

## Phenology of Flowering and Starch Accumulation in Grape (*Vitis vinifera* L.) Cuttings and Vines

G. LEBON<sup>1</sup>, E. DUCHÊNE<sup>2</sup>, O. BRUN<sup>3</sup> and C. CLÉMENT<sup>1,\*</sup>

<sup>1</sup>Laboratoire de Stress, Défenses et Reproduction des Plantes, URVVC UPRES EA 2069, Université de Reims Champagne Ardenne, UFR Sciences, Moulin de la Housse, BP 1039, F-51687 Reims Cedex 2, France, <sup>2</sup>INRA de Colmar, Unité de Recherche Vigne et Vin, 28 rue Herrlisheim, BP 507, F-68021 Colmar, France and <sup>3</sup>Mumm-Perrier-Jouet Vignobles et Recherches, F-51206 Epernay, France

Received: 8 September 2004 Returned for revision: 22 October 2004 Accepted: 20 January 2005 Published electronically: 4 March 2005

- **Background and Aims** A reliable protocol for flowering and fruiting in cuttings was developed with the aim of (a) studying inflorescence and flower development in grapevine cuttings and field plants, and (b) assisting haploid plant production.
- **Methods** Inflorescence and flower development was studied in ‘Gewurztraminer’ (GW) and ‘Pinot Noir’ (PN) grape vines and cuttings grown in a glasshouse, along with variations in starch in the flowers. As there is a strong relationship between flower development and starch, the starch content of reproductive structures was estimated.
- **Key Results** Inflorescence and flower development were similar in the vines and cuttings with consistent differences between the two cultivars. Indeed, the ontogenesis of male and female organs is not synchronous in GW and PN, with both female and male meiosis occurring earlier in PN than in GW. Moreover, changes of starch reserves were similar in the two plant types.
- **Conclusions** Cuttings have a similar reproductive physiology to vines, and can be used to study grape physiology and to develop haploid plants.

**Key words:** Cuttings, flowering, reproductive structures, starch, vines, *Vitis vinifera* L.

### INTRODUCTION

The life cycle of higher plants varies greatly, depending on the species. In small herbaceous plants such as *Arabidopsis thaliana*, the cycle from seed to seed is 6 weeks, whereas woody plants with a long juvenile phase may not start flowering for several decades. Hence, most of the information on reproduction in plants is obtained from herbaceous models using *Arabidopsis thaliana* (Boss *et al.*, 2004; Jack, 2004), *Nicotiana tabacum* or similar species (Rieu *et al.*, 2003).

There is rich interest in the reproductive physiology of woody plants. For example, it has been shown that there is a strong relationship between flower development and carbohydrates (Rodrigo *et al.*, 2000; Jean and Lapointe, 2001; Ruiz *et al.*, 2001; Iglesias *et al.*, 2003). In particular, the concentration of starch in the ovule at specific steps of development is closely correlated to fertility. In *Prunus armeniaca*, secondary ovules that degenerate often display starch degradation in their tissues (Rodrigo and Herrero, 1998). Moreover, carbohydrate physiology in flowers is affected by environmental stresses such as drought (Lalonde *et al.*, 1997), chilling (Ebadi *et al.*, 1995) or heat (Young *et al.*, 2004), which lead to partial or total sterility.

In *Vitis vinifera* L., the pathways of inflorescence and flower development contrast with those for many other species in several aspects (Boss *et al.*, 2003), with stresses delaying or stopping reproductive development. For example, low temperatures near flowering affect ovule development and pollen tube growth (Ebadi *et al.*, 1995). These responses are mediated by perturbations in carbohydrate

physiology. Indeed, shading the leaves reduces photosynthesis and carbohydrate supply to the developing inflorescences, causing flower abscission and lower yields (Jackson, 1991). The reaction to stresses differs according to the genotypes (Gu *et al.*, 1996; Zapata *et al.*, 2004). ‘Gewurztraminer’ and ‘Pinot Noir’ have different rates of flower abscission, related to the sugar content in the inflorescence during flower development (Lebon *et al.*, 2004). Efforts to understand this behaviour would be improved by developing a reliable model of flowering and carbohydrate physiology.

