Vitis **42** (1), 19–21 (2003)

Calcium accumulation and redistribution during the development of grape berry

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Summary

In this study, we investigated the evolution of calcium content during grape berry development (*Vitis vinifera* L.). In 5 years using several cultivars, the calcium content per berry was assessed in all compartments of the fruit. In the whole berry the calcium content increased from anthesis to ripeness. In the pericarp it decreased at the onset of ripening whereas it increased in seeds. Furthermore, during ripening, calcium was transported from the flesh to the skin. As calcium is a cation mobile in xylem, we compared our results with those on the functioning of xylem during the development of grape berries. We hypothesize that the calcium accumulation observed in the whole berry during ripening is due to its accumulation in the seeds.

K e y w o r d s : *Vitis vinifera*, grape berry, calcium, flesh, pericarp, seeds, skin, xylem.

Introduction

Calcium, an essential plant nutrient, is taken up by the root system and translocated to the aerial parts of plants, like the fruit, by symplastic and apoplastic transport via the xylem system (White 2001). In fleshy fruits, calcium deficiency can cause physiological disorders such as bitter-pit in apple, blossom-end rot in tomato, tip-burn in strawberry or melon watercore (Shear 1975; Odet and Dumoulin 1993; Wills *et al.* 1998). Furthermore, the calcium content is linked with resistance against pathogens. For example, in the grape berry, a high concentration of calcium contributes to delay senescence and to increase resistance to *Botrytis cinerea* (Chardonnet and Donèche 1995). Many studies have shown that calcium accumulates in grape berries throughout their development (Schaller *et al.* 1992; Ollat and Gaudillère 1996; Rogiers *et al.* 2000) whereas others indicate that calcium accumulation stops after veraison (Possner and Kliewer 1985; Creasy *et al.* 1993; Chardonnet 1994; Cabanne and Donèche 2001).

In plants, calcium is well known to move in xylem but to be substantially immobile in phloem (Marschner 1983). Many studies have been performed on developmental changes of phloem and xylem transport during grape berry ontogeny (Düring and Oggionni 1986; Düring *et al.* 1987; Findlay *et al.* 1987; Creasy *et al.* 1993; Greenspan *et al.* 1996; McCarthy and Coombe 1999; Coombe and McCarthy 2000). Using different techniques, these authors have shown that peripheral xylem flow in the berries ceases after veraison, *i.e.* at the onset of ripening (Creasy *et al.* 1993; McCarthy and Coombe 1999). Nevertheless, it has been shown that xylem bundles remain intact in the pedicel of berries (Rogiers *et al.* 2001). In dye uptake experiments, Findlay *et al.* (1987) found higher dye accumulation after veraison than before veraison, while no difference between pre- and post-veraison uptake of dye is reported by Creasy *et al.* (1993). Moreover, xylem flow has been shown to cease in the pericarp with a residual flow towards the pedicel as well as an increase in the calcium content of whole berries (Rogiers *et al.* 2001). It was concluded that in spite of an obvious xylem sap blockage in the pericarp, calcium continues to accumulate. Other authors have shown that at veraison peripheral xylem flow ceases while axial xylem flow continues (Düring *et al.* 1987), the latter primarily serving the seeds (Düring *et al.* 1987). The increase in the calcium content in the whole berry could thus be due to an accumulation of calcium in seeds. As suggested by Rogiers *et al.* (2000), it is important to localize calcium in the berry, in particular in the seeds, to clarify this issue. By using our numerous data on calcium distribution in berries, we describe here the fate of calcium in various compartments during berry development.

Material and Methods

P l a n t m a t e r i a l : Grape berries of *Vitis vinifera*, cvs Sauvignon blanc, Semillon, Merlot and Cabernet-Sauvignon, growing in a Graves vineyard (Bordeaux, France), were harvested at different stages of fruit development until maturity during the 1997 to 2001 seasons. Homogeneity of samples was previously checked by berry diameter and density (Barnavon 1999). For each date, two lots of washed berries were immediately frozen at -30 °C until used. The first lot was used for analysis of entire berries, the second lot for analysis of different compartments of the berry, *i.e.* seeds and pericarp; the latter is composed of flesh and skin. Each analysis was done in triplicate.

C a l c i u m c o n t e n t : To determine the calcium content of entire berries and of their compartments, they were separately homogenized at each sampling date with an Ultra-Turrax T 25 (IKA, Staufen, Germany) with ultrapure water. The homogenate was evaporated to dryness on a hotplate in porcelain capsules at 90 °C and heated in an oven at 550 °C; 3 ml of 0.1 N nitric acid were used to dissolve the ashes and the volume was adjusted to 25 ml with pure water.

