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REVIEW ARTICLE

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Bitter pit in apples: pre- and postharvest factors: A review

Tomislav Jemrić[1], Ivan Fruk[1], Mladen Fruk[1], Sanja Radman[2], Lovro Sinkovič[3] and Goran Fruk[1]

[1] *University of Zagreb, Faculty of Agriculture, Department of Pomology. Svetosimunska cesta 25. HR–10000 Zagreb, Croatia.* [2] *University of Zagreb, Faculty of Agriculture, Department of Vegetable Crops. Svetosimunska cesta 25. HR–10000 Zagreb, Croatia.* [3] *Agricultural Institute of Slovenia. Hacquetova ulica 17. SI–10000 Ljubljana, Slovenia*

Abstract

Bitter pit is a physiological disorder that significantly reduces the quality of apples. Although it has been detected since the beginning of the last century, still there is little known about the mechanism of its occurrence. According to numerous studies, bitter pit is formed as a result of calcium deficiency in the fruit. Some authors cite the high concentration of gibberellins, later in the production season, most probably caused by excessive activity of the roots, as the chief causative factor. Beside Ca, there are several factors that can also contribute to its development, like imbalance among some mineral elements (N, P, K and Mg), cultivar, rootstock, the ratio of vegetative and generative growth, post-harvest treatments and the storage methods. There are some prediction models available that can estimate the risk of bitter pit in apples, but even those are not always reliable. The aim of this review was to encompass the pre and postharvest factors which cause bitter pit and point out the directions for solving this problem.

Additional key words: *Malus* × *domestica* Borkh.; Ca absorption; $CaCl_2$; gibberellins, 1-MCP, DPA, controlled atmosphere, physiological disorder.

Abbreviations used: 1-MCP (1-methylcyclopropene); CA (controlled atmosphere); DPA (diphenylamine); GA (gibberellin); LOX (lipoxygenase).

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Correspondence should be addressed to Goran Fruk: gfruk@agr.hr

Introduction

According to FAOSTAT the worlds apple (*Malus* × *domestica* Borkh.) production was around 80.82 million tons in 2013, of which almost 11.74 million tons was produced in the European Union. Bitter pit is a physiological disorder associated with a calcium deficiency of the fruits that greatly reduces the quality of apples. It is formed as small brown accumulations of dead cells on apple skin. Shape is spherical with size of 1 to 4 mm and bitter in taste. They are located directly below the peel, but often found scattered in the flesh of the fruit, especially in the calyx. There is very little information available about the mechanisms of its formation (Ferguson & Watkins, 1989; Saure, 1996; Freitas *et al.*, 2010) despite the numerous studies that have been conducted for over a hundred years since the existence of bitter pits. Bitter pit develops mainly during storage, but the process that causes it usually starts during the growth period of the fruits (Ferguson & Watkins, 1989). This disorder causes dark depressions on the fruit surface that is associated with the collapse of flesh cells below the peel (Amarante *et al.*, 2013).

Bitter pit has been the subject of several review papers more than twenty years (Perring, 1986; Ferguson & Watkins, 1989). Ferguson *et al.* (1999) reviewed the influence of pre-harvest factors that caused the occurrence of physiological disorders after the harvest. They stated that air temperature before harvest, and the fruit position in the crown of the tree are among the most significant factors causing this problem.

In addition to Ca, there are other factors that are involved in stimulating the occurrence of bitter pit, such as mineral elements like P and Mg; cultivar, grafting rootstock, ratio of vegetative and generative growth and storage handling. This review aims to encompass the pre- and postharvest factors, involving in the formation of bitter pit and also to point directions for solution.

Pre-harvest factors affecting appearance of bitter pit

Bitter pit is associated with the imbalance of nutrients in the fruit, especially Ca deficiency (Val Falcón *et al.*, 2000). In addition, the role of other factors in the orchard, which may cause the aforementioned symptoms cannot be ignored. Telias *et al.* (2006) reported that the incidence of symptoms of bitter pit is determined by rootstock, cultivar, growth regulators, crop load, number of seed, harvest date, water availability, temperature and mineral composition, such as Ca and P concentration in the leaves and fruits, concentration of Mg in the leaves and yield parameters. Year and location are also significant factors for the occurrence of bitter pit (Volz *et al.*, 2006).