Grapevine cuttings can flower when grown under controlled conditions (Mullins, 1966; Mullins and Rajasekaran, 1981), although the available protocols are complicated. Moreover, the correspondence between the development of inflorescences *in vitro* and in the vineyard is not well explained. The objective of this work was to provide a simple protocol for flowering in *in vitro* cuttings that mimic sexual reproduction in the field. The development of male and female tissues and the presence of starch within the flowers were followed during ontogenesis in cuttings and the response compared in vineyards. The study was performed in ‘Gewurztraminer’ and ‘Pinot Noir’, which differ in fertility and sensitivity to flower abscission (Lebon *et al.*, 2004).

### MATERIALS AND METHODS

#### *Plant material*

Investigations were carried out on *Vitis vinifera* L. using the flower abscission-sensitive ‘Gewurztraminer’ (GW) and the non-sensitive ‘Pinot Noir’ (PN) varieties. Dormant cuttings,

\* For correspondence. E-mail christophe.clement@univ-reims.fr

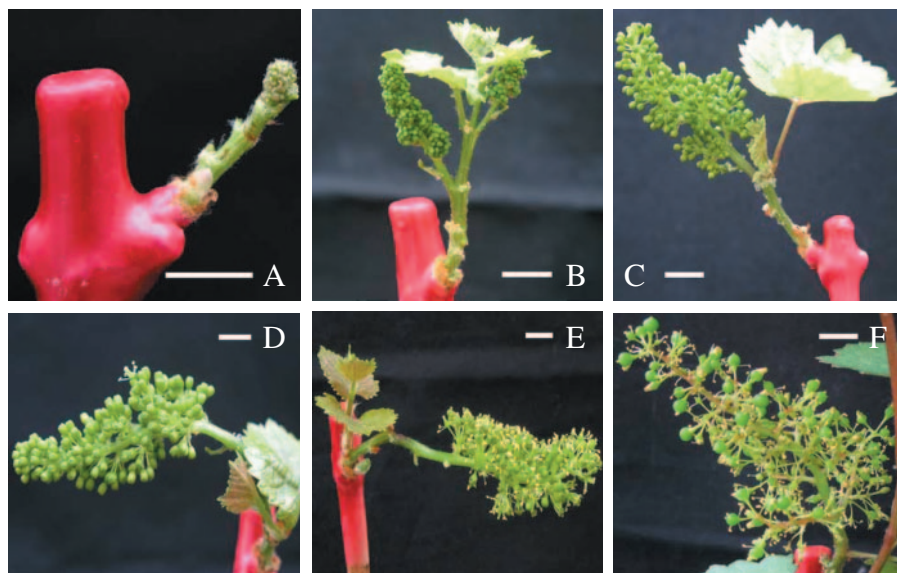


FIG. 1. Phenology of the 'Gewurztraminer' grape cuttings: (A) visible clusters (stage 12); (B) separated clusters (stage 15); (C) separated floral buds (stage 17); (D) flowering onset (stage 21); (E) end of flowering (stage 25); and (F) fruit set (stage 27). Scale bars = 1 cm.

which had been cane-pruned were collected from the INRA vineyard in Bergheim, France (Mullins and Rajasekaran, 1981).

Cuttings were collected at the proximal part of the cane and limited to three nodes, successively named N0 (the proximal), N1 and N2. A key point in the protocol is the sampling of the cane fragments generating the cuttings. The physiology of this fragment, in particular the amount of reserves in the wood, is important for obtaining predictable results. Harvesting fragments from any part of the annual cane provides cuttings with different sugar contents that vary in reproduction. Similarly, fragments from the distal part of the cane have low carbohydrate reserves and flower less (data not shown).

Cuttings were treated with cryptonol (2 % v/v) to prevent contamination, stored in the dark and forced for at least 2 weeks at 4 °C. After 16 h of hydration at 25–30 °C, N0 and N1 were removed and the proximal extremity of the cutting immersed in indole-3-butyric acid (IBA) at 1 g L<sup>-1</sup> for 30 s to promote rhizogenesis. This protocol was developed from Mullins (1966) and Mullins and Rajasekaran (1981) with IBA favouring rhizogenesis and thus eliminating the need for a 4-week pre-treatment in which the cuttings are warmed at the base. The IBA-treated cuttings developed within 1 week instead of 4 weeks.