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The calcium content was measured by capillary ion electrophoresis. A Waters Capillary Ion Analyzer (Quanta 4000, Waters, Saint-Quentin en Yvelines, France) was used throughout the investigation. The sample was loaded onto a 75 µm x 60 cm fused silica capillary using gravity in the hydrostatic injection mode for 30 s. Running conditions were 20 kV for the potential, resulting in an electrophoretic current of 11 µA at constant temperature of 25 °C. An electrolyte containing 50 mg tropolone, 50 mg UV-CAT2 and 50 mg Crown-6-Ether in 100 ml pure water was used (Waters, St Quentin en Yvelines, France). Detection was performed at a wavelength of 185 nm.

Electrophoregrams were recorded and processed with the Millennium data acquisition system (Waters). The calcium content was expressed in mg·berry^{-1}. Then the polynomial regression was performed by graphical determination with all data (Excel 97).

Results and Discussion

Over 5 years we gathered data from cvs Cabernet-Sauvignon, Sémillon, Merlot and Sauvignon for whole berry, flesh, skin, pericarp and seeds. To illustrate a general phenomenon, all data of calcium evolution were presented by polynomial regression. Optimum adjustment of the curve with the data was achieved by using second or third order polynomial equations. Fig. 1 represents calcium evolution in pericarp and seeds during development of grape berries. A polynomial equation of the order 2 was found, *i.e.* a parabola, with a low R^2 ($R^2 = 0.31$), whatever type of polynomial was used. This could be due to a poor fit between the equation and the data, or, to a wide variability of the data, limiting the determination of a regression curve. The latter assumption is more probable in view of the data and the bell-shaped curve which was also proposed by other authors (HRAZDINA 1984; CHARDONNET 1994). Moreover, the calcium content of grape berries depends on biological (cultivar, rootstock), edaphic (available soil cation and water content) and climatic factors. Consequently, the evolution of the calcium content in the various berry compartments can vary from year to year or with the grapevine cultivar (CHARDONNET 1994; CABANNE and DONÈCHE 2000). It appeared that the calcium content of the pericarp increased until veraison, then decreased during ripening. In plants, calcium is primarily transported in the xylem system. At veraison, a rupture of the xylem vessels occurs in the pericarp, and this is probably responsible for the halt of calcium accumulation in this compartment (DÜRING *et al.* 1987). It was interesting to note that the calcium content in the pericarp decreased by 0.05 mg·berry^{-1}. For the seeds, the curve was quite representative of the data since R^2 was 0.81. The calcium content in the seeds increased throughout the development of the berries including ripening. In general, xylem sap flow enters the berries in a peripheral vascular system leading to the pericarp and an axial system leading to the seeds. As reported previously, this peripheral flow disappears at veraison. The balance of calcium supply between flesh and seeds is then shifted entirely towards the seeds (DÜRING *etal.* 1987). The accumulation of calcium in the seeds thus persists until maturity, it becomes even more significant during ripening. It is possible that part of the calcium accumulating in the seeds during ripening (1 mg·berry^{-1}) is translocated from the pericarp to the seeds. Indeed, work is in progress in our laboratory to determine the capacity of seeds to absorb or release mineral cations.

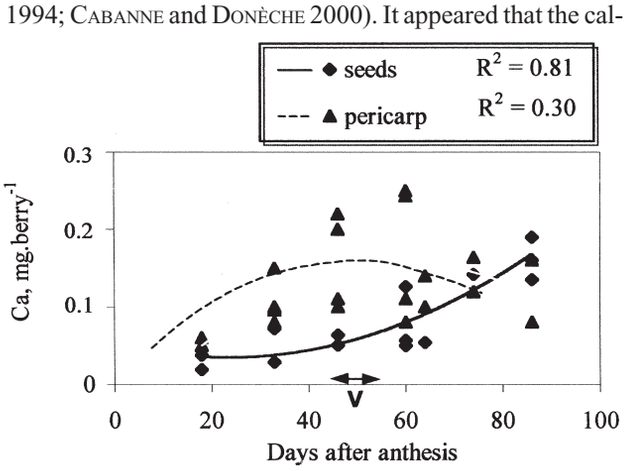

Fig. 1: Calcium evolution in seeds and pericarp from anthesis to maturity. Curves were obtained by polynomial regression of data. (V = veraison).

