorescences until fruit set (Candolfi-Vasconcelos and Koblet, 1990). However, sugars are also known to be involved in other species as regulators of (i) source–sink interactions in the whole plant under standard or stress conditions (Roitsch, 1999) and (ii) plant development and gene expression (Gibson, 2005).

Our aim here is to provide a systematic account of the currently available information about the various aspects

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of sugar involvement in the formation of reproductive organs in the grapevine, based on a detailed knowledge of the flowering process. This review includes most of information currently available in the grapevine varieties, although phenotypic differences between cultivars can be large and generate differential cultivar behaviours.

## The flowering process in grapevine

The various steps of inflorescence and flower formation have been extensively reviewed in previous papers (Srinivasan and Mullins, 1981; Boss *et al.*, 2003). The initiation of the inflorescence and the formation of flowers represent two distinct steps of sexual reproduction which occur over two successive years.

### Inflorescence formation

Inflorescences are initiated in the latent bud during the summer of the first year. A typical trait of *Vitis vinifera* is the simultaneous formation of both vegetative and reproductive forming organ primordia by the same apex (Boss *et al.*, 2003). The apex of the latent bud produces from three to eight leaf primordia depending on the variety, and then divides into two parts. The part opposite to the youngest leaf primordium, is a meristematic protuberance referred to as the uncommitted primordium, or *Anlage*, and its formation represents the first step of inflorescence initiation (Srinivasan and Mullins, 1976, 1981; Mullins *et al.*, 1992; Boss and Thomas, 2002; Boss *et al.*, 2003). Usually, the first uncommitted primordium appears during the summer in latent buds at the base of canes (Mullins *et al.*, 1992). A bract develops on the distal part of the *Anlage*. Two branches (or arms) are then formed from the apex of the *Anlage*: the internal one is close to the apex whereas the external arm is close to the bract. This is a key step in the formation of reproductive organs in the grapevine since these two arms may develop into inflorescence or tendril primordia (Srinivasan and Mullins, 1976, 1981; Mullins *et al.*, 1992; Boss and Thomas, 2002; Boss *et al.*, 2003) or less commonly into a shoot primordium. The fate of uncommitted primordia depends on environmental fluctuations such as light or temperature as well as internal factors such as growth regulators or sugar reserves (Srinivasan and Mullins, 1981). For example, cytokinins favour the formation of uncommitted primordia (Srinivasan and Mullins, 1978) and their development into inflorescences (Srinivasan and Mullins, 1980). The internal branch divides and produces several ramified globular primordia which will constitute the structural basis of the inflorescence (Scholefield and Ward, 1975). The intensity of ramification in the internal branch progressively decreases from the base to the apex and thus gives a conical form to the inflorescence primordium (Srinivasan and Mullins, 1981). The latent

bud enters winter dormancy when one to three ramified globular primordia are formed depending on the variety (Pratt, 1971). Carbohydrate physiology of the whole vine during the period of inflorescence initiation determines the number of bunches that will emerge the following year (Candolfi-Vasconcelos and Koblet, 1990).

### Flower development

The formation of flowers occurs during the following spring. Bud break is preceded by the activation of all structures in the latent bud, especially the differentiation of inflorescences and the first steps of floral organ development (Agaoglu, 1971). At each inflorescence forming organ primordium, from three to five flower primordia may develop depending on the variety. The successive development of floral organs is simultaneous in each flower of the inflorescence in the same primordium (Mullins *et al.*, 1992). There is an order of organ appearance that is similar to all angiosperms: five sepals appear first and form the calyx. Five petals which form the corolla then develop, followed by five stamens and then two carpels that form the pistil. The calyx has a ring feature (Gerrath, 1993) and might protect the internal organs from environmental fluctuations at the early stages of bud break. Petals and stamens develop from initially common primordia (Gerrath and Posluszny, 1988). During their growth, petals cover the sepals which degenerate and join at their top to form the cap (Srinivasan and Mullins, 1976, 1981) that will protect the fertile organs. The cap is dislodged at anthesis by the growth of stamen filaments (Pratt, 1971; Srinivasan and Mullins, 1976, 1981; Gerrath, 1993; Boss *et al.*, 2003). The gynoecium originates from the fusion of two carpels (Agaoglu, 1971; Considine and Knox, 1979; Posluszny and Gerrath, 1986). In each locule, two anatropous ovules develop and are inserted into the septum. Some nectaries may develop at the base of the ovary.

Due to the complexity of inflorescence and flower formation in grapevine, previous authors have described the successive phases to provide a scale of reproductive development. Lorenz *et al.* (1994) and Coombe (1995) summarized reproductive development into 22 successive stages encoded by numbers from 0 to 50 based on the external inflorescence characteristics. After leaf expansion (stage 09), the inflorescence appears from the bud at stage 12 and separates from the rest of the annual shoot at stage 15. At this stage, the flowers are packed together and are progressively separated by floral peduncle elongation (stage 17). Anthesis occurs at stage 19 and continues for about one week. The floral caps then fall off following the growth of stamen filaments (Gerrath, 1993; Boss *et al.*, 2003), cap fall marking the following stages: full bloom (stage 23) is reached when 50% of flower caps have fallen, whereas stage 25 is reached when 80% of caps

have fallen. Stage 27 marks the onset of berry development from the fertilized ovules. Stamens then degenerate and the young berry is now visible.

