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Abscission of grapevine fruitlets in relation to ethylene biosynthesis

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Summary

The ethylene content of grapevine (*Vitis vinifera*, cv. Chardonnay) flowers and fruitlets was followed during flowering, *i.e.* from stage 18 to stage 32 (Eichhorn and Lorenz 1977).

A strong rise of ethylene was observed at the beginning of abscission, corresponding to stage 23. High levels of ethylene were found in the whole period in which abscission occurred, indicating a possible role of ethylene in grapevine fruitlet abscission. The rise of ethylene was associated with a rise of 1-aminocyclopropane-1-carboxylic acid (ACC) content and ACC oxidase activity. At the end of abscission (stage 28-29), the levels of ethylene, ACC and ACC oxidase activity decreased to a level close to that at pre-bloom. The ethylene evolution in two clones with different sensitivity to berry drop revealed the relationship between ethylene and the intensity of abscission, and confirmed that the abscission promoting effect of ethylene depends on the phenological stage. Results are discussed with regard to possible hormonal interactions between ethylene, ACC, ACC oxidase and abscisic acid.

K e y w o r d s : abscission, *Vitis vinifera* L., ethylene, 1-aminocyclopropane-1-carboxylic acid, ACC oxidase, abscisic acid.

A b b r e v i a t i o n s : ABA = abscisic acid, ACC = 1-aminocyclopropane-1-carboxylic acid, ACO = 1-aminocyclopropane-1-carboxylate oxidase, cl. = clone.

Introduction

In grapevine (*Vitis vinifera* L.), random fruitlet abscission occurs after flowering leading to an unpredictable yield. Grapevine fruitlet abscission is a correlative phenomenon depending on many biotic and non-biotic factors (Carbonneau and Ollat 1993) and was demonstrated to be under hormonal control (Nitsch *et al.* 1960, Mullins 1986, Bessis and Fournioux 1992, Hofmaier 1993, Bessis *et al.* 2000, Colin 2000). Notably, as in many other species, ethylene was assumed to play a major role in the regulation of abscission. Grapevine fruitlet abscission was stimulated by exogenous application of ethylene and ethylene precursors (Bessis and Fournioux 1992, Hofmaier 1993, Bessis *et al.* 2000).

The aim of this work was to determine the relation between fruitlet abscission and endogenous ethylene concentrations. Ethylene evolution was followed from flowering to berry set; changes of ethylene were associated with those of ACC, the precursor of ethylene, and ACC oxidase, the enzyme converting ACC to ethylene.

Material and Methods

In a first experiment, evolution of ethylene was followed during flowering and compared with flower and fruitlet abscission. Two clones of Chardonnay with different sensitivity to berry drop were used. In a second experiment, changes in ethylene evolution, ACC content and ACC oxidase (ACO) activity were followed and compared with flower and fruitlet abscission. This experiment was done with only one clone of Chardonnay.

P l a n t m a t e r i a l : Grapevine flowers or fruitlets, including pedicels, of Chardonnay, clones 76 and 64-ATVB were used. Cl. 76 is a reference clone of Burgundy; clone 64-ATVB is a non-registered clone, with a high susceptibility to berry drop. Both clones were grown in a field experimental collection at the Association Technique Viticole de Bourgogne (Beaune, France).

C o l l e c t i o n o f p l a n t m a t e r i a l : Flowers or fruitlets were regularly collected in stages 18 to 30-32 (Eichhorn and Lorenz 1977). Samples always consisted of randomly collected groups of 5-10 flowers or fruitlets.

For each clone, 15 grapes were chosen and tagged before flowering. During sampling, floral abscission and developmental stage were determined. Flowers or berries were counted in a non-destructive way. Phenological stages were determined at the same time according to Eichhorn and Lorenz (1977).

A n a l y s i s o f e t h y l e n e , A C C a n d A C O : Determination of ethylene: Immediately after sampling, approximately 0.5 g fresh weight of flowers or fruitlets were enclosed in 20 ml sealed vials and heated in a double boiler at 100 °C for 90 min. After cooling, 1 ml gas sample was withdrawn from the headspace and the ethylene content was determined by a gas chromatograph equipped with a Porapack Q column and a flame ionization detector. Experiments were replicated 5 times.

Determination of ACC: Approximately 0.5 g fresh weight of flowers or fruitlets were ground in liquid nitrogen and homogenized in absolute ethanol with an Ultra-Turrax homogenizer for 5 min. ACC was extracted in boiling ethanol for 30 min. After centrifugation, the supernatant was removed and evaporated. Residues were dissolved in 1 ml distilled water and used for the ACC assay according to Lizada and Yang (1979). Experiments were replicated 5 times.