The occurrence of bitter pit is a common problem of acidic soils. The application of 200-300 g/m^2 lime reduces the incidence of bitter pits for 75.6% to 78.2% in apples (Wang *et al.*, 2005). Saure (1996) quoted that a major factor in the occurrence of bitter pit is high concentration of plant hormones gibberellins (GAs) late in the season, probably caused by excessive activity of roots. High concentration of GAs can increase permeability of cell membranes thus causing water stress which triggers development of bitter pit. Same author states that Ca deficiency could be a secondary factor which increases the risk of existing bitter pit. The role of Ca is to stabilize cell membrane and reduce its permeability, thus water stress could be prevented. The role of GAs could explain why the symptoms appear only in some parts of the treetops, and why, in the same flower cluster, some fruits appear with bitter pit and others without. The findings of Silveira *et al.* (2012) support the above mentioned statements. The authors achieved a reduction in the appearance of bitter pit by applying Prohexadione-Calcium, an inhibitor of GA synthesis, and gibberellin GA_3 on vegetative growth of the trees.

The role of calcium

Ca is the second most significant mineral of the fruits primarily associated with the cell walls, and essential for the appearance of plants and fruit quality. The last review article on the physiological activity of Ca in apples was published more than 30 years ago by Vang-Petersen (1980), which indicated the need to make a review of studies published in recent times on bitter pits.

The appropriate concentration of Ca helps to maintain the firmness of apple fruits and reduce the incidence of physiological disorders, such as water core, bitter pit and internal browning of apple flesh (Conway *et al.*, 2002). The Ca content in the fruits is usually much lower than in other parts of the plant (Saure, 2005). During the growing season Ca can be translocated from the fruits to the leaves and new shoots (Vang-Petersen, 1980), thus requiring a continuous supply of this mineral. This situation leads to a decrease in the concentration of Ca which can lead to the occurrence of bitter pits.

The main role of calcium is to maintain the structure of the membrane and to be a secondary messenger to various forms of stress, regulation of stomata and mechanical damages (Mahouachi *et al.*, 2006; McAinsh & Pittman, 2009).

As Ca enters the plant passively with water through the roots and is transported across xylem vessels, all the factors that affect the receiving of water, such as climatic conditions, the function of the roots, salinity, etc., have an effect on Ca intake (Napier & Combrink, 2006). Freitas & Mitcham (2012), based on a review of numerous studies of Ca deficiency factors, concluded that this phenomenon is the result of complex physiological processes which lead towards a decrease of Ca concentration in plant tissues, particularly the cell. Furthermore, the key role in the formation of bitter pit could have the translocation of Ca from the peduncle to the calyx part of the fruit. Translocation depends on the capacity of binding Ca^{2+} ions in the cell wall, adoption of Ca^{2+} at the peduncle end tissue of the fruit, the number of functional xylem tubes through which Ca^{2+} travels to the calyx, and the hydrostatic gradient of concentration that is required for the translocation process (Freitas & Mitcham, 2012). Freitas *et al.* (2015) suggested that Ca^{2+}, together with organic acids in vacuole, can form strong precipitates, thus making Ca^{2+} unavailable to other cell processes and also enhance susceptibility to form bitter pit.

Calcium in the fruits occurs in different forms. Some of the fractions, such as water-soluble Ca, are physiologically active and responsible for the development of bitter pit (Saks *et al.*, 1990). Insoluble Ca fraction is bound to the cell walls and cannot play an important physiological role, while water-soluble Ca is physiologically active fraction and can change activity of the different enzymes (Pavičić *et al.*, 2004). In bitter pit affected fruit, Ca is accumulated in the areas affected by bitter pit in surface tissue, but in unaffected fruit Ca is evenly concentrated throughout fruit surface. Bitter pitted fruit have more water insoluble Ca than water soluble Ca fraction. However, in unaffected fruit there is no difference in concentrations between these two Ca fractions (Val *et al.*, 2008).

When the concentration of Ca in the fruit falls below 5mg/100 g susceptibility to bitter pit occurrence is increased (Vang-Petersen, 1980; Dris & Niskanen, 2004; Amarante *et al.*, 2013). As Ca concentration varies depending on the cultivar (Delian *et al.*, 2011) it can cause different intensity of bitter pit formation in different cultivars. Concentration of Ca in the skin of the fruit is greater than in the flesh (Amarante *et al.*, 2009) and Ca can be translocated from outer to the interior parts of the fruit during storage (Perring & Pearson, 1986), which explains the fact that bitter pit usually occurs after harvest.