These morphological markers do not precisely reflect the internal development of reproductive organs, which may be affected by fluctuations of environmental factors such as temperature and light at the time of bud burst (Petrie and Clingeleffer, 2005). The kinetics of male and female reproductive development depend on variety and are not necessarily synchronous with the developmental scale defined by Lorenz *et al.* (1994). For example, in Merlot, Cabernet-Sauvignon, and Chardonnay, female meiosis occurs, respectively, 10, 15, and 16 d before anthesis (Fougère-Rifot *et al.*, 1993). Similarly, comparing Gewurztraminer and Pinot noir plants grown in the same vineyard, it was necessary to add intermediates stages between stages 15 and 17 (i.e. stages 15+2 d and 15+8 d) to indicate the time of meiosis accurately. It was thus established that both male and female meiosis occur one week earlier in Pinot noir than in Gewurztraminer (Lebon *et al.*, 2004, 2005): in Pinot noir, meiosis takes place between stages 12 and 15 in anthers and between stages 15+2 d and 15+8 d in ovules (Fig. 1). Meiosis is a key point in the accomplishment of sexual reproduction. Anthers and ovules show particular sensitivity at this stage to various kinds of stress, especially those leading to any form of carbohydrate deprivation (Saini, 1997; Jean and Lapointe, 2001). Most often, the lack of sugar supply to the flower bud at meiosis results in its abortion.

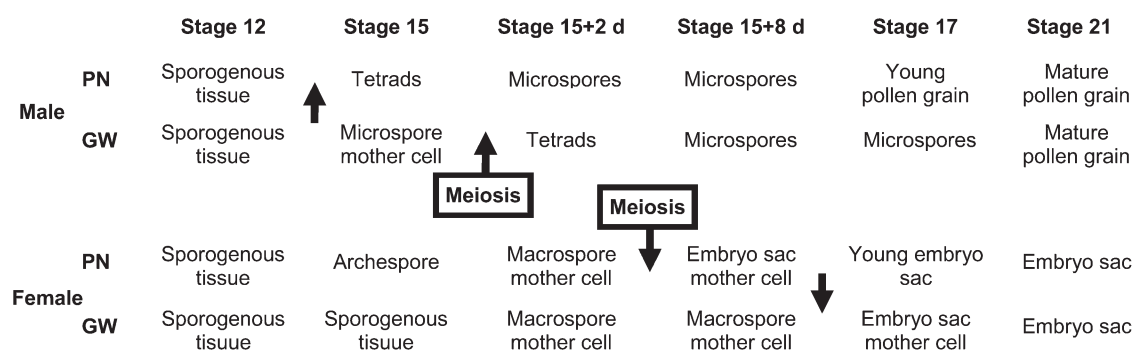
The process of reproductive development requires energy from floral initiation to fruit maturity. In woody plants, the energy devoted to reproduction originates from several sources, including reserves accumulated in perennial organs the year before, or photosynthesis performed either in the inflorescences themselves or in the leaves (Mullins *et al.*, 1992; Sauter and van Cleve, 1994; Rodrigo *et al.*, 2000; Hieke *et al.*, 2002). The total cost of energy can be calculated quite accurately, i.e. the energy

required for the development of a sole vine flower from evocation to anthesis corresponds to 37.6 Joules, which represents the use of 2 mg of carbohydrates (Blanke, 1990).

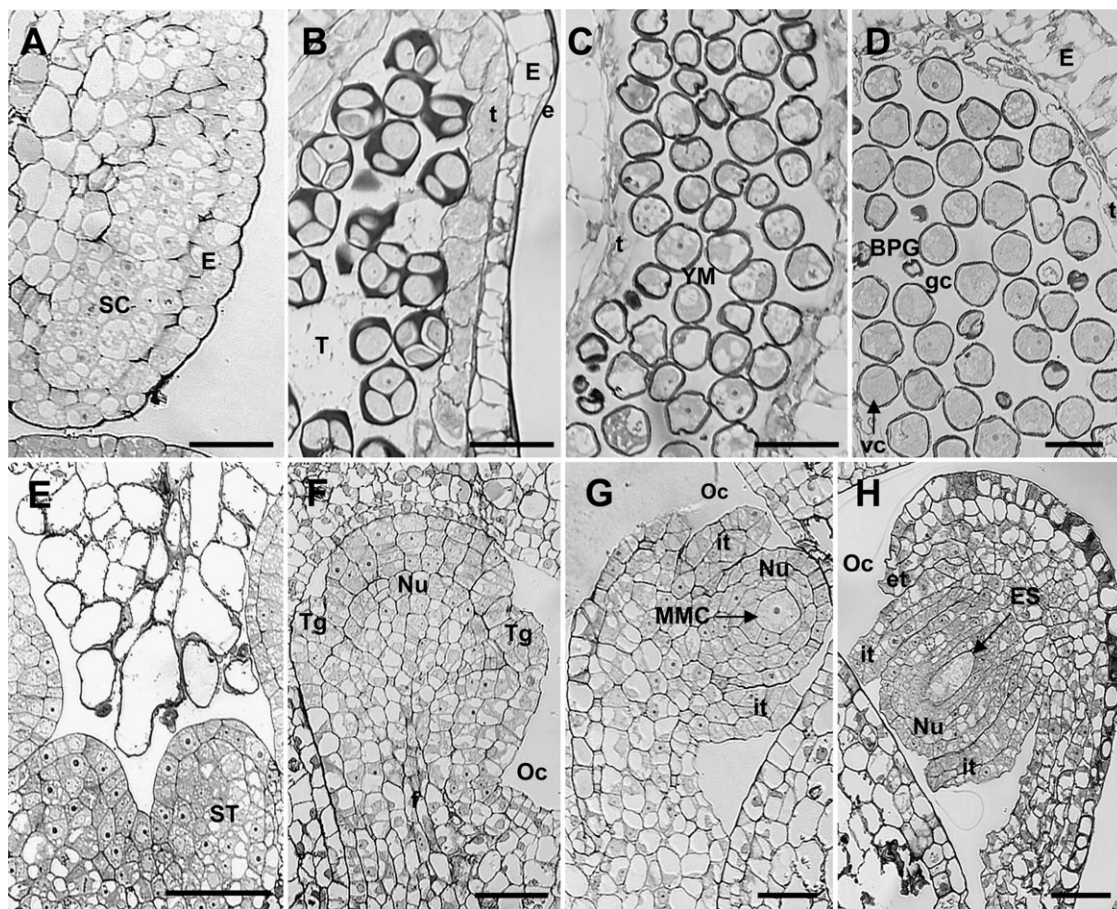
The supply of carbohydrates is thus crucial for the achievement of grapevine reproduction (Caspari *et al.*, 1998). They are involved in (i) the initiation of the inflorescence during the summer of the first year, (ii) the initiation of flowers on inflorescences during the second year, and (iii) at meiosis. This energy is provided by various successive organs during the annual cycle.