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Measurement of *in vivo* ACO activity: The ACO activity was determined by measuring ethylene production after incubation of plant material in a sealed vial in the presence of synthetic ACC. Approximately 0.5 g fresh weight were incubated in 1 ml of 10^{-2} M ACC and agar solution in a 20 ml sealed vial at 25 °C. Only pedicels were immerged in the ACC solution. After incubation for 90 min, 1 ml gas samples were withdrawn from the headspace and the ethylene content was determined as described above. A control consisting of plant material incubated without ACC allowed determination of natural and wound-induced ethylene evolution. Experiments were replicated 5 times.

Results

Ethylene content and its relation to abscission: For both clones, the ethylene content was low until stage 23 (Fig. 1 a). A distinct rise was observed thereafter and a maximum was reached in stage 27. In this stage, the ethylene content was 2.2 - 4.4-fold higher than in stage 18. In stage 27, the ethylene content of cl. 64 was 1.3-2.5-fold higher than of cl. 76.

These results were compared with floral abscission (Fig. 1 b). The increasing ethylene content was correlated with the onset of abscission. Notably, the ethylene content was very high in cl. 64 during the whole period in which abscission occurred.

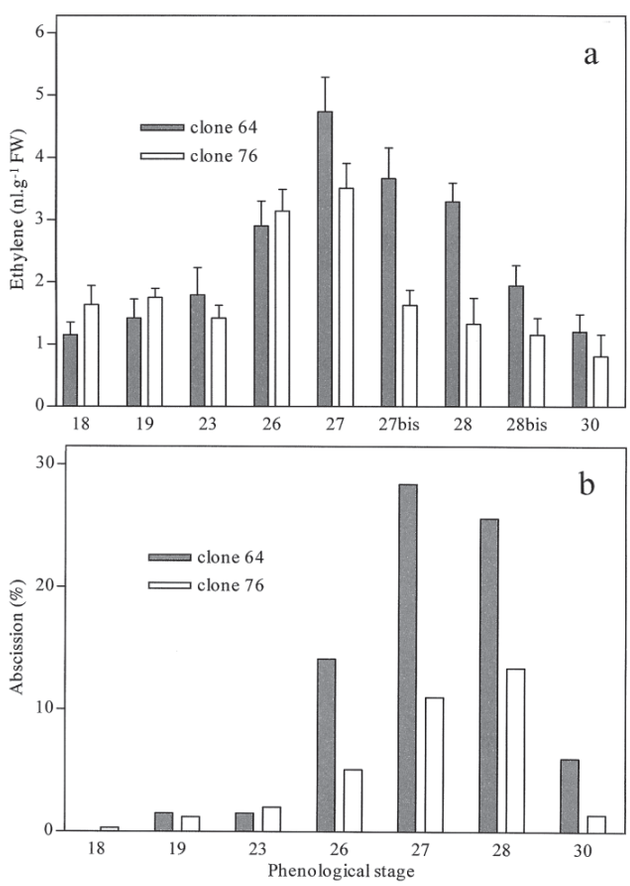

Fig. 1: **a**) The ethylene content of flowers and fruitlets of two clones of Chardonnay in different stages of development (EICHHORN and LORENZ 1977). **b**) Abscission of flowers and fruitlets of two Chardonnay clones in different stages of development. Results are percentages of the total amount of flowers before flowering.

The relationship between ethylene and the intensity of abscission was revealed by comparing both clones. When abscission was higher in cl. 64 than in cl. 76, the ethylene content was also greater. However, clone by clone comparison did not reveal a very close relationship between the ethylene content and the intensity of abscission, because for cl. 76, the highest ethylene content was not correlated with highest rates of abscission.

Ethylene and ACC contents and ACO activity: In this second experiment, results concerning ethylene evolution were in line with results described above (Fig. 2 a). The onset of abscission was associated with an increasing ethylene content. The ethylene content in stage 26 was 6-fold that in stage 18. After a peak in stage 26, the ethylene content remained high throughout the period of