Calcium affects the softening of fruit, because it is an essential part of the structure of cell walls, and the integrity of the cell membrane (Fallahi *et al.*, 1997). Its deficiency leads to the collapse of cells, which results in enzymatic browning of the tissue caused by polyphenol oxidase and peroxidase (Napier & Combrink, 2006). The activities of superoxide dismutase, peroxidase, catalase and ascorbate peroxidase were significantly lower in fruits with bitter pit than in the healthy fruits (Wang *et al.*, 2001). Sharma *et al.* (2012) found a strong positive correlation between the activity of lipoxygenase (LOX) and the occurrence of bitter pit. The activity of this enzyme has been negatively correlated with Ca content in the fruit (Sharma *et al.*, 2012). Wińska-Krysiak & Łata (2010) found increased LOX activity in fruit with bitter pit incidence.

Additional losses due to lack of Ca are caused by sensitivity of tissues to secondary infections, such as *Phytophthora* spp., *Erwinia* spp. and *Botrytis* spp. (Napier & Combrink, 2006). Similar effect on fruit firmness, thus reducing fruit sensitivity to pathogens shows microelement boron. Spraying the trees after full bloom increases the firmness of fruits after storage and reduces sensitivity to bitter pit and *Gleosporium* rots (Wójcik *et al.*, 1997). Therefore, these two elements (Ca and B) which combined to form of chelates were proved successful in controlling bitter pit (Wooldridge & Joubert, 1997).

Treatments with calcium

Spraying with Ca is a recommended action to reduce the appearance of bitter pit, but the effectiveness is not always consistent. For this application, various forms and combinations with Ca were used. The Ca concentration is usually found highest in young fruits, then decreases during fruit growth, followed by a dramatic decline before maturation (Zheng *et al.*, 2006). Increased Ca in the fruits can be achieved by foliar application of Ca salts during the growing season (Porro *et al.*, 2006; Lötze *et al.*, 2008), or by dipping the fruits, after harvest, in the solution of those salts (Conway *et al.*, 2002). Late application of calcium nitrate ($Ca(NO_3)_2$) –80 days after full blooming– increases the Ca content in the fruit more than the early one (6 days after full blooming); or middle early application –40 days after full blooming– (Lötze *et al.*, 2008).

Foliar application of calcium chloride ($CaCl_2$) improved the quality of cv. 'Jonathan' (Kadir, 2005), increased the level of Ca in the skin of the fruits and reduced the bitter pit in the cvs. 'Tsugaru Strait' and 'Fuji' (Lee *et al.*, 1997). Foliar spraying with Ca, extracted from oyster shellfish, effectively reduced the appearance of bitter pit in the cvs. 'Fuji' and 'Jonagold' (Moon *et al.*, 1999). Calcium carbonate ($CaCO_3$) reduced bitter pit only after a short storage (Guerra *et al.*, 2011). $CaCl_2$ applied one week before harvest, increased the firmness of 'Jonagold' after 120 days of storage by 20% (Peryea & Neilsen, 2006). Casero-Mazo (1996) found that in foliar application, except $CaCl_2$, Ca-amino-acids can be successfully used for reducing bitter pit and increasing fruit firmness.

According to Weibel *et al.* (2001), bitter pit appear more frequently in organic apple production because the treatments with $CaCl_2$ are rarely applied. Mostly, they are applied only as a matter of necessity, but then it is usually too late. Because of low mobility of Ca, damage cannot be prevented. Biškup *et al.* (2003a) reported the lowest percentage of bitter pit observed on fruits which were sprayed with 3% fertilizer with Ca (containing 12% CaO), while the highest percentage of bitter pit was recorded on untreated fruits (39.64%). Wójcik & Szwonek (2002) recommend use of the fertilizer with 22.5% $Ca(NO_3)_2$ for apple cultivars that are sensitive to physiological disorders related to a deficiency of Ca. In addition to spraying with Ca, a combination with P or self treatment with P is also effective (Tomala & Soska, 2004).

Application of Ca salts is not always effective (Robinson & Lopez, 2012). Conway *et al.* (2002) proposed that the major problem of foliar spraying is insufficient intake of Ca in the fruit, in order to achieve the desired effect. Low intake of Ca in the fruit will not lead to a reduction in the appearance of symptoms of bitter pit; on the other hand, outrageous Ca intake can lead to damage. The effectiveness of spraying with Ca varies depending on the soil, cultivars, climatic conditions and the time of application. Calcium nitrate is commonly used to influence bitter pit in apples and can cause leaf blight (Wooldridge *et al.*, 1997).