During berry development, the calcium content in flesh and skin was was determined as well (Fig. 2). Polynomials (order 3) were used for skin and flesh curves and relatively close relationships were found. Calcium accumulated in the flesh until veraison and then gradually decreased. However, the percentage of variance was relatively high. The use of polynomials of higher degree did not give a better fit. In the skin, the calcium content increased throughout berry development and then reached a plateau around maturity. During the "herbaceous" phase of growth (stage I), calcium accumulated in the flesh and skin. After veraison, calcium accumulation in the skin continued apparently to the detriment of the flesh. The variance observed for the flesh is significant and is probably responsible for that observed previously for the pericarp (Fig. 1). Furthermore, these observations confirmed an assumption according to which calcium is translocated from the flesh to the skin during ripening (CHARDONNET 1994; CABANNE and DONÈCHE 2001). However, calcium accumulation in the skin was slightly lower than its loss in the flesh. This consolidates our previous assumption of a calcium translocation from the pericarp (*i.e.* the flesh) to the seeds.

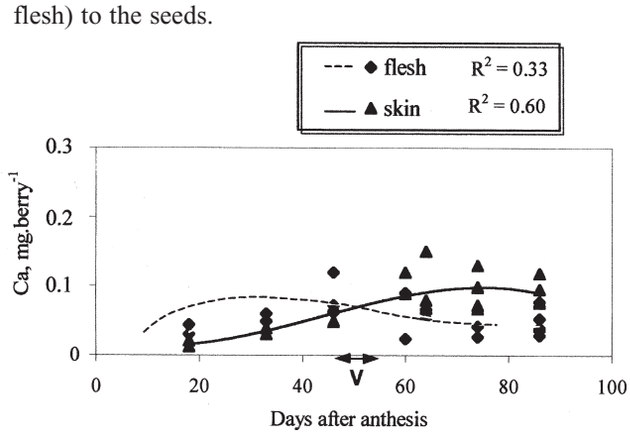

Fig. 2: Calcium evolution in flesh and skin from anthesis to maturity. For details: Fig. 1.

Calcium evolution in whole berries was compared to the sum of the data obtained in parallel for the pericarp and the seeds (Fig. 3). The calcium content in whole berries increased throughout their development; the increase seemed to be faster and more significant in stage I. During ripening, the increase was due exclusively to calcium accumulation in the seeds. The two curves are extremely close, thus validating the prediction for whole berries.

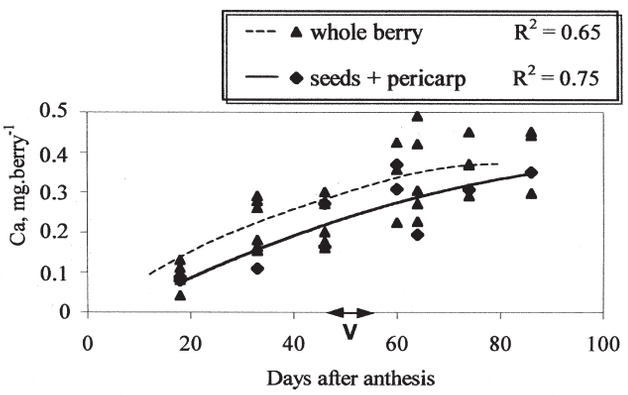

Fig. 3: Calcium evolution in the whole berry from anthesis to maturity. Comparison with data obtained separately for pericarp and seeds. For details: Fig. 1.

As shown previously, we added the calcium content of the flesh and skin and compared the results with those of the pericarp (Fig. 4). The two curves are almost identical thus validating the prediction despite the variance between some of them.

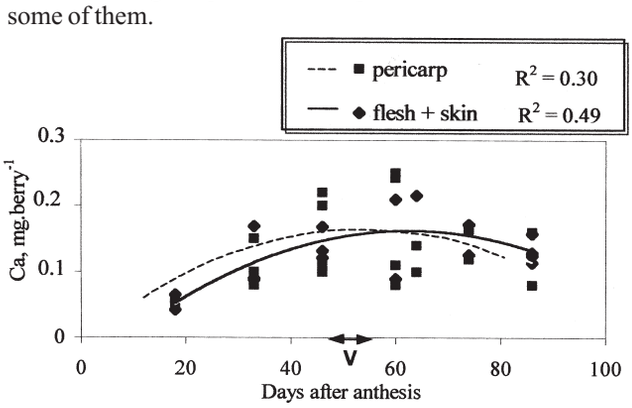

Fig. 4: Comparison of data obtained from pericarp with those obtained separately from flesh and skin from anthesis to maturity. For details: Fig. 1.

The aim of this work was to clarify some details concerning the calcium supply in berries. The metabolism of grape berries is now known to be a complex process where considerable compartmentation occurs between different types of tissues (Famiani *et al.* 2000). The calcium content and its evolution must be considered in the same way. Indeed, while calcium accumulation ceases at the onset of ripening in the pericarp, it persists in the seeds. At the same time, calcium is probably translocated from the flesh to the skin and seeds.

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Received December 4, 2002