Cuttings were planted in 0.5-L pots containing perlite : sand (1 : 1) and transferred to a greenhouse at 20/30 °C (night/day), with the pots placed on a 30 °C warming blanket. The photoperiod was 16 h using natural daylight or artificial 400 W 'Saudiclaude' lamps supplying 1000 μE m<sup>-2</sup> s<sup>-1</sup>, and a relative humidity of 50 %. Each pot was watered daily with 100 mL of Coïc and Lesaint medium (Coïc and Lesaint, 1971). Leaves were removed until the first inflorescence appeared (each cuttings was limited to four leaves).

Inflorescences were collected from vineyard and glass-house cuttings (Eichhorn and Lorenz, 1977), from the

visible cluster (stage 12) up to fruit set (stage 27). Since meiosis occurs in these cultivars between stages 15 and 17 in vineyards (Lebon *et al.*, 2004), this period was subdivided (15 + 2 d and 15 + 8 d). Development was quicker in the cuttings and the intermediate stages were defined as 15 + 1 d and 15 + 3 d.

Each developmental stage occurred when at least half of the cuttings were at this stage. The numbers of flowers, berries and seeds per fruit were counted on each cutting ( $n = 10$ ).

#### Microscopy

Samples were fixed in 2 % glutaraldehyde (v/v in a 0.1 M phosphate buffer) at pH 7.2 in 2 % sucrose (w/v) and 1 % Tween 20 (v/v) for 24 h and agitated at room temperature. After three rinses (5 min) in buffer, the flowers were post-fixed with 1 % osmium tetroxide (w/v) in the buffer without Tween 20 for 4 h. Flowers were then rinsed three times (5 min) in the buffer, dehydrated in an alcohol series, transferred to acetone, and embedded in Araldite.

For starch, 1 μm sections were collected on glass slides, bleached for 30 min with hydrogen peroxide (H<sub>2</sub>O<sub>2</sub> 10 volumes), and the periodic acid Schiff (PAS) polysaccharide-specific reaction carried out according to Clément *et al.* (1994). Sections were plunged in 1 % periodic acid (w/v) for 4 h, in Schiff's reagent without rinsing for 16 h, and in 5 % sodium metabisulfite (w/v) for 20 min. Sections were rinsed in distilled water, air-dried, and mounted in Eukitt<sup>®</sup>. For each stage, ten flowers from five inflorescences were used, each inflorescence sampling from a different vine or cutting.

#### Statistical analysis

For the number of flowers, berries and seeds per fruit, ten inflorescences were used, each inflorescence sampling from a different vine or cutting. Statistical analyses were carried

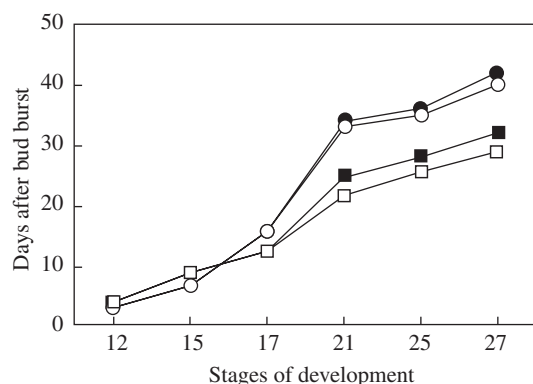


FIG. 2. Development of grape inflorescences in 'Gewurztraminer' (open symbols) and 'Pinot Noir' (closed symbols), in vines (circles) and cuttings (squares). Stage 12, visible clusters; stage 15, separated clusters; stage 17, separated floral buds; stage 21, early bloom (10 % opened flowers); stage 25, late bloom (90 % opened flowers); and stage 27, fruit set. Ten inflorescences were used, each inflorescence sampling from a different vine or cutting. Data are the mean of ten plants per treatment. The standard errors were smaller than the symbols, and therefore are not shown.

out using Student's *t*-test. A 5 % probability was considered significant. For morphology and starch content, ten flowers from five different inflorescences per stage were sampled, each inflorescence sampling from a different vine or cutting; for these parameters, no statistical analysis was performed because observations from the ten flowers considered were similar.