Spraying with $CaCl_2$ during the vegetation, sometimes beginning in June, proved to be the most economically efficient measure to increase the Ca in fruit and reduce the risk of bitter pit (Peryea *et al.*, 2007). Fertigation with $CaCl_2$ and $Ca(NO_3)_2$ before harvest significantly increases the amount of Ca and N in apple fruits (Johnson *et al.*, 2001; Moor *et al.*, 2005).

Cuticle penetration of Ca salts depends on their point of deliquescence (chemical absorption of water vapour from the air performed by hydrated salts) which is lower in chloride and nitrate salts than in organic Ca salts (Schönherr, 2001). Raese & Drake (2002) reported that most of the Ca preparations, exception calcium sulphate ($CaSO_4$), increase the concentration of Ca and reduce the incidence of bitter pit and scald. In contrast, the spraying with $CaSO_4$ increases the occurrence of bitter pit and reduces the concentration of Ca, especially in the cultivar 'Golden Delicious' (Raese, 2000). The possible explanation might be role of S in auxin synthesis (Falkenberg *et al.*, 2008). Higher level of auxin synthesis leads to higher vegetative growth thus consuming Ca leaving fruit Ca deficient.

Other mineral elements

Although the development of bitter pit is not fully understood yet, earlier studies showed relationship between bitter pit and nutrient levels in the fruit (Krishkov, 2007). It is believed that high-quality fruits usually contain a high proportion of K and Ca and low amount of Cu, Zn and Mn. Thus, the occurrence of bitter pit is closely associated with the amount of Ca and other mineral elements (Liu & Han, 1997).

The Ca concentration is not the only criterion for the occurrence of bitter pit. Pavičić (1993) observed that K as an individual element, can also affect the occurrence of bitter pit in apples. But, interaction of elements such as K/Ca and Na/Ca, P/Ca and Mg/Ca, showed closer connection with bitter pit than same elements individually (Ben, 1998). This was confirmed by numerous authors who concluded that relationship of Ca with other elements, such as K, N, P, Mg and Mn is important (Ben, 1995b; Biškup *et al.*, 2003b; Kim & Ko, 2004a, b; Dilmaghani *et al.*, 2005; Amarante *et al.*, 2006; Porro *et al.*, 2006; Martsynkevich & Krivorot, 2007; Guerra *et al.*, 2011). Intensive spraying with Ca preparations is recommended only when fruits establish a critical relation K/Ca, which leads to bitter pit formation (Drahorad & Aichner, 2001).

Dilmaghani *et al.* (2005) studied the K/Ca relationship and its impact on the quality of the fruit. They observed a negative correlation between the relationship of K/Ca and the firmness of fruits at the time of harvest and found that firmness of the fruit was higher in those treated with Ca. Similar result was reported by Biškup *et al.* (2003b). The content of Ca in the fruit was negatively correlated with the percentage of bitter pit and the relation of K/Ca and N/Ca. The fruits affected with bitter pit had significantly lower Ca content in the flesh and higher contents of N and K than fruits without them (Kim & Ko, 2004a, b). Bitter pit is associated with Ca content, as well as with the ratio of K/Ca, (K+Mg)/Ca and N/Ca in the fruits and leaves (Sió *et al.*, 1999).

Increased lipoxygenase activity in fruits with high K/Ca ratio was established by Marcelle (1989) and Wińska-Krysiak & Łata (2010) who also reported that higher lipoxygenase activity was found in fruit with bitter pit incidence, suggesting that high K/Ca ratio and high lipoxygenase activity plays important role in bitter pit incidence. Amarante *et al.* (2013) reported higher Mg/Ca ratio in the flesh than in the peel at the distal end of the fruit, regardless of the occurrence of bitter pit. La Grange *et al.* (1998) found that Ca levels were higher in fruits without bitter pit than in infected fruits. The concentration of minerals (N, P, K, Mg) did not affect the occurrence of bitter pit. Krishkov (2007) quoted the degree of occurrence of bitter pit and it is associated with the concentration of K and Mg, regardless of the content of Ca. The groups of medium and large size, healthy apples showed a smaller content of Mg than apple fruits with bitter pit (Ben, 1995b). Takac (1994) had proven earlier that fruits with physiological disorders have lower Ca content and higher content of K, and possibly N and Mg. The ratio of Ca/Mg and K/Mg varies significantly depending on the cultivar (Delian *et al.*, 2011). The K/Ca ratio in the flesh is an important indicator of bitter pit in the 'Fuji' cultivar, but for cv. 'Catarina' K/Ca ratio shows a correlation with bitter pit only if it is determined in the skin of the fruit (Miqueloto *et al.*, 2011). Robinson & Lopez (2012) reported an association between the appearance of bitter pit and P concentration in fruits, unlike the association with the Ca concentration.