### Source–sink fluctuation in the grapevine during the annual cycle in relation with flowering

During the annual cycle there is a complex flux of carbohydrates in the whole vine between annual (leaves, inflorescences, and berries) and perennial (roots, trunks, and canes) organs. The developing organs attract nutrients and represent sink organs. Conversely, the organs which are able to release/synthesize sugars to the sinks are the source organs, i.e. the mature leaves. In angiosperms, the reproductive organs are typically sink organs throughout their development, whereas vegetative organs may behave either as source or sink depending on the stage of the annual cycle (Ho, 1988). During the season, in the grapevine some organs accumulate or release sugar reserves and others assimilate carbon through photosynthesis (Fig. 2). The balance between those activities can influence the development of reproductive organs since the main steps of source–sink interactions in grapevine coincide with key points of reproductive development. More precisely, the decline of starch mobilization from the perennial organs is synchronous with (i) the onset of net positive photosynthesis in the leaves and (ii) female meiosis (Lebon *et al.*, 2004; Zapata *et al.*, 2004a). Starch metabolism and photosynthesis thus are the two key processes determining sugar availability.



**Fig. 1.** Determination of developmental stage in Pinot noir and Gerwurztraminer varieties underlining the occurrence of male and female meiosis according to morphological phenology. Intermediate stages were added between stages 15 and 17 (15+2 d and 15+8 d) to get the time of meiosis accurately. d, days; GW, Gewurztraminer variety; PN, Pinot noir variety.



**Fig. 2.** Development of reproductive organs in Gewurztraminer according to Lebon *et al.* (2005). (A–D) Anther. (A) Stage 12, sporogenous cells (bar=500  $\mu$ m); (B) stage 15+2 d, tetrads (bar=500  $\mu$ m); (C) stage 15+8 d, young microspores (bar=500  $\mu$ m); (D) stage 17, bicellular pollen grains (bar=500  $\mu$ m). (E–H) Ovule. (E) Stage 12, sporogenous tissue (bar=250  $\mu$ m); (F) stage 15, archesporial tissue (bar=250  $\mu$ m); (G) stage 17, macrospore mother cell (bar=250  $\mu$ m); (H) stage 23, embryo sac (bar=250  $\mu$ m). BPG, bicellular pollen grains; e, epidermis; E, endothecium; ES, embryo sac; et, external tegument; f, funicle; gc, generative cell; it, internal tegument; MMC, macrospore mother cell; Nu, nucellus; Oc, ovarian cavity; SC, sporogenous cells; ST, sporogenous tissue; t, tapetum; T, tetrads; Tg, tegument; vc, vegetative cell; YM, young microspores.

### Sugar mobilization from wood reserves

In all grapevine varieties studied, starch represents the most important part of sugar reserves and is mobilized or accumulated according to the plant needs (Winckler and Williams, 1938; Eifert *et al.*, 1960; Bouard, 1966; Mullins *et al.*, 1992; Huglin and Schneider, 1998; Zapata *et al.*, 2001). In Pinot noir and Merlot, starch is stored in woody organs, either the roots or the canes. During winter dormancy, starch is mainly located in the ray parenchyma of the roots (Zapata *et al.*, 2004a). At this time, 90% of starch is contained in the root system (Eifert *et al.*, 1960; Bouard, 1966; Bates *et al.*, 2002) and represents one-third of the root dry weight (Zapata *et al.*, 2001).

In early spring, when the soil temperature reaches 10–12°C, winter dormancy is over in most cultivars and plant metabolism is reactivated (Huglin, 1986). Then starch is the sole source of carbohydrates in grapevine (Scholefield *et al.*, 1978; Huglin and Schneider, 1998; Zapata *et al.*, 2004b), and is progressively mobilized by

the growth of annual vegetative and reproductive organs (Bates *et al.*, 2002; Zapata *et al.*, 2004a, b). Reserve mobilization in the grapevine extends approximately until anthesis, depending on varieties (Zapata *et al.*, 2003). Reserve retranslocation destined to feed the developing inflorescences and other annual organs stops at flowering (Yang *et al.*, 1980; Candolfi-Vasconcelos *et al.*, 1994; Caspari *et al.*, 1998). Starch then accumulates again in annual (Bates *et al.*, 2002) and perennial (Mullins *et al.*, 1992; Bates *et al.*, 2002; Zapata *et al.*, 2004a) tissues from fertilization until the beginning of berry ripening (*véraison*) (Candolfi-Vasconcelos *et al.*, 1994; Caspari *et al.*, 1998) and reserves are thus progressively restored (Fig. 3).

