Scilate Apple Mineral Distribution and Prediction of Bitter Pit

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Abstract

Calcium deficiency in apple fruit has been identified as a primary cause of bitter pit in apples, however some apples are not as susceptible as others. The apple cultivar 'Scilate' has been identified as less susceptible to bitter pit, but it is not known whether high calcium concentrations are what contribute this resistance. Because scilate is a more recent variety, little research has been done to observe the effects of crop load, fruit size, and vegetative growth on calcium concentrations. Cortical plug and peel samples from apples selected from heavy cropped trees grown on EMLA 26, and light cropped trees, grown as stock trees for scion wood, on EMLA 106 were analyzed for macro and micronutrient concentrations. We found that fruit from EMLA 26 had a higher calcium concentration, and a lower Mg:Ca ratio compared to fruit from EMLA 106. It could not be determined if high calcium caused resistance to bitter pit in scilate, however, apples from scionwood trees should not be commercially harvested due to lower calcium concentrations correlative with higher incidence of bitter pit.

Introduction

Bitter pit, a disorder common in apple fruits, is one of the largest nutritional problems that the apple industry and researchers have struggled to solve. Bitter pit results in dark necrotic spots found just under the skin within the first 0-5 millimeters (Ferguson 1983). All though it may occur before harvest, bitter pit more often occurs during storage. Bitter pit usually appears on the distal, or calyx end of the apple first, but in severe cases can appear surrounding the apple. Over the years, researchers have agreed that bitter pit is a result of calcium deficiency magnified

by environmental conditions such as alternating wet/dry conditions, excessive vegetative growth, light crop loads, and large fruit size.

The role of calcium in bitter pit has been investigated on a cellular level, and has indicated that calcium plays a role in binding cell membranes and maintaining their structure and function (Amarante 2013). Picchioni (1998) observed that calcium vacuum-infiltration led to a delay in membrane lipid catabolic processes as indicated by a delayed loss of membrane phospholipids, free sterols, and sterol conjugates. Picchioni (1998) also observed that calcium infiltration increased fruit firmness compared to the water-infiltrated control. Freitas (2010) hypothesized that bitter pit primarily occurs due to flux of free apoplasmic calcium to vacuoles and cell walls. Because of this flux away from the apoplasm, a reduction in free calcium occurs and the cell membranes become leaky and lead to cell death (Amarante 2013). High magnesium and potassium is often reported in apples susceptible to bitter bit and is thought to compete with calcium binding sites on the cell membrane (Amarante 2013). Magnesium vacuum-infiltration has been shown to increase bitter pit in apples, supporting the notion of competition for cell membrane binding sites (Ferguson 1983).

The distribution of mineral elements in apples has been studied extensively in relation to the development of bitter pit. In general, calcium concentrations decrease from the stem end to the calyx end of the apple (Hopfinger 1979, Perring 1986). The core has been shown to have the highest concentrations of calcium followed by the peel and then the flesh (Ferguson 1983, Perring 1986). The same trend is followed by magnesium but at higher concentrations (Ferguson 1983). During storage, calcium has been shown to re-distribute within the fruit. Ferguson (1983) observed that calcium and magnesium initially appeared to move from the core towards the skin. However, Ferguson (1983) observed that the Mg:Ca ratio increased within the outer flesh of the

apple, where bitter pit commonly occurs. When testing pitted tissue exclusively, the concentration of magnesium was much higher than non-pitted tissue, supporting the idea that high magnesium competes with calcium (Hopfinger 1979). The date the apples were picked had a significant effect on the translocation of calcium. Early picks showed an increase in core calcium (decrease in flesh) followed by a gradual outward movement away from the core, while later picks only showed translocation away from the core (Perring 1986). Bitter pit incidence was higher in earlier picks, which may indicate that the initial calcium movement out of the flesh led to the development of bitter pit (Perring 1986).