A high proportion of N reduces the content of Ca (Recasens *et al.*, 2004) and fruit firmness, as well as increases incidence of physiological disorders (Takac, 1994; Kim & Ko, 2004a, b). Apple fruits with a higher content of N have a higher concentration of ethylene and level of respiration (Fallahi *et al.*, 2006; Sharma *et al.*, 2012). Pavičić *et al.* (2004) reported that the level of N in the fruit cannot be used as an indicator of bitter pit in apple cultivar 'Idared'. Miqueloto *et al.* (2011) found that fruits with symptoms of bitter pit have a higher ethylene production, respiration and titratable acidity, but lower pH, firmness, texture and skin colour. Firmness was proved to be more important for the cv. 'Fuji' while titratable acidity was more essential for cv. 'Catarina'.

Application of B in the soil increases the concentration of Ca in apple fruits and reduces the proportion of the fruits with the bitter pit, internal flesh browning and *Gleosporium* rotting (Wójcik *et al.*, 1999). As mentioned before, spraying the trees with Bafter full blooming has similar effect (Wójcik *et al.*, 1997). Wójcik & Mika (1996) indicated that cv. 'Elstar' is more sensitive for bitter pit and *Gleosporium* rotting during storage and has less firmness when B is applied after blooming. After five months of cold storage, the occurrence of bitter pit reduced slightly due to the B treatment in the middle of the growing season (Zude *et al.*, 1997). Sanz & Machín (1999) pointed out asymptomatic Fe deficiency, which corresponds to the flower dry matter 310-400 ppm Fe, causing bitter pit. It is obvious that there are unsolved dilemmas about the exact role of mineral elements in the occurrence of bitter pit symptoms.

Cultivar

Bitter pit appears on the cvs. 'Golden Delicious', 'Goldspur' and 'Champion' (Takac, 1994), and other cultivars as well. The cultivar 'Golden Delicious' showed a higher incidence for bitter pit compared to 'Red Delicious' (Khan *et al.*, 2006), and cv. 'Catarina' than 'Fuji' (Miqueloto *et al.*, 2011). Volz *et al.* (2006) studied the genetic variability in sensitivity and found that there is a strong genetic influence for the occurrence of bitter pit, though interaction with the environment is also significant. Therefore, the new cultivars should be tested at various locations, in order to evaluate their sensitivity to bitter pit. The explanation of different cultivar susceptibility was stated by Miqueloto *et al.* (2014) who found that different cultivars lose their xylem functionality at different stages of fruit growth. Since Ca^{2+} ions are transported mainly through the xylem (Saure, 2005), this is logical explanation why some cultivars have greater bitter pit susceptibility.

The incidence of bitter pit is associated with the K/Ca ratio in fruits, but varies with cultivar which suggests that there are no general optimum K/Ca ratio; but optimal values of K/Ca depends on the cultivar (Porro *et al.*, 2006; Delian *et al.*, 2011). These facts indicate the necessity to breed the cultivars resistant to bitter pit and storage diseases, but contain all the valuable features. The fruits of cv. 'SPA440' are resistant to the development of scald, bitter pit, internal flesh browning and loss of aroma during storage (Hampson *et al.*, 2005).

Eijden (1993) found that though the cv. 'Delcorf' (hybrid 'Stark Jon Grimes' × 'Golden Delicious') is less productive than cv. 'James Grieve', it is not sensitive to bitter pit. In current context, it is necessary to start breeding programs in order to expand the cultivation of apple cultivars resistant to bitter pit.