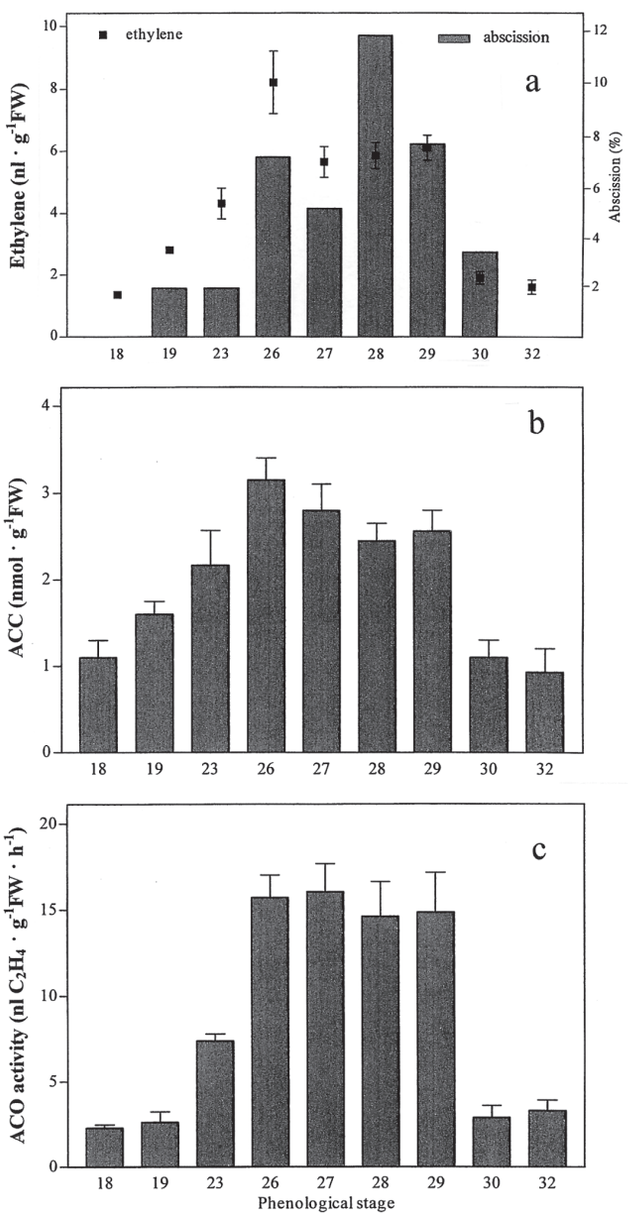

Fig. 2: **a**) The ethylene content and abscission rate of flowers and fruitlets in different stages of development. The abscission rate (%) is derived from the total amount of flowers before flowering. **b**) The ACC content of flowers and fruitlets in different stages of development. **c**) The ACO activity in flowers and fruitlets in different stages of development.

abscission. The ethylene content was not correlated with abscission intensity.

ACC levels increased between stages 18 and 26 (from 1.1 to 3.2 nmol·g^{-1}). After a stabilization period until stage 29, the ACC level decreased to a level close to that at pre-bloom (Fig. 2 b).

A similar pattern was observed for the ACO activity (Fig. 2 c), *e.g.*, a fast increase at the onset of flowering, followed by a stabilization period until the end of abscission and finally a return of the ACO activity to the pre-bloom level.

Discussion

Our results revealed a relationship between ethylene produced by the floral organs and their abscission. The abscission promoting effect of ethylene supplied to grapevine flowers and young berries has already been described (Bessis and Fournioux 1992, Hofmaier 1993, Bessis *et al.* 2000), but a direct role for ethylene in natural floral abscission of grapevine has not been demonstrated. Our results provide evidence for such a function of ethylene.

The ethylene increase during flowering was associated with a similar increase of ACC and the ACO activity. The high levels of ACC may be linked either to an activation of ACC-synthase, or to a higher availability of S-adenosylmethionine, or to a translocation of ACC from unknown origin. The presence of elevated ACC was however consistent with a rise of ethylene. In stage 19, the ACO activity increased significantly and persisted at a high and steady level until the end of abscission. This enzyme seems to play a major role in grapevine floral abscission. Recent studies on peach have shown that a specific ACO gene, *PP-ACO1*, was preferentially expressed in abscission zones of young fruit and was paralleled by an accumulation of its RNA (Ruperti *et al.* 2001). These data indicate that studies on ACO in grapevine floral organs should be continued.

Even if the period in which the abscission rate was highest is identical with a phase of intense ethylene production, the highest levels of ethylene were sometimes not correlated with highest abscission rates. A previous report showed that the capability of exogenous ethylene to promote grapevine fruitlet abscission depended on the developmental stage (Bessis and Fournioux 1992). Thus, in the first experiment, from stage 28, the ethylene level of clone 76 was close to the pre-bloom ethylene level but abscission was still high. So it seems that ethylene is not the only regulator of grapevine fruitlet abscission. Many authors have shown that the auxin/ethylene ratio plays a decisive role in the regulation of fruit abscission (Sexton *et al.* 1985, Brady and Speirs 1991, Sexton 1997). Concerning grapevine, recent studies reported peaks of free abscisic acid (ABA) at about full bloom (Baigorri *et al.* 2000, Colin 2000) and a coincidence between very high levels of ABA and "coulure" (*i.e.* excessive berry drop) (Colin 2000). Interactions between ABA and ethylene in regulating fruit abscission may be possible. Preliminary results obtained with an *in vitro* model described by Hilt and Bessis (2000), indicated that ABA was able to stimulate the ethylene biosynthesis, notably by acting on ACC-oxidase (Hilt, unpubl.). For fruitlet abscission of citrus induced by carbohydrate shortage, ABA might be implicated in the sensing of carbohydrate deficiency and might mediate ethylene synthesis *via* synthesis or transport of ACC (Gomez-Cadenas *et al.* 2000). These reports and our data support the assumption that ABA and ethylene interact in regulating grapevine fruitlet abscission. If so, ethylene would coordinate the abscission process itself, mainly by triggering the synthesis of hydrolases (Brown 1997, Sexton 1997).

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