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 $2.0\,m \times 6.0\,m$. 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 ($\sim$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 $25 \pm 2\,°C$ and $70 \pm 10\%$ 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
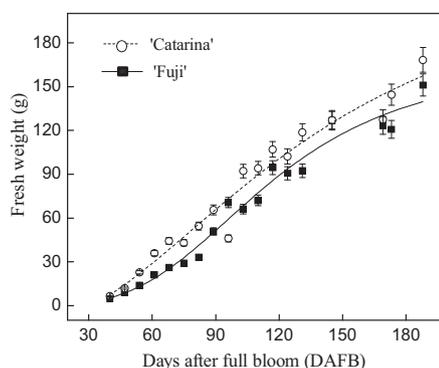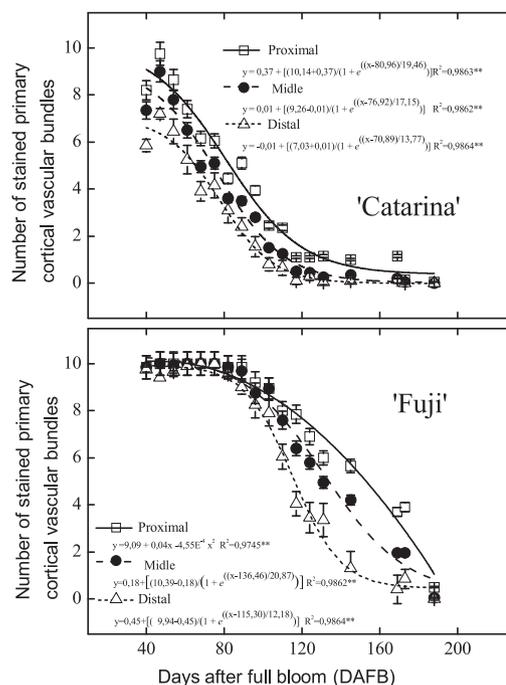

Fig. 1. Fresh weight (g) of 'Fuji' and 'Catarina' apples during fruit development. Each point represents the average of 20 fruit samples. Vertical bars indicate the standard error of the mean.

analysis. All statistical analyses were conducted using SAS software, version 9.1 (SAS Institute, 2009).

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.
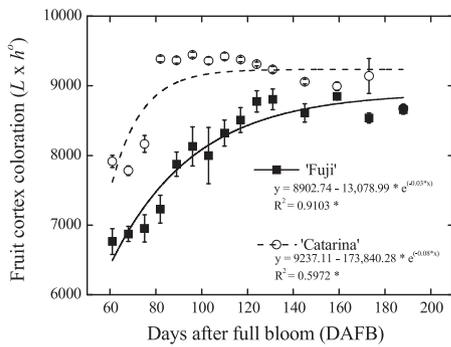
Fig. 3. Intensity of cortex staining ($L \times h°$) indicating xylem function in the secondary cortical system, during 'Fuji' and 'Catarina' apples development. Increased $L \times h°$ values indicate a reduction in the staining of the vascular bundles and loss of xylem function. Each point represents the average of three slices (proximal, midle and distal) of 20 fruit samples. $*p \leq 0.05$ for nonlinear models.

distal), during the period of 40 to 188 DAFB in both 'Catarina' and 'Fuji' apples (Fig. 2). The number of stained vascular bundles from the beginning to the end of the evaluation period was reduced by 93–100% in 'Catarina' apples, however this decrease only occurred after 80 DAFB. For 'Fuji' apples the number of stained vascular bundles was reduced by 80–85% (Fig. 2). The maintenance of xylem functionality until 80 DAFB in 'Fuji' apples may be due to a greater number and/or higher viability of procambial cells, compared to 'Catarina' apples. Procambial cells give rise to new elements of the xylem (protoxylem and metaxylem) (Chatelet et al., 2008) that are able to replace and perform the function of collapsed xylem elements. Therefore, the two cultivars may differ in the number of procambial cells and/or their longevity, which would affect the development the elements of the xylem during fruit growth.

At commercial harvest (188 DAFB), 'Fuji' apples showed ~15% stained primary functional vascular bundles in the three sections evaluated. Meanwhile, in 'Catarina' apples, 7% functional vascular bundles were observed at commercial harvest in the proximal portion, with total loss of xylem functionality (0%) in the middle and distal portions (Fig. 2).