### Leaf photosynthesis

In all cultivars of grapevine, leaf photosynthesis increases from bud break until flowering and then regularly decreases until leaf senescence (Stoev, 1952). Although grapevine leaves may assimilate CO<sub>2</sub> as early as bud

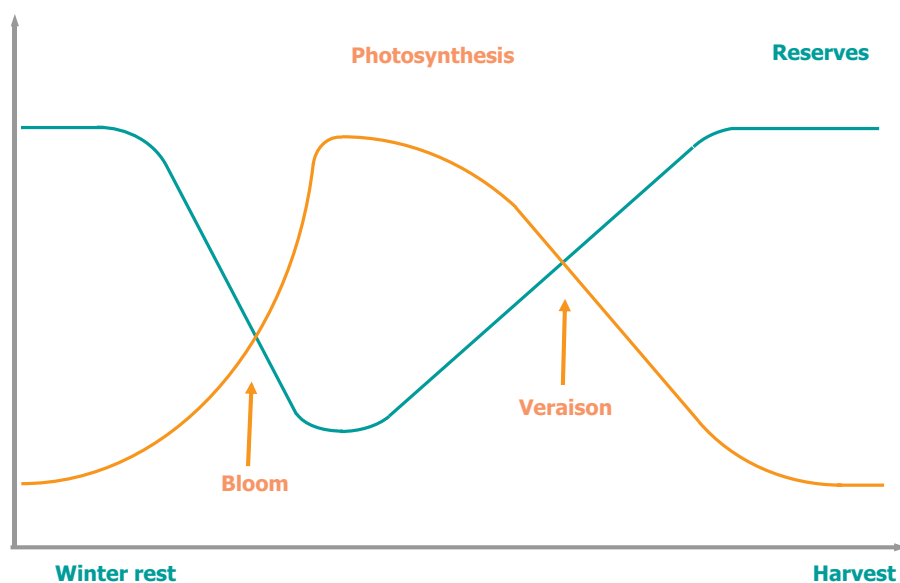


Fig. 3. Reserve and photosynthesis fluctuation in the grapevine during the annual cycle. Véraison is the beginning of berry ripening.

break (Griffon, 1905; Kriedemann, 1968), leaves remain sink organs during the early steps of their development. They become source organs when they reach between one-third (Koblet, 1969; Alleweldt *et al.*, 1982; Petrie *et al.*, 2000) and one-half (Stoev, 1952; Hale and Weaver, 1962) of their final size. At the whole plant scale, the maximum photosynthesis occurs before and during flowering, but some slight differences may appear according to the variety (Zufferey *et al.*, 1999; Zufferey and Murisier, 2002). For example, in Chasselas, maximum leaf photosynthesis is registered when the leaves reach 75–100% of their final size and this maximum level is maintained for approximately 30 d (Zufferey *et al.*, 1999). In Chasselas (Zufferey *et al.*, 1999) and Riesling (Schultz *et al.*, 1996), carbon dioxide fixation remains higher than 70% of the maximum during the next 100 d, supporting berry development. In other varieties such as Chardonnay, CO<sub>2</sub> assimilation rates reach a maximum in late spring to early summer (Chaumont *et al.*, 1994).

During the day, photosynthesis fluctuates depending on environmental conditions, in particular, temperature variations (Schultz, 2003) that influence stomatal opening and the rate of net photosynthesis (Roper and Williams, 1989; Maroco *et al.*, 2002; Patakas *et al.*, 2002; Medrano *et al.*, 2003). Under natural conditions, photosynthesis is greatest during the first hours of the day, in the morning reaching approximately 10–15  $\mu\text{M CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  (Chaumont *et al.*, 1994; Medrano *et al.*, 2003).

The allocation of photoassimilates fluctuates during the year. From flowering to véraison, the flux of fixed CO<sub>2</sub> is oriented towards both developing annual organs and perennial organs. By véraison, the pool of starch reserves is restored in the wood of most varieties (Stoev and

Ivantchev, 1977) and the main part of photoassimilates is then directed to berry maturation, at least in Pinot noir and Sauvignon blanc (Candolfi-Vasconcelos *et al.*, 1994; Caspari *et al.*, 1998). Later, photosynthetic activity progressively declines in parallel with leaf senescence (Stoev, 1952; Bertamini and Nedunchezian, 2003). This decline is mostly due to a reduction in stomatal conductance (Schultz *et al.*, 1996) and in protein content (Bettner *et al.*, 1986), especially Rubisco (Hunter *et al.*, 1994).

The annual cycle of grapevine sugar physiology may be separated into two successive phases. Phase one corresponds to the mobilization of starch from woody organs which supply the annual organs with carbohydrates during their early growth. Phase two coincides with net leaf photosynthesis which supports both the continuation of annual organ development and the replenishment of reserves. The transition between the two phases is progressive but occurs during various stages of flowering, depending on the variety. In Pinot noir, Gewurztraminer, and Merlot, it occurs at the time of female meiosis (Zapata *et al.*, 2004a, b), meaning that the flower bud is particularly vulnerable. During this delicate period, any interruption or partial decline of sugar supply may result in excessive flower abortion (Merjanian and Ravaz, 1930; Keller and Koblet, 1994, 1995; Caspari *et al.*, 1998).

### Carbohydrate supply to the inflorescence during flower development

In woody plants, vegetative and reproductive organs compete for resources provided by either reserve mobilization or photosynthesis (Wardlaw, 1990). In the grapevine, such competition is directly involved in fruit set, as

indicated by the beneficial effect of tipping the main shoot during flowering (Coombe, 1962; Koblet, 1966; Smithyman *et al.*, 1998), or of removing laterals (Vasconcelos and Castagnoli, 2000). During the first stages of inflorescence growth and flower development, leaves and stems have a diminishing effect on inflorescences by causing carbohydrate retention (Mullins, 1967, 1968; Mullins and Rajasekaran, 1981). In the meantime, emerging inflorescences have a limited ability to attract assimilates because of both their small size when compared to the leaves (Mullins *et al.*, 1992) and their partial autonomy through effective photosynthesis in the inflorescence tissues (Keller and Koblet, 1994; Lebon *et al.*, 2005).

