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Relationship between xylem functionality, calcium content and the incidence of bitter pit in apple fruit

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ABSTRACT

The objective of this study was to evaluate the relationship between xylem functionality, calcium (Ca) deficiency and the incidence of bitter pit (BP) in 'Fuji' and 'Catarina' apples (low and high susceptibility to BP, respectively). Fruits were assessed for fresh weight, xylem functionality (of primary and secondary cortical vascular bundles) and mineral content (Ca, Mg, K, and N) during development (40–188 days after full bloom DAFB), as well as for the incidence (%) and severity of BP at commercial harvest (188 DAFB). During fruit development, 'Catarina' apples demonstrated an earlier loss of xylem functionality, lower Ca content, higher K content, and higher K/Ca, (K + Mg)/Ca and (K + Mg + N)/Ca ratios compared to 'Fuji' apples. The large loss in xylem functionality in 'Catarina' apples which led to a higher (K + Mg + N)/Ca ratio in the fruit seems to explain the higher susceptibility to BP as compared to 'Fuji'. Showing with this, the xylem functionality may be a key physiological to infer about this disorder.

1. Introduction

Bitter pit (BP) is a physiological disorder that causes high postharvest losses in apples. However the physiological causes of its development are poorly understood. This disorder is characterized by the breakdown of cells in the flesh just beneath the peel, giving rise to small dark depressions especially in the distal portion of the fruit (Amarante et al., 2006a).

The incidence of BP is related to low calcium (Ca) content and a high magnesium (Mg), potassium (K) and nitrogen (N) content in the fruit (Saure, 2005). These mineral elements are transported via vascular bundles from the roots to the fruit (Lang, 1990). According to Dražeta et al. (2004), these vascular bundles are arranged in two systems within the fruit, known as cortical vessels and carpels. The cortical vascular system consists of ten primary bundles, surrounding the fruit carpel that branch toward the epidermis to form a secondary cortical vascular system. The carpel vascular system contains ten ventral bundles and five dorsal bundles, which emerge from the peduncle and pass through carpels in anastomosis where it concludes in the pistil.

Vascular bundles are comprised of two vascular tissues: xylem and phloem. The phloem is living tissue that carries water and solutes (both organic and inorganic), while xylem vessels consist of dead cells that transport water and inorganic solutes (minerals). Ca is translocated throughout the xylem, via a series of charge exchanges across negatively charged sites within the cell walls. These sites are associated with multiple divalent cations and the chelation of Ca ions on xylem walls (Hanger, 1979). However, only minute quantities are translocated via the phloem (Saure, 2005).

During fruit growth and development in some fruit species, such as apples (Dražeta et al., 2004) and kiwi (Dichio et al., 2003), loss of xylem functionality may occur. This loss in xylem functionality may be associated with an increase in the number (Rančić et al., 2010) and/or elongation of parenchyma cells, which compresses xylem vessels (Lang and Ryan, 1994) without affecting the functionality of phloem vessels. This causes a reduction of Ca flow to the fruits, but does not affect K, Mg and N flow (Dražeta et al., 2004), which can compromise the postharvest quality of the fruits (Amarante et al., 2012). However, the fact that calcium reduction may cause a disruption of cell membranes, leading to cell death and tissue collapse resulting in BP. In order to clarify the hypothetic relationship between the functionality of xylem vessels and the BP postharvest disorder, the aim of this study was to evaluate the relationship of xylem functionality with Ca deficiency in 'Fuji' (lower susceptibility to BP) and 'Catarina' (higher susceptibility to BP), both apples grown in the south of Brazil.

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2. Materials and methods

The fruits were harvested during the 2009–2010 apple season from a commercial orchard located in the municipality of São Joaquim-SC (28°11′19, 66″ S, 49°59′42, 60″ W and 1219 m altitude). The fruits were grown on thirteen year-old 'Fuji' and 'Catarina' apple trees grafted onto 'Marubakaido' rootstock, with EM-9 filter. The trees were trained to a central leader and planted at medium-density with 2.0 m × 6.0 m. The soil Ca, Mg and K content was quantified at 11, 5 and 0.51 $cmol_c/dm^3$ respectively in 0–30 cm of soil depth.

Fruits were harvested weekly until 131 days after full bloom (DAFB). After this period, samples were collected at intervals of 15 days prior to the commercial harvest (~13.5°Brix and/or 4.5 N, 188 DAFB). Fruits were harvested early in the morning when plant transpiration is minimal and the water potential of the plant is similar to the soil water potential. The fruits were placed into plastic bags with distilled water to prevent xylem embolism, and taken to the laboratory. The fruits were then assessed for fresh weight, functionality of xylem and mineral content.