Since calcium has been identified as the main nutrient deficiency in bitter pit, many practices are utilized to increase calcium in fruits by soil fertilizer, foliar fertilizer, and cultural practices. In the xylem, calcium moves by mass flows to areas of high transpiration. Because fruits have low transpiration through their waxy cuticle, little calcium is transported to the fruit by the xylem. Calcium is mostly immobile in the phloem, so transportation to fruit in total is limited. Wilkinson (1968) showed that the concentration of calcium in apples decreased as the apple grew, which indicates that most of the calcium is transported to the fruit early in the season. Calcium fertilizers applied to the soil have given mixed results since calcium is mostly transported to the leaves. In soils with adequate calcium, bitter pit may even get worse upon addition of calcium because high soil salts limit transport to low transpiring parts by guttation. Foliar applications of calcium are widely utilized with eight or more sprays applied early in the growing season (Dave Cammack, personal communication). However, foliar applications have some conflicting results concerning their effectiveness since absorption of foliar calcium by leaves and transportation to fruit is minimal (Ferguson 1983, Val 2008). Foliar applied strontium, which has been used as a tracer for calcium, showed that only small amounts of strontium were

transported from the leaves to the fruit and that application to fruit was crucial (Rosen 2006). Wilkinson (1968) observed that some calcium might even move out of the fruit, which counteracts the benefits of foliar sprays. Cultural practices such as decreasing the relative humidity around the apple increased its calcium concentration by increasing fruit transpiration or decreasing the transpiration of leaves (Cline 1992). Thinning the crop, thought to make more calcium available to the left over fruit, negatively effected the calcium concentration in fruit and increased bitter pit (Telias 2006).

Sampling Methods to Predict Bitter Pit

The research on the mineral distribution of calcium and magnesium has lead to a standardized approach in New Zealand for sampling apple fruit to predict future bitter pit (Ferguson 1979). In developing a standardized sampling method four factors have been considered to reduce variability and improve the predictive power of reported calcium levels. The four factors for sampling include the processes and methods for sampling the fruit for mineral analysis, the size of the fruit selected, the cropping load of the trees sampled, and the part of the tree the fruit are picked.

Various methods for sampling fruit have been used to analyze mineral concentrations including whole fruit samples with seeds and stems removed, slices of whole fruit including portions of the core, slices without the core, segments of flesh and peel together, plugs of flesh, or the peel. Whole fruit samples, and samples that include portions of the core often give higher calcium limits and are less effective in predicting bitter pit because they don't discriminate for difference in calcium distribution within the fruit (Ferguson 1979). Segments of flesh and peel sampled together report higher calcium limits because the peel has more calcium and thus increases the concentrations identified (Wills 1976, Turner 1977). Cortical plugs of flesh and

samples of peels have been used fairly successfully to generate a predictive incidence of bitter pit because the samples focus on areas where bitter pit occurs (Turner 1977, Ferguson 1979, Telias 2006). Samples with peels may vary depending on whether the samples were washed or not, for example Wills (1976) reported high calcium concentrations in samples that were unwashed compared to fruit that were washed. In a recent study, sampling the soluble fraction, or extract, of the flesh or peel at the calyx end was most effective in predicting bitter pit based on the calcium concentration or Mg:Ca ratio (Amarenta 2013). Amarente (2013) indicated that the next most effective way was to sample the total fraction of a plug of flesh for calcium and the Mg:Ca ratio.

Variability between different apples on a tree occurs due to different sizes. Ferguson (1990, 1992) observed that as fruit size increases, calcium concentration decreases, which increased the incidence of bitter pit. Ferguson (1990) reported that calcium concentrations decreased by .05 milligrams of calcium per 100 grams of fresh weight for every 10-gram increase in fruit weight. This finding was in correlation with a 5% increase in the incidence of bitter pit for every 10-gram increase in fruit weight. Magnesium and potassium concentrations stayed constant or increased as the fruit weight increased, which lead to an increase in the Mg:Ca and K:Ca ratios supportive of increased bitter pit (Ferguson 1990).