Sucrose, glucose, and fructose are the major sugars of both xylem and phloem sap which feed the developing inflorescence. Sugars are only present in the xylem around bud burst time (Campbell and Strother, 1996). Although sucrose is supposed to be the major sugar transport form in grapevines (Mullins *et al.*, 1992), the sap composition analysis at bud break has revealed that hexoses are the major sugars of the xylem sap: sugar concentration is approximately 550  $\mu\text{M}$ , consisting of 250  $\mu\text{M}$  glucose, 250  $\mu\text{M}$  fructose, and 50  $\mu\text{M}$  sucrose (Glad *et al.*, 1992a; Campbell and Strother, 1996). In the phloem sap, up to 75 mM sugars are transported each hour in sap flow towards the inflorescence. Sugar concentration continuously increases until fertilization, representing 70% of compounds in sap flow at the beginning of flowering and 85% at full bloom and declines thereafter down to 60% at the end of fertilization (Glad *et al.*, 1992b). The occurrence of sugar transport from the phloem into the cells of reproductive organs has been investigated in some detail. Although most of the available data concern berry ripening (Fillion *et al.*, 1999; Davies *et al.*, 1999; Atanassova *et al.*, 2003; Vignault *et al.*, 2005), it has been shown that genes encoding membrane sucrose- or hexose-transporters are also expressed in flowers. *VvSuc11*, *VvSuc12*, and *VvSuc27* genes are involved in grapevine sucrose/ $\text{H}^+$ -transport, but are differentially expressed in plant tissues. In flowers there is a preferential expression of the *VvSuc27* gene which declines until berry ripening, whereas *VvSuc11* and *VvSuc12* are mostly expressed after berry ripening, when the rate of sugar import into the berries rapidly increases and hexoses begin to accumulate (Davies *et al.*, 1999). Regarding glucose and fructose, the hexose transporter gene *VvHT1* is mainly expressed during berry ripening (Atanassova *et al.*, 2003), but its expression could also be detected in flowers. More detailed analysis of sugar transport during the formation of individual reproductive organs may be of help in further understanding the accurate regulation of sugar supply to the grapevine flowers and its capacity to overcome various forms of stress.

During flower development, sugar distribution in the inflorescence fluctuates according to variety. Comparing

Gewurztraminer and Pinot noir, significant differences were observed between stages 15 and 17 at the time of female meiosis. Inflorescences of Gewurztraminer exhibited higher concentrations of starch and sucrose, whereas those of Pinot noir presented higher levels of glucose and fructose (Lebon *et al.*, 2004). However, despite higher starch concentrations in Gewurztraminer inflorescences, starch reserves were present in the ovules and the anthers of Pinot noir but were absent in those of Gewurztraminer (Lebon *et al.*, 2004). The differences of sugar distribution in these two varieties coincide with (i) an earlier transition between the phase of reserve mobilization and the phase of leaf assimilate export in Pinot noir (Zapata *et al.*, 2004b) and (ii) a better capacity of Pinot noir to overcome abiotic stress at the time of female meiosis. Although it has not been demonstrated, it seems that the variation in sugar composition in the inflorescence at this crucial stage may interfere with the final berry yield in the grapevine, as already shown in *Prunus armeniaca* (Rodrigo and Herrero, 1998; Rodrigo *et al.*, 2000) and in *Citrus* (Ruiz *et al.*, 2001; Iglesias *et al.*, 2003).

The successive sources of carbohydrates that contribute to the whole process of reproduction are then (i) leaf photosynthesis during the summer of the previous year, (ii) reserve mobilization from the perennial organs during the year of flowering, followed by (iii) leaf photosynthesis during the same year. In order to understand further the respective contribution of each of these parameters, a number of studies have been carried out, aiming to alter either reserve replenishment or photosynthesis.

### Involvement of reserves

Using radiolabelled  $^{14}\text{CO}_2$ , it was shown in the 'Delaware' variety that up to 28% of the photoassimilates are stored in the perennial organs, being partitioned as follows: 67% in the roots, 23% in the trunk, and 10% in the canes (Yang *et al.*, 1980). These assimilates are devoted to the growth of these organs and to reserves that will support the early development the following year (Scholefield *et al.*, 1978). Interruption of reserve replenishment has a direct impact on the parameters of reproduction in the following year. Owing to the chronology of reproductive organ formation, both the number of inflorescences per plant and the number of flowers per inflorescence are affected (Duchêne *et al.*, 2003a, b; Bennett *et al.*, 2005).

The numerous papers dealing with artificial modification of reserve replenishment show that the percentage of bud break (Mansfield and Howell, 1981; Candolfi-Vasconcelos and Koblet, 1990; Howell *et al.*, 1994) and the number of inflorescences per plant (Candolfi-Vasconcelos and Koblet, 1990; Bennett *et al.*, 2002, 2005; Duchêne *et al.*, 2003a, b; Poni *et al.*, 2006) depend on the intensity of assimilation during the previous year. The main target

for modifying reserve content in the roots, trunk, and canes is photosynthesis. Disturbing photoassimilation during reserve replenishment may be performed either by leaf removal (Candolfi-Vasconcelos and Koblet, 1990; Koblet *et al.*, 1994; Petrie *et al.*, 2003; Bennett *et al.*, 2002, 2005), modifying the leaf/fruit ratio (Duchêne *et al.*, 2003a, b), root pruning (McArtney and Ferree, 1999a) or shading (McArtney and Ferree, 1999b).