## RESULTS AND DISCUSSION

It is possible to mimic flowering and fruit set in the woody grapevine (*Vitis vinifera*) planted under controlled conditions. Both the male and female flowers were similar in the cuttings and field vines.

### Phenology

Previous studies on cuttings of grapevine (Mullins, 1966; Mullins and Rajasekaran, 1981) and apple (Höfer and Lespinasse, 1996) were not focused on the ontogenesis of reproductive organs, although it is a key issue in models of flowering. The grapes could be distinguished at stage 15 (Fig. 1B), whereas each flower bud was separated at stage 17 (Fig. 1C). At least 10 % and 90 % of the flowers were opened, respectively, at stages 21 and 25 (Fig. 1D and E), and thereafter fruit set occurred (Fig. 1F). Development was similar in the two cultivars, and both in vineyards and cuttings (Fig. 2). However, development after stage 17 was more rapid in the field than in the glasshouse.

The PN cuttings had more flowers than the vines, whereas there were no differences in the two types of GW plants (Table 1). There were also no differences between the two cultivars within a plant type. In contrast, the GW plants had more berries than the PN plants, and a higher rate of fruit set. The number of seeds per berry was not affected by cultivar or plant type, with general means of  $1.35 \pm 0.49$  and  $1.79 \pm 0.97$ , respectively (Table 1). Final yield was

TABLE 1. Number of flowers, berries per bunch, seeds per berries, and fruit set in 'Gewurztraminer' (GW) and 'Pinot Noir' (PN) grapes

	PN	GW
No. of flowers		
Vine	$206 \pm 24^a$	$231 \pm 32^a$
Cutting	$295 \pm 28^b$	$255 \pm 40^{ab}$
No. of berries		
Vine	$135 \pm 20^c$	$188 \pm 18^d$
Cutting	$162 \pm 3^c$	$226 \pm 38^d$
Fruit set (%)		
Vine	$65 \pm 3^e$	$82 \pm 6^f$
Cutting	$57 \pm 6^e$	$94 \pm 6^f$
Number seeds per berry		
Vine	$1.8 \pm 0.8^g$	$1.6 \pm 0.7^g$
Cutting	$1.4 \pm 0.5^g$	$1.8 \pm 1.0^g$

For each stage, ten flowers from five inflorescences were used, each inflorescence sampling from a different vine or cutting.

Values are means of ten inflorescences  $\pm$  s.e.

Statistical analyses were carried out using Student's *t*-test. Means for a considered parameter were not significantly different when followed by the same letter ( $P \geq 0.05$ ).

TABLE 2. Reproductive stages according to the classification of Eichhorn and Lorenz (1977)

	Developmental stage					
	12	15	15 + 2 d 15 + 1 d	15 + 8 d 15 + 3 d	17	21
Male						
GW						
Vine	SC	mMC	T	m	Vm	MPG
Cutting	SC	mMC	T	T	Vm	MPG
PN						
Vine	SC	T	m	Vm	YPG	MPG
Cutting	SC	T	m	m	YPG	MPG
Female						
GW						
Vine	ST	ST	MMC	MMC	ESMC	ES
Cutting	ST	ST	A	A	MMC	ES
PN						
Vine	ST	A	MMC	ESMC	ES	ES
Cutting	ST	A	MMC	MMC	ES	ES

A, Archeospore; SC, sporogenous cells; m, microspores; mMC, microspore mother cell; MMC, macrospore mother cell; Vm, vacuolated microspores; MPG, mature pollen grain; ESMC, embryo sac mother cell; ES, embryo sac; ST, sporogenous tissue; T, tetrads; YES, young embryo sac; YPG, young pollen grain.

For vine and cutting, data are the means of ten flowers from five different inflorescences, and each inflorescence was sampled from a different vine or cutting. For each stage of development, the ten flowers considered show similar results.

similar in the cuttings and vineyard, with respect to number of flowers, number of fruits and seed set.