Differences in crop load introduce variability in calcium between different trees sampled. In general, lighter crop loads have lower calcium concentrations and higher incidence in bitter pit in comparison to heavier crop loads in the same orchard (Ferguson 1990, Ferguson 1992, Turner 1977). The increased fruit size of light crop loads was thought to be the cause of the decreased calcium concentrations; however, Ferguson (1992) observed that bitter pit levels were different between fruit of the same size but from different crop loads. Ferguson (1992) reported that bitter

pit levels were 1% and 17% in heavy and light crop loads of 117-gram fruit. Levels were 3% and 29% for heavy and light crops of 168-gram fruit. Ferguson (1990) observed that the calcium concentration of light cropped trees decreased twice as fast as heavy cropped trees with increasing fruit size. Turner (1977) thought that the increased vegetative growth often observed on light cropping trees lead to less calcium transport to the fruit as it grew. Ferguson (1992) suggest that both size and cropping are independent factors that influence each other in determining calcium levels and incidence of bitter pit.

The position of the fruit on the tree also correlates with varying levels of calcium. No significant difference was found between fruit sampled from the inner part of the tree and the outer part (Wills 1976, Ferguson 1990). However, calcium concentrations in the fruit significantly decreased from the lower to the upper portions of the tree (Ferguson 1990). Ferguson (1990) suggested that sampling procedures should be consistent on what height the fruit are picked.

Objective

Recently, the apple cultivar known as Honeycrisp has been gaining increased attention by many growers; however, the production of Honeycrisp has proven to be a challenge due to its high susceptibility to bitter pit (Rosenberger 2004). Another new apple, out of New Zealand, that is gaining interest among club growers is Scilate, which is known to not be very susceptible to bitter pit (Brookfield 2010). The objective of this study was to identify if high calcium concentrations are the reason Scilate tends to be resistant to bitter pit by comparing results from mineral analysis to those reported in other studies. In the process we also wanted to validate higher concentrations of calcium in the peel compared to the flesh. Lastly, we wanted to see how

the combination of vegetative growth, fruit size, and crop load affects the calcium concentration of Scilate grown on different rootstocks.

Materials and Methods

The apples used for this study were apples picked from Scilate grown on either EMLA 26 or EMLA 106. EMLA 26 is a higher cropping rootstock that requires support for the crop load, while EMLA 106 is a more vigorous rootstock grown primarily for vegetative growth and thus has a low crop load. All of the trees received the same irrigation, fertilizer, and foliar calcium sprays. Early in the growing season, eight foliar sprays of 30% calcium were applied at five lbs/acre. Apples from each type were picked and stored in plastic bags in a refrigerator at 4.5 Celsius for two weeks.

After two weeks, 10 apples were selected from each bag that were similar in size and lacked abnormalities. Each apple was washed in distilled water and allowed to dry. After drying, each apple was weighed and an initial fresh weight was recorded. The bottom one centimeter of the apple was cut off and then another one-centimeter disk was cut from the bottom of the apple. Three to four cortical tissue plugs were taken with a cork borer just inside the skin and then weighed. The peel around the disk was then removed and weighed. This process was repeated for all 20 apples. Samples were then placed in a drying oven set to 80 Celsius for 48 hours. Once dry, each sample was then weighed, bulked with like samples, and ground to fine powder in a coffee grinder. This process was repeated for all four sample types (EMLA 106 flesh and peel, EMLA 26 flesh and peel). Samples were then sent to USU and analyzed in a thermo electron iCAP ICP (inductively-coupled plasma spectrophotometer), which reports micronutrients in milligrams per kilogram dry weight and macronutrients in percent. For comparison to values reported in other literature, concentrations of calcium, magnesium, and potassium were

converted to milligrams per 100 grams fresh weight. In order to predict bitter pit, data reported by several studies on Cox's Orange Pippin apples were compiled and the calcium concentration, magnesium concentration, and Mg:Ca ratio were plotted over bitter pit incidence (Figure 1)