The intensity of the disturbance may be controlled either by the number of leaves removed or the developmental stage at which the experiment is performed. In this respect, the threshold of grapevine sensitivity to photosynthesis disturbance is quite high. Depending on the variety, at least 50% of leaves must be removed to obtain a significant impact on the numbers of inflorescences emerging the year after (Howell *et al.*, 1994). Also, the reduction of inflorescence number is higher when (i) the number of leaves removed is higher (Koblet *et al.*, 1994) or (ii) leaf removal is performed earlier (Mansfield and Howell, 1981; Candolfi-Vasconcelos and Koblet, 1990; Bennett *et al.*, 2005). For example, in Chardonnay, defoliation (except for four basal leaves) decreases starch content in the roots, from 17% dry weight in control plants down to 1.5% in the most severe treatments, leading to the loss of half of the following year's inflorescences (Bennett *et al.*, 2005). The post-harvest period also contributes to reserve replenishment: in the variety Semillon, total defoliation at harvest causes 21% of yield reduction the year after. A cumulative effect is registered when the experiment is performed twice, reaching a 50% decrease in inflorescence number (Holzapfel *et al.*, 2006).

While the number of inflorescences that appear in spring is dependent upon the extent of reserve replenishment during the previous year, the number of flowers per inflorescence is regulated in most cases by parameters fluctuating during the year of their development. There is a positive correlation between starch content in the wood at bud break and the number of flowers per inflorescence (Bennett *et al.*, 2002), showing that alteration of reserve replenishment may also affect the number of flowers per inflorescence (Duchêne *et al.*, 2003a, b).

Fruit set does not generally seem to be affected by the carbohydrate content in the perennial organs, although information to the contrary has been published for a few varieties (Petrie *et al.*, 2003). It has been demonstrated that fruit set remains similar in any particular variety, independently of reserve level in perennial organs and inflorescence number (Duchêne *et al.*, 2003a; Bennett *et al.*, 2002, 2005).

### Influence of leaf photosynthesis

As reported above, young leaves are capable of photosynthesis very early during their growth period, although they become the source of assimilates for the other organs of

the plant only when they reach at least one-third of their final size. In the whole vine, leaf photosynthesis regularly increases during the growth of the inflorescence. In Gewurztraminer and Pinot noir, net photosynthesis is enhanced respectively 2-fold and 4-fold between stage 12 and flower opening (Lebon *et al.*, 2005). The maximum is reached around flowering, which synchronizes more or less with the decline of sugar mobilization from the perennial organs (Zapata *et al.*, 2004a).

The contribution of photosynthesis to inflorescence and flower development during the same year is poorly documented, although sugars from current photosynthesis control fruit set (Hunter and Visser, 1990; Caspari *et al.*, 1998; Bennett *et al.*, 2002; Duchêne *et al.*, 2003a). Most of the studies in this field deal with berry feeding during growth and maturation. Up to 44% of the assimilated carbon by the whole plant is destined for reproduction including inflorescence, flower, and berry development (Candolfi-Vasconcelos *et al.*, 1994). It has been demonstrated that phyllotaxy regulates the fate of photoassimilates. Indeed, 89–93% of assimilates reaching the inflorescence originate from leaves on the same side of the cane (Motomura, 1993).

Indirect evidence of the role of current photosynthesis in reproduction has been provided. Assimilation disturbance by decreasing the efficient leaf surface area before flowering severely affects flower development, leading to necrosis and yield decreases (Candolfi-Vasconcelos and Koblet, 1990, 1991). It is possible to restore flower development by providing glucose to developing inflorescences (Jackson, 1991). Photosynthesis decrease may be induced either by progressive (Caspari *et al.*, 1998) or 90% leaf removal (Chaumont, 1995; Ollat, 1997). When leaf removal is less severe, it may be compensated by increased photosynthesis in the remaining leaves (Candolfi-Vasconcelos and Koblet, 1990) or by subsequent reserve mobilization as in the cherry (Layne and Flore, 1993).

### Photosynthesis in the inflorescence

In the grapevine, reproductive organs contain chlorophyll from the emergence of inflorescence until berry ripening, suggesting that photosynthesis occurs within those organs and partly contributes to the reproductive effort. The occurrence of photosynthesis in the inflorescence is now quite well documented.

Chlorophyll content in the inflorescence declines regularly during flower development (Lebon *et al.*, 2005): in both Pinot noir and Gewurztraminer inflorescences, chlorophyll level is comparable to that in the leaf at stage 12 and then rapidly decreases during the next steps, i.e. by 50% at stage 15. However, the chlorophyll *a/b* ratio remains constant and compatible with efficient photosynthesis during the whole of flower development (Blanke

and Leyhe, 1989a; Lebon *et al.*, 2005). Similar results were obtained in another species of *Vitis*, *V. labruscana* (Niimi and Torikata, 1978). Stomata are present in various organs of the inflorescence, including stalks, calyx, cap, and ovaries (Blanke and Leyhe, 1988; Blanke, 1991). The highest density was found on the calyx and the flower cap (Pallioti and Cartechini, 2001), suggesting that gas exchange occurs mainly in those organs.

The estimation of net photosynthesis has provided various results according to the variety and the way of measurement. In Riesling and Müller Thurgau varieties (Blanke and Leyhe, 1989b; Leyhe and Blanke, 1989), as well as in Pinot noir and Gewurztraminer (Lebon *et al.*, 2005), net photosynthesis was measured in the inflorescences at various stages of flower development (Fig. 4). It was significant during the early phases of inflorescence development and then declined in parallel with chlorophyll decrease and was insignificant at flowering. In Müller Thurgau net photosynthesis was 0.2–0.8  $\mu\text{g CO}_2$  fixed per bud and per hour (Leyhe and Blanke, 1989). Considering the whole inflorescence, net photosynthesis reached up to 4  $\mu\text{g CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  in Pinot noir and Gewurztraminer, which was comparable to the value measured in the leaf at stage 12 (Lebon *et al.*, 2005). Conversely, no net photosynthesis could be measured in the inflorescence of Chasselas until flower opening (Pallioti and Cartechini, 2001).