Both cultivars showed a faster reduction in the number of stained primary vascular bundles in the distal portion, followed by the middle and proximal portions (Fig. 2). The loss of function of the xylem primary vascular bundles was greater in the distal portion, which could be the main reason of the lower Ca content generally
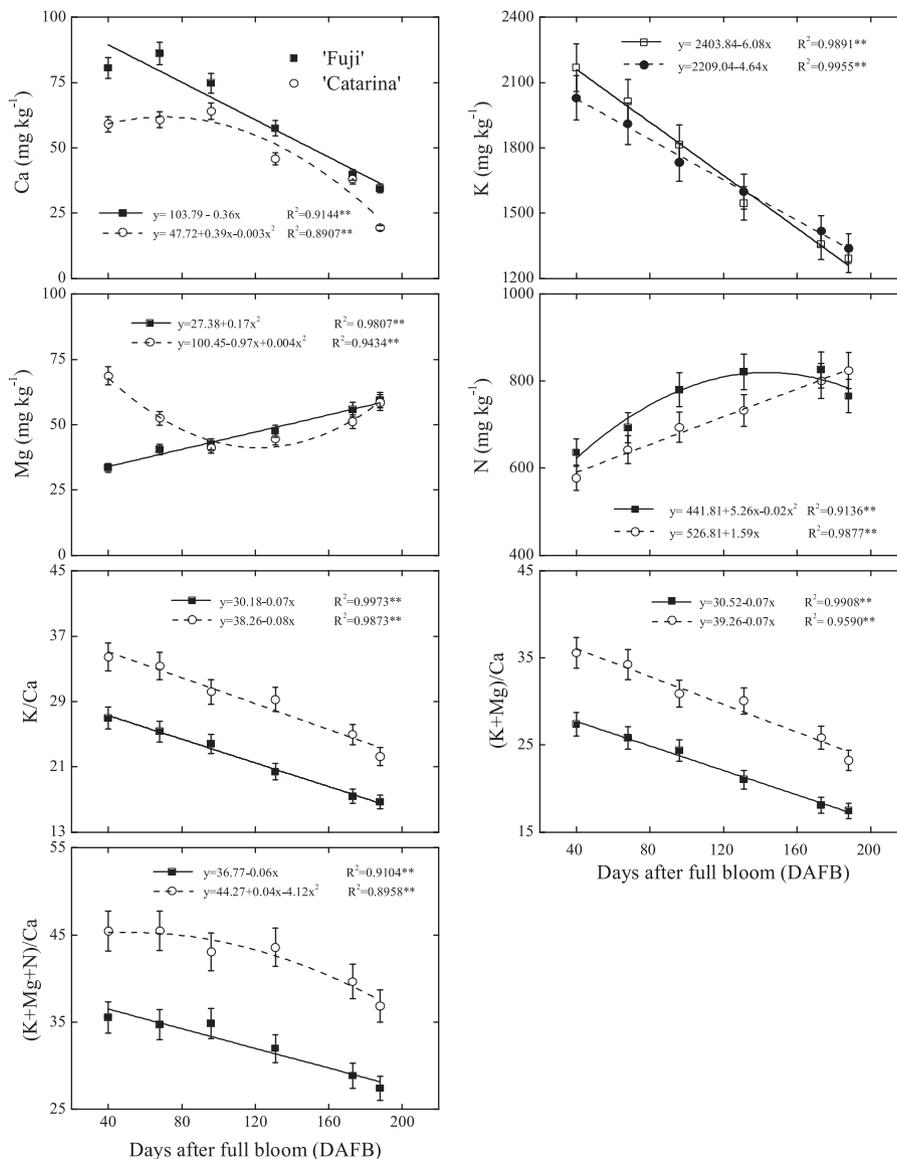
Fig. 4. Levels of Ca, K, Mg and N (mg kg^{-1} fresh wet weight), and K/Ca, (K + Mg)/Ca and (K + Mg + N)/Ca ratios in 'Fuji' and 'Catarina' apples during fruit development. Vertical bars indicate the standard error of the mean. $**p \leq 0.01$ for linear and nonlinear models.

observed in this portion of the fruit where BP incidence is usually observed (Amarante et al., 2012).

An increase in the $L \times h^{\circ}$ number, indicating a reduction in the staining of vascular bundles in the secondary cortical system and a loss of xylem function, was observed throughout fruit development in both cultivars (Fig. 3). However, 'Catarina' apples showed a dramatically loss of functionality in the secondary vascular bundles compared to 'Fuji' apples throughout the growing season. 'Catarina' apples showed a loss of functionality of the secondary vascular bundles from 61 to 80 DAFB, whereas 'Fuji' apples showed a gradual loss of functionality of these vascular bundles until 188 DAFB (Figs. 2 and 3). This loss of xylem function may partly be attributed to fruit growth in the 'Catarina' cultivar as compared to the 'Fuji' cultivar.

There was a reduction in Ca and K content during fruit development in both cultivars (Fig. 4). The N content increased during the fruit development for 'Fuji' apples until 188 DAFB, while "Catarina 'apples had the rise of the N content until 120 DAFB, followed by decline of 120–188 DAFB. However, in the commercial harvest both cultivars showed the same content of N (Fig. 4). During fruit development, Mg levels increased linearly in 'Fuji', but showed little change in 'Catarina' apples (Fig. 4). There was a reduction in K/Ca, (K+Mg)/Ca and (K+Mg+N)/Ca ratios during fruit development in both cultivars (Fig. 4). 'Catarina' apples showed lower Ca levels and higher K/Ca, (K+Mg)/Ca and (K+Mg+N)/Ca ratios throughout fruit development (40–188 DAFB), when compared with 'Fuji' apples.