Results

Scilate from trees grown on EMLA 26 rootstock had a lower average fresh weight of 225.4 grams. These fruits were visually smaller and tended to have more green undertones. Scilate from trees grown on EMLA 106 had a higher fresh weigh of 304.9 grams. The fruit from these trees were visually larger and tended to have more yellow undertones and less reddening. The difference in color is most likely due to differences in shading, since EMLA 106 trees are grown as stock plants for scion wood and thus provide much more shade.

A complete mineral analysis on the fruit (excluding nitrogen) reported several differences between flesh and peel, and rootstock (Table 1). The same trends occurred from flesh to peel regardless of the rootstock. The peel had higher concentrations of boron, calcium, iron, magnesium, manganese, molybdenum, and sulfur. The flesh had higher concentrations of copper, potassium and zinc, while phosphorus was similar in the flesh and peel. EMLA 106 saw a greater percent increase of calcium and magnesium from flesh to peel compared to EMLA 26. The Mg:Ca was lower in the peel compared to the flesh regardless of fruit source (Table 2).

Differences in EMLA 26 and EMLA 106 are more informative in relation to bitter pit. EMLA 106 had higher concentrations of boron, iron, potassium and molybdenum in the flesh, while copper, magnesium, phosphorus, sulfur, and zinc were similar between EMLA 106 and EMLA 26. However, EMLA 26 had a significantly higher concentration of calcium (.015% vs. .008%) and manganese in the flesh. These same trends were observed in the peels of both fruit

sources except that EMLA 106 had higher concentrations of magnesium than EMLA 26. The Mg:Ca ratio was much higher in the flesh and peel of EMLA 106 than in EMLA 26.

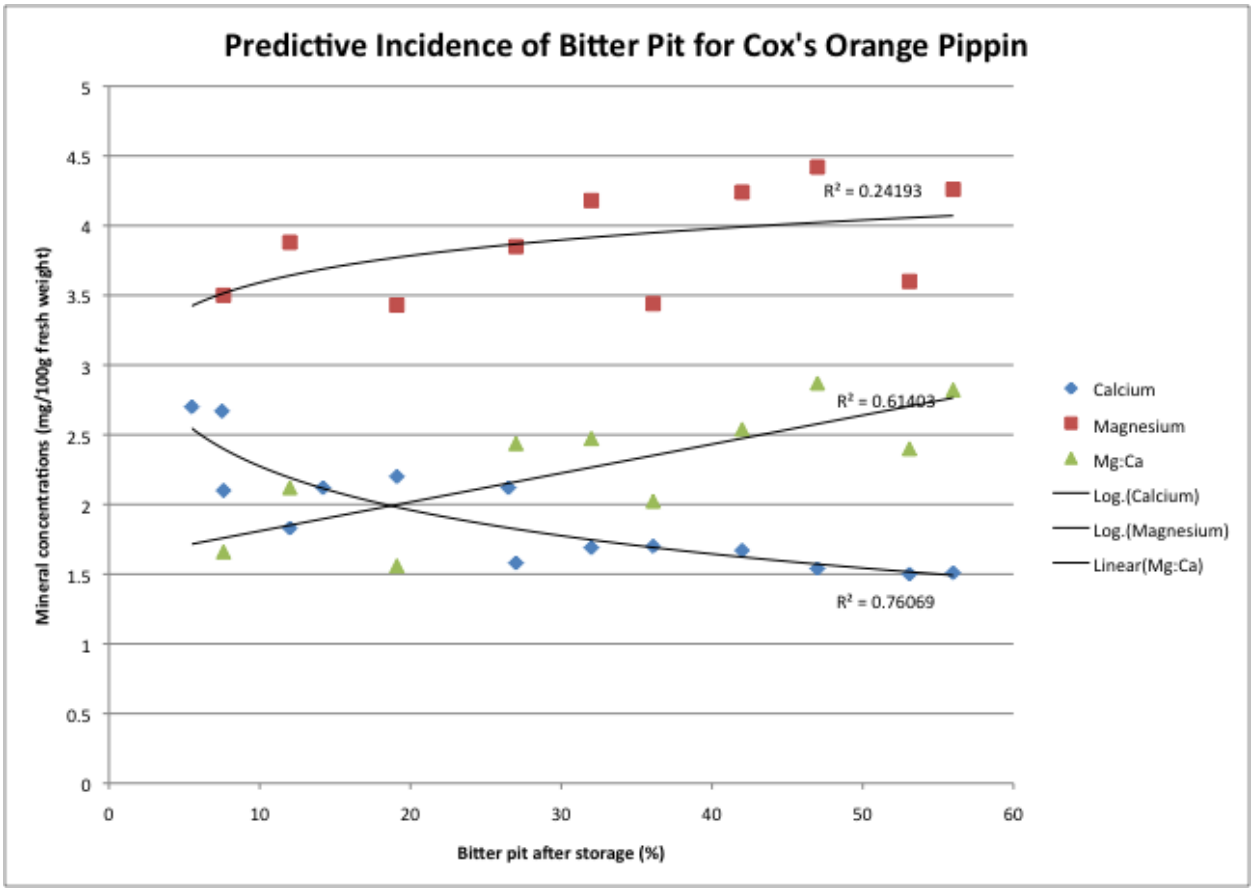
Table 1. Mineral analysis of EMLA 106 and EMLA 26 flesh and peel