Measuring the rate of  $\text{CO}_2$  fixation does not reflect the gross photosynthesis, but only the difference between  $\text{CO}_2$  fixation and respiration. It has been shown in all the varieties studied that the inflorescence exhibits high respiration, reflecting intense metabolic activity (Niimi and Torikata, 1978; Blanke and Leyhe, 1989b). Respiration

in the inflorescence regularly increases during flower formation, as revealed by the constant increase of the internal  $\text{CO}_2$  concentration (Lebon *et al.*, 2005).  $\text{CO}_2$  originating from cell respiration may be re-fixed by phosphoenolpyruvate carboxylase (PEPCase) (Pallioti and Cartechini, 2001). It has been shown that the PEPCase activity increases in the flower caps until cap fall (Blanke and Leyhe, 1989b), which is in accordance with a constant increase of internal  $\text{CO}_2$  within the inflorescence tissues (Lebon *et al.*, 2005).

Overall, the inflorescence of grapevine performs  $\text{CO}_2$  fixation during flower formation, using atmospheric or respiratory  $\text{CO}_2$ . It now needs to be determined to what extent this autotrophic activity contributes to the total nutrient requirement of the inflorescence until anthesis. Also, the capacity of this process to adapt to abiotic stresses may represent an important tool for the plant to overcome environmental stresses which induce sugar starvation of the reproductive organs at key steps of development.

### Flower abscission and sugars

In the grapevine, flower abscission (so-called coulure) has been thoroughly investigated due to its economic impact. The exact meaning of coulure has changed over the years, depending on the organs undergoing the phenomenon, either flowers (Branas, 1957; Bessis, 1965) or young fruits (Stoev, 1962; Marès, 1868). More recently, Bessis and Fournioux (1992) considered that coulure occurs between bloom and fertilization. Flower abscission occurs naturally in vineyards. In this case, the extent of flower transformation into berries depends on both the variety and the

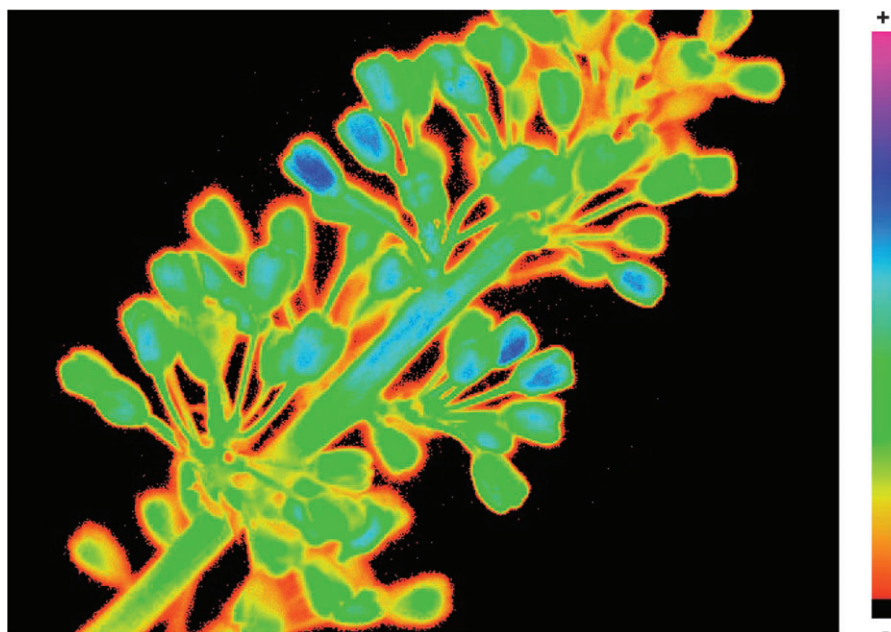


Fig. 4. Maximal PSII fluorescence of grapevine inflorescence at stage 17.



initial number on flowers in the inflorescence (Huglin and Schneider, 1998). For example, Pinot noir and Gewurztraminer grown in northern areas have between 210 and 230 flowers per inflorescence, providing, respectively, 135 and 188 berries (Lebon *et al.*, 2004). This means that, in this study, fruit set was 65% in Pinot noir against 82% in Gewurztraminer, or that flower abscission was 35% in Pinot noir and 18% in Gewurztraminer under optimal cultural conditions. However, under stress, flower abscission may increase dramatically, reaching up to 80% in sensitive varieties such as Gewurztraminer and much less in resistant varieties such as Pinot noir (Huglin and Schneider, 1998).

In the grapevine, coulure may be caused by several parameters. This includes environmental factors such as temperature (Buttrose and Hale, 1973; Ebadi *et al.*, 1995), drought (Ussahatanonta *et al.*, 1996), excessive humidity (Toussaint, 1983), or light (May and Antcliff, 1963; Ollat, 1997; Ebadi *et al.*, 1996). It has also been demonstrated that a disturbance in concentration of growth regulators as auxins (May, 2004; Roberts *et al.*, 2002), cytokinins (Boss and Thomas, 2000; Ollat *et al.*, 2002), gibberellins (Boss and Thomas, 2000; Dokoozlian *et al.*, 2001), ethylene (Hilt and Bessis, 2003), and polyamines (Colin, 2000; Aziz *et al.*, 2001) have an important influence on fruit set. Among these, polyamines are of particular interest in further understanding relationships between sugars and flowering in the grapevine. It has been shown in Pinot noir that free polyamines fluctuate in parallel with sugars in the inflorescence during development (Aziz, 2003). Shading the plants at full bloom causes a decrease in both sugar and free polyamines and leads to coulure, which can be prevented by application of spermidine prior to darkening (Aziz, 2003). These results suggest that spermidine participates in the regulation of flower abscission by modulating concentrations of sugars.