Rootstocks

Rootstock is one of the factors in the yield (orchard) which can affect the quality of the fruit (Skrzyński, 2007). In addition to the cultivar, the rootstock can also affect the occurrence of bitter pit. The fruits from the trees grafted on rootstock M26 had high acidity, and a low concentration of Ca which resulted in increased occurrence of bitter pit (Tatarinov, 1992). Fruit of cvs. 'Jonagold' and 'Elstar' showed higher incidence of bitter pit when grown on rootstock M26 than on M9 or P22 (Ben, 1995a). According to Skrzyński & Gąstoł (2007) the physiological disorders, such as bitter pit and flesh browning, were observed during storage when M9 and M26 rootstocks were used. The cultivar 'Fuji' grafted onto rootstock M26 showed a lower incidence of bitter pit as onto rootstock MM106 (Kim & Ko, 2004b). The fruits of cv. 'Fuji' grown on M26 together with interstem 'Golden Delicious' had more bitter pit as with interstem 'Granny Smith' (Drake *et al.*, 1997). Fruits of 'Red Delicious' grown on rootstock M7 had higher concentration of Ca in the skin and a lower occurrence of bitter pit, but the fruits were smaller compared to those grown on the rootstock M26 (Raese & Drake, 2000). Ben (1995a) reported the occurrence of bitter pit of cv. 'Gloster' was lower in fruits which grew on less vigorous rootstock. These findings were confirmed by Kim & Ko (2004a) who concluded that incidence of bitter pit is more intensive on moderate, vigorous rootstocks compared to less vigorous rootstocks.

The fruits from the trees grafted onto rootstocks M9 and P14 were mostly affected by physiological disorders and other diseases during storage (Skrzyński, 2007). Higher incidence of bitter pit occurs in the less fertile seasons (Ferguson & Watkins, 1992), as a result of large fruits and high leaf/fruit ratio (Ernani *et al.*, 2002). Thus, it can be concluded that growing apple is more suitable on the low vigorous rootstocks.

Ratio of vegetative and generative development

Sió *et al.* (1999) reported that shoots growth and crop are associated with the appearance of symptoms of bitter pit on apple fruits. Bitter pit cause major losses during storage, when apples come from orchards with strong vegetative growth and low yields; and such problems lead to increased secondary infections (Frasnelli & Casera, 1996) with *Phytophthora* spp., *Erwinia* spp. and *Botrytis* spp. (Napier & Combrink, 2006).

Engel & Lenz (1998) reported that organically fertilized trees had good growth, but the fruits were poorly coloured and bitter pit had increased by 40% after storage. Therefore, it is important not to exaggerate with N application, because this encourages the formation of bitter pit. This happens due to high fertilization with N causing greater vegetative growth which, causes Ca translocation from fruit to new shoot (Vang-Petersen, 1980).

Ernani *et al.* (2002) noted that disorders which are associated with a Ca deficiency in apple cv. 'Gala' on soils with high pH in southern Brazil appear only in smaller yield seasons, as a result of large fruits and high ratio of leaf/fruit. An increasing workload of trees with fruits reduced the appearance of bitter pit, in case of the cv. 'Honeycrisp' (Robinson & Lopez, 2012), as well as the appearance of rot or bad fruits.

The fruits of the less productive trees develop up to 65 % damage (bitter pit, stains, core redness and browning disorders of cv. 'Braeburn'), compared to the fruits of the trees with standard load yields (Tough *et al.*, 1998). Hand thinning at the end of June mainly increases the size of fruits, firmness and sugar content, but also incidence of the of bitter pit during storage (Basak, 1999). Summer pruning in July resulted in higher concentration of Ca in fruit, better colouring of the fruits and reduced physiological disorders such as bitter pit, internal flesh browning and weight loss of apple fruits during storage at 4°C (Struklec, 1994). As mentioned earlier, Ca can be translocated from fruits to shoots during growing season (Vang-Petersen, 1980). Thus, aforementioned positive effect of summer pruning on decreasing bitter pit incidence can be explained by less shoots through which Ca could be translocated. Thus, Ca stays in fruit being available for binding with different enzymes and maintaining regular physiological activity of cell.

Wójcik *et al.* (2001) found that the fruits from more severely thinned trees contain less Ca, more K and are more sensitive to bitter pit than those from less thinned trees. Therefore, the intensity of the bitter pit corresponding to the high relation of K/Ca is usually stronger in bigger fruits (Ben, 1999). Prange *et al.* (2011) reported that bitter pit is specially expressed in the fruits of cv. 'Cox's Pippin' when they are bigger than 250 g. Therefore, bigger fruits need higher Ca content to maintain good Ca concentration for regular physiological processes. Since bigger fruits are usually developed on trees with low yield, where vegetative growth is stronger than generative, they might suffer from the competitiveness of shoots which take more Ca.