		EMLA 26			EMLA 106		
		Flesh	Peel	% change flesh to peel	Flesh	Peel	% change flesh to peel
BORON	mg/kg dry	18.98	20.01	5.41	42.57	49.08	15.30
CALCIUM	mg/kg dry	150	640	326.67	80	390	387.50
COPPER	mg/kg dry	9.80	3.09	-68.48	9.27	3.66	-60.53
IRON	mg/kg dry	9.61	21.76	126.40	12.75	28.40	122.84
POTASSIUM	mg/kg dry	6880	6080	-11.63	8050	6860	-14.78
MAGNESIUM	mg/kg dry	280	760	171.43	280	870	210.71
MANGANESE	mg/kg dry	1.38	4.68	239.15	1.17	4.21	260.70
MOLYBDENU	mg/kg dry	<0.05	0.17		0.19	0.31	65.36
SODIUM	mg/kg dry	<0.05	12.07		<0.05	<0.05	
PHOSPHORU	mg/kg dry	720	700	-2.78	660	680	3.03
SULFUR	mg/kg dry	240	360	50.00	230	440	91.30
ZINC	mg/kg dry	285.67	16.86	-94.10	290.23	16.07	-94.46

Table 2. Summary of mineral analysis related to bitter pit

Scilate mineral analysis (mg/100g fresh weight)							
Rootstock	Crop load/source	Avg. Fruit weight (g)		Ca	Mg	K	Mg:Ca
EMLA 26	Heavy/orchard	225.4	Flesh	2.24	4.19	102.87	1.87
			Peel	13.88	16.48	131.85	1.19
EMLA 106	Light/stock plant	304.87	Flesh	1.25	4.38	125.95	3.50
			Peel	9.09	20.28	159.92	2.23

Figure 1. Bitter pit incidence in relation to calcium, magnesium and Mg:Ca



(Taken from: Turner 1977, Ferguson 1979, Ferguson 1990, Ferguson 1992)