According to Nachtigall and Dechen (2006), apples have three distinct phases of mineral element fluctuation. The first one occurs until 20 DAFB, when the fruit is in a period of constant cell division and nutrient content is rapidly reduced. The second one occurs from 20 to 70 DAFB, when the fruits are in the cell expansion period, and there is a slow, steady decline in the concentration of minerals. The third one occurs after 70 DAFB, with stabilization in the nutrient content in the fruits. However, in our study, no stabilization was observed in K/Ca, (K+Mg)/Ca and (K+Mg+N)/Ca ratios during the final stage of fruit development for either cultivar.

Regarding BP incidence, at commercial harvest it was remarkably higher in 'Catarina' apples ($12.7 \pm 2.5\%$) than in 'Fuji' apples ($2.7 \pm 0.4\%$) (data not shown). There was a positive relationship between the ratio of (N+K+Mg)/Ca in the flesh and BP severity (number of pits/fruit) in the fruit of both cultivars (Fig. 5). However, 'Catarina' apples showed greater BP severity, and also had the highest ratio of (N+K+Mg)/Ca (Fig. 4). This result agrees with Amarante et al. (2006b), who also observed a significant increase in the severity of BP due to the reduction in Ca content in peel and flesh, and high ratios of Mg/Ca, (K+Mg)/Ca and (K+Mg+N)/Ca in peel of 'Catarina' apples.

The high loss of xylem functionality seen herein in 'Catarina' apples may be related to the damage caused to the xylem element walls as a result of fruit growth, as previously described in grapes (Bondada et al., 2005). During fruit development, 'Catarina' apples had a greater increase in size, as expressed by fresh weight (Fig. 1), than 'Fuji' apples. The larger size of 'Catarina" apples may be attributed to a greater number and/or size of parenchyma cells in the cortex of the fruit. The greater elongation of parenchyma cells promotes compression, collapse and loss of functionality in the xylem vessel elements (Dražeta et al., 2004). In addition, loss of xylem functionality has been associated with occlusions in the xylem vessel elements or reduction in the hydrostatic pressure gradient between the xylem vessels of the peduncle and the fruit flesh (Chatelet et al., 2008).

The loss of xylem functionality implies a reduction in the absorption and transport of Ca within the fruit and an uneven distribution of mineral elements throughout the fruit, which favors the development of BP. Phloem functionality remains unchanged because it is made of living cells that remain differentiated and continue
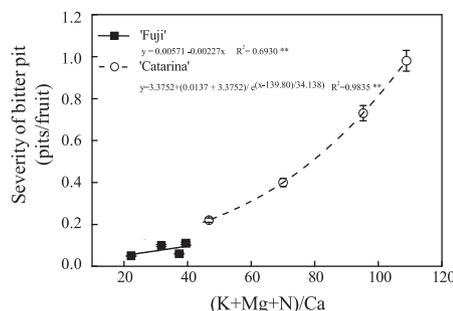

Fig. 5. Relationship between BP severity in 'Fuji' and 'Catarina' apples that were harvested at commercial harvest (188 DAFB) and the ratio (K+Mg+N)/Ca in the fruits. Vertical bars indicate the standard error of the mean. ** $p \leq 0.01$ for linear and nonlinear models.

to transport K, Mg and N during the entirety of fruit development, ensuring the supply of these minerals over that of Ca (Tromp and Oele, 1972). This may explain lower Ca content, higher K content, higher K/Ca, (K+Mg)/Ca e (K+Mg+N)/Ca ratios, and higher incidence and severity of BP in 'Catarina' apples compared to 'Fuji' apples.

This results suggest that Ca content in 'Catarina' apples dropped rapidly during the stage of cell division (40–60 DAFB) and cell enlargement (after 60 DAFB) and continued until commercial harvest in function of xylem losses as compared 'Fuji' apples, and then may explain why 'Catarina' apples have higher susceptibly of BP. Therefore, the key to comprehension of this physiological disorder may be linked to the loss of xylem functionality which reduce of the Ca content in the fruit and the occurrence of BP, how showed in this research.

Remarking, this study indicates that 'Catarina' apples show a high and precocious loss of xylem functionality and have a lower supply of Ca and higher K/Ca, (K+Mg)/Ca and (K+Mg+N)/Ca ratios, resulting in an increased susceptibility to BP compared to 'Fuji' apples. Thus, future studies should be conducted to determine whether this reduction in xylem functionality is due to anatomical changes in xylem vessels, resulting from the disruption and/or occlusion of vessel elements, by modifications in the hydrostatic pressure gradient within the fruit, or by the ratio of auxin/gibberellin between the two cultivars. There are some hormones, such as auxin and gibberellins, may affect the differentiation of xylem and phloem, cell elongation and thus, the mineral composition of fruits. Then, 'Catarina' and 'Fuji' apples may differ in the synthesis and/or metabolism of auxins and/or gibberellins, affecting xylem and phloem development and function during fruit growth resulting in changes in the Ca, K, Mg and N in the flesh, which are associated with the development of BP.

4. Conclusions

In 'Catarina' apples loss of xylem functionality is the factor responsible for reduced intake of Ca in the fruits and the increased the incidence of BP. Loss in xylem functionality in 'Catarina' apples seems to explain the higher susceptibility to BP for this cultivar as compared to 'Fuji'.

Acknowledgements

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