Flower abscission can be modified by variations in incident radiation (Ferree *et al.*, 2001), leaf area (Coombe, 1962; Koblet, 1966; Caspari *et al.*, 1998) or competition between vegetative and reproductive organs (Coombe, 1962; Koblet, 1966; Smithyman *et al.*, 1998; Vasconcelos and Castagnoli, 2000). Reduction of leaf surface area during flower formation leads to a decrease of nutrient availability for developing organs and to abnormal flower abscission (Candolfi-Vasconcelos and Koblet, 1991). However, leaf removal must reach 90% so that photosynthesis is so much reduced that additional reserve mobilization cannot compensate for the lack of autotrophy (Caspari *et al.*, 1998; Duchêne *et al.*, 2003b); these findings are therefore informative but unrelated to natural conditions.

Independently of its origin, the first indication of flower abscission consists of abnormalities in ovary development at the time of meiosis (Fougère-Rifot *et al.*, 1993). It seems that flower necrosis may be provoked by an

alteration of carbon metabolism (Gu *et al.*, 1996) as a result of physiological disturbance due to environmental or hormonal imbalance. The relationships between sugar physiology and flower abscission in the grapevine deserve further investigation, due to (i) the important role of sugars in the process of grapevine reproduction (Caspari *et al.*, 1998), (ii) the physiological transition of the whole plant physiology from reserve mobilization to leaf photosynthesis at the crucial female meiosis stage (Zapata *et al.*, 2004a, b), and (iii) the capacity to overcome bunch necrosis by providing sugars to the developing inflorescence (Jackson, 1991; Keller and Koblet, 1994).

It has been shown that soluble and insoluble sugar concentrations are higher in a coulure-resistant variety such as Chardonnay than in a coulure-sensitive variety such as Riesling (Vasudevan *et al.*, 1998). Similarly, the coulure-sensitive Gewurztraminer exhibits stronger vegetative growth than the coulure-resistant Pinot noir, suggesting that coulure sensitivity is related to lower sugar availability for developing flowers (Duchêne *et al.*, 2003b).

## Conclusions and future prospects

The formation of reproductive organs in the grapevine is a complex phenomenon extending over two successive years and interrupted by winter dormancy in temperate areas. Undoubtedly, the success of the reproductive process in the grapevine is dependent on sugar availability, although other nutrients such as nitrogen may be involved (Duchêne *et al.*, 2003a). Under optimal growing conditions, two key steps of reproductive organ formation should be considered. First, the physiological status of the plant during the initiation of inflorescence primordia during the summer preceding flowering; this regulates the number of inflorescences that will develop the year after, as shown by experimental disturbance of both photosynthesis and reserve replenishment. Second, the sugar content in the whole plant at bud break most likely participates in controlling the number of flowers on each inflorescence. Whatever the number of inflorescences per plant and the number of flowers per inflorescence, fruit set remains constant in the same variety when no additional stress occurs during the development of reproductive organs. Female meiosis has been identified as a third key stage in grapevine reproduction in relation to environmental stresses. Indeed, at this stage the reproductive organs of many angiosperms are sensitive to stress leading to flower sugar deprivation, even temporarily (Petrie and Clingeleffer, 2005). In the case of the grapevine, stress vulnerability is enhanced because female meiosis occurs when the whole plant physiology is switching its carbon nutrition from the mobilization of wood reserves towards photosynthesis.

Sugar metabolism during berry formation has been studied in great detail from fruit set until maturation, including the key step véraison. However, rather limited information is available about sugar's specific influence on inflorescence initiation or flower formation. For example, floral transition in the grapevine seems to be controlled by endogenous signals (Boss *et al.*, 2004), among which sugars, i.e. hexoses, may be involved (Roitsch and Gonzalez, 2004). Also, the regulation of sucrose or hexose transport has been characterized in detail during berry ripening (Davies *et al.*, 1999; Atanassova *et al.*, 2003; Cakir *et al.*, 2003; Letterrier *et al.*, 2003; Vignault *et al.*, 2005), but not during flower formation. Regulation at the female meiosis step may be of considerable importance for further understanding of flower sensitivity to environmental stresses such as drought or coldness, which are well known to interrupt photosynthesis and carbon metabolism (de Souza *et al.*, 2005; Ait Barka *et al.*, 2006). In this respect, determining the respective contribution of reserves, leaf and inflorescence photosynthesis to inflorescence growth using radiolabelled elements (vanden Heuvel *et al.*, 2002; Morinaga *et al.*, 2003) and the capability of the inflorescence to compensate fluctuations of carbon supply by regulating its own photosynthesis may be useful.

Grapevine flowering is currently being studied at the transcriptome level, following the expression of flowering-related genes (Boss *et al.*, 2006; Carmona *et al.*, 2008), or characterizing mutants in which flower development is affected (Chatelet *et al.*, 2007). Sugars are reliable markers of environmental stresses in the grapevine (de Souza *et al.*, 2005; Ait Barka *et al.*, 2006) and, in other plants, sugars participate in the regulation of numerous genes, including those involved in the flowering process (Koch, 1996; Roldan *et al.*, 1999; Ohto *et al.*, 2001; Gibson, 2005) and in source–sink regulation related to stress response (Roitsch, 1999). Therefore, in addition to their role as an energy source, sugars may be involved in the regulation of reproductive development in the grapevine as signal molecules. The recent optimization of genetic transformation in the grapevine (Bouquet *et al.*, 2006; Vidal *et al.*, 2006) offers new opportunities in terms of metabolic engineering of the carbohydrate supply of inflorescences and flowers (Goetz *et al.*, 2001) that may help to clarify the overall influence of sugars in the reproductive process of grapevine.

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