### Development of reproductive organs

Inflorescences in the cuttings developed more rapidly than those from the vineyard plants, with a difference of 12 and 13 d in GW and PN, respectively (Fig. 2). The rate of flower development is dependent on temperature, with

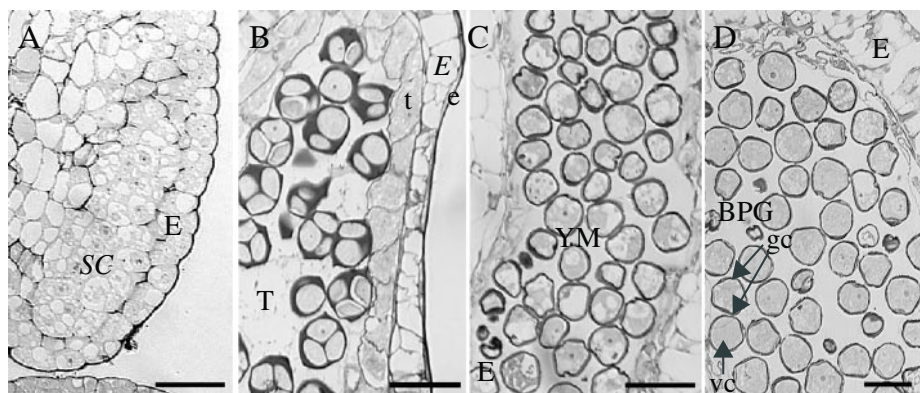


FIG. 3. Development of anthers in 'Gewurztraminer' (GW) cuttings: (A) stage 12, sporogenous cells; (B) stage 15 + 2 d, tetrads; (C) stage 15 + 8 d, young microspores; and (D) stage 17, bicellular pollen grains. BPG, Bicellular pollen grains; e, epidermis; E, endothecium; gc, generative cell; SC, sporogenous cells; t, tapetum; T, tetrads; vc, vegetative cell; YM, young microspores. Scale bars = 500  $\mu\text{m}$ .

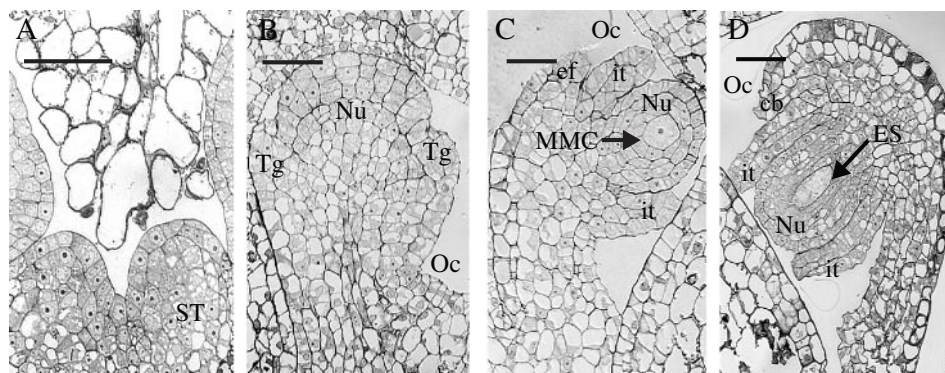


FIG. 4. Development of female flower parts in 'Gewurztraminer' cuttings: (A) stage 12, sporogenous tissue; (B) stage 15, archesporium; (C) stage 17, macrospore mother cell; and (D) stage 23, embryo sac. Arrows in C and D indicate starch grains. ES, Embryo sac; ef, external tegument; f, funicle; it, internal tegument; MMC, macrospore mother cell; Nu, nucellus; Oc, ovarian cavity; ST, sporogenous tissue; Tg, tegument. Scale bars = 250  $\mu\text{m}$ .

extreme temperatures damaging these tissues (Ebadi *et al.*, 1995; Young *et al.*, 2004). The vineyards are exposed to daily fluctuations of temperature, with relatively low temperatures in the night and early morning, whereas the cuttings were exposed to constant temperature ranging from 20–30 °C.