Freitas *et al.* (2013) reported that shading of apple trees not only increased total tissue fruit Ca^{2+} content, but also surprisingly increased fruit susceptibility to bitter pit. Authors suggested that this susceptibility to bitter pit probably happened because of higher Ca^{2+} binding to cortical tissue which resulted in lower content of available Ca^{2+} for other cellular functions. Defoliation (loss of leaves in a tree) increases the concentration of P and reduces the concentration of Ca (Wójcik & Mika, 1998).

Post-harvest factors affecting appearance of bitter pit

Harvest time

The fruits harvested before optimum harvest date were more sensitive to developing bitter pit than those harvested later (Perring & Pearson, 1986; La Grange *et al.*, 1998). The occurrence of bitter pit is lowest when the fruits are picked just before strong synthesis of ethylene starts (Prange *et al.*, 2011).

Storage of fruits

The fruits which had an adequate concentration of Ca in the tissues were firmer and able to be stored for a longer period than those with poor Ca content (Hisaw, 1991). Ca concentration decreases during storage (Sharma *et al.*, 2012), which is the reason bitter pit usually occurs after several months in the cold storage. According to Chen & Zhou (2004), a decreased ability to eliminate oxygen free radicals, and accelerate the disintegration of the cell wall due to lack of Ca were the main causes of metabolic disorders of apple fruits during storage.

Guerra *et al.* (2008) compared the quality of apple fruits treated with fungicides and anti scald agents before they were stored in different conditions: cold storage (1°C and 95% RH) and under controlled atmosphere, CA (1°C and 95% RH, 1% CO_2 and 3% O_2). Fruit firmness and titratable acidity decreased during storage, while bitter pit incidence was similar in both techniques. According to Jankovic & Drobnjak (1994), the fruits of cvs. 'Idared', 'Cacanska Pozna', 'Jonagold' and 'Melrose' stored in the CA did not show any physiological disorder, while bitter pit was observed on the cv. 'Melrose' stored under normal atmospheric conditions. The occurrence of bitter pit is sometimes increased with delayed cooling and storage at higher temperatures (Watkins *et al.*, 2004). Modified atmosphere packaging has been proven effective in the management of disorders like bitter pit and keeping quality (Khan *et al.*, 2006).

Treatments of fruits after harvest

Neven *et al.* (2000) noted that the physiological disorders (bitter pit) were exacerbated by the application of heat treatment. The effectiveness of dipping fruits in a solution of calcium chloride, after harvest, was studied for over three years by Dierend & Rieken (2007). Dipping of apple fruits in a solution of $CaCl_2$ increased the Ca content and fruit firmness, compared with untreated control and reduces bitter pit occurrence (Ait-Oubahou *et al.*, 1995). The best results were obtained when fruits were dipped in $CaCl_2$ for two minutes at the concentration of 7-7.5%. Furthermore, the entry of Ca in the fruit depends on the following factors: a) concentration of $CaCl_2$ in the solution for dipping; b) dipping time; c) apple cultivar; d) adding a dipping agent; and e) ripening stage.

Calvo (2005) reported that fruits treated with 1-methylcyclopropene (1-MCP) and diphenylamine (DPA) had a reduced incidence of bitter pit. DPA is a synthetic antioxidant that reduces the development of symptoms, but its use is limited due to the potentially negative effects on human health, and consumer preferences for fruits without chemical additives (Frasnelli *et al.*, 1995; Chapon & Bony, 1997; Kim-Kang *et al.*, 1998). The fruits treated with 1-MCP had a reduced incidence of bitter pit, in addition to minimal decline in the quality associated with ripening during storage, especially during shelf life (Zanella *et al.*, 2005). The efficiency of 1-MCP significantly affects harvest time and the quality of the fruits at harvest (Reed, 2002).

The alternative to the 1-MCP treatment is low O_2 storage for 10 days at 20 °C, before long-term storage at low temperature (0-4 °C) (Pesis *et al.*, 2010; Val *et al.*, 2010).

Methods for predicting the formation of bitter pit

Upon the early diagnosis of bitter pit (30-40 days before harvest), a Ca spraying programme implementation is recommended (Tagliavini & Marangoni, 2002; Torres *et al.*, 2015). Early seasonal mineral analysis is a method of K/Ca relationship fruits prediction during the harvest, which can be used as an index for assessing the risk of bitter pit (Porro *et al.*, 2006). Aichner & Drahorad (2003) showed the relationship of Ca and K in fruits providing useful information about the risk of formation of bitter pit, and other physiological disorders. Analysis of Ca concentration in the skin of the harvested fruits allows for better prediction of bitter pit than concentration of Ca in apple flesh (Amarante *et al.*, 2005; 2009). The ratio (K+Mg)/Ca of the leaves and fruits during harvest can be used as reliable indicator of the development of bitter pit during storage (Pavičić & Miljković, 1991).