Discussion

All though there are many differences in nutrient concentrations of the several nutrients included in the analysis, only calcium and magnesium will be discussed here due to their known relationship with bitter pit. Because this experiment didn't control for variability from fruit size or from rootstock effects, we can only make general observations and minor conclusions. As observed in table 1, most nutrients increase or stay constant in the peel compared to the flesh, including calcium and magnesium. This observation occurred regardless of the rootstock the tree grew on and agrees with data reported by Perring (1986). Elevated levels of calcium in the peel may be partially due to foliar applications of calcium (Val 2008). However, Perring (1986) observed that the peel had more calcium regardless of whether it was sprayed or not. The trend towards lower concentrations of calcium in the flesh is the basis for the relationship between bitter pit and its location in the fruit (Ferguson 1983).

Calcium inhibition by magnesium in the flesh is thought to lead to the development of bitter pit (Amarante 2013). The mineral analysis reported more magnesium than calcium in both the flesh and the peel of the apples. This is most likely due in part by the limited movement of calcium in the phloem. Redmund (1975) observed that water and nutrient flow to the apple occurred through the xylem during early stages of fruit development in June and July, but later became almost exclusively fed through the phloem (Wilkison 1968, Telias 2006). Magnesium is found in higher concentrations in Scilate apples, which supports greater mobility of magnesium in the phloem . In EMLA 26, a higher concentration of calcium was identified along with a reduced Mg:Ca ratio compared to EMLA 106 (Table 2). The higher concentrations of calcium are most likely due to smaller fruit size and heavier crop load similar to results observed by Ferguson (1990, 1992). If calcium transport into the fruit is decreasing as the fruit continues to

grow, then the overall calcium concentration will be less depending on the end size of the fruit. Magnesium follows a similar trend of decreasing concentrations overtime, but because magnesium is more phloem mobile, this decrease is much less compared to calcium (Telias 2006).

Scilate is a fairly new cultivar, and thus little research has reported optimum calcium levels or a Mg:Ca ratio that contributes to bitter pit resistance; however, Scilate is reported to be resistant to bitter pit (Brookfield 2010). In order to identify if calcium is the major contributor to resistance, mineral concentrations from our analysis were converted into units commonly reported from research in New Zealand on Cox's Orange Pippin (Table 2, Figure 2). When comparing the calcium concentrations (2.24 mg/100g fresh) and Mg:Ca ratios (1.87) to the values reported in New Zealand (Figure 1), we predict that about 10% of the Scilate on EMLA 26 will develop bitter pit in storage. In comparison, Scilate on EMLA 106 had calcium and levels of 1.25 mg/100g fresh and a Mg:Ca ratio of 3.5 that leads to a prediction of >50% bitter pit. As mentioned above, this is most likely due to the large fruit size, light crop load, and inhibition of foliar calcium contact with the fruit due to vegetative growth. Accuracy in this prediction is limited since different cultivars have different nutrient levels and susceptibility to bitter pit. However, of the left over fruit, only the apples from EMLA 106 developed bitter pit after a month of storage. The fruit grown on EMLA 106 should not be picked with the fruit grown on EMLA 26 because they are more likely to develop bitter pit. Since the reported calcium concentration is not high compared to other reported values, we could not conclude that high calcium concentration contributes to Scilates resistance to bitter pit.

Conclusion

Bitter pit has been researched for many years; however, it continues to plaque the apple fruit industry. Our research is very limited due to the lack of controls for variability from crop size, fruit size, rootstock effects, and a test for bitter pit incidence. However, in our analysis of calcium in and magnesium in Scilate, we found our data to confirm that concentrations of calcium and magnesium are higher in the peel, and that a combination of fruit load and fruit size affects these concentrations. Our data does not prove that Scilate is resistant to bitter pit because of high calcium concentrations, and thus research with more controls and a measure of actual bitter pit should be done to better identify the cause of the reported resistance to bitter pit from Brookfield (2010). A comparative study of different apple cultivars would also be useful for creating a concentration reference for growers trying to reach optimal levels to reduce bitter pit.

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