The chronology of pollen development was identical in the vines and cuttings (Table 2). In GW, stage 12 sporogenous cells (Fig. 3A) multiplied and gave rise to microspore mother cells at stage 15. Male meiosis occurred between stages 15 and 15 + 2 d (Fig. 3B) with microspores released from the tetrads at stage 15 + 8 d (Fig. 3C). Microspores vacuolated thereafter and pollen mitosis took place between stages 17 and 21 (Fig. 3D). At stage 21 (anthesis) mature pollen grains were released from the anthers. Pollen development occurred earlier in PN than in GW (Table 2). Meiosis was registered between stages 12 and 15, whereas pollen mitosis took place between stages 15 + 8 d and 17. These results indicate that cuttings grown under controlled conditions can be used to study pollen development in grape.

There was a slight delay in female meiosis in the cuttings compared with the vines (Table 2). In GW cuttings, within the sporogenous tissue (Fig. 4A) an archesporium developed at stage 15 + 1 d (Fig. 4B), which remained until stage

15 + 3 d. Stage 17 was characterized by the presence of the macrospore mother cell (Fig. 4C) and meiosis occurred thereafter. The embryo sac was formed at stage 21 (Fig. 4D). In the GW vines, the sporogenous tissue multiplied at early stages of development. The macrospore mother cell was seen from stage 15 + 2 d to stage 15 + 8 d. Between stages 15 + 8 d and 17 meiosis occurred, and the three micropylar macrospores degenerated, leading to a single macrospore generating the whole embryo sac at stage 17. At anthesis, the embryo sac contained eight cells and was ready for fertilization.

Female development was faster in PN than in GW (Table 2). In vines, the archesporium appeared at stage 15 and meiosis occurred between stages 15 + 2 d and 15 + 8 d. Afterwards, the embryo sac developed between stages 15 + 3 d and 17, but the other steps of development were similar to those in vines.

#### Location of starch in the anther and the ovule

Changes of starch reserves were similar in the two plant types. Starch was found in all the sporophytic tissues of the anther (epidermis, endothecium and tapetum) in the

TABLE 3. Presence (+) or absence (0) of starch in Gewurztraminer (GW) and Pinot Noir (PN) vines and cuttings

	Gewurztraminer (vine/cutting)						Pinot Noir (vine/cutting)					
	Developmental Stages											
	12	15	15 + 2 15 + 1	15 + 8 15 + 3	17	21	12	15	15 + 2 15 + 1	15 + 8 15 + 3	17	21
Male structures												
Epidermis	+/0	+/0	+/0	+/0	+/0		+/0	+/0	+/0	+/0	+/0	
Endothecium	+/0	+/0	+/0	+/0	+/0		0/0	+/0	+/0	+/+	+/+	
Tapetum	+/0	+/0	+/0	+/0	+/0		+/0	+/0	+/0	+/0	+/0	
Pollen	0/0	+/0	+/0	0/0	+/0		+/0	+/0	+/0	+/0	+/0	
Female structures												
Ovary	0/0	0/0	+/0	+/0	+/+	+/+	+/0	+/0	+/0	+/+	+/+	+/+
Nucellus	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	+/0	0/0	0/0
Tegument	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	+/0	0/0	0/0
Embryo sac						0/0					0/0	0/0

For vine and cutting, ten flowers from five different inflorescences were analysed to study the presence of starch, and each inflorescence was sampled from a different vine or cutting. For each stage of development, the ten flowers considered show similar results for starch content. Structures are scored a '+' if the ten inflorescences had starch, and a '0' if the ten inflorescences had no starch.

vines (Table 3). In the gametophytic tissue, starch could be seen in PN microspores at all stages, but not at stages 12 and 15 + 8 d in GW (Table 3). Later on, amylogenesis/amyolysis occurred in the young pollen grains. In the cuttings, starch grains were not detected in sporophytic tissues, except in the endothecium at stages 15 + 3 d and 17 in PN, and only in stage 17 in GW (Table 3). No starch was detected in the gametophytic tissue (Table 3). The anther sporophytic tissues influence the concentrations of sugars in the anther by storing/mobilizing starch and regulate the volume of sugar reaching the pollen grain during development (Clément and Audran, 1995). The difference reported here between vines and cuttings may correspond, in terms of starch in the anther sporophytic tissues, to such buffering action of the sporophytic tissues and obviously does interfere with pollen development.