Weibel *et al.* (2001) reported how in mature fruits there is a potential of symptoms of bitter pit and relation of K/Ca based on the content of Ca/K in pistils of T-stage development of fruit. They found that in cvs. 'Boskoop' and 'Maigold' the K/Ca ratio above 5.8 indicates a significant risk for bitter pit phenomenon. When it reaches a critical threshold in relation K/Ca, growers are recommended to apply special measures, such as increasing the frequency of Ca spraying, in order to prevent the formation of bitter pit (Aichner & Drahorad, 2003).

Analysing the Ca/N ratio in 'Gala' apple fruit 20 days before harvesting, or during the harvest, is suitable for prediction of bitter pit occurrence (Amarante *et al.*, 2010). There is a small risk to fruits which have less than 400 mg/kg of N and more than 42 mg/kg of Ca; a medium risk when 400-500 mg/kg N and 36-42 mg/kg Ca; and high risk with more than 500 mg/kg of N and less than 36 mg/kg of Ca.

Infiltration of Mg represents valuable tool of bitter pit risk assessment of the cv. 'Gala' (Amarante *et al.*, 2005; 2009; 2010). After 10 years of experimental studies in Chile and USA, the Mg infiltration into fruits showed high possibility of bitter pit prognosis for different cultivars, locations, seasons and harvest periods (Retamales *et al.*, 2000). The accuracy of this method is satisfactory and can be used to predict the incidence of bitter pit only if fruits are sampled 20 days before harvest.

However, none of the methods was effective for bitter pit prediction after fruits were already stored under CA (Sestari *et al.*, 2009a). Therefore, it is better to rely on the analysis made 20 days before harvest. For 'Royal Gala' apples, a better prediction method was immersion into ethephon solution (Sestari *et al.*, 2009b). This was also advocated by Lötze *et al.* (2010), who concluded that Bangerth´s (1970) method was more effective in predicting bitter pit incidence in 'Braeburn' and 'Golden delicious' apples than Mg infiltration. Bangerth´s method implies that fruits are picked 14 day before expected harvest date and immersed in a water solution containing 0.2% ethephon (Bangerth, 1970; Lötze, 2005).

An alternative to the above-mentioned methods is the "passive method" (Torres *et al.*, 2015), that can also be used on fruits that are picked two to three weeks before estimated harvest date and left in room temperature for 20 days; during this time, the first visible symptoms of bitter pit should appear after seven days. This method was tested by Torres & Alegre (2012) and Torres *et al.* (2015) who concluded that it is a good, cheap and easy method of predicting bitter pit incidence in 'Golden Smoothee' apples.

Some microelements can also be used for prediction of risk of bitter pit. Sanz & Machín (1999) reported that bitter pit is formed when iron concentration in flowers was 310-400 ppm of dry matter.

Conclusion

Despite numerous studies on bitter pit phenomenon, there is still some lack of information to understand the complete physiological mechanism of its development. Furthermore, factors that affect the reliability of prognostic models still need to be better understood. Ca content in the cells play a major role in bitter pit development, especially water soluble Ca. Treatments with 1-MCP are very effective in maintaining the quality of apples after harvest as well as in reducing the incidence of bitter pit, but are not effective if applied after climacteric. The development of new, bitter pit resistant cultivars, and a better understanding of the physiological processes that lead to the occurrence of bitter pit, will reduce losses and improve the quality of apples. Besides these preventive methods, there are not much curative measures that can be taken (postharvest dipping in solution of Ca agents, 1-MCP, DPA, etc.) because they are not effective, or have negative effect on human health. Postharvest use of chemicals in many countries is very limited or even forbidden. Therefore, prediction methods have huge importance to minimizing fruit losses caused by bitter pit development, allowing us to act on time to prevent bitter pit development or to consume fruits before its development.

The reasons behind this huge problem in apple with their respective phenomenon and their possible solutions were discussed thoroughly in this review study. This will open fresh insights for other researchers and overcome these issues that will lead to the better understanding of bitter pit.

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