Xylem functionality was assessed according to the methods of Dražeta et al. (2004). Each fruit was sectioned at the base of its peduncle (approximately 1 mm), followed by immediate immersion of the peduncle into a staining solution (1% acid fuchsin). The fruits were infiltrated with the stain solution for approximately 8 h, with transpiration under normal conditions (temperature of 25 ± 2 °C and 70 ± 10% RH), using a fan to remove the boundary layer. Each fruit was cut into transverse 10 mm-thick slices from the calyx: distal (blossom end), middle (equatorial region) and proximal (peduncle end) portions. Each slice (20 samples both for each cultivar and each evaluated date) was assessed for the number of vascular bundles and their staining intensity in the primary and secondary cortical vascular system. The counting method through visual analysis was used to determine the number of functional xylem vascular bundles in the primary cortical system. Lightness (L) and hue angle ($h°$) of the cortex (in proximal + middle + distal regions) were measured to determine the staining intensity of the xylem in the secondary vascular system, using a Minolta model CR 400 colorimeter. It was possible to quantify the red staining intensity of the cortex that resulted from the transport of the stain solution via the xylem vessels by multiplying $L \times h°$. High values of $L \times h°$ indicate higher lightness (stronger white staining and lighter red staining in the cortex), which indicates lower functionality of the xylem in the secondary vascular system.

The contents of Ca, Mg, K and N were determined in the fruits harvested at 40, 68, 96, 131, 173 and 188 DAFB, according to the methodology described by Miqueloto et al. (2011). For mineral analysis, the fruits were cut at proximal, middle and distal portions, and a 5 mm layer from each portion was removed from the flesh, just beneath the peel as suggested by Amarante et al. (2006a). The Ca and Mg contents were determined in this portion of the flesh using emission spectrophotometry induced by plasma, K by flame photometry, and N by the semi micro-Kjeldahl method.

Four samples of 150 fruit each cultivar, harvested during commercial harvest (188 DAFB), were used to assess the incidence (%) and severity (number of pits/fruit) of BP. The visual severity of BP was determined using a 0 to 6 scale (0 – no pits, 1 – one pit, 2 – two pits, 3 – three pits, 4 – four pits, 5 – five pits and 6 – six or more pits).

The data were subjected to Bartlett's test for homogeneity of variances and, the Shapiro–Wilk test for normality of residuals, when the data serving the assumptions they were submitted of analysis of variance. An analysis of variance (ANOVA) was used to analyze fresh weight, xylem functionality and mineral content; and fruit development was subjected to linear and nonlinear regression analysis. All statistical analyses were conducted using SAS software, version 9.1 (SAS Institute, 2009).

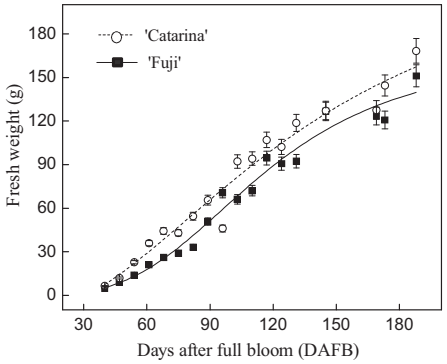

Fig. 1. Fresh weight (g) of 'Fuji' and 'Catarina' apples during fruit development. Each point represents the average of 20 fruit samples. Vertical bars indicate the standard error of the mean.

3. Results and discussion

The fresh weight of 'Catarina' and 'Fuji' apples increased exponentially during the period of 40 to 60 DAFB and partially linear after 60 DAFB (Fig. 1). For the 40–60 DAFB the growth was only by cell division, after this period the growth occurred by cell division and cell expansion and the rest of the season occurred by cells expanding (Fig. 1). It was observed that during commercial harvest (188 DAFB), 'Catarina' apples showed 6.7% higher fresh weight than 'Fuji' (Fig. 1), showing 'Catarina' apples had larger size of the fruit. According Saure (2005), larger apples have lower Ca concentration due to occur a dilution of Ca in such tissues of the fruit and higher susceptibility to BP.

A reduction in stained primary cortical vascular bundles was observed in all three portions assessed (proximal, middle and

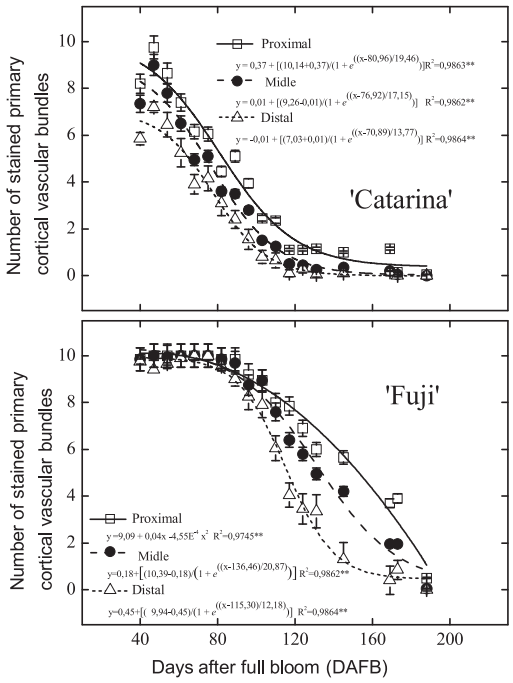

Fig. 2. Number of stained primary cortical vascular bundles in the proximal, middle and distal portions of 'Fuji' and 'Catarina' apples, during fruit development. The average number of stained vascular bundles in each cultivar per day was obtained from 20 fruit samples. Vertical bars indicate the standard error of the mean. $^{**}p \leq 0.01$ for nonlinear models.