In the ovules of the vines, concentrations of starch were low except at stage 15 + 8 d in the teguments and nucellus of the PN ovules (not in GW) (Table 3). Nevertheless, amylose reserves were detected in ovaries of both cultivars from stage 12 and 15 + 2 d in PN and GW, respectively, to anthesis. In contrast, starch was not detected in the nucellus and teguments of the cuttings during flower development, except at stage 15 + 3 d for PN (Table 3), same as in the vines. In the cuttings, starch accumulation in the ovaries was delayed until stage 17 in GW and stage 15 + 3 d in PN. In grapevine (Lebon *et al.*, 2004), as in other fruit trees (Rodrigo and Herrero, 1998; Rodrigo *et al.*, 2000), the presence or absence of starch in the ovule at meiosis has a key influence on fertilization and fruit set. In the cuttings and vines, the variations in concentrations of starch in the inflorescences and ovules were similar.

There were differences in reproduction between GW and PN grown in the vineyard; GW exhibits faster inflorescence development (Fig. 2), more flowers per inflorescence (Table 1), but a lower flower abscission rate, percentage of fruit set and fewer seeds per fruit (Table 1). Moreover, starch has a greater incidence in PN ovules (Lebon *et al.*, 2004). These differences were also detected in the cuttings

grown under the conditions described here, further showing that the protocol used is reliable.

In grapevine, uncommitted primordium in the latent bud developed into inflorescence primordium and a potential fully developed inflorescence next spring, depending on environmental conditions (Boss *et al.*, 2002). Rapid shoot growth can produce tendrils rather than inflorescences. Further development of the flowers is influenced by variety and environment (Boss *et al.*, 2003). Flower abscission is poorly described in grapevine but can dramatically diminish the yield (Huglin and Schneider, 1998). The supply of carbohydrates to the flower is a key factor in abscission (Gu *et al.*, 1996). A model of flower development in woody species should assist efforts to understand physiological traits that favour inflorescence initiation and flower development.

Cuttings can be used to develop doubled haploid grapevines. Until recently, attempts to obtain *in vitro* microspore embryogenesis from field plants generally resulted in diploid callus from the sporophytic tissues of the anther (Mauro *et al.*, 1986). In apple, the success of microspore embryogenesis is dependent on the donor material. Anthers collected from the field did not produce haploid embryos (Höfer and Lespinasse, 1996), whereas those from cuttings gave doubled haploids (Höfer *et al.*, 1999; Höfer, 2004).

#### LITERATURE CITED

- Boss PK, Bastow RM, Mylne JS, Dean D. 2004. Multiple pathways in the decision to flower: enabling, promoting, and resetting. *Plant Cell* 16: S18–S31.
- Boss PK, Buckeridge EJ, Poole A, Thomas MR. 2003. New insights into grapevine flowering. *Functional Plant Biology* 30: 593–606.
- Boss PK, Sensi E, Hua C, Davies C, Thomas MR. 2002. Cloning and characterisation of grapevine (*Vitis vinifera* L.) MADS-box genes expressed during inflorescence and berry development. *Plant Science* 162: 887–895.
- Clément C, Audran JC. 1995. Anther wall layers control pollen sugar nutrition in *Lilium*. *Protoplasma* 187: 172–181.
- Clément C, Chavant L, Burrus M, Audran JC. 1994. Anther starch variations in *Lilium* during pollen development. *Sexual Plant Reproduction* 7: 347–356.

- Coïc F, Lesaint L. 1971.** Comment assurer une bonne nutrition en eau et en ions minéraux en horticulture. *Horticulture Française* **8**: 11–14.
- Ebadi A, May P, Sedgley M, Coombe BG. 1995.** Effect of low temperature near flowering time on ovule development and pollen tube growth in the grapevine (*Vitis vinifera* L.), cvs Chardonnay and Shiraz. *Australian Journal of Grape and Wine Research* **1**: 11–18.
- Eichhorn KW, Lorenz DH. 1977.** Phänologische Entwicklungsstadien. Der rebe. Nachrichtenb. *Deutsch Pflanzenschutz (Braunschweig)* **29**: 173–180.
- Gu S, Lombard P, Price S. 1996.** Effect of shading and nitrogen source on growth, tissue ammonium and nitrate status and inflorescence necrosis in Pinot noir grapevine. *American Journal of Enology and Viticulture* **47**: 173–180.
- Höfer M. 2004.** *In vitro* androgenesis in apple—involvement of the induction phase. *Plant Cell Reports* **22**: 365–370.
- Höfer M, Lespinasse Y. 1996.** Haploidy in apple. In: Jain SM, Sopory SK, Veilleux RE, eds. *In vitro haploid production in higher plants, Vol. 3*. Dordrecht: Kluwer, 259–274.
- Höfer M, Touraev A, Heberle-Bors E. 1999.** Induction of embryogenesis from isolated microspores. *Plant Cell Reports* **18**: 1012–1017.
- Huglin P, Schneider C. 1998.** *Biologie et écologie de la vigne*. Paris: Tech and Doc, Lavoisier eds.
- Iglesias DJ, Tadeo FR, Primo-Millo E, Talon M. 2003.** Fruit set dependence on carbohydrate availability in citrus trees. *Tree Physiology* **23**: 199–204.
- Jack T. 2004.** Molecular and genetic mechanisms of floral control. *Plant Cell* **16**: S1–S17.
- Jackson DI. 1991.** Environmental and hormonal effects on development of early bunch stem necrosis. *American Journal of Enology and Viticulture* **42**: 290–294.
- Jean D, Lapointe L. 2001.** Limited carbohydrate availability as a potential cause of fruit abortion in *Rubus chamaemorus*. *Physiologia Plantarum* **112**: 379–387.
- Lalonde S, Beebe DU, Saini HS. 1997.** Early signs of disruption of wheat anther development associated with the induction of male sterility by meiotic-stage water deficit. *Sexual Plant Reproduction* **10**: 40–48.
- Lebon G, Duchêne E, Brun O, Magné C, Clément C. 2004.** Flower abscission and inflorescence carbohydrates in sensitive and non-sensitive cultivars of grapevine. *Sexual Plant Reproduction* **17**: 71–79.
- Mauro MC, Nef C, Fallot J. 1986.** Stimulation of somatic embryogenesis and plant regeneration from anther culture of *Vitis vinifera* cv. Cabernet-Sauvignon. *Plant Cell Reports* **5**: 377–380.
- Mullins MG. 1966.** Test plants for investigations of the physiology of fruiting in *Vitis vinifera* L. *Nature* **209**: 419–420.
- Mullins MG, Rajasekaran K. 1981.** Fruiting cuttings: revised method for producing test-plants of grapevine cultivars. *American Journal of Enology and Viticulture* **32**: 35–40.
- Rieu I, Wolters-Arts M, Derksen J, Mariani C, Weterings K. 2003.** Ethylene regulates the timing of anther dehiscence in tobacco. *Planta* **217**: 131–137.
- Rodrigo J, Herrero M. 1998.** Influence of intraovular reserves on ovule fate in apricot (*Prunus armeniaca* L.). *Sexual Plant Reproduction* **11**: 86–93.
- Rodrigo J, Hormaza JI, Herrero M. 2000.** Ovary starch reserves and flower development in apricot (*Prunus armeniaca*). *Physiologia Plantarum* **108**: 35–41.
- Ruiz R, Garcia-Luis A, Honerri C, Guardiola JL. 2001.** Carbohydrate availability in relation to fruitlet abscission in Citrus. *Annals of Botany* **87**: 805–812.
- Young LW, Wilen RW, Bonham-Smith PC. 2004.** High temperature stress of *Brassica napus* during flowering reduces micro- and megagametophyte fertility, induces fruit abortion, and disrupts seed production. *Journal of Experimental Botany* **55**: 485–495.
- Zapata C, Déléens E, Chaillou S, Magné C. 2004.** Partitioning and mobilization of starch and N reserves in grapevine (*Vitis vinifera* L.). *Journal of Plant Physiology* **161**: 1031–1040.